RESEARCH ARTICLE

NMDA receptor activation induces long-term potentiation of glycine synapses

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Abstract

Of the fast ionotropic synapses, glycineric synapses are the least well understood, but are vital for the maintenance of inhibitory signaling in the brain and spinal cord. Glycinergic signaling comprises half of the inhibitory signaling in the spinal cord, and glycineric synapses are likely to regulate local nociceptive processing as well as the transmission to the brain of peripheral nociceptive information. Here we have investigated the rapid and prolonged potentiation of glycineric synapses in the superficial dorsal horn of young male and female mice after brief activation of NMDA receptors (NMDARs). Glycinergic inhibitory postsynaptic currents (IPSCs) evoked with lamina II-III stimulation in identified GABAergic neurons in lamina II were potentiated by bath-applied Zn²⁺ and were depressed by the prostaglandin PGE₂, consistent with the presence of both GlyRα₁- and GlyRα₃-containing receptors. NMDA application rapidly potentiated synaptic glycineric currents. Whole-cell currents evoked by exogenous glycine were also readily potentiated by NMDA, indicating that the potentiation results from altered numbers or conductance of postsynaptic glycine receptors. Repetitive depolarization alone of the postsynaptic GABAergic neuron also potentiated glycineric synapses, and intracellular EGTA prevented both NMDA-induced and depolarization-induced potentiation of glycineric IPSCs. Optogenetic activation of trpv1 lineage afferents also triggered NMDAR-dependent potentiation of glycineric synapses. Our results suggest that during peripheral injury or inflammation, nociceptor firing during injury is likely to potentiate glycineric synapses on GABAergic neurons. This disinhibition mechanism may be engaged rapidly, altering dorsal horn circuitry to promote the transmission of nociceptive information to the brain.
Introduction

In the superficial dorsal horn, thermal, mechanical, and nociceptive information is processed and then conveyed to the brain via ascending inputs. The wiring diagram of nociceptive information flow in the dorsal horn is far from complete, but inhibitory synapses have long been recognized as important points of control restricting the transmission of pain information to the brain [1–7]. Pharmacological disinhibition allows peripheral afferents from low-threshold mechanosensory cells that usually activate lamina III-V neurons to drive lamina I projection neurons in vitro [2, 8]. Glycine receptors (GlyRs) are most prevalent in caudal brain regions and in the spinal cord, where they mediate a large proportion of inhibitory neurotransmission [9, 10]. Either acute blockade [6, 11] or chronic loss [12–14] of glycineric transmission in the spinal cord results in allodynia, hyperalgesia, and itch, while a GlyR allosteric modulator reduces neuropathic pain [15].

Glycinergic neurons in the dorsal horn and brainstem trigeminal nucleus innervate both excitatory and inhibitory neurons in the superficial layers [16, 17]. GlyRs are ligand-gated ion channels, members of the cys-loop superfamily that includes GABA_A receptors, 5HT_3 receptors, and nicotinic nACh receptors. Like GABA_A receptors, glycine receptors are chloride channels and generally act to hyperpolarize cells and stabilize the membrane potential. Glycine receptors exist as heteromeric pentamer complexes of alpha (α1–4) and β subunits. The β subunits bind to the scaffolding protein, gephyrin to stabilize the receptors at synapses [18]. After very early postnatal development, synaptic GlyRs in the dorsal horn are heteromers composed of α1, α3, and β subunits [19–21], and α3 subunits in the dorsal horn may be preferentially required for nociception [15, 19].

Synaptic plasticity in the nociceptive circuitry has the potential to switch the circuit rapidly from a resting state to a pain state, and understanding mechanisms of plasticity is therefore of interest both in normal nociception and pain states and in pathological pain such as neuropathic pain. Long-term potentiation (LTP) is a characteristic of many excitatory brain synapses, and has also been reported at glutamatergic synapses made by primary afferents in the dorsal horn [22–24], and GABAergic synapses in the dorsal horn have been shown to undergo LTP as well [25]. However, nearly nothing is known about mechanisms of plasticity at glycineric synapses. We reported previously that LTP is induced at glycineric synapses on GABAergic neurons in the dorsal horn by the proinflammatory cytokine interleukin 1 β (IL-1β) [26], which is released in the dorsal horn following injury [27–29]. The same glycineric synapses were maximally potentiated shortly after in vivo inflammation, and we hypothesized that glycine receptor LTP in this model was caused by local release of IL-1β during peripheral inflammation. Here we have identified other mechanisms that potentiate glycineric synapses.

In cultured spinal cord neurons, NMDA can increase glycineric currents [30]. Single-particle tracking experiments showed that clusters of GlyRs and miniature IPSC amplitudes are markedly increased after treatment with NMDA, but receptor clustering is prevented if Ca^{2+} is chelated [31]. Elevation of intracellular Ca^{2+} was also reported to potently increase glycine receptor single channel openings [32] in cultured cells or when heterologously expressed. Because of the relative paucity of information about glycineric synapse plasticity and its potential importance in modulating nociception, we are interested in characterizing glycineric synapses and the control of their synaptic strength in situ in the dorsal horn. We find that bath-applied NMDA causes a long-lasting potentiation of these glycineric synapses through a postsynaptic mechanism. Simply depolarizing GABAergic neurons repetitively also potentiates glycineric synapses, and both depolarization- and NMDA-induced potentiation are prevented by chelation of postsynaptic Ca^{2+}. Furthermore, NMDAR activation by primary nociceptors also potentiated glycineric synapses. Together, our findings suggest that glutamate...
released from primary nociceptive afferents during peripheral damage could act at NMDARs on inhibitory dorsal horn neurons to promote persistent potentiation of glycineric synapses. These rapid onset synaptic changes are likely to contribute to nociceptive processing during normal pain states.

Materials and methods

Animals

All experiments were conducted in strict adherence to the National Institutes of Health Guide for the Care and Use of Laboratory animals and as approved by the Brown Institutional Animal Care and Use Committee. Animals included Tg(Gad2-EGFP)DJ31Gsat/Mmucd mice (GENSAT project, Rockefeller University; http://www.gensat.org), backcrossed more than ten times on the Swiss Webster background prior to use in this study. Hemizygous GAD65-EGFP mice were mated to Swiss Webster mice in each generation and were used as hemizygotes. Trpv1-Cre and lox-STOP-lox-ChR2-EYFP mice were purchased from The Jackson Laboratory. For optogenetic experiments, trpv1-Cre<sup>+/+</sup> mice were mated with ChR2-EYFP<sup>+/+</sup> mice to generate trpv1<sup>+/−</sup>/ChR2-EYFP<sup>+/−</sup> offspring (referred to here as TRPV1/ChR2). Both male and female mice of all genotypes (p25-p40) were maintained on a 12h light/dark cycle and were provided food and water ad libitum. Data taken from both male and female mice were included in this study, and no significant sex differences were identified. Animals were deeply anesthetized with isoflurane and then injected with a terminal dose of ketamine (75 mg/kg) and dexmedetomidine (1 mg/kg). Mice were then transcardially perfused with cutting solution containing (in mM): 92 choline chloride, 1.2 NaH<sub>2</sub>PO<sub>4</sub>, 1.2 NaHCO<sub>3</sub>, 20 HEPES, 25 dextrose, 5 Na-ascorbate, 2 thiourea, 3 Na-pyruvate, 10 MgSO<sub>4</sub>, 0.5 CaCl<sub>2</sub> [33, 34] that was bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Animals were then decapitated, and the spinal cord was rapidly dissected from the ventral aspect. Transverse lumbar spinal cord slices (300 μm thick) were prepared as described previously [26]. Slices were incubated at 34˚C for 1 hour prior to recording in oxygenated recording ACSF containing (in mM) 119 NaCl, 2.5 KCl, 2.5 CaCl<sub>2</sub>, 1 NaH<sub>2</sub>PO<sub>4</sub>, 5 MgSO<sub>4</sub>, 26 NaHCO<sub>3</sub>, 1.3 Na-ascorbate, 25 dextrose) and then stored at room temperature (RT) until use. Maintaining slices at RT improved their viability over time.

Electrophysiology and optogenetics

Slices were continuously perfused with oxygenated ACSF at room temperature at a rate of 1 ml/min. To limit heterogeneity, recordings were restricted to the lateral area of dorsal horn lamina II. GABAergic neurons were visually identified, and only recordings from neurons that expressed GFP are included in this study, with the exception of experiments from trpv1-ChR2: optogenetic experiments were made from unlabeled lamina II neurons in slices from these mice. Current-clamp recordings were made at the start of every experiment to observe action potential firing patterns in response to current steps of 50 pA delivered at resting membrane potential every 10 seconds. Neurons with resting membrane potentials less than -55 mV or with holding currents greater than 50 pA were not considered healthy and were eliminated from further study.

Cells were voltage-clamped at -70 mV, and glycineric IPSCs were evoked at 0.1 Hz using a stainless steel stimulating electrode placed lateral to the recording site in lamina II, and isolated using bicuculline (30 μM) and 6,7-dintroquinaloxine-2,3-dione (DNQX, 10 μM) to block GABA<sub>A</sub>R and AMPAR currents, respectively. Remaining synaptic currents could be entirely blocked by strychnine confirming that they are glycineric (see also Chirila et al., 2014). IPSCs were recorded as inward currents using pipettes filled with KCl-based internal solution containing (in mM): 125 KCl, 2.8 NaCl, 10 HEPES, 2 MgCl<sub>2</sub>, 4 Na-ATP, 0.3 Na-GTP, 10 Na-phosphocreatine, 0.6 EGTA. In some experiments EGTA was increased to 15 mM in the pipette
solution, as noted. In these experiments, neurons were held for at least 20 minutes after breaking into whole-cell mode before another manipulation. For occlusion experiments NMDA (50μM) was bath applied for 5 min. If glycine inputs potentiated by more than 20%, IL-1β (10ng/ml, 10 min) was added to the bath at least 20 min following NMDA washout when IPSCs reached a steady state. For optogenetic experiments with low-frequency stimulation (LFS) of nociceptive inputs in TRPV1/ChR2 animals, an optic fiber (230μm diameter) was placed at the dorsal slice edge to stimulate TRPV1/ChR2 primary afferents with light (Plexon LED, 465nm, 0.5 - 1ms, 1-9mW). Presence of TRPV1/ChR2 input was determined in each slice/cell before bath application of DNQX. LFS was carried out in presence of bicuculline and DNQX; we stimulated each cell with a light train of 2 Hz for 2 min while voltage-clamped at -40mV at a 2ms light pulse duration. All pharmacological agents, including NMDA, were bath-applied at known concentrations unless otherwise indicated. Bicuculline and PGE₂ were dissolved in DMSO; the final concentration of DMSO in experimental ACSF solution was 0.03% and 0.1%, respectively. All other pharmacological agents were dissolved in water.

Statistical analysis
All data are presented as mean ± SEM of the percent change in IPSC amplitude. Potentiation was measured at 12–17 minutes after NMDA application for NMDA-induced Gly LTP, or 15 minutes after the start of drug application. For PPR analysis, 60 IPSCs before and at 10–20 min after the start of NMDA or after depolarization were averaged; cells were included in PPR analysis if they exhibited at least 20% LTP above baseline values. We evaluated the effect experimental manipulation using one-way ANOVA (Fig 1) or paired t-tests (Figs 2–7) of non-normalized raw data at indicated time points. All statistical analyses were performed using GraphPad Prism software. Statistical tests were considered to be significant at a 95% confidence interval, with p values reported in the Results section.

Results
We recorded from eGFP-labeled GAD-65 neurons in lamina II of the dorsal horn. This population of cells exhibited three main firing patterns in response to direct current injection (Fig 1A and 1B). The majority of neurons fired 1–3 action potentials in 300 ms (initial firing type, 60/141), while other cells exhibited either tonic firing throughout the pulse (54/141) or a delayed or “gap” mode of firing that is characteristic when A-type K⁺ currents are present (27/141)(Yasaka et al., 2010). Cells we included in the gap/delay class sometimes fired an initial spike followed by a gap before resuming. The action potential threshold was significantly lower for tonic firing cells than for initial or gap/delay cells (Fig 1C; initial vs tonic, p < 0.0001, gap vs. tonic, p<0.0001, one-way ANOVA, Tukey’s multiple comparisons test; n = 60 initial, n = 54 tonic, n = 27 gap/delay), while the input resistance, and the rise time and decay time of evoked Gly IPSCs did not significantly differ among the classes (Fig 1D–1F).

GlyRs in lateral dorsal horn lamina II contain both α1 and α3 subunits
Both GlyRα1 and GlyRα3 subunits have been reported in lamina II, and GlyRα3 in particular has been implicated in inflammatory pain [19]. Previous work has shown that Zn²⁺ is an allosteric modulator of glycine receptors, transiently potentiating glycinergic synapses containing GlyRα1 [35, 36], while prostaglandin E₂ (PGE₂) depresses glycinergic synapses containing GlyRα3 [19]. Using these tools, we tested which receptor subunits were present in glycinergic synapses on the GABAergic neurons of lamina II. Glycinergic inhibitory postsynaptic currents (Gly IPSCs) were evoked using a stimulating electrode placed laterally nearby in lamina II. Bath-application of ZnCl₂ (1 μM) increased glycinergic IPSC amplitudes (Fig 2A–2C; 7/8
GABAergic neurons, 144±12.7% of baseline, p = 0.04, n = 8), while PGE2 (10 μM) depressed glycinergic IPSCs in some but not all cells (Fig 2D–2F; 6/9 GABAergic neurons; 74±0.9% of baseline, p = 0.11, n = 9)(Fig 2). These data suggest that the majority of GABAergic neurons in our study are likely to have both GlyRα1- and α3-containing receptors at their synapses, while some may have only GlyRα1-containing receptors.

NMDA potentiates Gly IPSCs

NMDAR activation increases glycineric miniature IPSC amplitudes in cultures from embryonic spinal cord [30, 31]. To determine whether NMDAR activation similarly potentiates
glycinergic synapses in situ in the dorsal horn, we bath applied NMDA (50 μM, 5–10 minutes) and recorded evoked Gly IPSCs. NMDA application potentiated evoked Gly IPSC amplitudes within minutes (Fig 3A–3C; IPSC amplitudes: 147±7.6% vs. control amplitudes, p<0.0001, n = 40). Notably, NMDA potentiated Gly IPSCs in neurons of all three types of cell identified by action potential firing pattern (Fig 3D). Potentiation in most cells persisted throughout the recording period (up to 2 hours) after wash-out of NMDA; we therefore refer to this potentiation as NMDA-induced Gly LTP. The average decay time constant of Gly IPSCs was unchanged after NMDA, suggesting that the potentiation does not result from a decreased glycine transport (baseline τ = 6.98±0.43, post-NMDA τ = 7.46±0.45; p = 0.16, n = 7). To confirm that the potentiation was indeed produced via NMDA receptor activation, we bath-applied NMDA in the presence of the non-competitive NMDAR antagonist, 7-chlorokynurenic acid (100 μM) (Fig 3E–3G). As expected, NMDA did not potentiate glycine IPSCs under these conditions (IPSCs after 7-CK, 106±15.8% of baseline, p = .33, n = 4). In previous work, we reported that bath application of interleukin-1β (1L-1β) potentiates Gly IPSCs in lamina II gad2+ neurons (Chirila et al., 2014). We therefore tested whether NMDA-induced Gly LTP shares synaptic mechanisms with IL-1β-induced potentiation. We first bath-applied NMDA,
Fig 3. NMDA receptor activation potentiates Gly IPSCs. A. The mean IPSC before and after bath application of 50μM NMDA, 10 minutes. Inset: average of 5 IPSCs just before (black) and at 5 minutes after the start of NMDA application (blue). B. Raw data from all experiments of this type; bold bar and symbols represent the mean IPSC before and after NMDA. C. Average of 40 NMDA experiments. Fig 3B-C include experiments in which NMDA alone was bath-applied. Some of these
and then upon stable potentiation of Gly IPSCs, we applied IL-1β. As shown in Fig 3H–3J, after NMDA-induced potentiation (135±40%, n = 6, paired t-test baseline vs. NMDA: p = 0.03) IL-1β produced no further significant potentiation (93±32%, n = 6, paired t-test NMDA vs. IL-1β: p = 0.63), suggesting a shared underlying mechanism.

NMDA-induced Gly LTP is mediated postsynaptically

LTP can result either from an increase in presynaptic neurotransmitter release or from an increase in postsynaptic receptor number or function, and the short-term dynamics of synaptic activation can be used to infer the locus of synaptic change. While measurements of miniature IPSCs can be used to suggest the locus, we found that mIPSCs occur so rarely in our recordings that they are not a very reliable method for collecting sufficient data. Instead, we measured the paired-pulse ratio (PPR), which generally decreases if the probability of neurotransmitter release increases during LTP [37–39], as well as postsynaptic responses to bath-applied glycine. On average, during Gly LTP the PPR remained unchanged after NMDAR application, suggesting that LTP is not caused by an increase in transmitter release, but instead by increased postsynaptic glycine receptor number or conductance (Fig 4A and 4B; PPR control, 1.18±0.05; post-NMDA, 1.17±0.07; p = .86, n = 26). To test this more directly, we measured whole-cell postsynaptic currents evoked by exogenous bath-applied glycine. These glycine currents are independent of presynaptic glycine release, thus any increase after NMDA can only result from postsynaptic changes. Glycine (3 mM) was applied for 30 s every ten minutes, and the resulting inward currents were recorded [26]. Control measurements were acquired by washing on glycine 2–3 times, and then NMDA (50 μM) was bath applied for five minutes. Following application of NMDA, exogenous glycine currents were significantly increased (Fig 4D and 4E; glycine currents after NMDA: 275±30.5% of baseline, p = 0.011, n = 10). Together, our results suggest that NMDA Gly LTP depends upon an increase in number and/or function of GlyRs and is independent of glycine release.

Postsynaptic calcium is required for NMDAR Gly LTP and sufficient to potentiate Gly IPSCs

Many forms of synaptic plasticity require a rise in postsynaptic calcium [26, 40, 41], and the high Ca2+ permeability of the NMDAR is required for the majority of signaling through this channel. To determine whether NMDA Gly LTP is calcium-dependent, we included EGTA (15 mM) in the recording pipette to chelate postsynaptic intracellular calcium. Compared with same-day control recordings, high intracellular EGTA blocked NMDAR Gly LTP (0.6 mM EGTA, 134±12.06% of baseline, p = 0.03, n = 7; 15 mM EGTA, 95±12.6% of baseline, p = 0.73, n = 8; Fig 5A–5C) suggesting that elevated intracellular calcium is necessary for NMDAR Gly LTP. To determine whether elevated Ca2+ alone is sufficient to potentiate Gly synapses, GABAergic neurons were repetitively depolarized to -10 mV at 0.5 Hz for 10 minutes, to open VGCCs and elevate postsynaptic calcium [42, 43]. Synaptic stimulation was halted during this depolarization period. Following the depolarization protocol, Gly IPSCs were significantly increased (Fig 6A–6C; 150±18.9% of baseline values, n = 11). Like NMDA-
induced Gly LTP, depolarization-induced potentiation was blocked by 15 mM intracellular EGTA (Fig 6B and 6C; 94 ± 10% of baseline values, n.s., n = 5; unpaired t-test depolarization alone vs. depolarization + 15 mM EGTA: p = 0.023). Together, the results suggest that postsynaptic Ca^{2+} is necessary for NMDAR Gly LTP, and that postsynaptic calcium entry is also sufficient to potentiate glycinergic synapses.

Brief low-frequency stimulation of nociceptor afferents potentiated Gly IPSCs through NMDA receptors

Sensitization of nociceptors is typically accompanied by an increased spontaneous discharge pattern of peripheral nociceptors, which induces changes in synaptic strength with dorsal horn neurons [23, 44]. While GlyR LTP was elicited by either bath-applied NMDA or experimenter-induced postsynaptic depolarization, both methods represent relatively unphysiological stimuli. To test our observations in a more physiological context, we next investigated whether Gly IPSCs could be potentiated upon activation of primary nociceptor afferents at frequencies occurring during injury or inflammation. We used TRPV1/ChR2 transgenic mice as recently described [44, 45] to optogenetically activate nociceptor afferents at a frequency that has been suggested to occur naturally during painful peripheral stimuli [23, 46]. Stimulation of nociceptors with light evoked glutamatergic synaptic events but not direct release of glycine [44]. We recorded Gly IPSCs in lamina II neurons in the presence of bicuculline and DNQX to allow isolated Gly IPSC recordings, however NMDARs were not blocked to permit activation of NMDARs by primary afferents. After a stable period of electrically-evoked Gly IPSCs, electrical stimulation was paused, and for 2 minutes 2 Hz light pulses were delivered (2ms, 9mW). Driving primary afferents in this manner potentiated Gly IPSCs evoked electrically once light stimulation had ended (Fig 7A, 129±17% of baseline values, n = 12, paired t-test baseline vs. after LFS: p = 0.002). The same experiment carried out in the presence of d-APV, however, did not trigger Gly LTP (Fig 7B, 98±26% of baseline values, n = 8, paired t-test baseline vs. LFS + APV: p = 0.87) and differed significantly from LFS-induced potentiation (unpaired t-test LFS vs. LFS + APV: p = 0.03).

Discussion

Inhibitory synapses in the dorsal horn are prime potential sites of modulation during nociception. Our previous work demonstrated that glycine receptor synapses on GABAergic neurons in lamina II are potentiated 90 minutes after peripheral inflammation in vivo. Here we report that activation of NMDARs in spinal cord slices also potentiates glycinergic synapses on the same cells. The NMDA-induced potentiation is maintained by postsynaptic alterations in glycine receptors, as demonstrated by increased responsiveness to exogenously applied glycine.
NMDA induced glycine potentiation requires a rise in intracellular Ca\(^{2+}\), and repetitive depolarization alone also potentiates the synapses in a Ca\(^{2+}\)-dependent manner. Finally, NMDAR activation induced by synaptic stimulation of trpv1-lineage nociceptor afferents also potentiates glycinerergic synapses.

Fig 5. NMDA Gly LTP is blocked by chelating postsynaptic Ca\(^{2+}\). A. Left panel, example experiment showing Gly IPSC potentiation by NMDA in a control cell recording with 0.6 mM EGTA in the pipette solution. Inset, average of 5 IPSCs just before and 5 min after NMDA (blue). Right panel, raw data from 7 similar experiments. B. Left panel, example experiment showing Gly IPSCs before and after NMDA in a recording with 15 mM EGTA in the pipette solution. Inset, average of 5 IPSCs just before and 5 min after NMDA (blue). Right, raw data from 8 similar experiments. C. Averaged time course data from these experiments (0.6 mM EGTA, n = 7; 15 mM EGTA, n = 8). Experiments with each concentration of NMDA were carried out in alternation.

https://doi.org/10.1371/journal.pone.0222066.g005

NMDA induced glycine potentiation requires a rise in intracellular Ca\(^{2+}\), and repetitive depolarization alone also potentiates the synapses in a Ca\(^{2+}\)-dependent manner. Finally, NMDAR activation induced by synaptic stimulation of trpv1-lineage nociceptor afferents also potentiates glycinerergic synapses.
Firing properties of GAD-65 labelled lamina II neurons

Dividing dorsal horn neurons into functionally relevant subgroups has been crucial in beginning to define the circuitry of this complex and heterogeneous structure [47, 48]. Recent studies have used genetically labeled mouse lines to study a more homogeneous group and to begin to categorize distinct cell properties. In our experiments, we used neurons in lamina II labeled genetically with eGFP under the GAD-65 promoter that exhibited three distinct firing modes in response to depolarizing pulses (initial, tonic, and gap/delay). Previous work using either genetic labeling or peptide co-localization suggested that delayed or gap firing is a hallmark of excitatory interneurons, while tonic firing is more characteristic of inhibitory interneurons [49–52]. However, our data indicate that some neurons labeled in the GAD-65 reporter mouse exhibit delayed/gap firing, which has also been reported for a small subset of genetically identified inhibitory neurons in superficial layers of the spinal cord [51]. Here we have referred to these neurons as GABAergic for convenience, but recognize that approximately 20% of our recordings may represent another cell class since only 80% of neurons labeled in this mouse are GABA immunopositive [53]. Notably, in GAD-65 mice, only 60% of all lamina II GABAergic neurons are labelled, suggesting that studies using GAD-67 labeled neurons likely only sample a partially overlapping population [53]. GAD-65 labeled neurons (unlike most GAD-67 labeled cells) co-express c-fos after treatment with peripheral capsaicin, however, emphasizing the likely participation of the GAD-65 neurons we used in peripheral inflammatory processes [52].

Glycine receptors on lamina II GABAergic neurons

Using Zn$^{2+}$ to probe for α1-containing receptors and PGE$_2$ to probe for α3-containing receptors, we found that all but one of our recorded glycineric synaptic currents potentiated with Zn$^{2+}$, and the majority but not all exhibited synaptic depression with PGE$_2$. Our results suggest that glycineric synapses on this GABAergic population have α1-containing receptors, but
may also contain α3-containing receptors, either as α1/α3/α heteromers or as α1 or α3 homomeric channels. Using immunocytochemistry, previous work indicated that approximately half of lamina II neurons appear to co-express both subunits, and our data in the GAD-65 cell population are consistent with these results [19]. Recent work in cultured neurons indicates that IL-1β does not affect GlyRα3-containing receptors, although IL-1β potentiation of GlyRα1-containing receptors was not observed in this study [54]. Our stimulation site in lamina II could activate glycinergic synapses from multiple sites, so it is also possible that distinct sets of afferents innervate synapses with differing GlyRα subtypes.

**NMDA-induced Gly LTP mechanisms**

Brief bath-application of NMDA effectively potentiated glycinergic synapses on lamina II GABAergic neurons. The potentiation typically began within minutes of NMDA application, and persisted long after NMDA was washed out. We observed NMDA LTP in GAD-65-labeled neurons exhibiting a range of firing properties, including tonic firing and delayed firing. If single-spiking, tonic, and gap/delay cell populations in our study indeed represent functionally distinct groups, our results suggest that multiple postsynaptic cell types (including gap/delay cells) can exhibit postsynaptically-mediated glycinergic LTP. A non-competitive NMDAR
antagonist completely prevented LTP, indicating that LTP was not caused by off-target effects of NMDA. The potentiation was not accompanied by a significant decrease in the paired-pulse ratio, as expected if it were caused by an increased probability of glycine release. Instead, the LTP appears to result from a postsynaptic increase in glycine receptor number or function, as exogenously applied glycine currents were robustly increased after brief NMDA application. In this condition, the presynaptic release of glycine is not a factor, and instead this experiment confirms that NMDA treatment increases the postsynaptic response to glycine. The extracellular application of glycine might be expected to sample both synaptic and extrasynaptic glycine receptors. In considering the mechanism of potentiation, it is surprising to think of extrasynaptic glycine currents being enhanced after NMDA, as previous studies using single-particle tracking and other approaches have strongly suggested that glycine receptors are inserted and confined to synaptic regions where the scaffolding protein, gephyrin, is clustered [55, 56]. This observation of heterosynaptic plasticity is similar to the underlying mechanism of NMDAR-dependent homosynaptic LTP at AMPAR synapses, with receptors immobilized by synapse-to-cytoskeletal scaffolds [57]. The potentiation of bath-applied glycine responses we observe could therefore reflect insertion of glycine receptors at gephyrin-enriched synaptic sites. Alternatively, after NMDA application, extrasynaptic glycine receptors may also be inserted at sites expected to have low gephyrin levels. It is also possible that glycine receptors at all sites undergo an increase in single channel open times or affinity [32]; more work will be needed to distinguish these possibilities.

**Postsynaptic Ca$^{2+}$ and NMDA Gly LTP**

Bath-application of NMDA has often been used to mimic neuronal activation and to induce NMDAR-dependent synaptic plasticity. For example, NMDA induces LTD or LTP at excitatory synapses [58–60] and can also potentiate GABAergic synapses heterosynaptically, via Ca$^{2+}$ [61–64] and calcium/calmodulin-dependent protein kinase II (CaMKII) [61, 64]. Similarly, glycinergic synapse strength is heterosynaptically potentiated by Ca$^{2+}$ influx and CaMKII [30, 55, 65, 66]. In cultured spinal cord neurons, GlyR clusters and miniature Gly IPSC amplitudes are both markedly increased after NMDA treatment; moreover, clustering was prevented when Ca$^{2+}$ was chelated [31]. Consistent with a similar mechanism, we found that high intracellular EGTA prevented NMDA-induced GlyR LTP. Repetitive depolarization of the postsynaptic cell alone also proved sufficient to potentiate glycinergic synapses, as long as intracellular Ca$^{2+}$ was not chelated; similar repetitive depolarization potentiates GABAergic synapses in visual cortex slices [67]. Importantly, low-frequency synaptic stimulation of primary nociceptor afferents was sufficient to potentiate glycinergic synapses on lamina II neurons, indicating that heterosynaptic NMDAR-dependent GlyR LTP is elicited with physiological stimuli. Together our data are consistent with the idea that a rise in intracellular Ca$^{2+}$ through NMDARs at nociceptor synapses, or even during action potential firing of the postsynaptic cell driven by any mechanism, can potentiate glycinergic synapses, most likely by augmenting synaptic glycine receptor numbers/function.

In our occlusion experiments testing whether IL-1β and NMDA potentiate glycinergic synapses via a similar mechanism, potentiation by IL-1β always prevented further potentiation by NMDA. We showed previously that potentiation by IL-1β is also prevented by EGTA (Chirila et al., 2014), and NMDA potentiation was also prevented by chelation of Ca$^{2+}$ by intracellular EGTA. These observations are consistent with a common final pathway for potentiation by both agents. Ninety minutes after peripheral inflammation of the paw, glycinergic synapses on GAD-65 labeled neurons are potentiated, via a postsynaptic mechanism [26]; we originally attributed this to local release of IL-1β after injury [27, 29]. Having shown here that NMDAR
activation either by NMDA or during primary afferent stimulation also potentiates these synapses, however, the inflammation-induced potentiation could occur through multiple mechanisms. Glutamate released from primary afferents at 2 Hz may elicit both release of IL-1β from dorsal horn glial cells and direct activation of NMDARs on lamina II neurons; our results show that both are expected to potentiate glycineergic synapses.

**Circuit considerations**

What is the role of glycine receptor LTP in the synapses on lamina II GABAergic neurons in the nociceptive circuitry? We report that driving primary nociceptors for a brief period markedly potentiates glycine currents on lamina II neurons. Glycine receptor LTP in these neurons after peripheral inflammation [26] may serve to inhibit nociceptive information flow after injury, consistent with the fact that intrathecal strychnine promotes nocifensive behaviors [6, 68]. However, this method of applying strychnine increases the excitability of nearly all dorsal horn neurons; during NMDAR activation or inflammation, GlyR LTP occurring in GABAergic neurons may instead allow increased transmission of ascending nociceptive signals. The GABAergic interneurons of lamina II are often invoked as a component of the gate controlling the passage of peripheral nociceptive information to the brain [11, 69]. Potentiation of glycineergic synapses on inhibitory neurons by Ca$^{2+}$ influx via NMDARs or neuronal firing (or IL-1β release after inflammation) is expected to open the gate, effectively disinhibiting ascending nociceptive information flow. Reduced inhibition in the dorsal horn is observed in several animal models of persistent pain, suggesting that simply altering inhibitory synaptic function in the dorsal horn can mimic persistent pain syndromes [70–73]. Disinhibition also causes ascending projections that normally respond only to noxious stimuli to be activated by excitatory inputs carrying non-nociceptive signals (allodynia) [74, 75]. The capability of GABAergic interneurons to undergo Gly LTP, as well as their precise synaptic wiring and regulatory control of ascending neurons will determine the functional role of glycine receptor LTP in the dorsal horn. Our current understanding of the local circuit suggests that GAD-65 interneurons of lamina II sampled in our study could include islet cells or dynorphin-containing cells of lamina II, and possibly some parvalbumin neurons of lamina III [47]. Inhibition of any of these cell types could promote excitability of projection neurons in the dorsal horn, either directly or indirectly. If the gap/delay cells that underwent Gly LTP are excitatory interneurons as suggested by others [49–52], this adds another layer of complexity to the circuit possibilities. It will be critical in future work to identify how widespread the phenomenon of glycine receptor LTP is, as well as its behavioral consequences.

**Acknowledgments**

The authors would like to thank Kauer lab members for helpful suggestions. We also are grateful for technical assistance from Ms. Ayumi Tsuda.

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References

1. Latremoliere A, Woolf CJ. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. J Pain. 2009; 10(9):895–926. Epub 2009/08/29. https://doi.org/10.1016/j.jpain.2009.06.012 PMID: 19712899; PubMed Central PMCID: PMC2750819.

2. Torsney C, MacDermott AB. Disinhibition opens the gate to pathological pain signaling in superficial neurokinin 1 receptor-expressing neurons in rat spinal cord. J Neurosci. 2006; 26(6):1833–43. Epub 2006/02/10. https://doi.org/10.1523/JNEUROSCI.4584-05.2006 PMID: 16467532.

3. Baldo BA, Daniel RA, Berridge CW, Kelley AE. Overlapping distributions of orexin/hypocretin- and dopamine-beta-hydroxylase immunoreactive fibers in rat brain regions mediating arousal, motivation, and stress. J Comp Neurol. 2003; 464(2):220–37. https://doi.org/10.1002/cne.10783 PMID: 12898614.

4. Zeilhofer HU, Benke D, Yevenes GE. Chronic pain States: pharmacological strategies to restore diminished inhibitory spinal pain control. Annu Rev Pharmacol Toxicol. 2012; 52:111–33. https://doi.org/10.1146/annurev-pharmtox-010611-134636 PMID: 21854227.

5. Zeilhofer HU, Wildner H, Yevenes GE. Fast synaptic inhibition in spinal sensory processing and pain control. Physiol Rev. 2012; 92(1):193–235. https://doi.org/10.1152/physrev.00043.2010 PMID: 22298656.

6. Yaksh TL. Behavioral and autonomic correlates of the tactile evoked allodynia produced by spinal glycine inhibition: effects of modulatory receptor systems and excitatory amino acid antagonists. Pain. 1989; 37(1):111–23. Epub 1989/04/01. https://doi.org/10.1016/0304-3959(89)90160-7 PMID: 2542867.

7. Woolf CJ, Shortland P, Sivilotti LG. Sensitization of high mechanotreshold superficial dorsal horn and flexor motor neurones following chemosensitive primary afferent activation. Pain. 1994; 58(2):141–55. Epub 1994/08/01. https://doi.org/10.1016/0304-3959(94)90195-3 PMID: 7816483.

8. Baba H, Ji RR, Kohno T, Moore KA, Ataka T, Wakai A, et al. Removal of GABAergic inhibition facilitates polysynaptic A fiber-mediated excitatory transmission to the superficial spinal dorsal horn. Mol Cell Neurosci. 2003; 24(3):818–30. Epub 2003/12/11. PMID: 14664828.

9. Altshuler RA, Betz H, Parakkal MH, Reeks KA, Wenthold RJ. Identification of glycineergic synapses in the cochlear nucleus through immunocytochemical localization of the postsynaptic receptor. Brain Res. 1986; 369(1–2):316–20. https://doi.org/10.1016/0006-8993(86)90542-1 PMID: 3008938.

10. Alvarez FJ, Dewey DE, Harrington DA, Fyffe RE. Cell-type specific organization of glycine receptor clusters in the mammalian spinal cord. J Comp Neurol. 1997; 379(1):150–70. PMID: 9057118.

11. Foster E, Wildner H, Tudeau L, Haueter S, Ralvenius WT, Jegen M, et al. Targeted ablation, silencing, and activation establish glycineergic dorsal horn neurons as key components of a spinal gate for pain and itch. Neuron. 2015; 85(6):1289–304. Epub 2015/03/20. https://doi.org/10.1016/j.neuron.2015.02.028 PMID: 25789756; PubMed Central PMCID: PMC4372258.

12. Coull JA, Boudreau D, Bachand K, Prescott SA, Nault F, Sik A, et al. Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. Nature. 2003; 424(6951):938–42. https://doi.org/10.1038/nature01868 PMID: 12931188.

13. Sivilotti L, Woolf CJ. The contribution of GABAergic and glycine receptors to central sensitization: disinhibition and touch-evoked allodynia in the spinal cord. J Neurophysiol. 1994; 72(1):169–79. Epub 1994/07/01. https://doi.org/10.1152/jn.1994.72.1.169 PMID: 7965003.

14. Zeilhofer HU. Loss of glycineergic and GABAergic inhibition in chronic pain—contributions of inflammation and microglia. Int Immunopharmacol. 2008; 8(2):182–7. Epub 2008/01/10. https://doi.org/10.1016/j.intimp.2007.07.009 PMID: 18162224.

15. Huang X, Shaffer PL, Ayube S, Bregman H, Chen H, Lehto SG, et al. Crystal structures of human glycine receptor alpha3 bound to a novel class of analgesic potentiators. Nat Struct Mol Biol. 2017; 24(2):108–13. Epub 2016/12/20. https://doi.org/10.1038/nsmb.3329 PMID: 27991902.

16. Takazawa T, Choudhury P, Tong CK, Conway CM, Scherrer G, Flood PD, et al. Inhibition Mediated by Glycinergic and GABAergic Receptors on Excitatory Neurons in Mouse Superficial Dorsal Horn Is Location-Specific but Modified by Inflammation. J Neurosci. 2017; 37(9):2336–48. Epub 2017/01/29. https://doi.org/10.1523/JNEUROSCI.2354-16.2017 PMID: 28130358; PubMed Central PMCID: PMC5354347.

17. Takazawa T, MacDermott AB. Glycinergic and GABAergic tonic inhibition fine tune inhibitory control in regionally distinct subpopulations of dorsal horn neurons. J Physiol. 2010; 588(Pt 14):2571–87. Epub 2010/05/13. https://doi.org/10.1113/jphysiol.2009.176895.
2010/05/26. https://doi.org/10.1113/jphysiol.2010.188292 PMID: 20498232; PubMed Central PMCID: PMC2916898.

18. Tyagarajan SK, Fritschy JM. Gephyrin: a master regulator of neuronal function? Nat Rev Neurosci. 2014; 15(3):141–56. Epub 2014/02/21. https://doi.org/10.1038/nrn3670 PMID: 24552784.

19. Harvey RJ, Depner UB, Wassele H, Ahmadi S, Heinli C, Reinold H, et al. GlyR alpha3: an essential target for spinal PGE2-mediated inflammatory pain sensitization. Science. 2004; 304(5672):884–7. Epub 2004/05/08. https://doi.org/10.1126/science.1094925 PMID: 15131310.

20. Becker CM, Hoch W, Betz H. Glycine receptor heterogeneity in rat spinal cord during postnatal development. EMBO J. 1988; 7(12):3717–26. PMID: 2850172; PubMed Central PMCID: PMC454946.

21. Malosio ML, Marqueze-Pouey B, Kuhse J, Betz H. Widespread expression of glycine receptor subunit mRNAs in the adult and developing rat brain. EMBO J. 1991; 10(9):2401–9. Epub 1991/09/01. PMID: 1651228; PubMed Central PMCID: PMC452935.

22. Randic M, Jiang MC, Cerne R. Long-term potentiation and long-term depression of primary afferent neurotransmission in the rat spinal cord. J Neurosci. 1993; 13(12):5228–41. Epub 1993/12/01. PMID: 8254370.

23. Ikeda H, Heinke B, Ruscheweyh R, Sandkuhler J. Synaptic plasticity in spinal lamina I projection neurons that mediate hyperalgesia. Science. 2003; 299(5610):1237–40. Epub 2003/02/22. https://doi.org/10.1126/science.1080659 PMID: 12595694.

24. Liu XG, Sandkuhler J. Long-term potentiation of C-fiber-evoked potentials in the rat spinal dorsal horn is prevented by spinal N-methyl-D-aspartic acid receptor blockade. Neurosci Lett. 1995; 191(1–2):43–6. Epub 1995/05/19. https://doi.org/10.1016/0304-3940(95)11553-0 PMID: 7659287.

25. Fenselau H, Heinke B, Sandkuhler J. Heterosynaptic long-term potentiation at GABAergic synapses of spinal lamina I neurons. J Neurosci. 2011; 31(48):17383–91. Epub 2011/12/02. https://doi.org/10.1523/JNEUROSCI.3076-11.2011 PMID: 22131400; PubMed Central PMCID: PMC6623805.

26. 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; 1183:221–42. Epub 2014/07/16. https://doi.org/10.1007/978-1-4939-1096-0_14 PMID: 25023312; PubMed Central PMCID: PMC4219416.

27. Lynch JW, Jacques P, Pierce KD, Schofield PR. Zinc potentiation of the glycine receptor chloride channel is mediated by allosteric pathways. J Neurochem. 1998; 71(5):2159–68. Epub 1998/11/03. https://doi.org/10.1046/j.1471-4159.1998.71052159.x PMID: 9798943.
36. Miller PS, Da Silva HM, Smart TG. Molecular basis for zinc potentiation at strychnine-sensitive glycine receptors. J Biol Chem. 2005; 280(45):37877–84. Epub 2005/09/08. https://doi.org/10.1074/jbc.M508302020 PMID: 16144831.

37. Staubli U, Larson J, Lynch G. Mossy fiber potentiation and long-term potentiation involve different expression mechanisms. Synapse. 1990; 5(4):333–5. Epub 1990/01/01. https://doi.org/10.1002.syn.890050410 PMID: 23620200.

38. Zalutsky RA, Nicoll RA. Comparison of two forms of long-term potentiation in single hippocampal neurons. Science. 1990; 248(4963):1619–24. Epub 1990/06/29. https://doi.org/10.1126/science.2114039 PMID: 2114039.

39. Salin PA, Scanziani M, Malenka RC, Nicoll RA. Distinct short-term plasticity at two excitatory synapses in the hippocampus. Proc Natl Acad Sci U S A. 1996; 93(23):13304–9. Epub 1996/11/12. https://doi.org/10.1073/pnas.93.23.13304 PMID: 8917586; PubMed Central PMCID: PMC240888.

40. Malenka RC, Kauer JA, Zucker RS, Nicoll RA. Postsynaptic calcium is sufficient for potentiation of hippocampal synaptic transmission. Science. 1988; 242(4875):81–4. Epub 1988/10/07. https://doi.org/10.1126/science.2845577.

41. Herring BE, Nicoll RA. Long-Term Potentiation: From CaMKII to AMPA Receptor Trafficking. Annu Rev Physiol. 2016; 78:351–65. Epub 2016/02/11. https://doi.org/10.1146/annurev-physiol-021014-071753 PMID: 26683325.

42. Wylie DJ, Nicoll RA. A role for protein kinases and phosphatases in the Ca(2+)-induced enhancement of hippocampal AMPA receptor-mediated synaptic responses. Neuron. 1994; 13(3):635–43. Epub 1994/09/01. https://doi.org/10.1016/0896-6273(94)90031-0 PMID: 7917294.

43. Gutlerner JL, Penick EC, Snyder EM, Kauer JA. Novel protein kinase A-dependent long-term depression of excitatory synaptic transmissions. Neuron. 2002; 36(5):921–31. Epub 2002/12/07. https://doi.org/10.1016/s0896-6273(02)01051-6 PMID: 12467595.

44. Pradier B, Shin HB, Kim DS, St Laurent R, Lipscombe D, Kauer JA. Long-Term Depression Induced by Optogenetically Driven Nociceptive Inputs to Trigeminal Nucleus Caudalis or Headache Triggers. J Neurosci. 2018; 38(34):7529–40. Epub 2018/07/29. https://doi.org/10.1523/JNEUROSCI.3032-17.2018 PMID: 30054391; PubMed Central PMCID: PMC610432.

45. Cavanaugh DJ, Chesler AT, Jackson AC, Sigal YM, Yamana H, Grant R, et al. Trpv1 reporter mice reveal highly restricted brain distribution and functional expression in arteriolar smooth muscle cells. J Physiol. 2004; 560(Pt 1):249–66. Epub 2004/07/31. https://doi.org/10.1113/jphysiol.2004.083959 PMID: 15284347; PubMed Central PMCID: PMC1665197.

46. Heinken B, Ruschefwych R, Forsthuber L, Wunderldinger G, Sandkühler J. Physiological, neurochemical and morphological properties of a subgroup of GABAergic spinal lamina II neurons identified by expression of green fluorescent protein in mice. J Physiol. 2004; 560(Pt 1):249–66. Epub 2004/07/31. https://doi.org/10.1113/jphysiol.2004.070545 PMID: 15284347; PubMed Central PMCID: PMC1665197.

47. Punnakall P, von Schoultz C, Haenraets K, Wildner H, Zeilhofer HU. Morphological, biophysical and synaptic properties of glutamatergic neurons of the mouse spinal dorsal horn. J Physiol. 2014; 592(4):759–76. Epub 2013/12/11. https://doi.org/10.1113/jphysiol.2013.264937 PMID: 24324003; PubMed Central PMCID: PMC3934713.

48. Nowak A, Mathieson HR, Chapman RJ, Janzso G, Yangawa Y, Obata K, et al. Kv3.1b and Kv3.3 channel subunit expression in murine spinal dorsal horn GABAergic interneurons. J Chem Neuroanat. 2011; 42(1):30–8. Epub 2011/03/29. https://doi.org/10.1016/j.jchemneu.2011.02.003 PMID: 21440618; PubMed Central PMCID: PMC3161392.

49. Cui L, Kim YR, Kim HY, Lee SC, Shin HS, Szabo G, et al. Modulation of synaptic transmission from primary afferents to spinal substantia gelatinosa neurons by group III mGluRs in GAD65-EGFP transgenic mice. J Neurophysiol. 2011; 105(3):1102–11. https://doi.org/10.1152/jn.00108.2010 PMID: 21177998.
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54. Patrizio A, Renner M, Pizzarelli R, Triller A, Specht CG. Alpha subunit-dependent glycine receptor clustering and regulation of synaptic receptor numbers. Sci Rep. 2017; 7(1):10899. Epub 2017/09/09. https://doi.org/10.1038/s41598-017-11264-3 PMID: 28883437; PubMed Central PMCID: PMC5589798.

55. Charrier C, Machado P, Tweedie-Cullen RY, Rutishauser D, Mansuy IM, Triller A. A crosstalk between beta1 and beta3 integrins controls glycine receptor and gephyrin trafficking at synapses. Nat Neurosci. 2010; 13(11):1388–95. https://doi.org/10.1038/nn.2645 PMID: 20935643.

56. Specht CG, Grunewald N, Pascual O, Rostgaard N, Schwarz G, Triller A. Regulation of glycine receptor diffusion properties and gephyrin interactions by protein kinase C. Embo J. 2011; 30(18):3842–53. https://doi.org/10.1038/emboj.2011.276 PMID: 21829170.

57. Elias GM, Nicoll RA. Synaptic trafficking of glutamate receptors by MAGUK scaffolding proteins. Trends Cell Biol. 2007; 17(7):343–52. Epub 2007/07/24. https://doi.org/10.1016/j.tcb.2007.07.005 PMID: 17644382.

58. Lee H, Kameyama K, Huganir RL, Bear MF. NMDA induces long-term synaptic depression and dephosphorylation of the GluR1 subunit of AMPA receptors in hippocampus. Neuron. 1998; 21:1151–62. https://doi.org/10.1016/s0896-6273(00)80632-7 PMID: 9856470.

59. Sharma K, Fong DK, Craig AM. Postsynaptic protein mobility in dendritic spines: long-term regulation by synaptic NMDA receptor activation. Mol Cell Neurosci. 2006; 31(4):702–12. Epub 2006/03/01. https://doi.org/10.1016/j.mcn.2006.01.010 PMID: 16504537.

60. Moreau AW, Kullmann DM. NMDA receptor-dependent function and plasticity in inhibitory circuits. Neuropharmacology. 2013; 74:23–31. Epub 2013/03/30. https://doi.org/10.1016/j.neuropharm.2013.03.004 PMID: 23537500.

61. Marsden KC, Beattie JB, Friedenthal J, Carroll RC. NMDA receptor activation potentiates inhibitory transmission through GABA receptor-associated protein-dependent exocytosis of GABA(A) receptors. J Neurosci. 2007; 27(52):14326–37. Epub 2007/12/28. https://doi.org/10.1523/JNEUROSCI.4433-07.2007 PMID: 18160640.

62. Bannai H, Levi S, Schweizer C, Inoue T, Launey T, Racine V, et al. Activity-dependent tuning of inhibitory neurotransmission based on GABAAR diffusion dynamics. Neuron. 2009; 62(5):670–82. https://doi.org/10.1016/j.neuron.2009.04.023 PMID: 19524526.

63. Bannai H, Niwa F, Sherwood MW, Shrivastava AN, Arizono M, Miyamoto A, et al. Bidirectional Control of Synaptic GABAAR Clustering by Glutamate and Calcium. Cell reports. 2015; 13(12):2768–80. https://doi.org/10.1016/j.celrep.2015.12.002 PMID: 26711343; PubMed Central PMCID: PMC4700050.

64. Petrizzi EM, Rasavenga T, Hausrat TJ, Iurilli G, Olice U, Racine V, et al. Synaptic recruitment of gephyrin regulates surface GABAA receptor dynamics for the expression of inhibitory LTP. Nat Commun. 2014; 5:3021. Epub 2014/06/05. https://doi.org/10.1038/ncomms4921 PMID: 24894704; PubMed Central PMCID: PMC4059940.

65. Yamanaka I, Miki M, Asakawa K, Kawakami K, Oda Y, Hirata H. Glycinergic transmission and postsynaptic activation of CaMKII are required for glycine receptor clustering in vivo. Genes Cells. 2013; 18(3):211–24. Epub 2013/01/26. https://doi.org/10.1111/gtc.12032 PMID: 23347046.

66. Kirsch J, Betz H. Glycine-receptor activation is required for receptor clustering in spinal neurons. Nature. 1998; 392(6677):717–20. Epub 1998/05/16. https://doi.org/10.1038/33694 PMID: 9565032.

67. Kurotani T, Yamada K, Yoshimura Y, Crair MC, Komatsu Y. State-dependent bidirectional modification of somatic inhibition in neocortical pyramidal cells. Neuron. 2009; 57(6):905–16. Epub 2008/03/28. https://doi.org/10.1016/j.neuron.2008.01.036 PMID: 18967091; PubMed Central PMCID: PMC2880402.

68. Lu Y, Dong H, Gao Y, Gong Y, Ren Y, Gu N, et al. A feed-forward spinal cord glycineergic neural circuit gates mechanical allodynia. J Clin Invest. 2013; 123(9):4050–62. Epub 2013/08/28. https://doi.org/10.1172/JCI70026 PMID: 23979158.

69. Melzack R, Wall PD. Pain mechanisms: a new theory. Science. 1965; 150(3699):971–9. Epub 1965/11/19. https://doi.org/10.1126/science.150.3699.971 PMID: 5308016.

70. Laird JM, Bennett GJ. Dorsal root potentials and afferent input to the spinal cord in rats with an experimental peripheral neuropathy. Brain Res. 1992; 584(1–2):181–90. Epub 1992/07/03. https://doi.org/10.1016/0006-8993(92)90893-e PMID: 1515937.

71. Ibuki T, Hama AT, Wang XT, Pappas GD, Sagen J. Loss of GABA-immunoreactivity in the spinal dorsal horn of rats with peripheral nerve injury and promotion of recovery by adrenal medullary grafts. Neuroscience. 1997; 76(3):845–58. Epub 1997/02/01. https://doi.org/10.1016/s0306-4522(96)00341-7 PMID: 9135056.

72. Moore KA, Kohno T, Karchewski LA, Scholz J, Baba H, Woolf CJ. Partial peripheral nerve injury promotes a selective loss of GABAergic inhibition in the superficial dorsal horn of the spinal cord. J Neurosci. 2002; 22(15):6724–31. Epub 2002/08/02. https://doi.org/10.1523/JNEUROSCI.2155-02.2002 PMID: 12151551.
73. Muller F, Heinke B, Sandkuhler J. Reduction of glycine receptor-mediated miniature inhibitory postsynaptic currents in rat spinal lamina I neurons after peripheral inflammation. Neuroscience. 2003; 122 (3):799–805. Epub 2003/11/19. https://doi.org/10.1016/j.neuroscience.2003.07.009 PMID: 14622922.

74. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. Pain. 2011; 152(3 Suppl):S2–15. Epub 2010/10/22. https://doi.org/10.1016/j.pain.2010.09.030 PMID: 20961685; PubMed Central PMCID: PMC3268359.

75. von Hehn CA, Baron R, Woolf CJ. Deconstructing the neuropathic pain phenotype to reveal neural mechanisms. Neuron. 2012; 73(4):638–52. Epub 2012/03/01. https://doi.org/10.1016/j.neuron.2012.02.008 PMID: 22365541; PubMed Central PMCID: PMC3319438.