Glutamate transporter dysfunction associated with nerve injury-induced pain in mice

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Napier IA, Mohammadi SA, Christie MJ. Glutamate transporter dysfunction associated with nerve injury-induced pain in mice. J Neurophysiol 107: 649–657, 2012. First published November 9, 2011; doi:10.1152/jn.00763.2011.—Dysfunction at glutamatergic synapses has been proposed as a mechanism in the development of neuropathic pain. Here we sought to determine whether peripheral nerve injury-induced neuropathic pain results in functional changes to primary afferent synapses. Signs of neuropathic pain as well as an induction of glial fibrillary acidic protein in immunostained spinal primary afferent synapses. Dysfunction at glutamatergic synapses has been proposed as a mechanism. Inflammation within the spinal cord sections 4 days after partial ligation of the sciatic nerve indicated induction of glial fibrillary acidic protein in immunostained spinal primary afferent synapses. Signs of neuropathic pain as well as an induction of glial fibrillary acidic protein in immunostained spinal primary afferent synapses. Dysfunction at glutamatergic synapses has been proposed as a mechanism in the development of neuropathic pain. Here we sought to determine whether peripheral nerve injury-induced neuropathic pain results in functional changes to primary afferent synapses. Signs of neuropathic pain as well as an induction of glial fibrillary acidic protein in immunostained spinal primary afferent synapses.
(2.5% in oxygen), the biceps femoris muscle was blunt dissected to reveal the sciatic nerve proximal to its trifurcation. Connective tissue surrounding the nerve was carefully removed to improve access. A spinal hook was then used to lift the nerve without compression or stretching so that a single 6-0 silk suture could be threaded through approximately one-half of the nerve. This single suture was tied tightly before the nerve was returned to its original position. The musculature was then sewn together with a single suture, and the skin was brought together and closed with cyanoacrylate glue (Vetbond, 3M). The impact of nerve injury on hind paw weight bearing was assessed with a Linton Incapacitance Tester (Linton Instrumentation) before and 1, 4, and 7 days following nerve injury (Strickland et al. 2008). Measurements were taken by averaging the instantaneous force (measured as weight, in grams) applied to each hind-paw at presurgery (baseline) and each day postsurgery. Each datum is the average of 4 measurements taken at 10-s intervals. An incapacitation ratio was therefore derived as the force applied by the injured hind paw/uninjured paw. Animals that failed to show a 25% or greater reduction in their incapacitation ratio (7 of the 91 mice) were not used for biochemical or electrophysiological experiments and were euthanized.

Immediately following incapacitation measurements, each animal was placed into a plexiglass observation chamber (40 × 20 × 20 cm) and was monitored for 5 min for signs of spontaneous pain by scoring the number of events of hind-paw lifting, hind-paw flicking or shaking, and hind-paw tending. A composite score for each animal and was monitored for 5 min for signs of spontaneous pain by scoring the number of events of hind-paw lifting, hind-paw flicking or shaking, and hind-paw tending. A composite score for each animal was calculated by summing each score. As the mice were able to move freely about the enclosure, no scoring bias was applied to the affected paw.

**Immunofluorescent detection of spinal glutamate transporters.** Following pentobarbital sodium administration, mice were transcardially perfused with 10 ml of a heparin containing (3,000 IU/l) flush solution (in mM; 154 NaCl and 58.8 NaHCO3) before perfusion with 100 ml of the fixation solution; 4% formaldehyde prepared in PBS (in mM; 25.3 NaH2PO4·H2O, 108 Na2HPO4, and 154 NaCl). Spinal cords were removed following fixation and postfixed for 1 h before being transferred to a recording chamber where Dod-Chrome contrast optics was used to identify large I/Ib superficial dorsal horn neurons for patch-clamp electrophysiology. The internal solution of the recording pipette contained the following (in mM): 140 CsCl, 10 EGTA, 2 CaCl2, 2 MgATP, 0.3 NaGTP, and 5 QX314 chloride and had an osmolality of 290 mosmol/kg H2O. Drugs were superfused onto slices at a rate of 2 ml per min in normal aCSF at a nominal 33°C. Whole cell voltage clamp was performed using a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale CA) interfaced to an intel processor-based iMac computer (Apple, Cupertino, CA; Mac OS X 10.5) via an ITC-18 digitiser (Heka Elektronik, Ludwigshafen Germany). Electrically evoked excitatory postsynaptic currents (eEPSCs) were elicited by stimulating dorsal roots attached to the slice at 0.03 Hz with bipolar tungsten electrodes so that an appropriately sized current was produced in recorded neurons in the range of 100–800 pA. eEPSCs were sampled at 10 kHz and filtered at 4 kHz using Axograph X (Axograph Scientific). N-methyl-D-aspartate receptor (AMPA) mediated currents were obtained by clamping the membrane potential at −60 mV in the presence of 100 μM picrotoxin, 5 μM strychnine, and 100 μM DL-AP5. N-methyl-D-aspartate receptor (NMDAR)-mediated currents were obtained by clamping the membrane potential at +40 mV in the presence of 100 μM picrotoxin, 5 μM strychnine, and 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). Miniature EPSCs (mEPSCs) were filtered (4-kHz low-pass filter) and sampled at 10 kHz for online and later offline analysis and were recorded in the presence of 100 μM picrotoxin, 5 μM strychnine, and 1 μM tetrodotoxin to block GABAA, glycine, and sodium channels, respectively. mEPSCs above a preset threshold (4 SD above baseline noise) were automatically detected by a sliding template algorithm and then manually checked offline. mEPSCs were then counted in 4-s epochs every 2 s to construct rate-time plots. Data presented represent the average of ≥800 captured events over a minimum 5-min interval for each condition (drug or vehicle) for each cell recorded (n = 4). Series resistance (<20 MΩ) was compensated by 70–80% in all patch-clamp experiments. Recordings were stopped if series resistance deviated by >20% of baseline.

**Data analysis and statistics.** Densitometry of immunohistochemistry was performed using ImageJ (National Institutes of Health). Behavioral testing was recorded by the observer in a notebook before being manually entered into Prism (Version 5 for Windows; GraphPad Software) for statistical analysis and generation of graphs. Illustrations were prepared using Photoshop CS4 and Illustrator CS4 for Windows. Kinetics of AMPAR and NMDAR evoked eEPSCs were determined offline using the “fit exponential” tool in Axograph on the assembled average of 10 consecutive eEPSCs for each condition. The kinetics of unperturbed (in the absence of uptake inhibitors) eEPSC decay were best described by a single exponential. As with previous reports, the addition of a second exponential did not improve the quality of the fit (Grosskreutz et al. 2003). Rise and decay kinetics for each recording were only included for analysis if the assembled average trace was smooth enough to confidently measure these parameters. The area under the curve was also determined for each trace using Axograph. Statistical differences were determined using one-way ANOVA with Dunn’s multiple comparisons posttest (Western...
blots and behavioral tests) and Student’s t-test (immunohistochemistry and electrophysiology) in Prism.

Drugs. Unless otherwise stated, all reagents were obtained from Sigma-Aldrich (St. Louis, MO). Disodium CNQX, picrotoxin, strychnine, dl-threo-β-benzoxyspartic acid (TBOA), and dl-AP5 were purchased from Tocris (Bristol, UK). Tetrodotoxin and QX314 chloride were obtained from Alamone Labs (Jerusalem, Israel).

RESULTS

Nerve injury-induced signs of neuropathic pain and astrocyte activation. In agreement with previous reports, PNL induced reliable signs of pain (Seltzer et al. 1990) as well as increased staining of GFAP (Coyle 1998; Narita et al. 2006). From as early as 24 h postsurgery, nerve-injured mice displayed a significant reduction in the force applied to the injured paw compared with baseline (Fig. 1A, left-to-right ratio, baseline = 1.08 ± 0.03 vs. day 1 = 0.44 ± 0.02; P < 0.0001; ANOVA). This change continued up to and beyond day 7 postsurgery (day 4 = 0.51 ± 0.04 and day 7 = 0.58 ± 0.07; P < 0.0001; ANOVA). Similarly, signs of paw lifting, flicking, and guarding remained elevated during the 7-day postsurgical period (Fig. 1B, left vs. right, days 1, 4, and 7; P < 0.0001; ANOVA). We examined the well-established activation of astrocytes following PNL by examining GFAP staining. As shown in Fig. 1C the GFAP immunoreactivity was significantly elevated after nerve injury with the greatest effect being observed 4 days postinjury.

Effect of PNL on expression of spinal glutamate transporters. Following nerve injury, perfusion-fixed mouse spinal cord was sectioned for immunohistochemically stained sections of lumbar spinal cord at days 4 and 7 postinjury. The glial transporters EAAT1 and EAAT2 (Fig. 2, A and B) showed small, persistent decreases in ipsilateral dorsal horn with contralateral dorsal horn. For EAAT1, the ~7% decrease in optical density in the dorsal horn was not significant at either day 4 or 7 after injury. However, when both postinjury days were pooled, the decrease was significant (P < 0.05). For EAAT2, the 9% decreases in optical density for ipsilateral vs. contralateral dorsal horn were significantly reduced on both day 4 and day 7 after injury. The small reduction in EAAT2 immunohistochemical staining was then further explored by Western blot on crudely isolated dorsal horn soluble protein extracts in a separate group of animals. In these experiments, no significant changes to total EAAT2 levels were found at either postinjury time point (data not shown). As shown in Fig. 2D, no trends for changed expression of EAAT3 were detected immunohistochemically. We should note, however, that the antibody used to detect EAAT3 was from the same species (mouse) as the experimental animals and is most likely the cause of some non-specific staining, especially in the ventral horn. However, in concurrent experiments performed without primary antibody (negative controls), nonspecific staining was not observed in the superficial dorsal horn where analysis was performed (data not shown). Collectively, these data suggest that nerve injury associated with development of neuropathic pain has only a modest impact on the expression of glutamate transporters in the spinal dorsal horn.

**Fig. 1.** Effect of nerve injury on the development of neuropathic pain. A: hind-paw incapacitance test revealing significant weight shift from left (injured) to right hind paw. Numbers in columns represent number of animals tested. B: partial sciatic nerve ligation produces significant increase in the rate of spontaneous behaviors indicative of neuropathic pain. C: glial fibrillary acidic protein (GFAP) immunoreactivity in a spinal cord section from a nerve-injured mouse (inj, injured/ipsilateral) and histogram revealing significant increase in GFAP densitometry at day 4 postinjury (n = 4 in each group) *P < 0.05, ***P < 0.001.
Effect of nerve injury on AMPAR synaptic current kinetics.

If the small reduction in immunohistochemical staining for the astrocyte glutamate transporters excitatory amino acid transporters (EAAT) 1 (A), EAAT2 (B), and EAAT3 (C) following nerve injury reveals significant reduction of EAAT1 and EAAT2 between 4 and 7 days (*P < 0.05; n = 4 in each group).

Example AMPAR- and NMDAR-mediated eEPSCs 4 days after injury because maximal effects on GFAP, as well as decreased expression of EAAT1 and EAAT2, were observed at that time.

Example AMPAR- and NMDAR mediated eEPSCs are shown in Fig. 3. A and B. AMPAR and NMDAR eEPSC decay time constants were best fit by single exponentials as previously described (Feldmeyer et al. 2002; Grosskreutz et al. 2003; Stubblefield and Benke 2010). Fitted time constants did not differ significantly for either the rise (naive: 0.95 ± 0.1 ms, n = 12;...
injured: 1.0 ± 0.15 ms, n = 10) or decay (naive: 6.15 ± 0.74 ms, n = 23; injured: 6.65 ± 0.95 ms, n = 20) of AMPAR-mediated eEPSCs in naïve vs. injured animals, measured in the presence of DL-AP5 (100 µM), picrotoxin (100 µM), and strychnine (5 µM). Similarly, NMDAR rise time and decay kinetics, measured in the presence of CNQX (10 µM), picrotoxin (100 µM), and strychnine (5 µM), were not affected by nerve injury (rise, naïve: 6.29 ± 1.09 ms, n = 8; injured: 6.63 ± 1.69 ms, n = 6; decay, naïve: 195.6 ± 24.18, n = 7; injured: 187.8 ± 28.82, n = 7). These findings suggest that impaired glutamate transporter activity might not contribute greatly to primary afferent synaptic transmission after nerve injury.

Effect of nerve injury on sensitivity of glutamate transporters to inhibition. Measures of unperturbed decay time constants could be an insensitive measure of reduced glutamate reuptake in the vicinity of synapses because decay kinetics of both AMPAR- and NMDAR-mediated synaptic currents are dominated by channel kinetics rather than glutamate reuptake (Dingledine et al. 1999). Such measures could also be confounded by possible changes in subunit composition of AMPARs or NMDARs in pain states (Vikman et al. 2008), although our data suggest that no such changes occur in mouse dorsal horn 4 days after nerve injury. Therefore, to further explore whether enhanced spillover of synaptic glutamate develops after nerve injury, eEPSCs were recorded in the presence of a moderate concentration of a nonspecific glutamate transport inhibitor, TBOA (Shimamoto et al. 1998). By partially blocking glutamate reuptake to induce detectable spillover from excitatory synapses, any pathologically enhanced spillover of glutamate produced by nerve injury should be observed as an enhancement of these effects as previously reported at other central nervous system synapses (Nie and Weng 2010). As expected in naïve animals, TBOA (30 and 100 µM) potentiated the duration but not amplitude of AMPAR eEPSCs in a concentration-dependent manner by introducing the appearance of a second, late phase to the decay of the eEPSC without affecting the early decay phase (Fig. 4, A and B). As shown in Fig. 4B, 100 µM TBOA significantly increased the eEPSC late time constant (P = 0.015, from 36.5 ± 8.1 to 125.4 ± 20.4 ms, n = 6), as well as the area under the curve (P < 0.05, from 1688 ± 373 to 6,013 ± 1,269 pA/ms, n = 6) in naïve animals. Both measures presumably reflect spillover of glutamate from subsynaptic to extrasynaptic sites and adjacent synapses. In dorsal horn neurons from untreated animals, transporter inhibition had no significant effect on the early decay time constant (control = 5.1 ± 0.5 ms, 30 µM = 5.6 ± 1.6 ms, and 100 µM = 6.0 ± 1.2 ms), which is presumably dominated by decay kinetics of channel opening (see also below).

In the presence of TBOA, the addition of CNQX (10 µM in the continued presence of DL-AP5, picrotoxin, and strychnine) potentiated the duration but not amplitude of AMPAR eEPSCs in a concentration-dependent manner by introducing the appearance of a second, late phase to the decay of the eEPSC without affecting the early decay phase (Fig. 4, A and B). As shown in Fig. 4B, 100 µM TBOA significantly increased the eEPSC late time constant (P = 0.015, from 36.5 ± 8.1 to 125.4 ± 20.4 ms, n = 6), as well as the area under the curve (P < 0.05, from 1688 ± 373 to 6,013 ± 1,269 pA/ms, n = 6) in naïve animals. Both measures presumably reflect spillover of glutamate from subsynaptic to extrasynaptic sites and adjacent synapses. In dorsal horn neurons from untreated animals, transporter inhibition had no significant effect on the early decay time constant (control = 5.1 ± 0.5 ms, 30 µM = 5.6 ± 1.6 ms, and 100 µM = 6.0 ± 1.2 ms), which is presumably dominated by decay kinetics of channel opening (see also below).

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blocked all slow synaptic currents and nearly abolished the fast component \( (n = 3) \). This suggests that the enhanced slow components of the eEPSC induced by TBOA are mediated exclusively by AMPARs without any contribution from metabotropic glutamate receptors \((\text{mGluRs})\). mGluR-mediated slow eEPSCs have been reported in dorsal horn in the presence of TBOA but only after high frequency stimulation of primary afferent fibres \((\text{Galik et al. } 2008)\). TBOA \((100 \ \text{\mu M} \text{ but not } 30 \ \text{\mu M})\) also produced a small but significant inhibition of the amplitude of eEPSCs \((14 \pm 1.8\%)\), which could be mediated by spillover of glutamate to presynaptic mGluR \((\text{Drew et al. } 2008)\) or kainate receptors \((\text{Perrais et al. } 2010)\). A 30-\mu M concentration of TBOA was therefore selected for subsequent experiments in nerve-injured tissue because it produced only a moderate enhancement of the eEPSC late decay time constant without affecting amplitude.

If nerve injury does induce EAAT down-regulation in the vicinity of dorsal horn primary afferent synapses, glutamate spillover might be enhanced by moderate EAAT inhibition \((30 \ \text{\mu M} \text{TBOA})\) to a greater extent in nerve injured than control animals \((\text{Fig. 3C})\). As shown in Fig. 3D, TBOA significantly increased the early decay time constant in dorsal horn neurons from nerve-injured animals, suggesting that transporters in close proximity to glutamate release sites might be functionally modified to increase spillover. Alternatively, nerve-injured mice could express more extrasynaptic AMPA receptors in close proximity to release sites. Nerve injury did not greatly influence distal extrasynaptic receptor activation as no change to area under the curve or late decay time constant was observed following transporter inhibition. These findings suggest that reduced EAAT activity in the vicinity of primary afferent synapses, if it occurs after nerve injury, is not due to a widespread reduction in glutamate uptake capacity but is restricted to proximal extrasynaptic sites.

To ensure that the enhancing effects of TBOA on AMPAR kinetics were not due to presynaptic actions, mEPSCs were examined in naive animals. In the presence of 1 \mu M tetrodotoxin, no significant changes to mEPSC decay, amplitude, or frequency were observed during glutamate transporter inhibition in naive animals \((100 \ \text{\mu M} \text{TBOA}; \ n = 4; \text{ Fig. } 5)\). This is consistent with the finding that the early decay time constant of the eEPSC in uninjured mice was unaffected by TBOA and the expectation that mEPSCs, being sporadic quantal events, should produce little spillover to extrasynaptic AMPARs.

If reduced perisynaptic glutamate transporter activity does indeed explain the increased early decay time constant of AMPAR-mediated synaptic events via increased spillover, then it might also be possible to detect increased spillover to NMDA receptors. To address this, we utilized the use-dependent NMDA receptor blocker \( (+) \)-MK-801 to block subsynaptic NMDARs before enhancing glutamate spillover with TBOA, as reported at this synapse \((\text{Nie and Weng } 2009)\). In this experiment, stable baseline NMDAR-mediated eEPSCs \((\text{in the presence of CNQX})\) for dorsal horn neurons in whole cell voltage-clamp \( (+40 \text{ mV})\) were established by electrically stimulating the dorsal roots at 0.03 Hz \((\text{Fig. 6Aii})\). Then, active/open NMDARs were blocked during a 10-min superfusion of 50 \mu M MK-801. At this time, subsynaptic NMDAR eEPSCs were completely blocked \((\text{Fig. 6Aii})\). A 10-min wash was then performed to remove unbound MK-801 while stimulation continued \((\text{Fig. 6Aiii})\). Following this, 100 \mu M TBOA was superfused onto slices to generate glutamate spillover \((\text{Fig. 6Aiii})\). As shown in Fig. 6A, this approach successfully induced glutamate spillover and activation of extrasynaptic NMDARs. When performed in slices from nerve-injured mice, no change in time to peak was observed \((\text{Fig. 6B})\). However, a small nonsignificant trend for increase in peak amplitude and area under the curve were observed. Collectively, however, these findings suggest that no significant increase of spillover to extra-
DISCUSSION

The present study suggests that nerve injury associated with allodynia and astroglial activation produces only small changes to expression and function of glutamate transporters in the vicinity of primary afferent synapses. Our findings therefore argue against a major role for a contribution of glutamate transporter dysfunction and glutamate spillover in the development of neuropathic pain. It is well established that peripheral nerve injury leads to changes in the expression and function of markers of glutamatergic neurotransmission in the spinal dorsal horn. These changes are also thought to be responsible, in part, for the development and maintenance of neuropathic pain (Tsuda et al. 2005; Sandkuhler 2007; Scholz and Woolf 2007; Vikman et al. 2008). One of the ways in which this has been proposed to occur is a generalized down-regulation of glutamate transporters in the vicinity of synapses and a concomitant increase in the expression and function of glutamate receptors (Harris et al. 1996; Popratiloff et al. 1998; Garry et al. 2003; Sung et al. 200, 2007; Yang et al. 2004; Wang et al. 2006, 2008; Tawfik et al. 2008). For example, in rats, PNL induced an ~51 and 40% reduction in EAAT1 (Xin et al. 2009) and EAAT2 (Maeda et al. 2008; Xin et al. 2009), respectively, expression at day 7. In another model of neuropathic pain, chronic constriction injury, these losses are preceded by an increase in EAAT1–3 at day 4 (Sung et al. 2003; Wang et al. 2006). However, despite an overall increase in total protein at this time, glutamate reuptake activity was in fact lower than in sham-operated animals (Sung et al. 2003). In contrast to the studies above, we found that nerve injury resulted in modest decreases in expression over the same 4- to 7-day period. When we looked at EAAT2 expression further by Western blot, these reductions were lost. This may be due to transporter redistribution in astrocytes, exposing changes in epitope availability in immunohistochemical studies. It is hard to reconcile the differences in EAAT expression observed between ours and previous studies. Others have reported increased expression of all three transporters up to 4 days after chronic constriction injury in rats (Sung et al. 2003) but decreased expression after 7 days (Sung et al. 2003; Wang et al. 2006). Explanations could be that of species differences, as our experiments were performed in mice and not rat, or models used (partial nerve ligation vs. chronic constriction injury). For example, in mice mechanical allodynia and expression changes of nociceptive markers (e.g., NK1) are typically restricted to the ipsilateral hind paw (Malmberg and Basbaum 1998), whereas in rat these changes are bilateral. It has been shown previously that PNL and chronic constriction models induce contrasting phenotypes of hot and cold hyperalgesia as well as mechanical allodynia (Bennett and Xie 1988; Seltzer et al. 1990). These adaptations were thought to create conditions whereby synaptic glutamate has the potential to both persist within the synapse but also to diffuse out of the synapse to bind extrasynaptic receptors thereby enhancing primary afferent synaptic transmission and plasticity.

To test whether or not this occurs at functioning synapses, we made use of the broad-spectrum glutamate transport inhibitor TBOA to drive moderately increased glutamate spillover in the spinal dorsal horn. By inhibiting all three transporters with 100 μM TBOA, a substantial late phase decay time constant for AMPARs was produced. Furthermore, the area under the curve of the AMPAR current (current density) increased from 249% in 30 μM TBOA to 384% of control in 100 μM TBOA, suggesting a significant role for EAAT1 and EAAT2 in glutamate reuptake in the dorsal horn. Due to the rapid desensit-
zation of AMPARs following agonist binding, the facilitation of the synaptic current under these conditions is likely due to diffusion of synthetically released glutamate to proximal (early time constant) extrasynaptic AMPARs and more remote extrasynaptic receptors and synapses (Beurrer et al. 2009).

If nerve injury results in a basal increase in synaptic and perisynaptic glutamate concentrations, then eEPSCs from nerve-injured mice should display slower decay kinetics than naïve mice, similar to that observed for TBOA-induced spillover. In the absence of TBOA, decay time constants did not differ between naïve and nerve-injured mice, suggesting AMPAR composition in the vicinity of synapses is not greatly altered and spillover, if present, is modest. Interestingly, nerve injury was associated with an increased early decay time constant of AMPARs, suggesting increased perisynaptic glutamate spillover or, alternatively, increased perisynaptic AMPAR density.

This increased early decay time constant after nerve injury in the presence of TBOA (30 μM) does not appear to be due simply to increased sensitivity to TBOA. Firstly, the late decay time constant was unaffected after nerve injury in this concentration of TBOA but was greatly enhanced in control tissue by a higher concentration of TBOA (100 μM). Secondly, the early decay time constant was completely unaffected by the higher concentration of TBOA (100 μM) in control spinal cord. These findings strongly suggest the increased early decay time constant observed in TBOA reflects either an increased density of extrasynaptic AMPARs that are silent in the absence of transporter inhibition or, alternatively, impaired transporter (EAAT1 and/or EAAT2) activity in close proximity to synapses that enhances sensitivity to TBOA.

In a similar study in rats by Nie and Weng (2010), an EAAT2-specific blocker, dihydrokainate, significantly increased NMDAR EPSC amplitude, latency, duration, and decay time in naïve rats but failed to elicit any response in nerve-injured rats, suggesting complete loss of EAAT2 function despite expression levels of ~40% compared with controls. This finding is hard to reconcile with our results. However, it should be noted that these experiments were performed at 8–14 days postinjury in rats, whereas our electrophysiological recordings were made at day 4 postinjury in mice.

The possibility that perisynaptic spillover is increased can also be tested by examining the effect of TBOA on NMDARs after blockade of active subsynaptic receptors with MK-801. In this case, there was no significant difference to the degree of extrasynaptic NMDAR activation at primary afferent synapses from nerve-injured animals. This experiment therefore failed to confirm the interpretation that the increased early decay time constants induced by TBOA in nerve-injured animals was due to increased perisynaptic glutamate spillover. There was, however, a nonsignificant trend for increased peak amplitude of the extrasynaptic NMDAR-mediated eEPSC, which is consistent with a small increase in spillover. There are other potential explanations for the discrepancy between the AMPAR- and NMDAR-mediated results. It is possible that the increased early decay time constant of the AMPAR-mediated eEPSC in the presence of TBOA reflects spillover to very proximal extrasynaptic AMPARs or there may be an increase in extrasynaptic AMPAR density following nerve injury rather than increased spillover per se. There is evidence for AMPAR subunit adaptations during inflammatory pain that could be associated with extrasynaptic insertion of AMPARs (Tao 2010) but whether or not similar adaptations develop in nerve injury-induced pain is unknown. It is also possible that spillover to extrasynaptic NMDARs in the presence of TBOA (100 μM) is mediated more by receptors more distal to release sites than the AMPARs contributing to the early decay phase enhanced by nerve injury. If so, measured NMDAR spillover may be similar to the late decay phase of the AMPAR-mediated eEPSC in the presence of TBOA that is unaffected by nerve injury.

In conclusion, the present study suggests that peripheral nerve injury at a time associated with neuropathic pain and astrocyte activation does not greatly influence the expression of astrocytic glutamate transporters in the superficial dorsal horn nor does it produce substantial extrasynaptic spillover of glutamate from primary afferent synapses. However, in the close vicinity of synapses, glutamate transporter function may be prone to ineffective uptake under extreme circumstances and lead to enhanced receptor activation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: I.A.N. and S.A.M. performed experiments; I.A.N. and S.A.M. analyzed data; I.A.N. and M.J.C. interpreted results of experiments; I.A.N. prepared figures; I.A.N. drafted manuscript; I.A.N. and M.J.C. edited and revised manuscript; M.J.C. conception and design of research; M.J.C. approved final version of manuscript.

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