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Prefrontal cortex and spinal cord mediated anti-neuropathy and analgesia induced by sarcosine, a glycine-T1 transporter inhibitor ☆

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ABSTRACT

Sarcosine is a competitive inhibitor of glycine type 1 transporter. We hypothesized that it may have analgesic and anti-neuropathic efficacy by a dual action: affecting neurotransmission in the prefrontal cortex as well as within the spinal cord. In rats with spared nerve injury (SNI) oral sarcosine reduced mechanical sensitivity for the injured limb (anti-neuropathy or anti-allodynia) as well as for the uninjured limb (analgesia), showing better dose efficacy for the injured limb. Intrathecal administration of sarcosine was more effective in reducing mechanical sensitivity for the uninjured paw. In contrast, prefrontal cortex infusions of sarcosine acutely reduced mechanical sensitivity for the injured paw. Repeated daily oral sarcosine induced anti-neuropathy, observed only after days of repeated treatment; this long-term effect disappeared a few days after treatment cessation. The findings indicate that manipulating glycine-T1 transporter at multiple central sites can induce acute analgesia, as well as acute and long-term reduction in neuropathic pain behavior. Analgesic effects seem primarily mediated through spinal cord circuitry while anti-neuropathic effects seem mediated through prefrontal cortex circuitry, most likely through distinct molecular pathways. The results suggest that such an approach may provide a novel venue for treating clinical pain conditions.

1. Introduction

Chronic neuropathic pain conditions have a huge impact on society and on health care. Mechanisms underlying such conditions, especially in human clinical states, remain poorly understood, as a result available therapeutic approaches are limited and lack efficacy. Studies in animal models have demonstrated a long list of changes in the periphery as well as in the spinal cord that ensue following a neuropathic injury and are potential therapeutic targets [30,34]. Additionally, accumulating evidence from human brain imaging studies point towards cortical reorganization in chronic pain, many of which tightly correlate to clinical characteristics [1–3,14,19,29,32], implying that targeting components of this circuitry may also have therapeutic benefits. The present study attempts to take advantage of this new knowledge and tests the efficacy of manipulating neuropathic pain behavior by modulating glycinergic pathways in the cortex as well as the spinal cord.

We recently demonstrated that manipulating glycine availability at the NMDA receptor in the cortex, specifically in the medial prefrontal cortex (mPFC) and the amygdala, can give rise to long-term reduction in neuropathic pain behavior [26]. The study used oral or central infusions of D-cycloserine (DCS), a partial agonist at the strychnine-insensitive glycine-recognition site on the NMDA receptor complex [13], and showed that oral and mPFC, but not intrathecal, DCS reduce tactile sensitivity in rat models of neuropathic pain. DCS appears to modulate tactile sensitivity only for the neuropathic injured limb, in a dose-dependent manner and with increasing efficacy for up to 3 weeks of oral treatment. The study demonstrates a potential therapeutic drug for chronic pain with a purely supraspinal target. Here we study manipulating the glycine transporter as an alternate route with which availability of glycine in the central nervous system can be altered, and test its effects on pain behavior acutely and during long-term treatment.

Glycine is a major inhibitory neurotransmitter in the spinal cord and the brainstem, and participates in excitatory neurotransmission by modulating NMDA receptors throughout the central

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nervous system [15]. Extracellular glycine concentration is regulated by glycine transporters (GlyTs), and pharmacological and genetic studies show that both glycinergic inhibitory and glutamatergic excitatory neurotransmissions are regulated by GlyTs [15]. Two GlyT subtypes have been identified: GlyT1 is localized mostly on glia, and GlyT2 is localized on presynaptic terminals of glycinergic inhibitory interneurons. GlyT1 reduces glycine concentrations at NMDA receptors [5,7] and eliminates glycine from the synaptic cleft terminating glycinergic neurotransmission [11]. A number of antagonists to GlyTs have been identified, and recent studies have explored their effects on neuropathic behavior, concentrating on spinal cord modulation following acute, single dose treatment [17,27,31]. Given that DCS in the cortex shows anti-neuropathic effects [26], we reasoned that a GlyT1 antagonist could have a dual action of potentiating anti-neuropathic effects by NMDA-mediated processes in the cortex and by enhancing inhibitory circuitry in the spinal cord. Therefore, we tested the efficacy of sarcosine, a preferential GlyT1 inhibitor [22,28], on spared nerve injury (SNI) animals when administered through different routes, acutely as well as repeatedly over a two-week period.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats (obtained from Harlan, Indianapolis, IN; 200–250 g) were housed in groups of two or three and kept on a 12-h light/dark cycle with food and water available *ad libitum*. Experimental procedures were in accordance with the policies and recommendations of NIH guidelines (NIH publication No. 86–23, 1996), IASP guidelines for use of conscious animals in pain research [35], and all experiments were approved by the Northwestern University Institutional Animal Care and Use Committee. A single experimenter performed the behavioral measures, blinded to treatment groups, and using the method of equal blocks to minimize environmental variation of response and expectation bias.

2.2. Drugs

Sarcosine (*N*-Methylglycine), purchased from Sigma–Aldrich, USA, was used in these experiments. Sarcosine was administered orally (p.o.), intrathecally (i.t.) (while under light gas anesthesia), or infused into the right mPFC through implanted cannula.

2.3. Neuropathic pain – spared nerve injury (SNI)

The method used to induce nerve injury has been previously described in detail [9]. Rats were anesthetized with 5% isoflurane and a mixture of 30% N_2O and 70% O_2. The left sciatic nerve was exposed at the level of its trifurcation into the sural, tibial, and common peroneal nerves. The tibial and common peroneal nerves were tightly ligated and then completely severed between the ligations, leaving the sural nerve intact. Behavioral experiments began at least one week after SNI surgery.

In sham animals the same surgical procedure way used, however, the sciatic nerve was not manipulated.

2.4. Experimental design

The effects of varying doses of sarcosine on neuropathic pain behavior were assessed for the following conditions: single oral application at varying concentrations, single i.t. application, repeated oral application over several weeks, and infusion directly into the right mPFC.

2.5. Acute oral doses

The effects of oral administration of saline or sarcosine (50, 250, or 500 mg/kg) on pain behavior were observed at 1, 2, 3, and 24 h after gavage. The different concentrations of sarcosine (in 2 ml of saline) were administered at fixed times using stainless steel 16 G gavage needle. Animal weights were measured the day before each drug administration to accordingly adjust the volume of oral treatments.

2.6. Intrathecal injections

Rats were lightly anesthetized (isoflurane 3–5%, 3–5 min) and given i.t. injections of saline or sarcosine (250 μg) at the L5–L6 level. The effects of i.t. injections of saline or sarcosine on pain behavior were observed for up to 80 min after injection.

Intrathecal injections were made following the techniques described in the past [12,24]. Briefly, a 26 G needle (one inch in length) connected to a 25 μL Hamilton syringe was positioned with the bevel side facing upward and toward the head, and inserted in the L5–6 intervertebral space at a 75–80° angle. Once the needle made contact with bone, its incline was changed to about 30° to access the subarachnoid space. A flick of the rat's tail provided the indication that the needle had penetrated the dura. At this point 10 μl of the drug solution was injected into this presumed subarachnoid space.

2.7. Acute brain infusions

Rats were anesthetized and implanted with a guide cannula (26 gauge, Plastics One Inc., Canada) using the following stereotaxic coordinates for the right mPFC (contralateral to SNI): anteroposterior +2.9 mm from bregma; mediolateral −1 mm from midline; dorsoventral −4.1 mm from skull surface, with a mediolateral angle at 11° (the same coordinates as in [26]). SNI surgery took place about 6 days after cannulation.

Implanted animals were infused with either saline or sarcosine (50 μg in 0.5 μl in 2 min) and the effects on pain behavior were tested over a 24-h period, two weeks after SNI or sham surgeries.

2.8. Repeated oral doses

Rats were treated twice a day orally with either saline or sarcosine at a dose of either 250 or 500 mg/kg for 14 consecutive days. Solutions (in 2 ml saline) were administered by gavage at fixed times each day. Pain behavior was tested twice a week for two weeks during treatment, and followed for up to another two weeks after termination of treatment. Pain testing took place before the morning drug administration (about 15 h after last treatment) to minimize the contribution of acute sarcosine effects. Animal weights were measured the day before and seven days after start of treatment to accordingly adjust the volumes of oral treatments.

2.9. Behavioral tests: tactile sensitivity

Mechanical sensitivity of the hind paws was measured using hind paw withdrawal responses to the Von Frey filaments. Rats were placed in a Plexiglas box with a wire grid floor and allowed to habituate for 10–20 min to the environment. Filaments of varying forces (Stoelting, USA) were applied to the plantar surface of the hind paw. Filaments were applied in either ascending or descending strengths to determine the filament strength closest to the hind paw withdrawal threshold (as described in [6]). Each filament was applied for a maximum of 2 s at each trial; paw withdrawal during the stimulation was considered a positive response. The minimum stimulus intensity was 0.008 g with a maximum

cutoff of 45 g. Given the response pattern and the force of the final filament, 50% response threshold (in grams) was calculated according to [6].

2.10. Cold sensitivity

To measure sensitivity to cold, 1 drop (about 0.1 ml) of acetone solution was applied on the lateral hind paw of the rat. Rats were observed for 5 min and the duration of their withdrawal reaction (0–4 scale), as well as their withdrawal behavior (withdrawing, flicking, licking, and vocalizing) was recorded according to [8].

2.11. Motor activity

An open field apparatus (45 × 45 × 45 cm) was placed in a quiet room illuminated with dim white light. The floor of the apparatus was equally divided into nine squares (15 × 15 cm). Rats were individually placed into the open field on the central square and their spontaneous behavior was videotaped for 5 min. This was done 1½ h post-treatment. The total number of squares visited was used to assess general motor activity. The number of rearings, entries into the central square, and grooming episodes were analyzed as indirect markers of stress.

2.12. Statistical analyses

Statistical measures for tactile sensitivity thresholds were performed independently for each paw, as thresholds for injured and uninjured paws differ in the SNI model and the experimenter can visually identify the injured paw by its position. Mechanical response thresholds were measured for multiple doses at different time points, and for different routes of treatment. Responses were normalized relative to the baseline response for each animal, measured just before the drug administration. This corrects for deviations due to individual baseline differences and provides the opportunity to compare effect sizes across conditions, and for both injured and healthy paws. Drug effects were compared using a repeated-measures analysis of variance (RM-ANOVA) for dose, treatment (saline vs drug), surgery (sham vs SNI), and time from treatment as the repeated measure, and percent change in tactile threshold from baseline as the dependent variable. Cold allodynia and open field responses were analyzed similarly. Post hoc comparisons were tested using Fisher's LSD ($p < 0.05$). For the acute oral treatment 428 tactile response measures were collected. Of these nine were excluded as outliers (>2.5 standard deviations from the group mean). For the intrathecal study 192 tactile response measures were collected, four of which were excluded as outliers.

3. Results

3.1. Anti-nociceptive effects of acute oral sarcosine

The acute effects of oral administration of saline or sarcosine (50, 250, and 500 mg/kg) were tested in SNI rats at 1, 2, 3, and 24 h after administration. Each dose was tested in contrast to saline in separate weeks ($n = 8$ animals per group), starting two weeks after the SNI surgery. Tactile threshold changes relative to baseline were measured for the injured (left paw) and uninjured limbs (right paw).

For the SNI-injured paw, there were significant main effects of dose ($F_{2,43} = 3.7$, $p < 0.04$), time ($F_{3,129} = 8.1$, $p < 10^{-4}$), and a borderline treatment effect ($F_{1,43} = 3.5$, $p < 0.07$). The largest interaction was between time and treatment ($F_{3,129} = 6.1$, $p < 0.0007$), and a borderline interaction between time and dose ($F_{3,129} = 2.1$, $p < 0.06$). Fig. 1A–C shows efficacy of each dose separately, for the SNI-injured paw. At 500 mg/kg, sarcosine-treated animals showed robust increase in tactile threshold at 1, 2, and 3 h post-treatment. Post hoc analysis indicates a treatment difference only at 2 h post-treatment for 50 mg/kg, and no effect at any of the times tested for 250 mg/kg.

For the uninjured paw, there was a significant main effect of time ($F_{3,141} = 3.7$, $p < 0.01$), a borderline treatment effect ($F_{1,47} = 3.6$, $p < 0.06$), and significant interactions between time and treatment ($F_{3,141} = 2.7$, $p < 0.05$) as well as between treatment and dose ($F_{2,47} = 4.8$, $p < 0.01$). Fig. 1D–F shows efficacy of each dose separately, for the uninjured paw. Post hoc analysis shows significant differences between drug and saline only at the highest treatment dose (500 mg/kg), and at 1, 2, and 3 h after drug administration, relative to saline at corresponding times. In a separate group of animals ($n = 8$ per group) we tested the effects of acute oral saline or sarcosine only for 250 and 500 mg/kg. The results (not shown) indicated a small but significant increase in tactile threshold for the injured paw for the lower dose, a larger significant increase for the higher dose, and also a significant increase for the uninjured paw at the higher dose only. Taken together, acute oral sarcosine decreased tactile sensitivity for injured and uninjured limbs, but more efficiently for the injured limb.

3.2. Anti-nociceptive effects of acute application of sarcosine to the lumbar spinal cord

The acute effects of lumbar intrathecal administration of saline or sarcosine (250 μg in 10 μl) ($n = 7$ animals per group) were tested in SNI rats at 20, 40, 60, and 80 min after administration. Animals were tested starting at least two weeks after the SNI surgery. Intrathecal injections were given under gas anesthesia, and all animals were mobile within 5 min after the injection. Tactile threshold changes relative to baseline were measured for the injured (left paw) and uninjured limbs (right paw).

For the SNI-injured paw, there was only a borderline significant interaction effect between time and treatment ($F_{3,30} = 2.6$, $p < 0.07$). Fig. 2A shows efficacy of intrathecal sarcosine for the SNI-injured paw. According to post hoc analysis, 250 μg of sarcosine increased tactile threshold for the injured paw at 40 min following treatment. Fig. 2B shows efficacy of intrathecal sarcosine for the uninjured paw. Here, there were main effect differences for treatment ($F_{1,10} = 21.9$, $p < 0.001$), time ($F_{3,30} = 10.5$, $p < 0.0001$), and for the interaction between both factors ($F_{3,30} = 8.0$, $p < 0.0004$). Post hoc analysis indicates that 250 μg of sarcosine increased tactile threshold at 20 and 40 min after treatment. Thus, intrathecal sarcosine changes tactile thresholds more efficiently for the uninjured rather than the injured limb.

3.3. Anti-nociceptive effects of acute application of sarcosine to the medial prefrontal cortex

Thirty rats were initially implanted with prefrontal cortical cannulas. A week later half of these received SNI injury on the left limb, and the other half received sham left limb surgery. At least two weeks later cortical sarcosine infusion effects could only be tested in 13 sham and 11 SNI animals, as the cortical cannulas were not viable in the rest. A dose of 50 μg of sarcosine in 0.5 μl saline in contrast to saline, was tested in SNI and in sham animals at 1, 2, 3, and 24 h after the infusions, for tactile responses for the injured and uninjured limbs.

For the SNI-injured paw, there were borderline main effects for type of surgery (sham vs SNI; $F_{1,19} = 3.8$, $p < 0.07$) and for treatment (drug vs saline; $F_{1,19} = 3.8$, $p < 0.07$), a significant time effect ($F_{3,57} = 4.9$, p <0.004), a borderline time by type of surgery

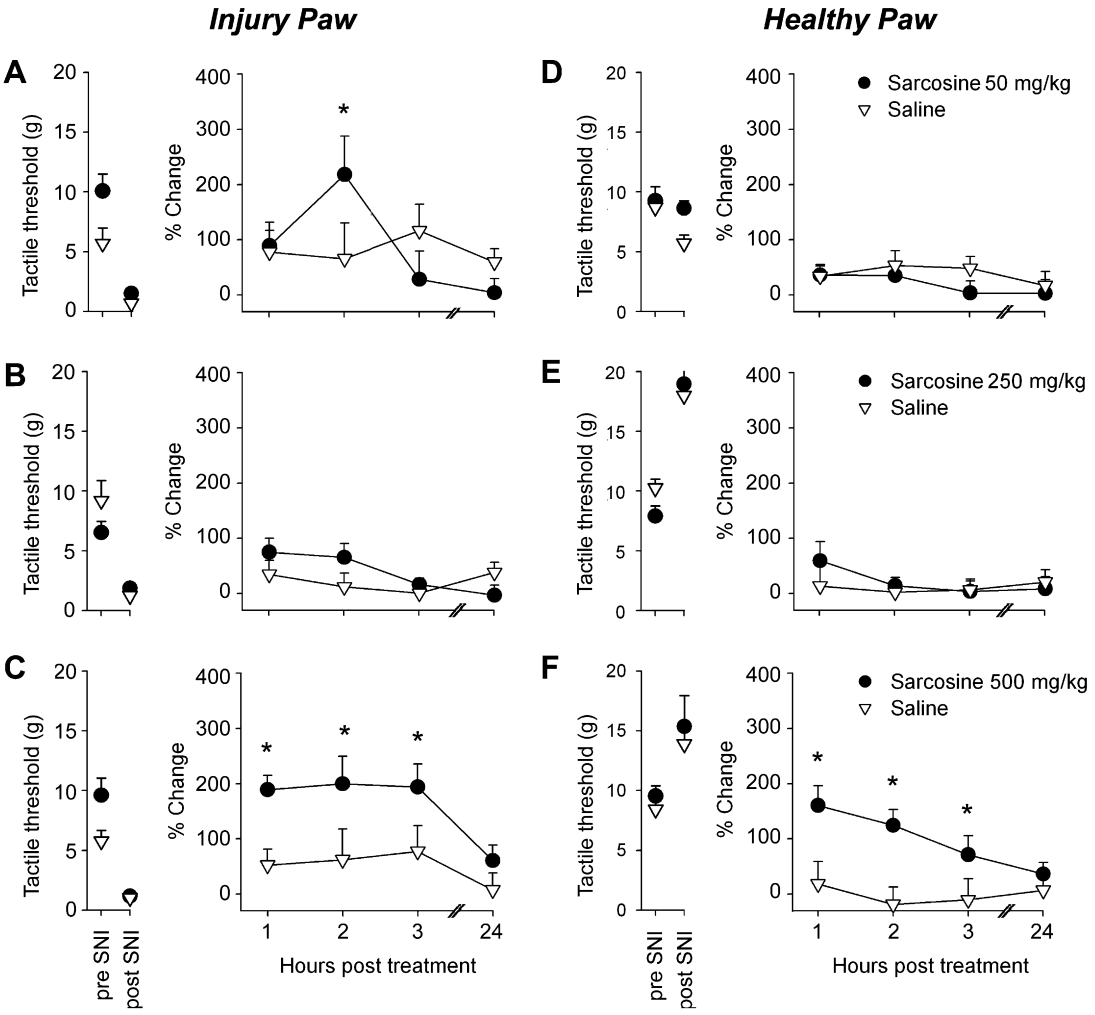

Fig. 1. Oral single dose of sarcosine reduced tactile sensitivity for the SNI-injured paw and for the uninjured paw, dose-dependently. Tactile responses were measured at baseline and 1, 2, 3, and 24 h after acute treatment ($n = 8$ per group) with saline (triangles) or sarcosine (circles). Average percent changes from baseline are shown for sarcosine doses of 50, 250, and 500 mg/kg in (A–C), respectively, for SNI-injured paw, and for the uninjured paw in (D–F) for the same corresponding doses. Tactile thresholds pre-SNI injury and at baseline (post-SNI, just before treatment) are shown in the left panels. Change in response threshold relative to the post-SNI values is shown in percentage, as a function of time. Asterisks indicate statistically significant differences when compared to the saline group. Error bars indicate SEM.

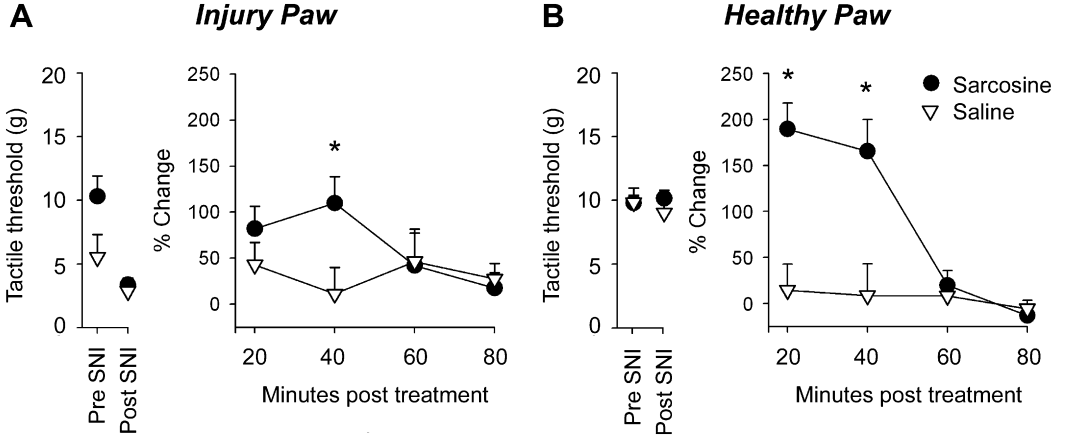

Fig. 2. Single intrathecal injection of sarcosine transiently reduced tactile sensitivity for the SNI-injured paw (A) and for the healthy paw (B), more effectively for the healthy paw. Tactile responses were measured for up to 80 min from treatment ($n = 7$ animals per group) with saline (triangles) or sarcosine (250 μg) (circles) in SNI rats. Tactile thresholds pre-SNI injury and at baseline (post-SNI, just before treatment) are shown in the left panels. Change in response threshold relative to the post-SNI values is shown in percentage, as a function of time. Asterisks indicate statistically significant differences when compared to the saline group. Error bars indicate SEM.

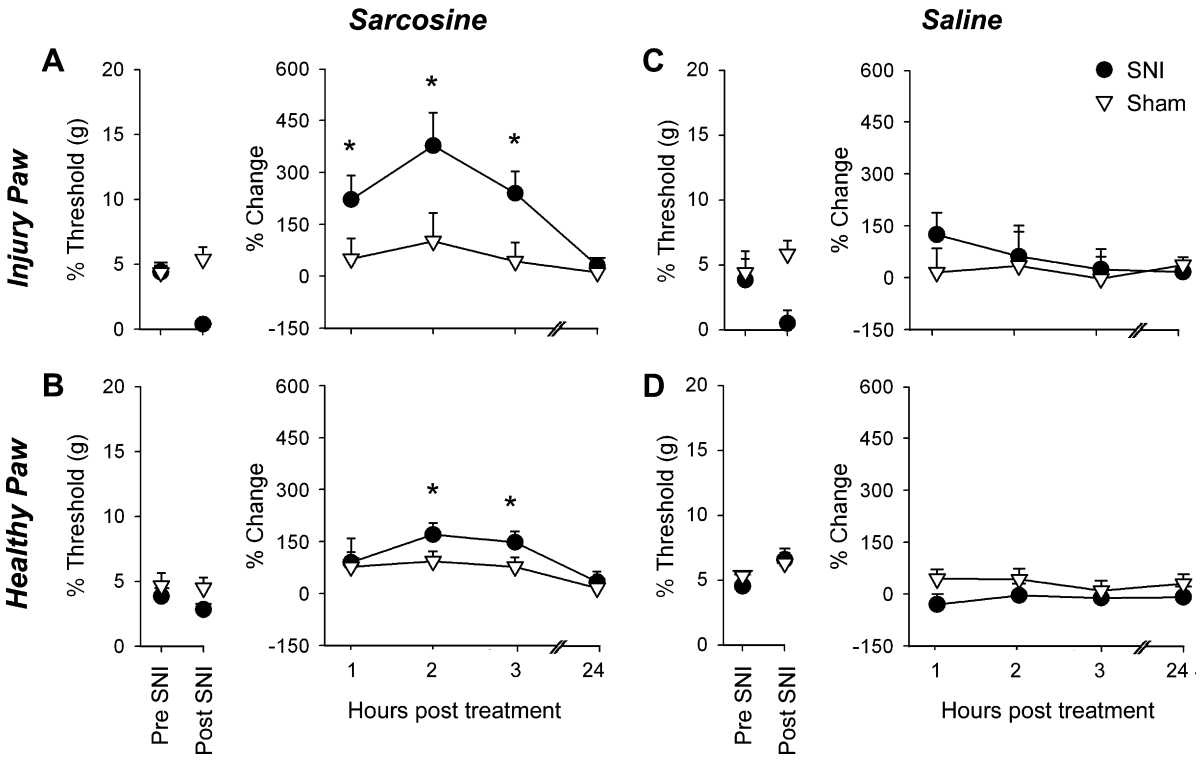

Fig. 3. Acute infusion of sarcosine in the medial prefrontal cortex induced increased tactile thresholds, mainly for the SNI-injured paw. Effects of infusing sarcosine (50 μg in 0.5 μl) (A and B) or saline (C and D) in medial prefrontal cortex were tested in SNI ($n = 11$) (circles) and sham ($n = 13$) (triangles) animals, on the SNI-injured (A and C) and healthy paws (B and D). Tactile sensitivity was monitored over 24 h. Tactile thresholds pre-SNI injury and at baseline (post-SNI, just before treatment) are shown in the left panels. Change in response threshold relative to the post-SNI values is shown in percentage, as a function of time. Asterisks indicate statistically significant differences when compared to the sham group. Error bars indicate SEM.

interaction ($F_{3,57} = 2.3$, $p < 0.08$), and a significant time by treatment interaction ($F_{3,57} = 3.5$, $p < 0.02$). Fig. 3A and C summarizes the results for the injured paw. At 1 h after sarcosine infusion in SNI animals, mechanical responses are higher than saline infusion in sham animals at 1 h. At 2 and 3 h the responses to sarcosine infusion in SNI animals were larger than all three control conditions (infusion of saline in SNI, infusion of sarcosine in sham, and infusion of saline in sham), and in comparison to responses at 24 h after sarcosine infusion. For the uninjured paw, we observe a main effect of type of treatment ($F_{1,19} = 11$, $p < 0.003$), time ($F_{3,57} = 5.3$, $p < 0.003$), a borderline interaction between surgery type and treatment type ($F_{1,19} = 3.6$, $p < 0.07$), and a significant interaction between time and surgery type ($F_{3,57} = 5.0$, $p < 0.004$). Fig. 3B and D summarize the results for the uninjured paw. Sarcosine-treated SNI animals showed an increase in mechanical threshold relative to saline-treated SNI animals at 1, 2 and 3 h, and an increase relative to saline-treated sham animals at 2 and 3 h. In the sham animals, infusion of sarcosine resulted in increased responses at 1, 2, and 3 h only in comparison to 24 h, but not relative to saline infusion. In summary, sarcosine infusion into the medial prefrontal cortex increases tactile thresholds for both the injured and the uninjured paw following SNI surgery. However, these anti-nociceptive effects were more robust for the injured paw.

3.4. Repeated oral sarcosine shows long-term anti-neuropathic effect

Nineteen animals underwent SNI surgery on the left limb and at least two weeks later they were treated orally with either saline ($n = 9$) or sarcosine (250 mg/kg) ($n = 10$), twice a day for 14 days. Tactile responses were measured relative to baseline on injured and uninjured limb twice per week over a month, at about 15 h after last treatment. At this dose repeated treatment effects were only observed on the injured paw and only at 10 and 14 days (Fig. 4A). For the SNI-injured paw, there was a borderline main effect of treatment ($F_{1,17} = 3.6$, $p < 0.06$), as well as a time effect ($F_{3,51} = 3.6$, $p < 0.02$). In contrast to saline, sarcosine treatment produced a borderline increase in tactile threshold for the injured paw at 10 days and a significant increase at 14 days. There was no significant difference for the uninjured paw between saline and sarcosine, and there were no significant differences for either limb over the 14 days of monitoring after cessation of treatment.

We repeated long-term sarcosine treatment study in a new group of animals at a higher dose. In this case we tested twelve animals after SNI injury, and another nine sham injury animals were studied. The SNI animals received either saline ($n = 6$) or sarcosine (500 mg/kg) ($n = 6$), sham injured animals received only sarcosine (500 mg/kg) ($n = 9$), twice a day for 14 days. Tactile responses were again measured about 15 h after last treatment. Treatment effects were only seen for in the SNI animals and only for the injured paw, at days 7, 10, and 14 (Fig. 4B). For the injured paw, comparing between sham animals, and SNI with saline or sarcosine treatment we observe a time effect ($F_{6,108} = 2.6$, $p < 0.3$) and a borderline interaction between time and groups ($F_{12,108} = 1.7$, $p < 0.08$). Comparison between SNI treated with saline or sarcosine for days 7, 10, and 14 shows a significant difference ($F_{1,18} = 6.9$, $p < 0.02$). There was no significant difference for the uninjured paw between saline and sarcosine.

3.5. Open field and cold sensitivity

Open field and cold sensitivity were studied in all the groups described above. Sarcosine treatment did not modulate responses in either test.

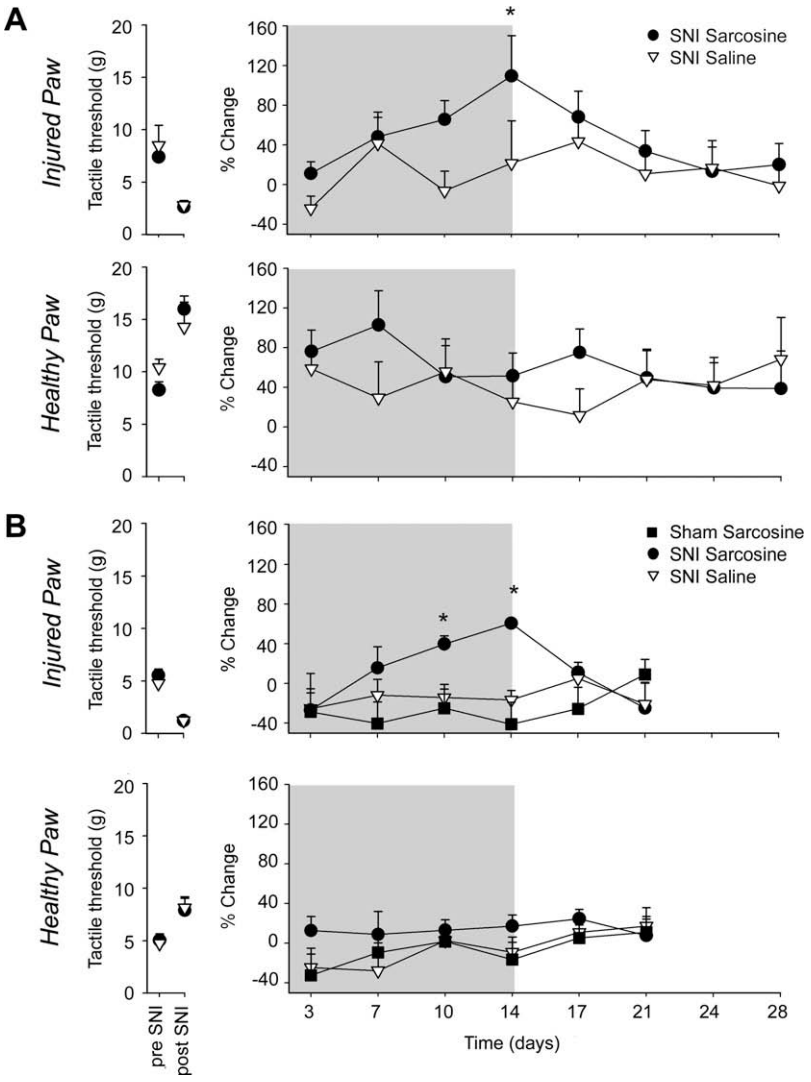

Fig. 4. Repeated oral sarcosine resulted in long-term increased tactile sensitivity only for the SNI-injured paw. Rats with SNI surgery or sham were treated twice daily with sarcosine or saline and tactile responses were assessed twice a week. (A) Rats with SNI injury were treated with sarcosine (250 mg/kg) ($n = 10$) (circles) or saline ($n = 9$) (triangles) for two weeks, and their responses were monitored over four weeks. (B) Rats with SNI injury received either saline ($n = 6$) or sarcosine (500 mg/kg) ($n = 6$) while rats with sham injury received sarcosine (500 mg/kg) ($n = 9$) (squares), twice a day for 14 days. Tactile responses were monitored for three weeks. Tactile thresholds pre-SNI injury and at baseline (post-SNI, just before treatment) are shown in the left panels. Change in response threshold relative to the post-SNI values is shown in percentage, as a function of time. Asterisks indicate statistically significant differences when compared to the sham group. Error bars indicate SEM.

4. Discussion

The main finding of this study is that treatment with sarcosine, a preferential GlyT1 inhibitor, reduces tactile sensitivity on the injured paw as well as on the uninjured paw in a rat model of neuropathic pain. We therefore interpret that sarcosine can lead to both acute analgesia and reduce the hypersensitivity occurring in the injured paw (i.e., an anti-allodynia effect). Both effects could be observed acutely and showed some dose dependence. Repeated sarcosine treatment effects were only observed on the injured paw, and continued to improve in efficacy over a two-week treatment period. As sarcosine treatment (acute and repeatedly over two weeks) did not change motor behavior, its effects cannot be attributed to neurotoxicity. Most intriguingly, the effects of sarcosine on pain behavior showed administration site specificity, with cortical infusion reducing mainly the injured paw sensitivity while spinal cord administration reducing more efficiently sensitivity for the uninjured paw. Given that GlyT1 inhibitors in the cortex can only act through NMDA neurotransmission while in the spinal cord they have access to NMDA as well as glycine inhibitory neurotransmission, we conclude that the distinct molecular pathways at the two sites differentially modulate anti-neuropathic and analgesic behavior.

Three studies have recently examined GlyT inhibitors as potential anti-nociceptive agents [17,27,31]. Generally all three studies show that various GlyTs may induce anti-nociception in rats and mice, in a variety of animal models of pain. None of these studies consider supraspinal effects of GlyTs. Our results best match the observations made by Tanabe et al. [31] where thermal and mechanical sensitivity was studied on streptozotocin-injected diabetes, partial nerve ligation induced neuropathy, and formalin-evoked nociceptive behavior in mice, following intrathecal sarcosine, glycine, or *N*-[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl] sarcosine. All three compounds showed efficacy in reducing thermal and mechanical hypersensitivity in concentrations and for durations comparable to the results we see for oral or intrathecal sarcosine in the rat. Yet, the study examined effects only for the injured limb, and thus did not differentiate injury-induced nociceptive behavior from analgesic effects. The study by Morita et al. [27] also reported on effects of sarcosine and related

chemicals (administered either intravenously or intrathecally) on mechanical sensitivity in partial peripheral nerve-ligated mice. At least the effects of sarcosine seem variant from our results and of those described by Tanabe et al., as Morita et al. observe much denser reversal of touch sensitivity observed for a period of days following single sarcosine injections. None of these studies explored the effects of repeated treatment of GlyTs on nociception.

The primary hypothesis of this study was that sarcosine should act at multiple central sites. We confirm this hypothesis and show that the two sites induce differential effects on nociceptive behavior, with the cortical site reducing neuropathic behavior and the spinal cord site showing better analgesic efficacy. Cortical infusion of sarcosine showed efficacy only on the injured paw, while spinal cord injection of sarcosine was more effective on the non-injured paw but also showed some efficacy on the injured paw as well. These results are distinct from the effects of DCS that we have described in the past [26], and the differences are informative regarding underlying mechanisms. Oral, intrathecal, or cortical administration of DCS, acutely or repeatedly, does not modify tactile sensitivity for the uninjured paw. Thus, DCS in contrast to sarcosine does not show analgesic effects. Oral or intrathecal single doses of DCS only minimally modify neuropathic behavior, while sarcosine administered similarly induces both analgesia and anti-neuropathic behavior. These results suggest that DCS and sarcosine modulate distinct molecular pathways in the spinal cord, although the effect size of sarcosine on tactile sensitivity seems similar to that of DCS.

The simplest candidate targets are the glycine site of the NMDA receptor for DCS, and potentiation of the glycinergic inhibitory neurotransmission by sarcosine [11,15]. On the other hand, cortical infusion of DCS or sarcosine induces only anti-neuropathic behavior, and repeated oral doses of both drugs result in continuously increasing anti-neuropathic behavior. Thus, the cortical site of action is most likely the same for both drugs. Given that cortical injections targeted the same coordinates as that used for DCS, and since DCS effects are most effective when injected in the prelimbic portion of the mPFC, we conclude that sarcosine is also acting at this cortical site. Moreover, given that anti-neuropathic effect of DCS infused in mPFC is enhanced following repeated oral exposure of the animal to DCS, we can infer that this brain site is where repeated sarcosine administration leads to long-term anti-neuropathic effects. Finally, blocking NMDA transmission in mPFC blocks DCS-induced anti-neuropathy, implying that this is also the molecular route of sarcosine effects on neuropathic behavior, especially its long-term effects with repeat administration. Although the similarities and differences between effects of sarcosine and DCS strongly imply these differential routes, they remain to be directly demonstrated for sarcosine.

The central bioavailability of sarcosine remains unclear, especially when administered orally. In this regard the observed effect sizes relative to the doses used are informative. In the repeat treatment studies we tested two doses, although the higher dose resulted in statistically stronger increases in tactile sensitivity, the overall effect size was very similar between the two doses. In contrast, DCS seems to have better central availability and in fact shows dose-dependent changes in effects size with oral repeat treatment [26]. The largest effect size for sarcosine was seen when the compound was acutely delivered to mPFC, an average increase of 400% in tactile thresholds only for the SNI-injured paw. In comparison to this effect, the repeat treatment effect sizes were about 100%, suggesting that a substantial amount of sarcosine is degraded prior to reaching central targets. Sarcosine is demethylated to glycine by sarcosine dehydrogenase, and it is possible that its central effects are mediated by its metabolite, glycine. However, sarcosine dehydrogenase is mostly expressed in the liver and its expression in the brain is minimal [4]. On the other hand, injection of glycine within mPFC increases tactile thresholds in SNI animals in an amount comparable to both sarcosine and DCS.

A recent study indicates that SNI injury induces reorganization of mPFC pyramidal neurons both in morphology and in relative ratio of NMDA to AMPA receptor expression within a week after the peripheral nerve injury, and this reorganization is correlated with increased tactile sensitivity of SNI [25]. This reorganization is observed for neurons located in a region that closely matches the coordinates where we observe the effects of cortical infusion of sarcosine in the present study, implying that the novel NMDA receptors associated with SNI may be the specific targets of action of sarcosine. Two recent rodent studies show that in a model of psychosis or of phencyclidine-induced cognitive deficits administration of GlyT1 inhibitors improves the related cognitive deficits as well as rescues long-term potentiation by indirectly modulating NMDA receptor function [16,23]. Given these observations and the evidence that mPFC controls reward-seeking behavior [18] and is thought to be involved in descending anti-nociceptive modulation [32], we propose that GlyT1 inhibition reduces pain-related cognitive decision-making and modulates nociceptive inputs to the spinal cord through both local inhibitory activity and the mPFC descending pathways.

Placebo-controlled clinical trials indicate the efficacy of sarcosine and related compounds for treatment of schizophrenia [20,33]. Sarcosine treatment seems well tolerated with few side effects. Long-term use of sarcosine is presumed to be safe given that sarcosenemia, a rare autosomal recessive metabolic disorder characterized by an increased concentration of sarcosine in blood and urine, is considered to be a relatively benign condition [10,21]. Therefore, characteristics of sarcosine in reducing nociceptive behavior in multiple rodent models, in inducing immediate analgesia and anti-neuropathy, together with potentiation of anti-neuropathic effects with repeated use, and coupled with human evidence of minimal side effects with long-term use at large doses, all suggest that it should be considered as candidate therapeutic approach for clinical management of chronic pain. Similar to DCS, we assume that its long-term efficacy should lead to decreasing the emotional suffering commonly observed in chronic pain, but unlike DCS, sarcosine should also result in immediate analgesia.

Conflict of interest

The authors claim no conflict of interest for this study.

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