Published in final edited form as:

J Pain. 2018 July ; 19(7): 727.e1–727.e15. doi:10.1016/j.jpain.2018.02.002.

Pain after spinal cord injury is associated with abnormal presynaptic inhibition in the posterior nucleus of thalamus

Anthony Park^a, Olivia Uddin^a, Ying Li^a, Radi Masri^{a,b}, and Asaf Keller^a

^aProgram in Neuroscience and Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, Maryland 21201 ^bDepartment of Endodontics, Periodontics and Prosthodontics, University of Maryland Baltimore, School of Dentistry, Baltimore, Maryland

Abstract

Pain after spinal cord injury (SCI-Pain) is one of the most debilitating sequelae of SCI, characterized as relentless, excruciating pain that is largely refractory to treatments. While it is generally agreed that SCI-Pain results from maladaptive plasticity in the pain processing pathway that includes the spinothalamic tract and somatosensory thalamus, the specific mechanisms underlying the development and maintenance of such pain are yet unclear. However, accumulating evidence suggests that SCI-Pain may be causally related to abnormal thalamic disinhibition, leading to hyperactivity in the posterior thalamic nucleus (PO), a higher order nucleus involved in somatosensory and pain processing. We previously described several presynaptic mechanisms by which activity in PO is regulated, including the regulation of both GABAergic and glutamatergic release by presynaptic GABA_B receptors. Using acute slices from a mouse model of SCI-Pain, we tested whether such mechanisms are affected by SCI-Pain. We reveal two abnormal changes in presynaptic signaling in the SCI-Pain condition. The substantial tonic activation of presynaptic GABA_B receptors on GABAergic projections to PO—characteristic of normal animals—was absent in mice with SCI-Pain. Also absent in mice with SCI-Pain was the normal presynaptic regulation of glutamatergic projections to PO by GABA_B receptors. The loss of these regulatory presynaptic mechanisms in SCI-Pain may be an element of maladaptive plasticity leading to PO hyperexcitability and behavioral pain, and may suggest targets for development of novel treatments.

Keywords

Synaptic regulation; GABA; glutamate; sensory processing; higher-order thalamus

---

Corresponding author: A. Keller, Dept. of Anatomy and Neurobiology, University of Maryland, School of Medicine, 20 Penn St., Baltimore, MD 21201 (asafkeller@fastmail.us).

Disclosures:

The authors declare no competing financial interests.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

INTRODUCTION

Chronic pain is one of the most debilitating disorders associated with spinal cord injury (SCI) and is estimated to affect up to 80% of all patients with SCI^{35,86}. Such central pain after spinal cord injury (SCI-Pain) typically presents as constant, diffuse, and bilateral pain commonly described as shooting, stabbing, or burning^{18,20,104}, and is generally refractory to treatment by conventional therapies^{6,22,35,78,97}. Thus, there is a critical need for the development of more efficacious therapeutics to ameliorate this crippling condition.

It has long been speculated that central pain can result from abnormal activity in thalamic nuclei that receive pain-related inputs from the spinothalamic tract^{15,16,29}. Consistent with this hypothesis, we and others have reported thalamic changes associated with SCI-Pain in rats^{43,47,65}, primates⁹⁸ and humans^{2,73}.

Previous work from our laboratory has expanded upon this hypothesis by showing that SCI-Pain is causally related to pathologically increased activity in the posterior thalamic nucleus (PO), a higher-order nucleus of the somatosensory thalamus⁶⁵. As a higher-order nucleus, PO is classically thought to mediate trans-cortical communication through reciprocal projections to and from cortical areas⁸⁵. However, there exist also dense projections from PO to regions implicated in emotional and pain processing, including the insular cortex and the amygdala^{41,60,61}. Indeed, Poggio and Moutcastle⁷⁶ demonstrated that PO neurons preferentially respond to noxious inputs, and similar findings have been confirmed by others^{26,74,99}. Thus, it has been proposed that PO plays an important role in the emotional-affective component of pain^{1,39,64}. It is through these interactions that PO can affect reflexive, affective and cognitive pain metrics.

We have further shown that the thalamic hyperactivity, in turn, is mediated by pathological suppression of inhibition from the zona incerta (ZI; this forms the incerto-thalamic circuit) that sends dense GABAergic projections to PO^{10,77}, and exerts both tonic and feed-forward inhibition of PO^{66,90,91,100}. Notably, inactivation of ZI results in immediate hyperalgesia in naïve rats, along with a concomitant increased activity of thalamic neurons¹⁰⁰. Furthermore, stimulation of ZI in SCI-Pain animals results in immediate reversal of behavioral signs of pain⁶³. Direct stimulation of ZI, however, is a highly undesirable clinical solution, not only due to its invasiveness, but also due to the large numbers of fibers of passage through the region. Instead, we endeavor to discover molecular targets for interventions, and, to this end, examine synaptic mechanisms regulating PO activity. In the present study we use a mouse model of SCI-Pain and acute slice electrophysiology to explore whether such presynaptic regulatory mechanisms are altered in, and contribute to SCI-Pain. Given our laboratory's overarching hypothesis that PO hyperactivity is a result of abnormal disinhibition, we first tested the specific hypothesis that presynaptic release from GABAergic terminals is decreased following SCI while glutamatergic release is unchanged. We also recently reported the presence of several presynaptic mechanisms that regulate release of GABA and glutamate in PO including presynaptic GABA_B-receptor mediated inhibition of release on both types of terminals and cholinergic inhibition of release on GABAergic terminals⁷², and explore whether such presynaptic regulatory mechanisms are altered in, and contribute to, SCI-Pain.

METHODS

We adhered to accepted standards for rigorous study design and reporting to maximize the reproducibility and translational potential of our findings, as described in Landis et al.⁵⁴ and in ARRIVE (Animal Research: Reporting In Vivo Experiments) Guidelines. Animals were randomly allocated to experimental or control groups, as described in Kim and Shin⁵¹. In all experiments the investigators were blinded to animal condition or to pharmacological agents applied *in vitro*. A coded key of all specimens evaluated was kept and was not shared with the investigators performing the experiments until data analyses were completed. Thus, allocation concealment, blinded conduct of the experiment and blinded assessment of the outcomes were performed.

We determined the sample size for all experiments achieve 0.8 power and alpha of 0.05 using G*Power software (Heinrich-Heine Universitat). For behavioral studies, we used data from preliminary studies in the laboratory that showed effect sizes $d > 0.7$, resulting in minimum total sample size of 68 for comparison of independent samples; however, greater number of animals underwent behavioral testing due to the necessity to produce sufficient number of animals for electrophysiological studies. For the electrophysiological studies, effect sizes were taken from our previously published synaptic studies in PO⁷², ranging from d of 0.9 to 1.5 for sample sizes between 6 to 11 to make comparison of dependent samples. All animals used in behavioral testing were also used for electrophysiological experiments; because multiple slices were obtained per animal, more than one type of experiment may have been performed on slices acquired from a single animal.

Animals

All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Maryland School of Medicine. Animals were housed with 2–5 same-sex animals for non-breeding experimental cages, while ratios of 1 male to 2 females were maintained for breeding cages. Food and water were available *ad libitum* and enrichment with shreddable paper nesting material was provided in all cages in addition to standard corn cob substrate bedding. Animals were kept in 12/12 light/dark cycles. For survival surgeries, strict aseptic surgical procedures were used, in accordance with the guidelines of the International Association for the Study of Pain. Gad2^{tm2(cre)Zjh/J} mice (The Jackson Laboratory, Bar Harbor, ME) of both sexes were used because our initial intent was to incorporate optogenetics in this study. For each set of experiments, both behavioral and neurophysiological, data were first tested for sex differences. For behavioral tests, two-way ANOVAs were performed with injury condition and sex as the two independent variables for each time point (3, 7, 14, and 21 days after surgery). For neurophysiological experiments, data were pooled across the drug conditions and two-way ANOVAs were performed with the injury and sex conditions as the two independent variables. Data from both sexes were pooled if there were no significant sex differences ($p > 0.05$).

Spinal Lesions and Sham Surgeries

Mice (42 to 70 days old) were randomly assigned to SCI or sham surgery groups. Under isoflurane anesthesia, the mice were head-restrained on a stereotaxic apparatus without

further spinal restraints. Skin and muscles of the back were dissected to expose the spinal column at C7, and the interspinous ligament and the ligamentum flavum between C6 and C7 were bluntly dissected to reveal the spinal cord without performing a laminectomy. Using fine scissors, the dural sac overlying the spinal cord was opened, providing an access point for the lesioning electrode. A quartz-insulated platinum electrode (5 μ m tip) was targeted to the spinothalamic tract based on stereotaxic coordinates (0.8 mm lateral from midline, 0.9 mm deep). Once entered, a direct current of +20 μ A was passed through the electrode for 20 seconds, repeated 3 times, using the 53500 Lesion Making Device (Ugo Basile, Gemonio, VA, Italy). The lesion was induced unilaterally. Sham animals underwent identical surgical procedures, up to the dissection of back muscles, exposing the C7 spinous process; however, the spinal column was left undisturbed.

Behavioral Assessments

All animals were tested before spinal lesion or sham surgery, and at 3, 7, 14, and 21 days following surgery. The animals were habituated to the testing environment (an elevated horizontal wire mesh stand with a Plexiglas box separated by opaque separators for individual animals) for at least 1 hour prior to testing. Behavioral results from post-surgery time points were normalized to baseline levels, and compared using independent t-tests for differences between SCI and sham-operated animals at each time point with correction for multiple comparisons.

Hindpaw mechanical withdrawal thresholds were determined using a series of calibrated von Frey filaments by Stoelting (Wood Dale, IL), ranging from 0.16 to 4 grams. The filaments were applied to the plantar aspect of the hindpaw, and flinching or rapid removal of the paw was counted as a positive response. The up-down method was used to determine the withdrawal threshold, as described previously²⁷

Cold allodynia was determined using the acetone evaporation test as previously described^{9,38}. Briefly, a drop (20 μ L) of acetone was applied with a small syringe to the center of the plantar surface of the hind paw, and the animals were observed for responses. Behaviors were scored according to a 4 point scale: 1, no response; 2, quick withdrawal, flick or stamp of the paw; 3, prolonged withdrawal or repeated flicking of the paw; and 4, repeated licking of the paw with persistent licking directed at the ventral side of the paw. Acetone was applied alternately, three times, to each hindpaw, with a minimum of 5 minutes between successive applications, and resulting scores were averaged for each paw.

Dynamic mechanical allodynia was assessed, as described previously³², by stimulating the lateral plantar region of the hindpaw with light stroking with a soft brush, in the direction from heel to toe. Resulting behavior was scored on a 4 point scale: 1, no response, walking away or occasionally very brief paw lifting; score 2, sustained lifting (more than 2 s) of the stimulated paw; 3, strong lateral lifting above the level of the body; 4, flinching or licking of the affected paw. The test was performed as three sets of three stimuli (nine total stimuli) at intervals of at least 10 seconds between stimuli in each set, and at least 5 minutes between sets. The scores were then averaged.

Solutions and Drugs for *In Vitro* Experiments

N-methyl-D-glucamine protective recovery method was used to acquire healthy adult slices, as described previously⁸⁹. Briefly, several types of modified ACSF were used: 1) Recovery ACSF: 92 mM NMDG, 2.5 mM KCl, 1.2 mM NaH₂PO₄, 30 mM NaHCO₃, 20 mM HEPES, 25 mM glucose, 2 mM thiourea, 5 mM Na-ascorbate, 3 mM Na-pyruvate, 0.5 mM CaCl₂·4H₂O and 10 mM MgSO₄·7H₂O. 2) Cutting ACSF: 92 mM NaCl, 2.5 mM KCl, 1.2 mM NaH₂PO₄, 30 mM NaHCO₃, 20 mM HEPES, 25 mM glucose, 2 mM thiourea, 5 mM Na-ascorbate, 3 mM Na-pyruvate, 0.5 mM CaCl₂·4H₂O and 10 mM MgSO₄·7H₂O, and 3) Normal ACSF: 119 mM NaCl, 2.5 mM KCl, 1.2 mM NaH₂PO₄, 24 mM NaHCO₃, 12.5 mM glucose, 2 mM CaCl₂·4H₂O and 2 mM MgSO₄·7H₂O. All ACSF pH were adjusted to 7.35–7.45 and osmolarity adjusted to 300–310 mOsm. Solutions were saturated with carbogen (95% O₂/5% CO₂) throughout use. All experiments were performed using a high-chloride intracellular solution: 140 mM CsCl, 10 mM HEPES, 10 EGTA, 1 mM CaCl₂, 2 mM Mg-ATP, 0.2 mM Mg-GTP, and 5 mM QX-314 in order to enhance the detection of GABA_A receptor mediated currents. QX-314 and Cs⁺ were used to suppress the effects of postsynaptic GABA_B receptor by blocking potassium channels^{3,70,82}.

Tetrodotoxin (0.5 μM, Sigma-Aldrich, St. Louis, MO), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μM, Sigma), gabazine (10 μM, Tocris Bioscience, Bristol, UK), R(+)-baclofen (10 μM, Sigma), and carbachol (2 μM, Sigma) were dissolved in dH₂O. CGP-55845 (2 μM, Sigma) was dissolved in DMSO. All drugs were bath applied. Drug concentrations were determined to be comparable to those found in literature (Baclofen^{33,58}; CGP-55845^{49,105}; Carbachol⁶⁶, and to significantly exceed known dissociation constants (Baclofen^{7,40,50}; CGP-55845²⁴; Carbachol^{17,45}, but see⁶⁷).

Slice Preparation

Slices were harvested from animals 21–35 days after SCI or sham surgery. Thus, recordings were obtained from slices of animals that were 63–105 days old. Animals were deeply anesthetized with ketamine (180 mg/kg) and xylazine (20 mg/kg), and the brains were rapidly removed following decapitation. Coronal slices through PO thalamus, 300 μm thick, were cut in ice-cold cutting ACSF using a Leica VT1200S vibratome (Leica Microsystems, Nussloch, Germany) and transferred to warm (32–34°C) recovery ACSF for 10–15 minutes. The slices were then transferred to normal ACSF at room temperature for at least 45 minutes before starting experiments. Slices were acquired from the contralateral side to the spinal lesion. The thickness of the slices allowed for acquisition of 3–4 slices per animal.

Electrophysiology

Whole cell patch-clamp recordings were obtained from neurons in PO, under visual guidance, with either a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA), low-pass filtered at 2 kHz with a 4-pole Bessel filter, and digitized with Digidata 1322A (Molecular Devices), or an EPC 10 amplifier (HEKA Elektronik, Lambrecht, Germany), digitized at 20 kHz using Pulse software (HEKA). The entire slice was first examined under a 4x objective on our visually guided patch clamp setup. Various landmarks, such as the border between PO and ventroposterior medial nucleus (VPM), the reticular nucleus of thalamus, and hippocampus were clearly visualized. The image was then centered on the

triangular region on the border between PO, VPM, and lateral posterior thalamic nucleus/dorsal lateral geniculate nucleus and switched to a 40x water immersion objective and scanned medially and inferiorly to find PO neurons. Impedance of patch electrodes was 4 to 6 M Ω . Once a G Ω seal was obtained, holding potential was set to -70 mV and was maintained for the duration of the experiment. Series resistance (<40 M Ω , compensated at least 60%) was monitored throughout the recording, and recordings were discarded if series resistance changed by more than 30%. Intracellular solution was allowed to equilibrate for at least 3 minutes before recording.

For quantal analyses, miniature inhibitory postsynaptic currents (mIPSCs) or miniature excitatory postsynaptic currents (mEPSCs) were recorded. To record mIPSCs, 0.5 μ M TTX and 20 μ M CNQX were bath applied. To record mEPSCs, 0.5 μ M TTX and 10 μ M gabazine were applied. Pharmacological agents to modulate presynaptic release (baclofen, CGP-55845, or carbachol) were applied. For experiments with antagonists, we made two types of recordings: one set in which antagonists were applied alone, and another set in which antagonists were applied after the agonist. Data from these experiments were pooled. All agents were applied for at least 5 minutes and CGP-55845 was applied for at least 10 minutes before acquiring a 3 minute long recording; application times were determined to be comparable to those found in literature as cited in the previous *Solutions and Drugs* section. Recordings were terminated with application of gabazine (10 μ M for mIPSC recordings) or CNQX (20 μ M for mEPSC recordings) to ensure that observed events were due to GABA_A or AMPAR mediated currents, respectively. To avoid sensitization or desensitization, each slice was discarded after one recording.

To determine whether tonic current was present, GABA_A receptor mediated tonic currents were first pharmacologically isolated by applying CNQX (20 μ M). Following a 1 minute baseline epoch, gabazine (10 μ M) was bath applied to the slice. Elimination of sIPSCs were taken as a positive control for gabazine application. Sustained changes to holding current following gabazine application were analyzed as described below. In these experiments, more than one recording was allowed per slice.

Data Analysis

For quantal analysis, we detected miniature events off-line with MiniAnalysis (Synaptosoft, Decatur, GA). Event characteristics were exported to MATLAB (Mathworks, Natick, MA) for further analysis. For each recorded cell, cumulative frequency plots were generated for miniature event amplitudes and inter-event intervals for each drug condition. These cumulative frequency plots were compared within-cell by Kolmogorov-Smirnov tests (K-S test). Proportions of cells that were significantly affected by a drug application were compared between SCI-Pain vs. sham with Fisher's exact tests for proportions.

For continuous population data, all results were first compared by one-way ANOVA; if results were significant, pairwise tests were performed with paired t-tests with Bonferroni corrections.

For determining tonic currents, a two-minute epoch that includes 30 seconds prior to, and 90 seconds following gabazine application, was selected in pClamp (Molecular Devices) and exported to MATLAB, and fitted to a logistic regression line:

$$I = \text{initial} + \frac{(\text{final} - \text{initial})}{(1 + 10^{\text{slope} \cdot (\text{inflection} - t)})}$$

The fitting algorithm used a nonlinear least squares regression with a termination tolerance on residual sum squares of $<10^{-8}$. The magnitude of the tonic current was taken as the difference between the parameters *initial* and *final*. We considered neurons non-responsive to gabazine if the algorithm was unable to satisfactorily optimize a parameter set within 100 iterations according to the termination tolerance, or if the fit found had non-physiologically small slopes ($<0.01/\text{sec}$).

RESULTS

Electrolytic lesion of spinothalamic tract produces chronic pain signs in mice

Figure 1A shows a representative micrograph of a coronal section through the spinal cord, depicting the effects of an electrolytic lesion in the spinothalamic tract. The typical lesion was vertically elongated along the lateral spinothalamic tract (arrowheads), and, in some cases, also affected the anterior spinothalamic tract. Typically, no damage was found along the path of the electrode, though the site of penetration near the dorsal horn often displayed signs of minor damage (arrow). The lesion often partially affected nearby white matter tracts, particularly those lateral to the lateral spinothalamic tract, such as the anterior spinocerebellar tract, but never extended far enough medially to affect gray matter.

Mechanical hyperalgesia—We performed a set of behavioral assays to test for the development of behavioral pain symptoms at 3, 7, 14, and 21 days following surgery. To determine whether SCI produced pain, we normalized each post-surgery outcomes to baseline and compared between SCI and sham conditions at each time point with independent t-tests. Hindpaw withdrawal thresholds were significantly and bilaterally decreased following SCI (n=40 mice), compared to sham (n=37; Fig 1B). Ipsilateral to the lesion, withdrawal thresholds in SCI animals were significantly lower than those of sham animals at 3, 14, and 21 days following injury (p=0.030, 0.003, and <0.0001 , respectively, by t-test). Contralateral to the lesion, the thresholds in SCI animals were significantly lower by 14 days and persisted to 21 days following injury (p=0.0026 and $p<0.0001$, respectively). By 21 days following SCI, withdrawal thresholds decreased from baseline of 2.3 ± 1.3 g (mean $\pm$ SD; ipsilateral) and 2.4 ± 1.3 g (contralateral) to 0.7 ± 0.4 g and 0.6 ± 0.4 g, respectively. We did not observe, in our mouse model, a transient hypoalgesia immediately following injury documented in both the human condition^{18,34,36}, and in our electrolytic SCI lesion model in rat⁶⁵. Of note, no sex differences were found in any of the three behavioral paradigms across all days tested (p>0.05).

Cold allodynia—Cold allodynia is a common complaint amongst SCI-Pain patients^{30,36}. We tested for cold allodynia, using acetone application to the hindpaw (Methods).

Analogous to changes in mechanical pain thresholds, SCI animals (n=40 mice) exhibited significantly increased cold allodynia scores compared to sham-operated animals (n=37 mice) following surgery (Fig. 1C): ipsilateral to the lesion, SCI animals exhibited significantly increased cold allodynia scores beginning at 7 days following surgery ($p=0.045$, t-test), and the differences were maintained until 21 days following surgery ($p<0.0001$, t-test), while contralateral to the lesion, scores were significantly increased on all testing days ($p=0.018$ at day 7, and $p<0.0001$ at 7, 14, and 21 days). By 21 days following SCI, cold allodynia scores increased from baseline of 0.26 ± 0.59 points (mean $\pm$ SD; ipsilateral) and 0.15 ± 0.34 points (contralateral) to 2.08 ± 0.83 points and 2.59 ± 0.58 points, respectively.

Dynamic allodynia—Dynamic mechanical allodynia (DMA) is a sensitive measure suggestive of neuropathic pain in both humans and animals^{32,84}. Briefly, dynamic allodynia is evoked by light brushing across the skin, and it is thought to be mediated by activating A β fibers. There was a significant increase in DMA scores after injury in SCI animals (n=40 mice) bilaterally, compared to sham-operated animals (n=37 mice; Fig. 1D). Ipsilateral to the lesion, animals with SCI exhibited significantly greater DMA scores compared to sham at 7, 14, and 21 days following surgery ($p=0.035$, 0.0054 , and 0.014 , respectively, by t-test). Contralateral to the lesion, DMA scores were significantly greater on days 14 and 21 ($p=0.0056$, 0.021 , respectively, by t-test). By 21 days after injury, DMA scores increased from baseline of 0.024 ± 0.067 points (ipsilateral) and 0.012 ± 0.047 points (contralateral) to 0.111 ± 0.162 points and 0.128 ± 0.206 points, respectively.

Thus, we confirm that the electrolytic model of SCI in mouse recapitulates several important behavioral manifestations of the human condition, and use the present model to test mechanisms that may underlie SCI-Pain.

Probability of GABA release in PO is unchanged in SCI

To test the hypothesis that release from GABAergic terminals is reduced in SCI-Pain, we recorded mIPSCs from PO neurons in acute slices from both SCI and sham operated animals in the presence of TTX and CNQX. As noted in our previous report⁷², we observed no NMDA receptor mediated currents, and all synaptic events were eliminated with application of gabazine (GZ, $10\text{ }\mu\text{M}$), a GABA_A receptor antagonist. Because changes in miniature event frequency without changes in amplitude reflect presynaptic probability of release^{see 5,106}, we predicted that mIPSC frequency would be lower in the SCI group compared to shams. Contrary to our prediction, however, we found no differences in mIPSC frequency between SCI and sham-operated conditions: Neurons from SCI animals (n=19 from 15 mice) displayed an average mIPSC frequency of 1.19 ± 0.82 Hz (mean $\pm$ SD), while neurons from sham-operated animals (n=14 from 10 mice) displayed an average frequency of 1.26 ± 0.85 Hz ($p=0.82$; t-test).

Thus, our hypothesis that presynaptic release is diminished in SCI was falsified. However, a number of powerful mechanisms exist that actively modulate presynaptic release. These mechanisms act across several time scales and underlie various functions, including synaptic homeostasis⁷⁹. Thus, it is possible that while SCI may have exerted an independent effect

on presynaptic release, overall mIPSC frequency was maintained due to compensatory homeostatic changes in regulatory presynaptic mechanisms. We have previously demonstrated the presence of presynaptic GABA_B receptors on GABAergic terminals, and have also shown that these autoreceptors are tonically, but sub-maximally, active at baseline, providing an effective mechanism by which release probability can be both increased, via reduction of tonic activation, or decreased, via further activation.⁷²; thus, we hypothesized that GABAergic terminals maintained normal levels of presynaptic release through a compensatory mechanism of down-regulating the activity of pre-synaptic GABA_B receptors.

Tonic activation of GABA_B autoreceptors is suppressed in SCI-Pain

We first confirmed the existence of functional GABA_B receptor mediated presynaptic regulation by recording mIPSCs in the presence of TTX and CNQX, as described above. After baseline recordings, we bath applied a GABA_B receptor agonist, baclofen (10 μ M). A representative recording of neurons recorded from SCI animals shows that baclofen suppresses mIPSC frequency (Fig. 2A, top-left: baseline, middle-left: baclofen). This baclofen-induced suppression of release is illustrated in the cumulative probability plots in Figure 2B. Baclofen caused a significant rightward shift in the inter-event interval plot (right), suggesting a decrease in mIPSC frequency compared to baseline. Indeed, in this neuron, baclofen reduced mIPSC frequency by 43.7%, from 0.88 to 0.57 Hz ($p=0.0009$, K-S test) without affecting amplitude ($p=0.30$, K-S test; Fig. 2B, left). The same was confirmed in neurons from sham-operated animals (representative traces in Fig. 2A, right); cumulative probability plots in Figure 2C show that baclofen causes a significant rightward shift in the inter-event intervals (right; $p=0.0004$, K-S test; frequency decreased from 0.81 to 0.49 Hz) without affecting amplitude ($p=0.84$, K-S test).

Overall, mIPSC frequencies were significantly reduced in 10 of 11 SCI-condition neurons (from 10 mice), and in all 7 sham-condition neurons (from 6 mice; Figs. 2D,E). On average, baclofen application caused a 58.6% decrease in mIPSC frequency from 1.54 ± 0.93 Hz to 0.64 ± 0.39 Hz ($p=0.0008$, paired t-test) in the SCI condition, and a 60.4% decrease from 1.00 ± 0.44 Hz to 0.40 ± 0.19 Hz ($p=0.002$, paired t-test) in the sham condition. We found no significant differences between these populations across injury condition: mixed-design ANOVA found no interactions between injury and drug conditions ($p=0.266$), and no difference in main effect across injury conditions ($p=0.155$), while the main effect across drug condition was significant ($p<0.00005$). These findings suggest that the capability of presynaptic GABA_B autoreceptor activation to suppress GABA release was unaffected by SCI.

We have previously shown, in naïve mice, that GABA_B receptors are tonically active⁷². Consistent with that report, application of CGP-55845 (2 μ M), GABA_B receptor antagonist, increased mIPSC frequency. In the representative neuron from the sham group (Fig. 2A, right), CGP-55845 caused not only a full reversal of the effect of baclofen, but a further increase in mIPSC frequency beyond that recorded at baseline, reaching 1.13 Hz (40.7% increase; $p=0.0024$; K-S test). This is illustrated as cumulative probability plots in Fig. 2C, in which the CGP-55845 condition frequency plot is significantly left shifted in comparison to both baclofen and baseline conditions. As a population, 5 of 7 neurons displayed

significant increases beyond baseline levels (Fig. 2E, right), suggesting that presynaptic GABA_B autoreceptors exhibit tonic activation, consistent with our previous report and expectations.

In contrast, in the SCI-condition, CGP-55845 only reversed the effect of baclofen, without causing a further increase in mIPSC frequency. In the representative neuron from the SCI group (Fig. 2A, left), CGP-55845 had no significant effect on mIPSC frequency ($p=0.27$, K-S test). Whereas CGP-55845 effectively antagonized the effects of baclofen in all eleven neurons from SCI mice, only one of these neurons displayed a significant increase in mIPSC frequency compared to baseline (Fig. 2D, right).

On average, in neurons from sham mice, CGP-55845 caused a 27.8% increase in mIPSC frequency relative to baseline, from 1.00 ± 0.44 Hz to 1.28 ± 0.39 Hz ($p=0.017$, paired t-test). In contrast, in neurons from SCI mice, mIPSC frequency was not significantly affected by CGP-55845 ($p=0.611$, paired t-test). As expected from these differences, Fisher's exact test found significant differences across injury conditions ($p=0.013$). Thus, these data demonstrate that GABA_B mediated tonic inhibition is suppressed in PO neurons from SCI-Pain mice. Of note, no sex differences were found in the present mIPSC frequencies, or in subsequently described mEPSC frequencies ($p>0.05$).

Cholinergic regulation of GABA release is unchanged in SCI-Pain

In addition to the GABA_B receptor mediated presynaptic regulation, we have also previously reported the presence of presynaptic suppression of GABA release^{66,72}. Because the reduction in tonic GABA_B activity accounted for the normal level of presynaptic GABA release, we hypothesized that SCI would not affect cholinergic regulation. To determine whether cholinergic regulation was affected, we recorded mIPSCs under recording conditions described above and, after collecting baseline mIPSCs, applied a cholinergic agonist, carbachol (2 μ M).

Representative recording of neurons recorded from SCI and sham-operated animals are shown in Figure 3A. The traces illustrate the suppression of release mediated by carbachol application in both conditions. This is further demonstrated through the cumulative probability plots in Figures 3B (SCI) and 3C (sham). In the representative neurons from the SCI condition, carbachol application reduced the frequency of mIPSCs by 21.3% from 0.60 to 0.47 Hz ($p=0.027$, K-S test) while having no effect on amplitude ($p=0.804$, K-S test). In the sham condition, carbachol application reduced the frequency of mIPSCs by 69.28% from 0.88 to 0.58 Hz ($p=0.003$, K-S test) while having no effect on amplitude ($p=0.058$, K-S test). Overall, mIPSC frequencies were significantly reduced in all six SCI condition (from 3 mice; Fig. 3D), and seven sham condition (from 4 mice; Fig. 3E) neurons tested. SCI neurons, on average, displayed a 40.5% decrease from 0.77 ± 0.16 to 0.45 ± 0.10 Hz ($p=0.0015$), while sham neurons displayed a 48.9% decrease from 1.51 ± 1.10 to 0.77 ± 0.57 Hz ($p=0.033$), confirming that cholinergic suppression of GABA release occurs in PO in both conditions. There was no significant difference in frequencies across injury conditions: mixed-design ANOVA showed no main effect differences across condition ($p=0.143$), and no interaction effects ($p=0.178$) while showing a significant main effect of carbachol

($p=0.0037$). Thus, we confirmed the presence of cholinergically mediated suppression of GABA release in PO, and that this effect is unchanged by SCI.

Probability of glutamate release in PO is unchanged in SCI

As previously discussed, one of our initial hypotheses was that presynaptic release in glutamatergic terminals would be unaffected by SCI, as SCI-Pain is proposed to be a disorder of disinhibition, rather than hyperexcitation⁶⁴. To test this hypothesis, we recorded mEPSC frequencies from neurons of SCI or sham-operated animals in the presence of TTX and gabazine. mEPSCs were confirmed to be AMPA receptor mediated, as application of CNQX eliminated all synaptic events. Consistent with our hypothesis, we found no differences in mEPSC frequency between SCI and sham-operated conditions. Neurons from SCI animals ($n=14$ from 10 mice) exhibited an average mEPSC frequency of 0.77 ± 0.37 Hz while those from sham-operated animals ($n=12$ from 7 mice) exhibited an average of 0.76 ± 0.51 Hz ($p=0.943$, t-test).

Regulation of glutamate release by GABA_B heteroreceptors is suppressed by SCI-Pain

As was with the GABAergic terminals, we previously documented that glutamatergic terminals are potently regulated by presynaptic GABA_B receptors⁷². Thus, analogous to the effects seen in the GABAergic counterparts, it is possible that SCI causes an increase in presynaptic release from glutamatergic terminals but simultaneously develops a compensatory mechanism to maintain normal levels. As our previous findings documented a lack of tonic GABA_B receptor activation on glutamatergic terminals, enhanced release may be compensated by new tonic activation of such presynaptic GABA_B receptors. To test this hypothesis, we performed similar experiments as those described for GABAergic terminals: We recorded mEPSCs in presence of TTX and gabazine, and after recording baseline activity, applied either baclofen or CGP-55845 to test the effects of GABA_B receptor activation and inactivation. If such compensatory mechanism is present, we predicted that we would observe an increase in mEPSC frequency with CGP-55845 application in the SCI, but not sham, condition.

As before, we first tested for the presence of functional GABA_B receptors by comparing mEPSC frequencies at baseline and with baclofen application. A representative trace of the sham-condition (Fig. 4A, right) illustrates the decreased mEPSC frequency with baclofen application (middle trace) compared to baseline (top trace). This is depicted as cumulative probability plots in Figure 4B, where the baclofen inter-event interval trace is significantly shifted to the right in comparison to baseline, demonstrating a decrease in frequency. Indeed, in the representative neuron, mEPSC frequency was decreased by 41.67% from 0.53 to 0.31 Hz ($p=0.0171$, K-S test), while amplitude was not affected ($p=0.125$, K-S test). Consistent with our previous results, 7 of 8 sham condition neurons (from 5 mice) responded with significantly decreased mEPSC frequencies ($p<0.05$, K-S test, Fig. 4C), and produced, on average, a 38.1% decrease from 0.88 ± 0.60 Hz to 0.54 ± 0.34 Hz ($p=0.011$, paired t-test).

Surprisingly, in contrast to neurons from sham animals, a majority of neurons from SCI animals did not exhibit significant suppression of release with baclofen application. A representative trace (Fig. 4A, left) and corresponding cumulative probability plots (Fig. 4D)

illustrate the lack of effects on both mEPSC frequency ($p=0.150$; K-S test) and amplitude ($p=0.122$, K-S test). Overall, only 2 of 12 neurons from the SCI condition (from 9 mice) produced significantly decreased ($p<0.05$, K-S test) mEPSC frequencies in response to baclofen (Fig. 4E). Further, the number of neurons responding to baclofen across injury conditions were significantly different by Fisher's exact tests ($p=0.0045$), and mixed-design ANOVA using mEPSC frequencies found a significant interaction between the injury and drug conditions ($p=0.041$), indicating that the response to baclofen is significantly different in SCI vs. sham, and suggesting that SCI-Pain results in suppressed presynaptic regulation of glutamate release.

Notably, we found that CGP-55845 application does not produce increases in mEPSC frequency compared to baseline in either condition: In the SCI condition, none of the 12 neurons tested displayed mEPSC frequencies significantly different from baseline after CGP-55845 application (Fig. 4E), and in the sham condition, only 1 of 8 neurons tested produced a significant increase (Fig. 4C). On average, CGP-55845 did not produce significant differences in neurons from sham ($p=0.587$, paired t-test) or SCI ($p=0.206$, paired t-test) animals. Consistent with this, the number of neurons responding to CGP-55845 was not significantly different between injury conditions ($p=1$), and mixed-design ANOVA found no interaction between injury and drug conditions ($p=0.527$), and no main effects of injury condition ($p=0.583$) or drug condition ($p=0.116$). Thus, we confirm here that GABA_B receptors on glutamatergic terminals in PO are not tonically active in either sham or SCI conditions, and rule out the possibility of a compensatory mechanism to suppress excitatory input.

Tonic GABA_A currents are unaffected by SCI

Tonic GABA_A currents, mediated by δ subunit-containing extra-synaptic receptors, have been reported in a number of brain regions, including thalamus^{reviewed in 23}. We previously reported the presence of such extrasynaptic GABA_A currents in a subpopulation of PO neurons⁷², and proposed that they may have significant functional implications, as small changes to resting membrane potential in thalamic neurons can have large effects on firing properties, due to their effects on t-type calcium channels^{31,48,92}. Similarly, PO hyperexcitability in SCI-Pain may be due to reduction in tonic GABA_A currents. To test for the presence of these tonic currents, we recorded from PO neurons in whole-cell voltage clamp configuration, and pharmacologically isolated GABA_A receptor mediated currents in the presence of CNQX (20 μ M). We recorded baseline holding current, then applied gabazine (10 μ M). We used the elimination of sIPSCs as positive control for the effect of gabazine. We continued to record changes in holding current for 2 additional minutes following elimination of all sIPSCs. Because we used a high-chloride intracellular solution, the reversal potential for GABA_A receptors was approximately 0 mV. Thus, in our recording conditions, GABA_A receptors mediated hyperpolarizing currents. If extrasynaptic GABA_A receptor mediated tonic currents were present in PO neurons, gabazine application would block such hyperpolarizing currents, causing a positive shift in holding current.

Bath application of gabazine resulted in a gradual depolarizing shift in holding current in 15 of 29 neurons (52%) from the SCI condition (from 19 mice), and 8 of 19 (42%) neurons

from the sham condition (from 13 mice). On average, the population of responsive neurons produced a change of 17.20 ± 7.79 pA (mean $\pm$ SD) in the SCI condition, and 17.85 ± 12.48 pA in the sham condition. When tested for differences across injury condition, neither the proportion of responsive neurons, nor the distribution of tonic current amplitudes among responsive neurons were statistically different (for proportions: $p=0.566$, Fisher's exact test; for amplitudes, $p=0.971$, MWU). There were no significant differences in input resistance of neurons in SCI and sham groups (SCI: 292 ± 193 M Ω vs. sham 258 ± 159 M Ω ; $p=0.68$ by two-sided t-test). Thus, we disprove our hypothesis that PO hyperactivity is mediated by suppression of GABA_AR mediated tonic currents in SCI-Pain.

DISCUSSION

SCI Model

The mouse model used in this study is adapted from our analogous rat model of electrolytic spinal cord injury^{65,96}. The electrolytic model has several advantages, including specific targeting of the spinothalamic tract—a necessary condition for development of SCI-Pain in humans^{29,29,30}, but see³⁷—while minimizing damage to motor pathways that may complicate analysis of pain behaviors. The mouse model recapitulates a number of important aspects of human SCI-Pain, including mechanical, cold, and dynamic allodynia, and development of bilateral distribution of pain, despite a unilateral SCI. Thus, we confirmed that a mouse model of SCI recapitulates important aspects of human SCI-Pain without motor complications, allowing us to test specific mechanisms underlying the pain syndrome, and enabling future studies to take advantage of powerful molecular and genetic tools available in this model animal¹⁰¹.

Compared to behavioral responses in the rat^{65,96}, the mouse showed higher variability in behavioral metrics. Such differences are not surprising, as a number of differences exist between the two species that are known to critically affect nociceptive behavioral assays for extensive discussion regarding nociceptive behavioral assays in mouse, see⁶⁸. For example, mice are more active than rats, and Mogil et al.,⁶⁸ have suggested that mice may require more training sessions before reaching stable levels of responses, and significantly longer habituation periods per testing session. Additionally, animals in the present study were tested at a relatively young age (baseline testing at 3 to 4 weeks) to minimize age related difficulties of *in vitro* patch-clamp experiments. Any of these factors may have further exaggerated behavioral variability in our animals. Despite this variability, our behavioral testing demonstrate statistically significant, and robust hyperalgesia in mice with SCI.

Regulation of transmitter release

We tested the overarching hypothesis that PO neurons in mice with SCI-Pain exhibit altered presynaptic release from GABAergic and glutamatergic afferents. Unexpectedly, we found that the release probabilities of both GABA and glutamate are unaffected in SCI-Pain. However, SCI-Pain produces changes to the regulatory mechanisms of presynaptic release. Specifically, we found that in SCI-Pain: 1) tonic activation of presynaptic GABA_B receptors in GABAergic terminals is suppressed, and 2) presynaptic GABA_B receptor mediated inhibition of glutamate release is suppressed.

Tonic GABA

We have previously reported the presence of tonic activation of presynaptic GABA_B receptors in GABAergic terminals in PO⁷², and we confirm their presence in sham-operated animals in the present study. The loss of tonic activation of these receptors in SCI-Pain can significantly affect synaptic transmission, as it limits the dynamic range of presynaptic regulation. In the normal condition, GABAergic synapses in PO both up and down-regulate the release of GABA by, respectively, reducing or enhancing the activation of the autoreceptors. The pathological absence of tonic activation of presynaptic GABA_B receptors in SCI-Pain results in GABAergic terminals that are only able to decrease GABA release from basal levels, but not to increase GABA release (Fig. 5A). Thus, the probability of release observed under baseline conditions represent the maximum level of GABA release in SCI-Pain, a level from which inhibition onto PO cannot be enhanced. As a result, the ability of GABAergic inputs to regulate PO activity is compromised, contributing to the hyperexcitability of PO neurons we previously described in SCI-Pain^{65,100}. The source of GABA responsible for the tonic activation is yet unknown. Significant evidence from various regions, including hippocampus and neocortex, suggest that the dominant source of GABA for activation of presynaptic autoreceptors is the GABAergic terminal itself^{42,52}. However, this may not be the case in PO, as we found that baseline levels of GABA release are unchanged in SCI-Pain, compared to sham; the loss of tonic activation in SCI-Pain, despite lack of differences in baseline release probability, is inconsistent with tonic activation of autoreceptors being primarily driven by GABA release from the same terminals. Thus, the GABA responsible for tonic activation of presynaptic GABA_B receptors may originate from other sources, such as glia. Glia are heavily involved in GABAergic signaling, either by directly releasing GABA⁵⁹, or indirectly through GABA uptake¹³. Further, glial regulation of GABA can maintain regionally restricted GABA levels, making such mechanisms more plausible⁵³. In addition, similar reductions to tonic activation of autoreceptors have been documented in a model of Huntington's disease, which implicated reduced capacity of astroglia to provide GABA¹⁰². Determining the source of GABA may be important for clinical translation of the present findings; if tonic activation is largely mediated by vesicular release, attempts to restore normal firing rates in GABAergic sources may also restore normal presynaptic regulation. On the other hand, if independent mechanisms are responsible for maintaining tonic activation, such as glia or molecular changes to the autoreceptors that decrease activation, they may potentially be targets for new therapies.

Regulation of excitation

In glutamatergic terminals we found that SCI-Pain abolishes presynaptic inhibition of release mediated by GABA_B receptors. As Figure 5B illustrates, the GABA_B heteroreceptor-mediated suppression of release from glutamatergic terminals that occurs in the normal condition provides a robust mechanism by which excitatory drive onto PO neurons can be suppressed. Thus, suppression of this mechanism in SCI-Pain restricts the capacity to down-regulate excitatory drive onto PO, and may ultimately contribute to PO hyperactivity and behavioral pain. Indeed, Wu et al.¹⁰³ have shown that directly enhancing presynaptic release of glutamate in the anterior cingulate cortex results in behavioral manifestations of pain,

providing precedence that enhancements in excitatory presynaptic release can underlie development of chronic pain.

Limitations

Our approach does not allow us to identify the origin of the miniature events recorded in this study. There are few, if any interneurons in rodent PO (Arcelli et al. 1997; Barbaresi et al. 1986; Ottersen and Storm-Mathisen, 1984), suggesting that GABAergic inputs are extrinsic. Indeed, PO receives converging GABAergic inputs from thalamic reticular nucleus (TRN), and other extrathalamic inhibitory inputs such as ZI, and anterior pretectal nucleus (APT)^{11,19,69,91}. Of these, the extrathalamic inputs have been proposed to exert stronger inhibitory influence on PO neurons, based on anatomical and electrophysiological evidence^{10,57}, and may contribute to the majority of events we recorded. Because these different sources of inhibition may mediate distinct computational processes, such as inhibition at different temporal scales⁴⁴, the functional implications of these findings may depend on identification of these source. Similarly, excitatory events observed may arise from several sources, including neocortex and the spinal cord.

We also acknowledge that it is difficult to extrapolate findings from acute slices to the intact brain^{see 87}. Of particular concern here are potential differences in levels of ambient GABA, which may have significant effects on both presynaptic regulation by GABA_B receptors and extrasynaptic receptors. However, we report that extrasynaptic GABA_A receptor-mediated tonic currents are unchanged in SCI, in either magnitude or the number of neurons that exhibit them. Given that these tonic currents are exquisitely sensitive to levels of ambient GABA, it seems unlikely that our experimental conditions cause ambient GABA levels to be differentially affected in SCI compared to sham. Furthermore, we have demonstrated that only presynaptic GABA_B receptors in GABAergic, and not glutamatergic, terminals are tonically active in the sham condition, suggesting that GABA levels affecting presynaptic regulation are likely modulated at a local level.

We considered PO neurons to be homogenous, due to the reported similarities in their morphology and membrane properties^{28,55}, the absence of interneurons in the ventrobasal thalamus^{4,8,46,71} and the uniformly elevated activity levels seen in previous *in vivo* recordings following SCI⁶⁵. However, we did observe a degree of heterogeneity in our neurophysiological measures, especially in tonic GABA_A mediated currents. This variability may suggest a hitherto unknown, intrinsic heterogeneity of PO neurons.

Functional and translational implications

Our previous work in elucidating the regulation of PO and its role in SCI-Pain have largely focused on a cellular level, investigating and manipulating the overall activity level of individual neurons^{for summary, see 64}. In the present study, we demonstrated that SCI leads to physiologically pertinent changes on the synaptic level that likely contribute to the changes in activity previously noted on the cellular level. We report also the loss of GABA_B mediated suppression of release from excitatory terminals, representing the first evidence of a mechanism by which changes to excitatory inputs to PO are also implicated in the development of SCI-Pain; Our previous work focused on the disinhibition hypothesis of

SCI-Pain in which pathological loss of inhibitory tone onto PO is the main driver of pathology. Thus, SCI-Pain is not simply a pathology of decreased inhibition, but a complex interplay of cellular and synaptic changes that result in PO hyperactivity.

Our findings of abnormal changes in presynaptic regulation in SCI-Pain suggest that targeting presynaptic release of neurotransmitters may be an effective way to address this condition. Targeting presynaptic release may be of particular advantage in thalamus, as direct pharmacological targeting of postsynaptic GABA receptors have the capacity to produce seizures^{21,80,94}. A number of both inhibitory and facilitatory presynaptic modulators that target various terminals are already in use. Well known examples include the adrenergic receptor agonist clonidine, serotonergic receptor agonist sumatriptan, and opioid agonist morphine^{notable examples reviewed in 56}.

Among these, the only modulator of presynaptic GABA_B receptors currently in use clinically is the GABA_B receptor agonist baclofen. Baclofen is typically used to treat spasticity, but also for pain^{75,83,88}. Whereas the efficacy of intrathecal baclofen as a treatment for SCI-Pain seemed promising in early studies⁶², more recent studies and meta-analyses have provided no evidence to support its use^{14,62,95}. Other modulators of presynaptic release, such as clonidine and morphine, have been proposed as potential treatments for SCI-Pain; however, their efficacy is also debatable^{14,95}.

These approaches may have been ineffective because of their method of delivery. Typically, these drugs are delivered directly into the central nervous system via intrathecal pumps to maximize delivery and minimize systemic side effects. However, limited cerebrospinal fluid flow in the intrathecal space likely prevents the drug from entering the brain, limiting its effects to the spinal cord^{12,25}. Additionally, there may be issues of specificity of drug targeting; most drugs that affect presynaptic release, including baclofen, act on both pre and postsynaptic targets, possibly complicating the overall effect of the drug. Further, receptors, such as GABA_B receptors, are found on various types of terminals that may have opposing effects. Thus, the feasibility of clinical use of modulators of presynaptic release to treat SCI-pain would be contingent on several requirements, including effective delivery and selective targeting. Interventional therapies also exist that exert significant effects on presynaptic probability of release. Some effects of deep brain stimulation across various brain regions have been attributed to changes in presynaptic release probability^{81,93}. However, it is unclear whether interventional approaches can selectively target presynaptic mechanisms, with minimal nonspecific effects.

Acknowledgments

Research reported in this publication was supported by the National Institute of Neurological Disorders and Stroke of the National Institutes of Health under award number R01NS051799 to A. Keller. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

1. Ab Aziz CB, Ahmad AH. The role of the thalamus in modulating pain. *Malays J Med Sci*. 2006; 13:11–18. [PubMed: 22589599]

2. Anderson WS, O'Hara S, Lawson HC, Treede RD, Lenz FA. Plasticity of pain-related neuronal activity in the human thalamus. *Prog Brain Res*. 2006; 157:353–364. [PubMed: 17046675]
3. Andrade R. Blockade of neurotransmitter-activated K⁺ conductance by QX-314 in the rat hippocampus. *Eur J Pharmacol*. 1991; 199:259–262. [PubMed: 1659537]
4. Arcelli P, Frassoni C, Regondi MC, Biasi SDE, Spreafico R. GABAergic Neurons in Mammalian Thalamus: A Marker of Thalamic Complexity. *Brain Research Bulletin*. 1997; 42:27–37. [PubMed: 8978932]
5. Atwood, HK., Malinow, Roberto, Manabe, Toshiya, Poo, Mu-ming, Sakmann, Bert, Angus Silver, P., Harold, Altman DSF., Thompson, Scott M., Stephen Redman Jennifer, S., et al. Central Synapses, Quantal Mechanisms and Plasticity (Human Frontier Workshop Reports, Workshop IV), Human Frontier Science Program; Strasbourg, France. 1998;
6. Baastrup C, Finnerup NB. Pharmacological management of neuropathic pain following spinal cord injury. *CNS Drugs*. 2008; 22:455–475. [PubMed: 18484790]
7. Baksh MM, Kussrow AK, Mileni M, Finn MG, Bornhop DJ. Label-free quantification of membrane-ligand interactions using backscattering interferometry. *Nat Biotechnol*. 2011; 29:357–360. [PubMed: 21399645]
8. Barbaresi P, Spreafico R, Frassoni C, Rustioni A. GABAergic neurons are present in the dorsal column nuclei but not in the ventroposterior complex of rats. *Brain Research*. 1986; 382:305–326. [PubMed: 2428443]
9. Bardin L, Malfetes N, Newman-Tancredi A, Depoortere R. Chronic restraint stress induces mechanical and cold allodynia, and enhances inflammatory pain in rat: Relevance to human stress-associated painful pathologies. *Behav Brain Res*. 2009; 205:360–366. [PubMed: 19616033]
10. Bartho P, Freund TF, Acsady L. Selective GABAergic innervation of thalamic nuclei from zona incerta. *Eur J Neurosci*. 2002; 16:999–1014. [PubMed: 12383229]
11. Bartho P, Slezia A, Varga V, Bokor H, Pinault D, Buzsaki G, Acsady L. Cortical control of zona incerta. *J Neurosci*. 2007; 27:1670–1681. [PubMed: 17301175]
12. Battal B, Kocaoglu M, Bulakbasi N, Husmen G, Tuba Sanal H, Tayfun C. Cerebrospinal fluid flow imaging by using phase-contrast MR technique. *Br J Radiol*. 2011; 84:758–765. [PubMed: 21586507]
13. Beenhakker MP, Huguenard JR. Astrocytes as gatekeepers of GABAB receptor function. *J Neurosci*. 2010; 30:15262–15276. [PubMed: 21068331]
14. Bensmail D, Ecoffey C, Ventura M, Albert T. SOFMER FSFPMAR. Chronic neuropathic pain in patients with spinal cord injury. What is the efficacy of regional interventions? Sympathetic blocks, nerve blocks and intrathecal drugs. *Ann Phys Rehabil Med*. 2009; 52:142–148. [PubMed: 19909704]
15. Beri A. Central pain and dysesthesia syndrome. *Neurol Clin*. 1998; 16:899–918. [PubMed: 9767069]
16. Beri A, Dimitrijevi MR, Lindblom U. Central dysesthesia syndrome in spinal cord injury patients. *Pain*. 1988; 34:109–116. [PubMed: 3174149]
17. Birdsall NJ, Hulme EC, Burgen A. The character of the muscarinic receptors in different regions of the rat brain. *Proc R Soc Lond B Biol Sci*. 1980; 207:1–12. [PubMed: 6102394]
18. Boivie, J. Central Pain. In: McMahon, S., Koltzenburg, M., editors. *Wall and Melzack's Textbook of Pain*. Elsevier Health Systems; 2005. p. 1057-1074.
19. Bokor H, Frere SG, Eyre MD, Slezia A, Ulbert I, Luthi A, Acsady L. Selective GABAergic control of higher-order thalamic relays. *Neuron*. 2005; 45:929–940. [PubMed: 15797553]
20. Bonica JJ. History of pain concepts and pain therapy. *Mt Sinai J Med*. 1991; 58:191–202. [PubMed: 1875956]
21. Bortolato M, Frau R, Orrù M, Fà M, Dessì C, Puligheddu M, Barberini L, Pillolla G, Polizzi L, Santoni F, Mereu G, Marrosu F. GABAB receptor activation exacerbates spontaneous spike-and-wave discharges in DBA/2J mice. *Seizure*. 2010; 19:226–231. [PubMed: 20233662]
22. Bowsher D. Termination of the central pain pathway in man: the conscious appreciation of pain. *Brain*. 1957; 80:606–622. [PubMed: 13499763]
23. Brickley SG, Mody I. Extrasynaptic GABA(A) receptors: their function in the CNS and implications for disease. *Neuron*. 2012; 73:23–34. [PubMed: 22243744]

24. Brugger F, Wicki U, Olpe HR, Froestl W, Mickel S. The action of new potent GABAB receptor antagonists in the hemisectioned spinal cord preparation of the rat. *Eur J Pharmacol.* 1993; 235:153–155. [PubMed: 8390938]
25. Bulat M, Klarica M. Recent insights into a new hydrodynamics of the cerebrospinal fluid. *Brain Res Rev.* 2011; 65:99–112. [PubMed: 20817024]
26. Casey KL. Unit analysis of nociceptive mechanisms in the thalamus of the awake squirrel monkey. *J Neurophysiol.* 1966; 29:727–750. [PubMed: 4961088]
27. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods.* 1994; 53:55–63. [PubMed: 7990513]
28. Chiaia NL, Rhoades RW, Fish SE, Killackey HP. Thalamic processing of vibrissal information in the rat: II. Morphological and functional properties of medial ventral posterior nucleus and posterior nucleus neurons. *J Comp Neurol.* 1991; 314:217–236. [PubMed: 1723993]
29. Cruz-Almeida Y, Felix ER, Martinez-Arizala A, Widerström-Noga EG. Decreased spinothalamic and dorsal column medial lemniscus-mediated function is associated with neuropathic pain after spinal cord injury. *J Neurotrauma.* 2012; 29:2706–2715. [PubMed: 22845918]
30. Defrin R, Ohry A, Blumen N, Urca G. Characterization of chronic pain and somatosensory function in spinal cord injury subjects. *Pain.* 2001; 89:253–263. [PubMed: 11166482]
31. Dreyfus FM, Tschertner A, Errington AC, Renger JJ, Shin HS, Uebele VN, Crunelli V, Lambert RC, Leresche N. Selective T-type calcium channel block in thalamic neurons reveals channel redundancy and physiological impact of I(T)window. *J Neurosci.* 2010; 30:99–109. [PubMed: 20053892]
32. Duan B, Cheng L, Bourane S, Britz O, Padilla C, Garcia-Campmany L, Krashes M, Knowlton W, Velasquez T, Ren X, Ross SE, Lowell BB, Wang Y, Goulding M, Ma Q. Identification of spinal circuits transmitting and gating mechanical pain. *Cell.* 2014; 159:1417–1432. [PubMed: 25467445]
33. Emri Z, Turner JP, Crunelli V. Tonic activation of presynaptic GABA(B) receptors on thalamic sensory afferents. *Neuroscience.* 1996; 72:689–698. [PubMed: 9157315]
34. Finnerup NB, Gyldensted C, Fuglsang-Frederiksen A, Bach FW, Jensen TS. Sensory perception in complete spinal cord injury. *Acta Neurol Scand.* 2004; 109:194–199. [PubMed: 14763957]
35. Finnerup NB, Jensen MP, Norrbrink C, Trok K, Johannesen IL, Jensen TS, Werhagen L. A prospective study of pain and psychological functioning following traumatic spinal cord injury. *Spinal Cord.* 2016; 54:816–821. [PubMed: 26927297]
36. Finnerup NB, Johannesen IL, Bach FW, Jensen TS. Sensory function above lesion level in spinal cord injury patients with and without pain. *Somatosens Mot Res.* 2003; 20:71–76. [PubMed: 12745445]
37. Finnerup NB, Sørensen L, Biering-Sørensen F, Johannesen IL, Jensen TS. Segmental hypersensitivity and spinothalamic function in spinal cord injury pain. *Exp Neurol.* 2007; 207:139–149. [PubMed: 17628539]
38. Flatters SJ, Bennett GJ. Ethosuximide reverses paclitaxel- and vincristine-induced painful peripheral neuropathy. *Pain.* 2004; 109:150–161. [PubMed: 15082137]
39. Friedman DP, Murray EA. Thalamic connectivity of the second somatosensory area and neighboring somatosensory fields of the lateral sulcus of the macaque. *J Comp Neurol.* 1986; 252:348–373. [PubMed: 3793981]
40. Froestl W, Mickel SJ, Hall RG, von Sprecher G, Strub D, Baumann PA, Brugger F, Gentsch C, Jaekel J, Olpe HR. Phosphinic acid analogues of GABA. 1. New potent and selective GABAB agonists. *J Med Chem.* 1995; 38:3297–3312. [PubMed: 7650684]
41. Gauriau C, Bernard JF. Posterior triangular thalamic neurons convey nociceptive messages to the secondary somatosensory and insular cortices in the rat. *J Neurosci.* 2004; 24:752–761. [PubMed: 14736861]
42. Glykys J, Mody I. The main source of ambient GABA responsible for tonic inhibition in the mouse hippocampus. *J Physiol.* 2007; 582:1163–1178. [PubMed: 17525114]
43. Hains BC, Saab CY, Waxman SG. Changes in electrophysiological properties and sodium channel Nav1.3 expression in thalamic neurons after spinal cord injury. *Brain.* 2005; 128:2359–2371. [PubMed: 16109750]

44. Halassa MM, Acsády L. Thalamic Inhibition: Diverse Sources, Diverse Scales. *Trends Neurosci.* 2016; 39:680–693. [PubMed: 27589879]
45. Hammer R, Berrie CP, Birdsall NJ, Burgen AS, Hulme EC. Pirenzepine distinguishes between different subclasses of muscarinic receptors. *Nature.* 1980; 283:90–92. [PubMed: 7350532]
46. Harris RM, Hendrickson AE. Local circuit neurons in the rat ventrobasal thalamus--a GABA immunocytochemical study. *Neuroscience.* 1987; 21:229–236. [PubMed: 3299139]
47. Hubscher CH, Johnson RD. Chronic spinal cord injury induced changes in the responses of thalamic neurons. *Exp Neurol.* 2006; 197:177–188. [PubMed: 16266704]
48. Hughes SW, Cope DW, Toth TI, Williams SR, Crunelli V. All thalamocortical neurones possess a T-type Ca²⁺ ‘window’ current that enables the expression of bistability-mediated activities. *J Physiol.* 1999; 517:805–815. [PubMed: 10358120]
49. Karson MA, Whittington KC, Alger BE. Cholecystokinin inhibits endocannabinoid-sensitive hippocampal IPSPs and stimulates others. *Neuropharmacology.* 2008; 54:117–128. [PubMed: 17689570]
50. Kaupmann K, Huggel K, Heid J, Flor PJ, Bischoff S, Mickel SJ, McMaster G, Angst C, Bittiger H, Froestl W, Bettler B. Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. *Nature.* 1997; 386:239–246. [PubMed: 9069281]
51. Kim J, Shin W. How to do random allocation (randomization). *Clin Orthop Surg.* 2014; 6:103–109. [PubMed: 24605197]
52. Kobayashi M, Takei H, Yamamoto K, Hatanaka H, Koshikawa N. Kinetics of GABAB autoreceptor-mediated suppression of GABA release in rat insular cortex. *J Neurophysiol.* 2012; 107:1431–1442. [PubMed: 22190629]
53. Kozlov AS, Angulo MC, Audinat E, Charpak S. Target cell-specific modulation of neuronal activity by astrocytes. *Proc Natl Acad Sci U S A.* 2006; 103:10058–10063. [PubMed: 16782808]
54. Landis SC, Amara SG, Asadullah K, Austin CP, Blumenstein R, Bradley EW, Crystal RG, Darnell RB, Ferrante RJ, Fillit H, Finkelstein R, Fisher M, Gendelman HE, Golub RM, Goudreau JL, Gross RA, Gubitza AK, Hesterlee SE, Howells DW, Huguenard J, Kelner K, Koroshetz W, Krainc D, Lazic SE, Levine MS, Macleod MR, McCall JM, Moxley RT, Narasimhan K, Noble LJ, Perrin S, Porter JD, Steward O, Unger E, Utz U, Silberberg SD. A call for transparent reporting to optimize the predictive value of preclinical research. *Nature.* 2012; 490:187–191. [PubMed: 23060188]
55. Landisman CE, Connors BW. VPM and PoM nuclei of the rat somatosensory thalamus: intrinsic neuronal properties and corticothalamic feedback. *Cereb Cortex.* 2007; 17:2853–2865. [PubMed: 17389627]
56. Langer SZ. Therapeutic use of release-modifying drugs. *Handb Exp Pharmacol.* 2008; 561–573. [PubMed: 18064425]
57. Lavalée P, Urbain N, Dufresne C, Bokor H, Acsády L, Deschenes M. Feedforward inhibitory control of sensory information in higher-order thalamic nuclei. *J Neurosci.* 2005; 25:7489–7498. [PubMed: 16107636]
58. Le Feuvre Y, Fricker D, Leresche N. GABAA receptor-mediated IPSCs in rat thalamic sensory nuclei: patterns of discharge and tonic modulation by GABAB autoreceptors. *J Physiol.* 1997; 502:91–104. [PubMed: 9234199]
59. Lee S, Yoon BE, Berglund K, Oh SJ, Park H, Shin HS, Augustine GJ, Lee CJ. Channel-mediated tonic GABA release from glia. *Science.* 2010; 330:790–796. [PubMed: 20929730]
60. Linke R, Braune G, Schwegler H. Differential projection of the posterior paralaminar thalamic nuclei to the amygdaloid complex in the rat. *Exp Brain Res.* 2000; 134:520–532. [PubMed: 11081834]
61. Linke R, Schwegler H. Convergent and complementary projections of the caudal paralaminar thalamic nuclei to rat temporal and insular cortex. *Cereb Cortex.* 2000; 10:753–771. [PubMed: 10920048]
62. Loubser PG, Akman NM. Effects of intrathecal baclofen on chronic spinal cord injury pain. *J Pain Symptom Manage.* 1996; 12:241–247. [PubMed: 8898508]
63. Lucas JM, Ji Y, Masri R. Motor cortex stimulation reduces hyperalgesia in an animal model of central pain. *Pain.* 2011; 152:1398–1407. [PubMed: 21396776]

64. Masri, R., Keller, A. Chronic Pain Following Spinal Cord Injury. In: Jandial, R.Chen, M.Chen, B., Ciacci, J., editors. *Regenerative Biology of the Spine and Spinal Cord*. Landes Bioscience; 2012.
65. Masri R, Quiton RL, Lucas JM, Murray PD, Thompson SM, Keller A. Zona incerta: a role in central pain. *J Neurophysiol*. 2009; 102:181–191. [PubMed: 19403748]
66. Masri R, Trageser JC, Bezdudnaya T, Li Y, Keller A. Cholinergic regulation of the posterior medial thalamic nucleus. *J Neurophysiol*. 2006; 96:2265–2273. [PubMed: 16870838]
67. Misgeld, U., Frotscher, M. *Central Cholinergic Synaptic Transmission*. Birkhauser; 1989.
68. Mogil, J., Wilson, S., Wan, Y. Assessing Nociception in Murine Subjects. In: Kruger, L., editor. *Methods in Pain Research*. CRC Press; 2001.
69. Murray PD, Masri R, Keller A. Abnormal anterior pretectal nucleus activity contributes to central pain syndrome. *J Neurophysiol*. 2010; 103:3044–3053. [PubMed: 20357063]
70. Nathan T, Jensen MS, Lambert JD. The slow inhibitory postsynaptic potential in rat hippocampal CA1 neurones is blocked by intracellular injection of QX-314. *Neurosci Lett*. 1990; 110:309–313. [PubMed: 2325903]
71. Ottersen OP, Storm-Mathisen J. GABA-containing neurons in the thalamus and pretectum of the rodent: An immunocytochemical study. *Anat Embryol*. 1984; 170:197–207. [PubMed: 6517354]
72. Park A, Li Y, Masri R, Keller A. Presynaptic and extrasynaptic regulation of posterior nucleus of thalamus. *J Neurophysiol*. 2017; 118:507–519. [PubMed: 28331010]
73. Pattany PM, Yezierski RP, Widerstrom-Noga EG, Bowen BC, Martinez-Arizala A, Garcia BR, Quencer RM. Proton magnetic resonance spectroscopy of the thalamus in patients with chronic neuropathic pain after spinal cord injury. *AJNR Am J Neuroradiol*. 2002; 23:901–905. [PubMed: 12063213]
74. Perl ER, Whitlock DG. Somatic stimuli exciting spinothalamic projections to thalamic neurons in cat and monkey. *Exp Neurol*. 1961; 3:256–296. [PubMed: 13734415]
75. Pistoia F, Sacco S, Sarà M, Franceschini M, Carolei A. Intrathecal baclofen: effects on spasticity, pain, and consciousness in disorders of consciousness and locked-in syndrome. *Curr Pain Headache Rep*. 2015; 19:466. [PubMed: 25416459]
76. Poggio GF, Mountcastle VB. A study of the functional contributions of the lemniscal and spinothalamic systems to somatic sensibility. Central nervous mechanisms in pain. *Bull Johns Hopkins Hosp*. 1960; 106:266–316. [PubMed: 14433641]
77. Power BD, Kolmac CI, Mitrofanis J. Evidence for a large projection from the zona incerta to the dorsal thalamus. *J Comp Neurol*. 1999; 404:554–565. [PubMed: 9987997]
78. Ravenscroft A, Ahmed YS, Burnside IG. Chronic pain after SCI. A patient survey. *Spinal Cord*. 2000; 38:611–614. [PubMed: 11093322]
79. Regehr WG. Short-term presynaptic plasticity. *Cold Spring Harb Perspect Biol*. 2012; 4:a005702. [PubMed: 22751149]
80. Richards DA, Lemos T, Whitton PS, Bowery NG. Extracellular GABA in the ventrolateral thalamus of rats exhibiting spontaneous absence epilepsy: a microdialysis study. *J Neurochem*. 1995; 65:1674–1680. [PubMed: 7561864]
81. Ross EK, Kim JP, Settell ML, Han SR, Blaha CD, Min HK, Lee KH. Fornix deep brain stimulation circuit effect is dependent on major excitatory transmission via the nucleus accumbens. *Neuroimage*. 2016; 128:138–148. [PubMed: 26780572]
82. Rossi P, Mapelli L, Roggeri L, Gall D, de Kerchove d'Exaerde A, Schiffmann SN, Taglietti V, D'Angelo E. Inhibition of constitutive inward rectifier currents in cerebellar granule cells by pharmacological and synaptic activation of GABA receptors. *Eur J Neurosci*. 2006; 24:419–432. [PubMed: 16903850]
83. Saltuari L, Kronenberg M, Marosi MJ, Kofler M, Russegger L, Rifici C, Bramanti P, Gerstenbrand F. Indication, efficiency and complications of intrathecal pump supported baclofen treatment in spinal spasticity. *Acta Neurol (Napoli)*. 1992; 14:187–194. [PubMed: 1442217]
84. Samuelsson M, Leffler AS, Johansson B, Hansson P. On the repeatability of brush-evoked allodynia using a novel semi-quantitative method in patients with peripheral neuropathic pain. *Pain*. 2007; 130:40–46. [PubMed: 17161534]
85. Sherman, SM., Guillery, RW. *Functional Connections of Cortical Areas: A New View from the Thalamus*. Cambridge, MA: The MIT Press; 2013.

86. Siddall PJ, McClelland JM, Rutkowski SB, Cousins MJ. A longitudinal study of the prevalence and characteristics of pain in the first 5 years following spinal cord injury. *Pain*. 2003; 103:249–257. [PubMed: 12791431]
87. Steriade, M. A Bradford Book. 2001. The Intact and Sliced Brain.
88. Tasséel Ponche S, Ferrapie AL, Chenet A, Menei P, Gambart G, Ménégalli Bogeli D, Perrouin Verbe B, Gay S, Richard I. Intrathecal baclofen in cerebral palsy. A retrospective study of 25 wheelchair-assisted adults. *Ann Phys Rehabil Med*. 2010; 53:483–498. [PubMed: 20829144]
89. Ting JT, Daigle TL, Chen Q, Feng G. Acute brain slice methods for adult and aging animals: application of targeted patch clamp analysis and optogenetics. *Methods Mol Biol*. 2014; 1183:221–242. [PubMed: 25023312]
90. Trageser JC, Burke KA, Masri R, Li Y, Sellers L, Keller A. State-dependent gating of sensory inputs by zona incerta. *J Neurophysiol*. 2006; 96:1456–1463. [PubMed: 16775205]
91. Trageser JC, Keller A. Reducing the uncertainty: gating of peripheral inputs by zona incerta. *J Neurosci*. 2004; 24:8911–8915. [PubMed: 15470158]
92. Tscherter A, David F, Ivanova T, Deleuze C, Renger JJ, Uebele VN, Shin HS, Bal T, Leresche N, Lambert RC. Minimal alterations in T-type calcium channel gating markedly modify physiological firing dynamics. *J Physiol*. 2011; 589:1707–1724. [PubMed: 21320888]
93. Veerakumar A, Challis C, Gupta P, Da J, Upadhyay A, Beck SG, Berton O. Antidepressant-like effects of cortical deep brain stimulation coincide with pro-neuroplastic adaptations of serotonin systems. *Biol Psychiatry*. 2014; 76:203–212. [PubMed: 24503468]
94. Vergnes M, Marescaux C, Micheletti G, Depaulis A, Rumbach L, Warter JM. Enhancement of spike and wave discharges by GABA-mimetic drugs in rats with spontaneous petit-mal-like epilepsy. *Neurosci Lett*. 1984; 44:91–94. [PubMed: 6425742]
95. von Heijne M, Hao JX, Sollevi A, Xu XJ. Effects of intrathecal morphine, baclofen, clonidine and R-PIA on the acute allodynia-like behaviours after spinal cord ischaemia in rats. *Eur J Pain*. 2001; 5:1–10.
96. Wang G, Thompson SM. Maladaptive homeostatic plasticity in a rodent model of central pain syndrome: thalamic hyperexcitability after spinothalamic tract lesions. *J Neurosci*. 2008; 28:11959–11969. [PubMed: 19005061]
97. Warms CA, Turner JA, Marshall HM, Cardenas DD. Treatments for chronic pain associated with spinal cord injuries: many are tried, few are helpful. *Clin J Pain*. 2002; 18:154–163. [PubMed: 12048417]
98. Weng HR, Lee JI, Lenz FA, Schwartz A, Vierck C, Rowland L, Dougherty PM. Functional plasticity in primate somatosensory thalamus following chronic lesion of the ventral lateral spinal cord. *Neuroscience*. 2000; 101:393–401. [PubMed: 11074162]
99. Whitlock DG, Perl ER. Thalamic projections of spinothalamic pathways in monkey. *Exp Neurol*. 1961; 3:240–255. [PubMed: 13784911]
100. Whitt JL, Masri R, Pulimood NS, Keller A. Pathological activity in mediodorsal thalamus of rats with spinal cord injury pain. *J Neurosci*. 2013; 33:3915–3926. [PubMed: 23447602]
101. Wilson SG, Mogil JS. Measuring pain in the (knockout) mouse: big challenges in a small mammal. *Behav Brain Res*. 2001; 125:65–73. [PubMed: 11682095]
102. Wójtowicz AM, Dvorzhak A, Semtner M, Grantyn R. Reduced tonic inhibition in striatal output neurons from Huntington mice due to loss of astrocytic GABA release through GAT-3. *Front Neural Circuits*. 2013; 7:188. [PubMed: 24324407]
103. Wu LJ, Steenland HW, Kim SS, Isiegas C, Abel T, Kaang BK, Zhuo M. Enhancement of presynaptic glutamate release and persistent inflammatory pain by increasing neuronal cAMP in the anterior cingulate cortex. *Mol Pain*. 2008; 4:40. [PubMed: 18823548]
104. Yezierski RP. Pain following spinal cord injury: pathophysiology and central mechanisms. *Prog Brain Res*. 2000; 129:429–449. [PubMed: 11098709]
105. Zheng F, Wess J, Alzheimer C. M2 muscarinic acetylcholine receptors regulate long-term potentiation at hippocampal CA3 pyramidal cell synapses in an input-specific fashion. *J Neurophysiol*. 2012; 108:91–100. [PubMed: 22490561]
106. Zucker RS, Regehr WG. Short-term synaptic plasticity. *Annu Rev Physiol*. 2002; 64:355–405. [PubMed: 11826273]

PERSPECTIVE

This manuscript presents synaptic mechanisms that may underlie the development and maintenance of SCI-Pain. Given the difficulty in treating SCI-Pain, a better understanding of the underlying neurobiological mechanisms is critical, and may allow development of better treatment modalities.

Highlights

- Electrolytic lesion of STT produces behavioral signs consistent with SCI-Pain
- Tonic activation of presynaptic GABA_B autoreceptors in PO is absent in SCI-Pain
- Regulation of glutamatergic release in PO by GABA_BR is absent in SCI-Pain

Figure 1.

Electrolytic lesion of spinal cord produces behavioral signs of pain. **A.** Representative histological section of mouse spinal cord with unilateral electrolytic lesion in the spinothalamic tract (C-7 level). Section has been Nissl stained with toluidine blue for visualization. Arrow indicates slight surface damage from where the electrode entered spinal tissue while arrowheads indicate electrolytic damage. **B:** Mechanical withdrawal thresholds are shown, normalized to each animal's baseline level. Note that a lower threshold represents hyperalgesia. Thresholds are decreased bilaterally in SCI animals (solid bars). In sham-operated animals (empty bars), there is a transient decrease in response threshold following surgery, but increases back to baseline after 14 days. **C:** Acetone induced cold allodynia test reveals enhanced cold allodynia in SCI animals bilaterally. Note that higher scores represent allodynia. **D:** SCI animals exhibit significantly increased dynamic mechanical allodynia bilaterally. As in C, higher scores represent allodynia. CC: central canal, DH: dorsal horn, VH: ventral horn, lst: lateral spinothalamic tract, vst: ventral spinothalamic tract, vsc: ventral spinocerebellar tract. Statistical comparisons are made

between the SCI and sham groups at each time point. * represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$. Lack of a symbol represents no significant difference. Error bars represent standard deviation.

Figure 2.

Tonic activation of GABA_B autoreceptors in PO are suppressed in SCI. **A.** Representative whole-cell voltage-clamp recordings of mIPSCs from PO neurons from either SCI condition (left), or sham condition (right) recorded with high intracellular chloride concentrations in the presence of 0.5 μ M TTX, 20 μ M CNQX during baseline (top), baclofen-applied (middle), and CGP-55845-applied (bottom) conditions. Baclofen (10 μ M) suppresses mIPSC frequency in both SCI and sham conditions. In the SCI condition, CGP-55845 application (2 μ M) reverses the effect of baclofen, while in the sham condition, CGP-55845 application further increases mIPSC frequency beyond baseline levels. **B.** Cumulative probability plots of the amplitude (left) and inter-event interval (right) of the representative traces from the SCI condition. Baclofen condition inter-event interval plot is significantly right-shifted compared to the baseline and CGP-55845 conditions. Baseline and CGP-55845 inter-event interval plots are not significantly different. No differences were observed in amplitude across conditions. **C.** Cumulative probability plots of representative traces from the sham condition, analogous to (B). Baclofen inter-event interval plot is significantly right-shifted, while CGP-55845 plot is significantly left-shifted compared to baseline. No differences were observed in amplitude. **D.** Group data plots depicting changes in mIPSC frequencies with application of baclofen (left) or CGP-55845 (right) in the SCI condition. Solid black lines indicate average, solid gray lines indicate statistically significant change from drug application within-neuron by K-S test, and red dotted lines indicate non-significant differences. Majority of neurons exhibited significantly decreased mIPSC frequencies with baclofen application, while majority of neurons exhibited no difference from baseline with CGP-55845 application. **E.** Group data plots depicting changes in mIPSC frequencies in the sham condition, analogous to (D). Majority of neurons exhibited significantly decreased mIPSC frequencies with baclofen, and significantly increased frequencies with CGP-55845.

Figure 3.

Cholinergic regulation of GABA release is unaffected by SCI. **A.** Representative whole-cell voltage-clamp recordings of mIPSCs from PO neurons from either SCI condition (left), or sham condition (right) recorded with high intracellular chloride concentrations in the presence of 0.5 μ M TTX, 20 μ M CNQX during baseline (top), and carbachol-applied (bottom) conditions. Carbachol (2 μ M) produces significant decreases in mIPSC frequency in both SCI and sham conditions. **B,C.** Cumulative probability plots of the amplitude (left) and inter-event interval (right) of the representative traces from SCI (B) and sham (C) conditions. In both conditions, carbachol inter-event interval plot is significantly right-shifted compared to the baseline. No differences were observed in amplitude across conditions. **D,E.** Group data plots depicting changes in mIPSC frequencies with carbachol application in the SCI (D) and sham (E) conditions. Solid black lines indicate average, solid gray lines indicate statistically significant change from drug application within-neuron by K-S test, and red dotted lines indicate non-significant differences. All neurons tested exhibited significantly decreased mIPSC frequencies with carbachol application in both conditions.

Figure 4.

Presynaptic GABA_B receptor mediated regulation of glutamate release in PO is suppressed in SCI. **A.** Representative whole-cell voltage-clamp recordings of mEPSCs from PO neurons from either SCI condition (left), or sham condition (right) in the presence of 0.5 μ M TTX, 10 μ M gabazine during baseline (top), baclofen-applied (middle), and CGP-55845-applied (bottom) conditions. In the SCI condition, neither baclofen nor CGP-55845 application has an effect on mEPSCs, while in the sham condition, baclofen significantly reduces mEPSC frequency compared to baseline. CGP-55845 application in sham condition reverses the effects of baclofen and brings mEPSC frequency back to baseline levels. **B.** Cumulative probability plots of the amplitude (left) and inter-event interval (right) of the representative traces from the sham condition. Baclofen inter-event interval plot is significantly right-shifted, while CGP-55845 plot is not significantly different from baseline. No differences were observed in amplitude. **C.** Group data plots depicting changes in mEPSC frequencies with application of baclofen (left) or CGP-55845 (right) in the sham condition. Solid black lines indicate average, solid gray lines indicate statistically significant change from drug application within-neuron by K-S test, and red dotted lines indicate non-significant differences. Majority of neurons exhibited significantly decreased mEPSC frequencies with baclofen. CGP-55845 did not produce further increases in mEPSC frequency compared to baseline in most neurons. **D.** Cumulative probability plots of representative traces from the SCI condition, analogous to (B). No differences are observed in either amplitude or frequency across conditions. **E.** Group data plots depicting changes in mEPSC frequencies in the SCI condition, analogous to (C). Majority of neurons did not produce significant changes to mEPSC frequency with either baclofen or CGP-55845 application.

Figure 5.

Schematic illustration of changes to presynaptic regulation of neurotransmitter release by SCI. **A.** In normal conditions, release from GABAergic terminals in PO is regulated by presynaptic GABA_B receptors that are tonically, but not maximally, active at baseline. Thus, release can be dynamically modulated to enhance release by reducing tonic activation (rightward arrow) or to decrease release by further activating the GABA_B receptors (leftward arrow). However, in SCI, tonic activation is suppressed, leading to the loss of ability to up regulate GABA release. **B.** In normal conditions, release from glutamatergic terminals in PO is also regulated by presynaptic GABA_B receptors; activation of these receptors leads to the suppression of glutamate release, reducing excitatory drive. In SCI, the ability to suppress release through this mechanism is lost.