European Journal of Pain

ORIGINAL ARTICLE

Loss of Glutaminase 1 in Small Sensory Neurons Prevents Nerve Injury Induced Mechanical Allodynia: Insights From Conditional Knockout Mice

Anabel Martínez-Padilla[1] | Javier Márquez[1] | Miguel Á. Huerta[2,3,4] | Carolina Roza[5,6] | Elsa Cisneros[6,7]

[1]Departamento de Biología Molecular y Bioquímica, Facultad de Ciencias e Instituto de Investigación Biomédica de Málaga y Plataforma en Nanomedicina (IBIMA Plataforma BIONAND), Universidad de Málaga, Málaga, Spain | [2]Department of Pharmacology, Faculty of Medicine, University of Granada, Granada, Spain | [3]Institute of Neuroscience, Biomedical Research Center, University of Granada, Granada, Spain | [4]Biosanitary Research Institute ibs. GRANADA, Granada, Spain | [5]Department of System's Biology, Medical School, University of Alcala de Henares, Alcalá de Henares, Spain | [6]Health Sciences School, Universidad Internacional de La Rioja (UNIR), Logroño, Spain | [7]Health Sciences School, Centro Universitario Internacional de Madrid (CUNIMAD), Madrid, Spain

Correspondence: Elsa Cisneros (elsa.cisneros@unir.net)

Received: 11 March 2025 | **Revised:** 7 June 2025 | **Accepted:** 12 June 2025

Funding: This work was supported by the MINECO (SAF2016-77585-R) and Universidad de Alcalá (CCG19/CCS-013).

ABSTRACT

Background: Glutamate, the primary neurotransmitter released by nociceptors, is predominantly synthesised by the enzyme Glutaminase 1 (GLS1). The involvement of GLS1 in pain pathways is well supported, as *Gls1* heterozygous mice exhibit altered nociception and GLS1 levels increase in the dorsal root ganglia (DRG) under chronic peripheral inflammation. However, the specific contribution of GLS1 in sensory neurons to the development and maintenance of chronic neuropathic pain remains unclear. To explore this, we specifically targeted GLS1 expression in nociceptors.

Methods: We used the Cre-LoxP system to generate a transgenic mouse with a specific deletion of *Gls1* gene in neurons expressing the Nav1.8 sodium channel. Gene deletion was assessed by genomic PCR and immunofluorescence. GLS1 conditional knockout (cKO) mice and control littermates, under naïve conditions or following spared nerve injury (SNI), were analysed for mechanical allodynia and for expression of GLS1 and other components of the glutamatergic system using real-time PCR and Western blotting.

Results: GLS1 cKO mice exhibited a significant reduction in GLS1 levels in the DRG, particularly in medium- to small-sized neurons. GLS1 deficiency prevented the development of mechanical allodynia following peripheral nerve injury. SNI induced GLS1 upregulation in the DRG of control mice, but not in cKO mice. In the spinal cord, NMDA receptor expression decreased after SNI only in naïve animals, while GLS1 and other glutamate receptors remained unchanged under all conditions.

Conclusions: Upregulation of GLS1 in sensory neurons after peripheral nerve injury contributes to mechanical allodynia. Targeting peripheral GLS1 could offer a potential analgesic strategy for neuropathic pain.

Significance Statement: We generated a transgenic mouse with a specific deletion of the *Gls1* gene in Nav1.8-expressing neurons to assess the role of peripheral GLS1 in pain transmission. GLS1 is not required for physiological pain but is essential for the development of mechanical allodynia after nerve injury. GLS1 is upregulated in nociceptors following nerve injury, suggesting enhanced glutamate signalling. Taken together, results suggest that targeting GLS1 expression in neuropathic conditions could be a potential therapeutic strategy.

Carolina Roza and Elsa Cisneros contributed equally to this study.

1 | Introduction

Glutamate is the primary neurotransmitter released by sensory afferent neurons and is essential for nociceptive signalling. In response to intense noxious stimuli or tissue damage, nociceptors release glutamate at central synapses to activate dorsal horn neurons within nociceptive circuits. Meanwhile, glutamate release at peripheral terminals, alongside vasoactive neuropeptides, promotes local sensitisation (Miller et al. 2011). Additionally, dysregulation of the glutamatergic system contributes to chronic pain conditions. For instance, elevated extracellular glutamate levels exacerbate neuropathic pain symptoms, including hyperalgesia and allodynia. Thus, modulating glutamatergic signalling may represent a promising strategy for neuropathic pain management. In this context, most research has focused on identifying pathophysiological mechanisms involving glutamate receptors and transporters (Malet and Brumovsky 2015; Temmermand et al. 2022), whereas the role of glutaminase enzymes remains less explored.

Glutaminase 1 (GLS1) is a mitochondrial enzyme that catalyses the hydrolytic deamidation of glutamine to produce glutamate. This enzyme requires inorganic phosphate for activation and is regulated by its end products, such as glutamate (Kvamme et al. 2001). In the nervous system, GLS1 is the primary source of glutamate and plays a critical role in maintaining homeostasis. Consequently, its dysregulation has been implicated in several neurological disorders (Márquez et al. 2013; Masson et al. 2006). The *Gls1* gene produces two mRNAs, which are processed into two isoenzymes named KGA and GAC. Both isoforms are expressed in most sensory neurons within the dorsal root ganglia (DRG), with the highest levels found in small- to medium-sized cells (Hoffman et al. 2010; Miller et al. 1993). GLS1 has also been detected in dorsal roots, the sciatic nerve and the trigeminal nerve, suggesting that local glutamate biosynthesis occurs at both peripheral and central nerve endings of primary afferents (Miller et al. 2011).

The role of GLS1 in nociception is evident, as heterozygous $GLS1^{+/-}$ mutants exhibit reduced nociceptive responses to mechanical, thermal and chemical inflammatory stimuli when compared to wild-type littermates. Additionally, the uncompetitive NMDA receptor antagonist memantine reduces nociceptive responses in wild-type mice but not in $GLS1^{+/-}$ mutants, suggesting that GLS1 downregulation impairs glutamatergic neurotransmission to such an extent that it masks any additional inhibitory effect of the receptor antagonist (Kayser et al. 2015). Furthermore, GLS1 levels increase in the DRG during chronic peripheral inflammation, with the most significant rise occurring in small- and medium-sized neurons (Hoffman et al. 2016; Miller et al. 2012). Nonetheless, the expression of GLS1 in sensory neurons, particularly in nociceptors, during neuropathy has not been studied.

We aimed to investigate the role of GLS1 in neuropathic pain. To achieve this goal, we generated a novel conditional transgenic mouse with a specific deletion of the *Gls1* gene in Nav1.8 neurons, a population of sensory neurons, most of which are nociceptors (Stirling et al. 2005). We analysed pain-related behaviours and GLS1 expression in control and GLS1 conditional knockout (cKO) mice under naïve conditions and following peripheral nerve injury. Our results suggest that GLS1 expression in nociceptors contributes to the development and/or maintenance of neuropathic pain. Therefore, targeting GLS1 activity in nociceptors may have therapeutic potential.

2 | Methods

2.1 | Animals

Mature adult male and female mice (used in approximately equal proportions, except for behavioural experiments, which exclusively involved females) were bred at the University of Málaga Animal House Central Facility. The animals were maintained at 22°C on a 12-h light/dark cycle with *ad libitum* access to food and water. All experimental protocols were approved by the University of Málaga Committee on Animal Research and by the Regional Government of Andalusia (project licence: 04/05/2021/070), in full compliance with European Union and national legislation.

2.2 | Generation of Conditional Knockout of GLS1 in Nav1.8^{+} Neurons

Female $GLS1^{lox/lox}$ mice, which harbour loxP sites flanking exon 1 of the Glutaminase 1 gene (generated as described in Mingote et al. 2016; JAX stock #017894, kindly donated by Dr. Massimo M. Santoro, Padua University) were crossed with male $Nav1.8^{Cre/+}$ mice, which carry the Cre recombinase gene under the control of the Nav1.8 promoter (Stirling et al. 2005; INFAFRONTIER EM:04582, kindly donated by Dr. Aziz Moqrich, Aix-Marseille University). Male $GLS1^{lox/+}$; $Nav1.8^{Cre/+}$ mice were then crossed with female $GLS1^{lox/lox}$ mice to obtain the cKO of GLS1 in Nav1.8+ neurons, $GLS1^{lox/lox}$; $Nav1.8^{Cre/+}$ mice. $GLS1^{lox/lox}$; $Nav1.8^{+/+}$ littermates were used as controls. Mice had a C57BL/6J background. The strategy for generating the cKO is depicted in Figure 1a.

For genotyping animals, tail genomic DNA was isolated according to standard protocols. The Cre allele was amplified by using the forward primer Cre Up 2 (GATCTCCGGTATTGAAACTCCAGC) and the reverse primer Cre Dn 2 (GCTAAACATGCTTCATCGTCGG), which yielded an amplicon of 650 pb (protocol standardised by the Mutant Mouse Resource & Research Centre at University of California, Davis). The 3′ loxP site was identified by PCR using the forward primer B (GGCCTGCTTAATGTTTCCTG) and the reverse primer C (GGCATATCCCTGAGTTCGAG), which produced an amplicon of 430 pb in floxed alleles (Mingote et al. 2016; Figure 1b).

2.3 | Induction of Neuropathy: Spared Nerve Injury

During all the surgical procedures, the mice underwent deep anaesthesia with 3%–4% sevoflurane (SevoFlo, Zoetis, US) in 100% oxygen. For spared nerve injury (SNI) (Shields et al. 2003), an incision was made in the skin on the lateral surface of the thigh, the sciatic terminal branches (sural, common peroneal and tibial nerves) were exposed, and the

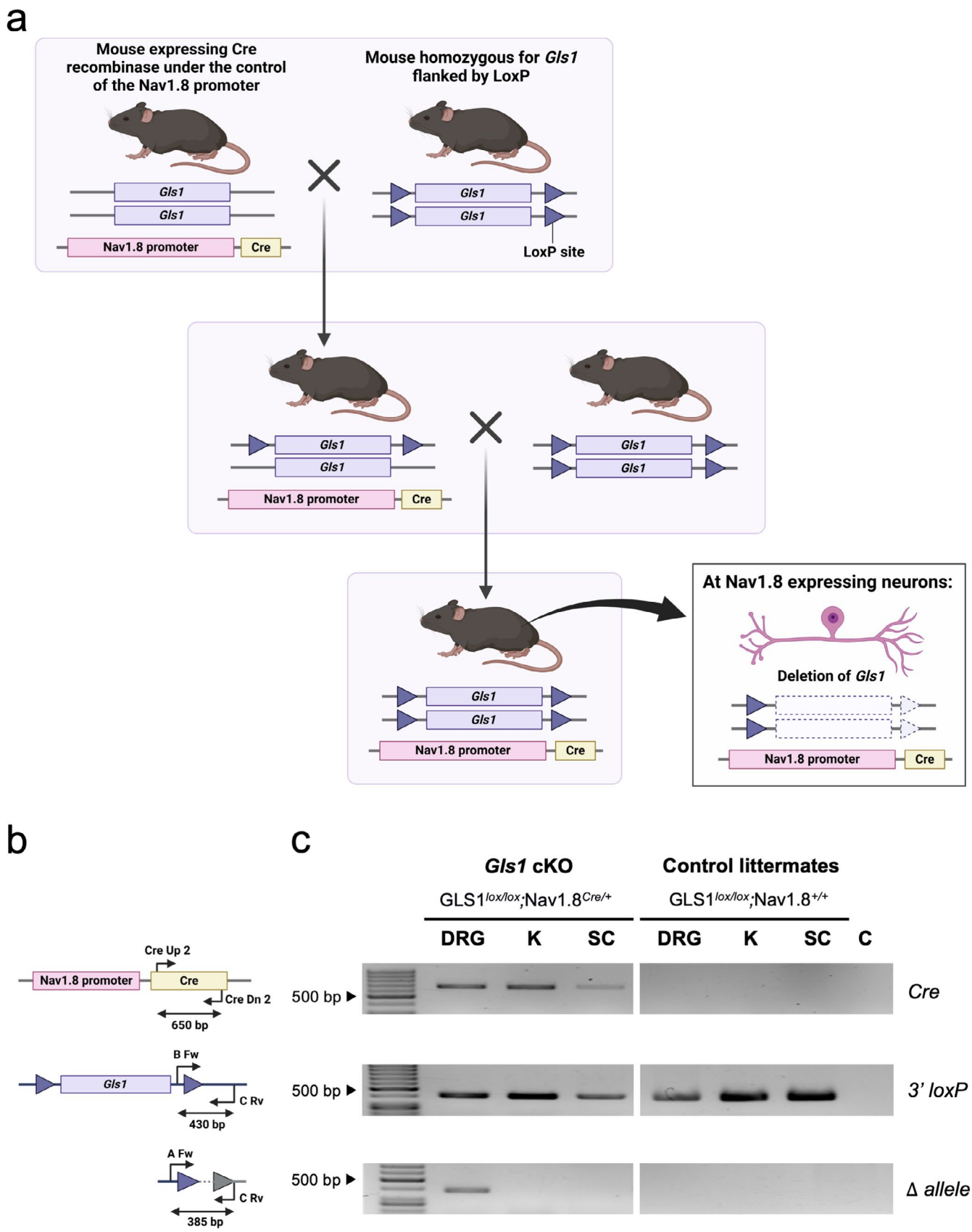

FIGURE 1 | Generation of sensory neurons-specific GLS1 conditional knockout. (a) Schematic diagram of the mating strategy to generate the GLS1 cKO. (b) Primer strategy used to determine the presence of Cre recombinase (amplified by primers Cre Up 2 and Cre Dn 2), 3′loxP (primers B and C) and recombined (Δ) Gls1 alleles (primers A and C). (c) PCR genotyping of DNA samples obtained from dorsal root ganglia (DRG), kidney (K), and spinal cord (SC) in GLS1 cKO and control littermates. The negative control is indicated as C.

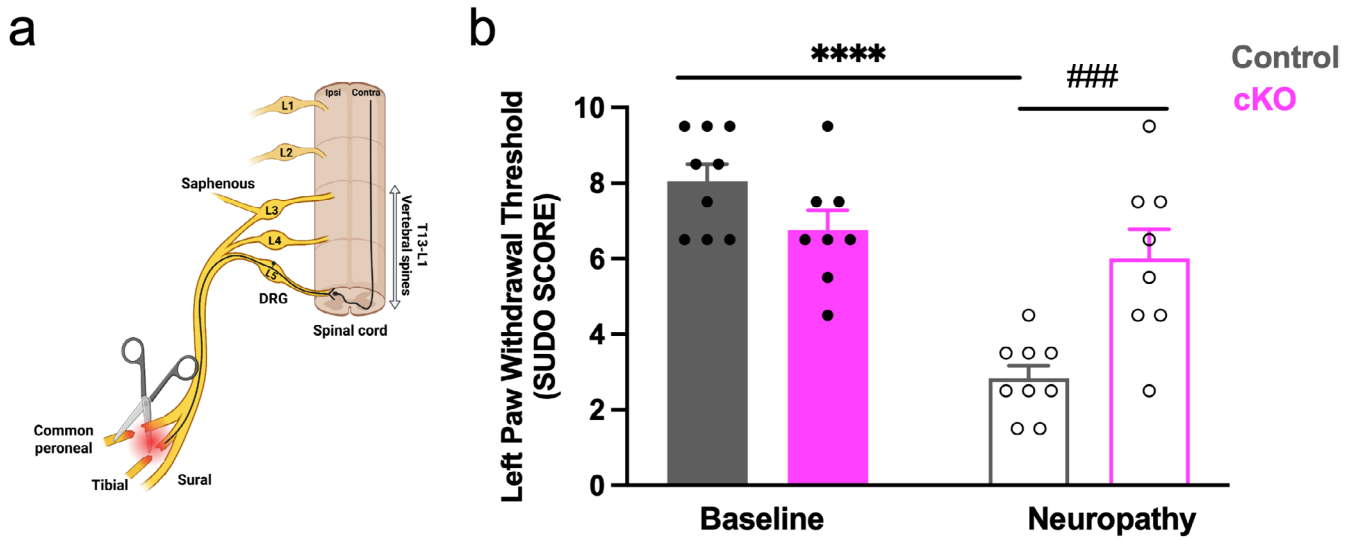

FIGURE 2 | Induction of neuropathy and testing of mechanical allodynia. (a) Schematic representation of the SNI procedure. The common peroneal and tibial branches of the sciatic nerve are ligated and transected, while the sural branch is left intact. The central axons of sensory neurons synapse with second-order interneurons in dorsal horn spinal cord, which sends its axons through spinothalamic spinal cord tract. For RNA and protein extraction we collected L4-L5 spinal cord segments that are enclosed between T3-L1 vertebral spines. (b) Calibrated von Frey filaments are applied to sural territory, and the paw withdrawal thresholds are measured using the simplified up-down method. Values are presented as SUDO scores at baseline and 14 days after surgery ($n = 9$ female mice in control group and $n = 8$ female mice in cKO group). Statistical analysis was performed using two-way ANOVA followed by Fisher's LSD test; ****$p < 0.0001$ indicates significant differences between baseline and neuropathy. ##$p < 0.001$ indicates significant differences between control and cKO.

common peroneal and tibial nerves were ligated with 8/0 nylon monofilament and sectioned distal to the ligature (schematised in Figure 2a). The sectioned end was inserted into an approximately 1–1.5 mm long polythene tube (0.28 mm internal diameter) to prevent lateral innervation of surrounding tissue. The tube was tied in place with the ligature piece of silk, and the incision was closed with 5/0 sutures or surgical staples (Fine Science Tools, Germany). Animals were then housed and inspected periodically for infections or abnormal behaviour.

2.4 | Behavioural Testing: Von Frey Test

Female GLS1 cKO and control littermates with or without neuropathy were tested for mechanical allodynia. An experimenter blinded to the conditions measured the threshold of paw withdrawal 14 days after injury, just before tissue extraction. Testing took place during the light phase (from 10:00 AM to 2:00 PM). Mice were acclimatised for at least 30 min to test chambers consisting of transparent Plexiglas cages (10 × 10 × 10 cm with a red frontal wall) on an elevated wire mesh floor. The sural territory of the hind paw was stimulated with calibrated von Frey filaments (0.02–2 g, 0.19–19.6 mN, Ugo Basile, Italy) following the simplified up-down method (SUDO; Bonin et al. 2014).

2.5 | Tissue Collection

Tissues were collected 14 days after surgery. The mice were deeply anaesthetised with 6% sevoflurane (SevoFlo, Zoetis, US) in 100% oxygen and perfused transcardially with ice-cold PBS. Then, L3-5 DRG, L4-L5 spinal cord segment, brain, liver and kidneys were extracted, frozen, and stored at −80°C until needed for RNA and protein analysis. After perfusion, some DRG were fixed in PFA (see below) and processed for immunostaining.

To identify the lumbosacral plexus and spinal cord segments, we used vertebral bodies as anatomical landmarks. In the C57BL/6 mice, L4–L5 spinal cord segments correspond to the region between the T13 and L1 vertebral spines (Harrison et al. 2013).

2.6 | Analysis of *Gls1* Deletion in cKO Mice

Deletion of *Gls1* exon 1 was assessed by PCR on genomic DNA extracted from different tissues using a forward primer A (GCCCCAAGCATCCTCATCTCGAATA) and the reverse primer C (GGCATATCCCTGAGTTCGAG). These primers produce a gel band of 385 bp (Δ allele) only in tissues where recombination takes place, that is, those expressing Nav1.8 (Figure 1b) (Mingote et al. 2016).

2.7 | RNA Extraction and Real-Time Reverse Transcription PCR

Total RNA was extracted with the PureLink RNA Mini Kit (Thermo Fisher Scientific, Waltham, MA). Only samples with 260/280 nm absorbance ratios of 2.0 ± 0.2 were used. cDNA was prepared using the QuantiTect Reverse Transcription Kit (Qiagen, Netherlands). The real-time reverse transcription PCR (RT-PCR) reactions were run in triplicate on 96-well reaction plates with SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, CA), using a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA). PCR reactions

were performed with 50 ng of cDNA using specific primers for mouse *Gls1*, *mGlu1*, *mGlu5*, *AMPA* and *β-actin* gene coding sequences (Table S1). 40 cycles of amplification were performed, including a denaturation (95°C; 15 s) and annealing/extension (60°C; 1 min) steps. Data acquisition and analysis of the assays were performed using the CFX Manager Software (Bio-Rad, Hercules, CA). The cycle threshold (Ct) values for *Gls1*, *mGlu1*, *mGlu5* and *AMPA* were normalised to those of the housekeeping gene *β-actin* (ΔCt). Relative fold changes in gene expression were calculated using the comparative $2^{-\Delta\Delta CT}$ method.

2.8 | Western Blotting

Tissues were homogenised with a pestle in RIPA buffer (50 mM Tris pH 8, 1% Nonidet P-40, 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate), supplemented with cOmplete Mini Protease Inhibitor cocktail (Roche, Basel, Switzerland), and centrifuged at 12,000×*g* for 20 min. Protein concentrations were measured with the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA) for normalisation purposes. After heating at 95°C for 5 min, total proteins (20 μg) were separated by SDS-PAGE and electrotransferred onto nitrocellulose membranes. Membranes were blocked in 5% non-fat milk in TBST buffer (20 mM Tris, 50 mM NaCl, 0.1% w/v Tween 20) at RT for 1 h and then incubated with the appropriate primary antibodies. The antibodies were rabbit antiserums for GLS isoform KGA (1:2500, kindly donated by Prof. Norman P. Curthoys, Colorado State University) and GLS2 isoform GAB (1:2000, ab113509, Abcam Cambridge, MA), NMDA receptor 1 (1:500, ab17345, Abcam Cambridge, MA) and β-Actin (1:1000, Merck, Darmstadt, Germany), used as a loading control. The membranes were washed and incubated with goat anti-rabbit IgG peroxidase antibody (1:80,000 dilution A0545, Merck, Darmstadt, Germany) in 5% BSA in TBST, followed by enhanced chemiluminescence (ECL) detection system (SuperSignal West Pico, Thermo Fisher Scientific, Waltham, MA). Immune-reactive bands were visualised in a ChemiDoc Gel Imaging System (Bio-Rad, Hercules, CA) and quantified using Image Lab software (Bio-Rad Hercules, CA). Values corresponding to the signal of each band were normalised to β-Actin signals.

2.9 | Immunostaining

Collected L3–L5 DRG were fixed by immersion in 4%PFA in 0.1 M phosphate buffer (PB) overnight at 4°C. Tissues were washed with PB 0.1 M, cryoprotected with 30% sucrose in PB, embedded in 7.5% gelatine/15% sucrose in PB, frozen and sectioned at 16 μm (Leica CM1950; Leica, Wetzlar, Germany). The sections were incubated in blocking solution (10% donkey serum in Tris-buffered saline, TBS) for 2 h and permeabilised in 0.5% Triton X-100 in TBS for 10 min. The samples were then incubated with primary antibodies diluted in TBS at room temperature (RT) for 18 h. The primary antibodies used were rabbit antiserum for GLS1 (1:100) and mouse anti-Nav1.8 (1:100; ab 93616; Abcam Cambridge, MA). The sections were washed in 0.2% Tween 20 in TBS and incubated with Alexa-Fluor 488-conjugated donkey anti-rabbit and Alexa-Fluor 594-conjugated donkey anti-mouse secondary antibodies (1:500 each secondary; Jackson ImmunoResearch Laboratories Inc., West Grove, PA) at RT for 1.5 h. After washing in TBS, the tissue was counterstained with DAPI (0.1 mg/mL; D9542, Sigma-Aldrich, St. Louis, MO) to label the nuclei. Sections were mounted in Mowiol and analysed by fluorescence microscopy (Olympus BX61, Tokyo, Japan).

All images were analysed using Fiji software (Schindelin et al. 2012). To quantify positively stained cells, a fluorescence intensity threshold was set and kept constant across all samples within each experiment. To avoid counting the same cell more than once, tissue sections were mounted consecutively onto five slides, ensuring that sections on the same slide were separated by at least 64 μm.

2.10 | Statistics

Statistical analyses were performed with Graph Pad Prism version 4 (GraphPad Software, San Diego, CA) and Microsoft Excel 365. All data are represented as mean ± standard error of the mean (SEM) for each group. Differences between mean values were analysed with unpaired Student *t*-test with Welch's correction or two-way ANOVA, followed by the appropriate post hoc test. A significant difference was accepted for $p < 0.05$.

3 | Results

3.1 | Generation and Characterisation of GLS1 Conditional Knockout Mice

To obtain clues about the role of the glutaminase GLS1 isoenzyme in nociception, we generated a mouse lacking the *Gls1* gene in cells expressing Nav1.8. This voltage-gated sodium channel is expressed in a subset of sensory neurons, more than 85% of which are nociceptors (Stirling et al. 2005). Mouse lines $GLS1^{lox/lox}$ and $Nav1.8^{Cre/+}$ were mated as shown in Figure 1a to obtain GLS1 cKO ($GLS1^{lox/lox}$; $Nav1.8^{Cre/+}$) and control littermates ($GLS1^{lox/lox}$; $Nav1.8^{+/+}$). GLS1 cKO mice were born at the expected Mendelian ratios and were indistinguishable from control littermates, indicating that the loss of *Gls1* in sensory neurons does not affect the viability or general development. Representative genotyping results for these two mouse lines are shown in Figure 1c. The Cre recombinase allele yielded a PCR band of 650 bp (only present in cKO mice), while homozygotes for the floxed alleles produced a band of 430 bp. To verify the specific deletion of *Gls1* exon 1 in cells expressing Nav1.8, we tested for the presence of the deletion allele (Δ), a 385 bp band produced only after Cre-mediated recombination of the floxed alleles. In control mice, Δ was absent, whereas in GLS1 cKO mice, it was amplified in genomic DNA from dorsal root ganglia (DRG) but not from other tissues, such as the kidney or spinal cord. Finally, to validate GLS1 deficiency in Nav1.8-expressing sensory neurons in the cKO mice, we performed double immunostaining of the DRG. In control mice, GLS1 protein was detected in neurons of all sizes, although its levels were higher in small-sized ones, where it co-localised with Nav1.8. Indeed, virtually all Nav1.8-positive neurons expressed GLS1 (93.30% ± 6.70%, $n = 4$). However, GLS1 was absent in all analysed Nav1.8-positive neurons from cKO mice (00.00% ± 00.00%, $n = 8$; unpaired Student's *t*-test with Welch's correction, $p < 0.0001$; Figure 3). These results confirm the successful generation of a conditional knockout

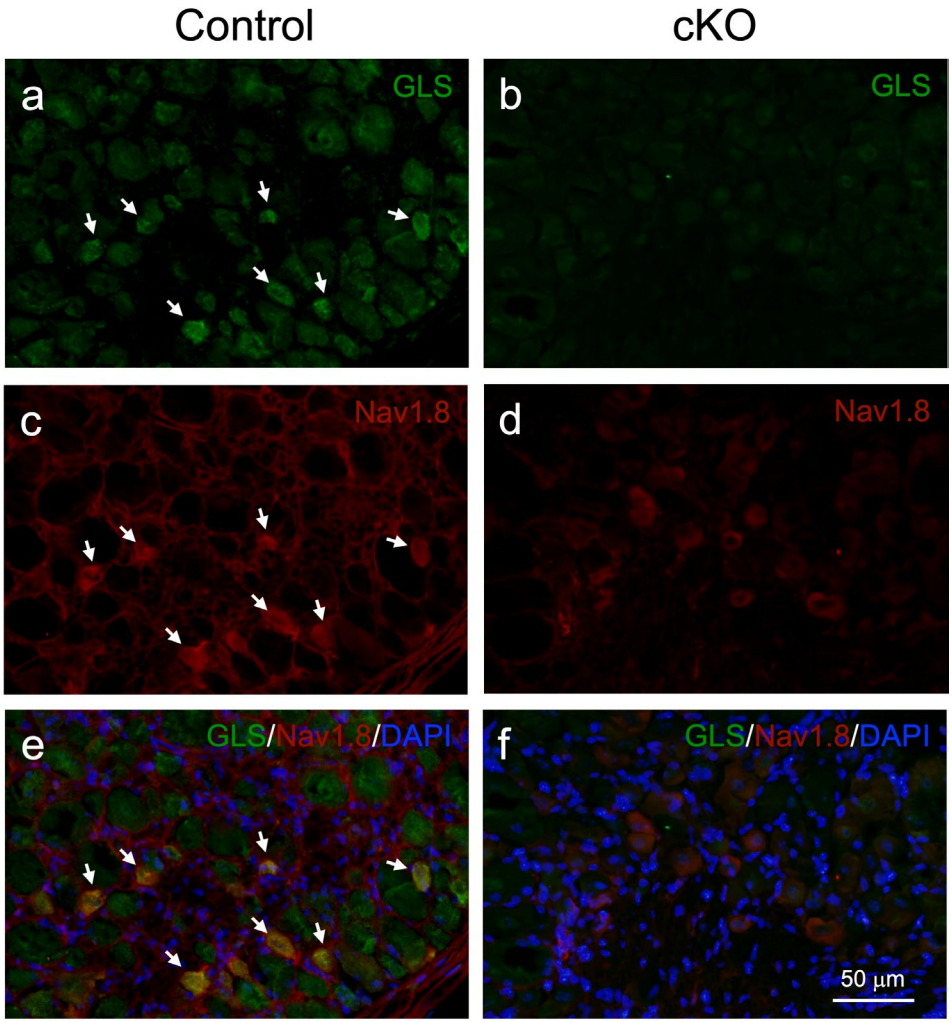

FIGURE 3 | GLS1 expression in DRG from conditional knockout mice. Representative cryostat sections of DRG of control littermates and GLS1 cKO mice were double-immunostained with GLS1 antiserum and Nav1.8 antibody, and counterstained with DAPI. (a, c) In control DRG, GLS1 immunoreactivity was observed in neurons of all sizes, with stronger signals in small-sized neurons, where it co-localised with Nav1.8-positive neurons (arrows). (b, d) In GLS1 cKO mice, GLS1 was absent in all Nav1.8-positive neurons and remained at low levels in the neurons of other sizes.

model for GLS1 in Nav1.8-expressing neurons, the majority of which are nociceptors.

3.2 | Nociceptive Behaviours in GLS1 Conditional Knockout Mice

Next, we investigated whether the GLS1 expression in Nav1.8-expressing sensory neurons is necessary for nociceptive or neuropathic pain behaviours. Nocifensive responses to mechanical stimulation of the hindpaws were similar in both control and GLS1 cKO naïve mice (see baselines in Figure 2; 8.00 ± 0.48 vs. 7.00 ± 0.50 SUDO scores, $n = 9$ and 8 females, respectively; two-way ANOVA followed by Fisher's LSD test, $p \geq 0.05$). Thus, the loss of GLS1 does not appear to affect physiological pain thresholds, at least in response to mechanical stimuli. As expected, 14 days after SNI induction, mechanical PWT decreased significantly in control animals (8.00 ± 0.48 to 2.80 ± 0.30 SUDO scores, $n = 9$ females for both naïve and SNI control mice; two-way ANOVA followed by Fisher's LSD test $p < 0.0001$). However, the PWT of GLS1 cKO mice showed a slight but non-significant decrease following SNI (7.00 ± 0.50 to 5.10 ± 0.60 SUDO scores, $n = 8$ both naïve and SNI cKO mice; $p \geq 0.05$). These results suggest that the loss of GLS1 in putative nociceptors prevents the induction and/or maintenance of mechanical allodynia, a hallmark symptom of neuropathic pain.

3.3 | Modulation of Glutaminases Expression in DRG Under Neuropathic Conditions

Because our previous findings indicate that GLS1 plays a role in neuropathic pain, we investigated whether its expression is regulated in sensory neurons in response to nerve injury. Real-time RT-PCR showed that in the DRG from control mice, *Gls1* mRNA levels increased ~threefold 2 weeks after SNI (2.03 ± 0.44 and 6.21 ± 3.45 in naïve and SNI control mice, respectively, $n = 5$ or 4 mice per group; two-way ANOVA followed by Fisher's LSD test, $p < 0.05$). Upregulated expression of *Gls1* correlates with an increase in protein levels, which double after SNI, as detected by Western blotting (Figure 4a; 0.55 ± 0.14 and 1.12 ± 0.16 in naïve and SNI control mice, respectively, $n = 5$ mice per group; two-way ANOVA followed by Sidak's post hoc test, $p < 0.001$). In DRG from naïve GLS1 cKO

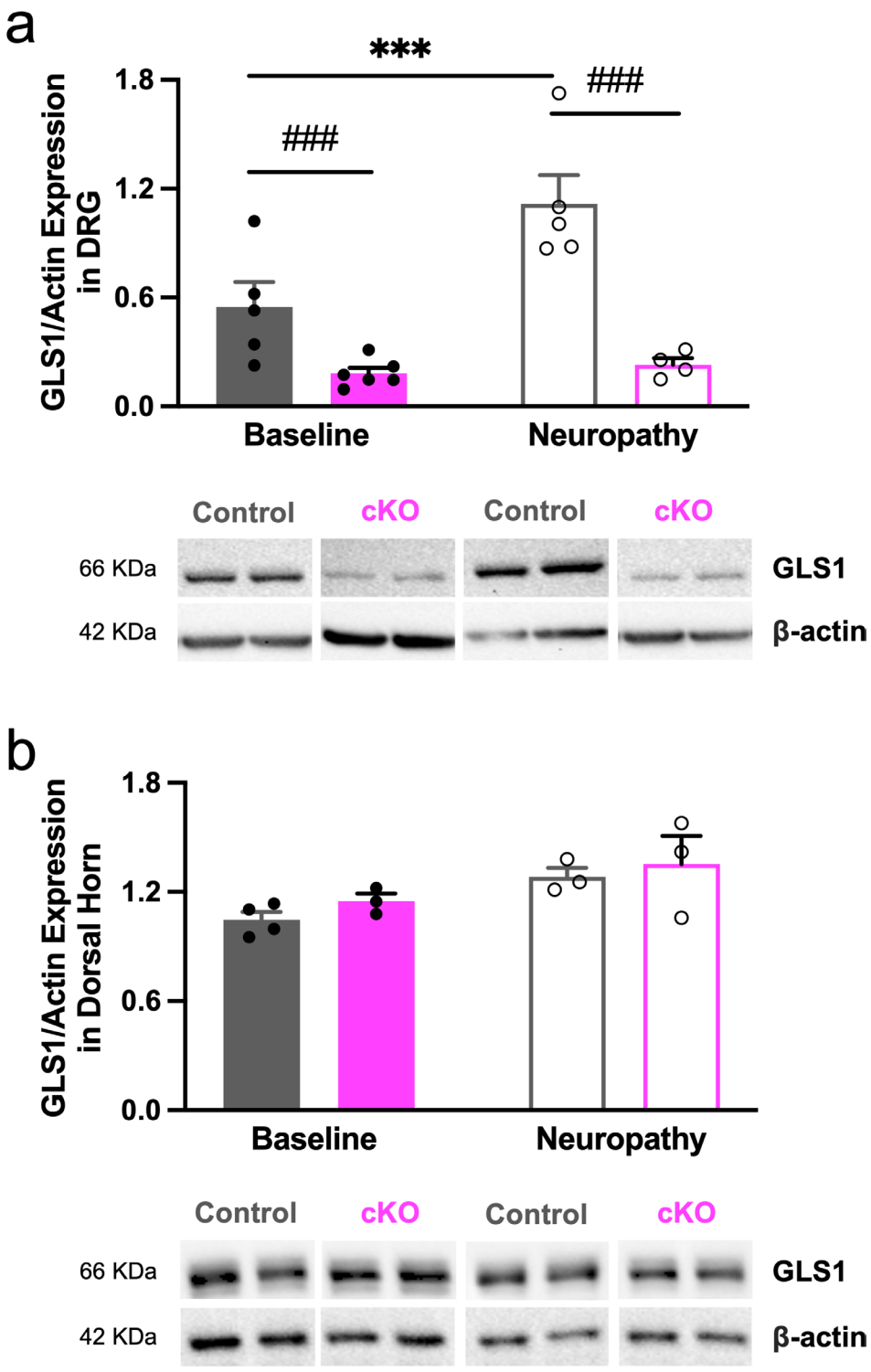

FIGURE 4 | Western blot analysis of GLS1 expression in control and GLS1 conditional knockout mice following nerve injury. Representative western blots and graphs of GLS1 protein levels normalised to β-Actin in naïve (baseline) or 14 days after SNI ($n = 4–6$ mice per group). GLS1 levels were measured in (a) L3-L5 DRG and (b) dorsal horn of the L4-L5 spinal cord segment. For neuropathic animals, we used DRG and hemi-spinal cords ipsilateral to the lesion. Statistical analysis was performed using two-way ANOVA followed by Sidak's post hoc test; ***$p < 0.001$ indicates significant differences between baseline and neuropathy. ###$p < 0.001$ indicates significant differences between control and cKO.

mice, GLS1 protein levels were significantly lower compared with control littermates (Figure 4a; 0.18 ± 0.03 vs. 0.55 ± 0.14, $n = 6$ and 5 mice per group; two-way ANOVA followed by Sidak's post hoc test, $p < 0.001$). This reduction aligns with the expected effect of *Gls1* deletion in Nav1.8-expressing sensory neurons. Nonetheless, GLS1 levels remain unchanged after nerve injury in GLS1 cKO (0.18 ± 0.03 and 0.23 ± 0.04 in naïve and SNI CKO mice, respectively, $n = 6$ and 4 mice per group; two-way ANOVA followed by Sidak's post hoc test, $p \geq 0.05$). Next, we analysed GLS2 expression, the other glutamine

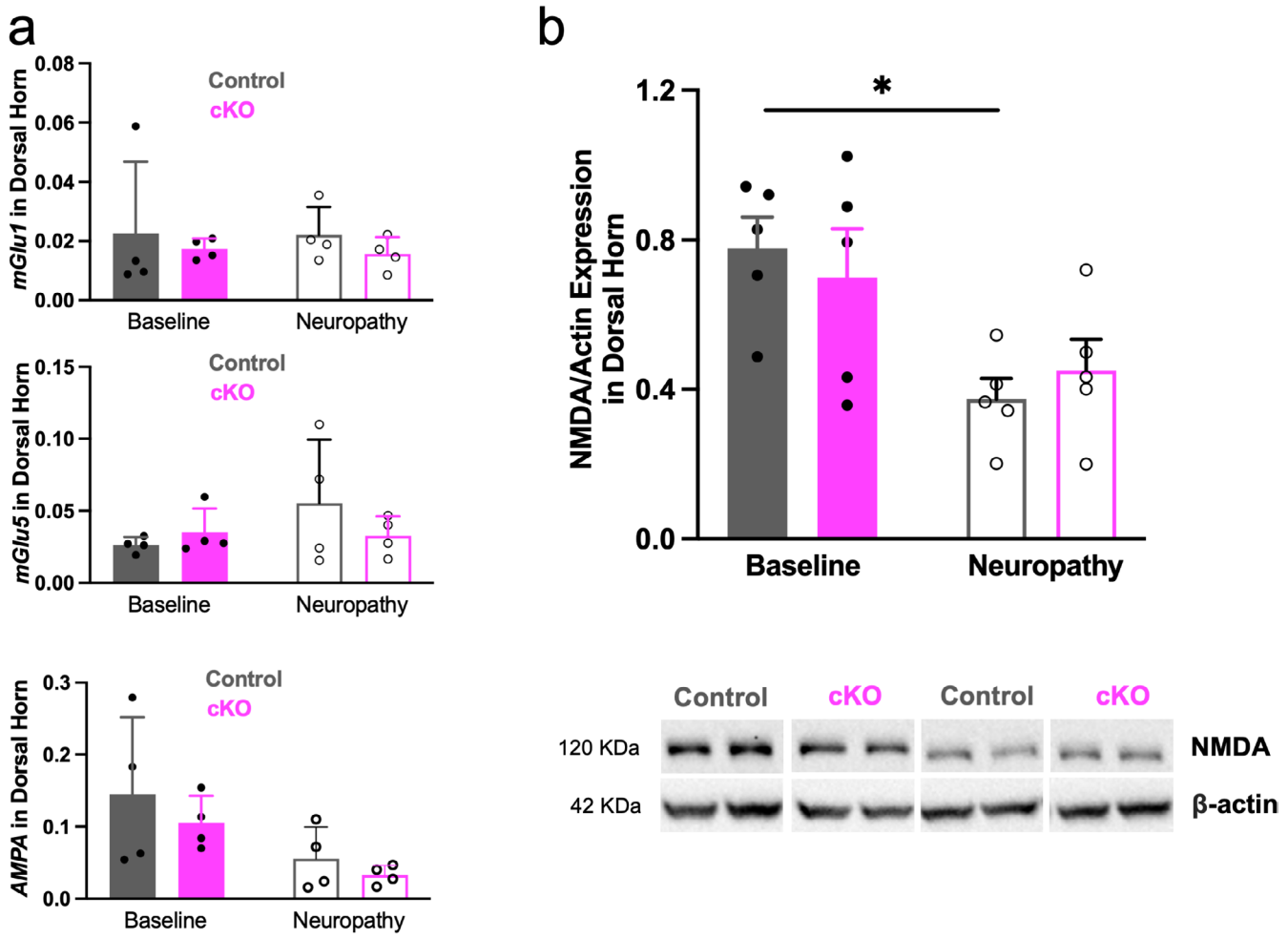

FIGURE 5 | Analysis of glutamate receptors in the spinal cord of control and GLS1 conditional knockout mice following nerve injury. (a) The *mGlu1*, *mGlu4* and *AMPA* receptor mRNA levels were measured and normalised to β-Actin expression in the dorsal horn of the L4-L5 spinal cord segment using real-time RT-PCR ($n = 4$ mice per group). (b) Representative Western blots and quantification of NMDA receptor 1 protein levels, normalised to β-Actin, in the dorsal horn of the L4-L5 spinal cord segment ($n = 5$ mice per group). The groups included control and GLS1 cKO mice under naïve conditions (baseline) or 14 days after SNI. Statistical analysis was performed using two-way ANOVA followed by Sidak's post hoc test; $^*p < 0.05$ indicates significant differences between baseline and neuropathy.

isoenzyme, to determine whether it could be also regulated after nerve injury or counterbalance for the loss of GLS1 in cKO mice. No significant changes in GLS2 levels were detected between any condition (1.10 ± 0.10 and 1.30 ± 0.30 for naïve and SNI control mice, and 1.00 ± 0.10 and 1.20 ± 0.30 for naïve and SNI CKO mice; $n = 4$ mice per group; two-way ANOVA followed by Sidak's post hoc test, $p \geq 0.05$). Together, these data suggest that GLS1 protein isoform is selectively upregulated in putative nociceptors following nerve injury, and that it's enhanced expression in this neuronal population is critical for the development of mechanical allodynia.

3.4 | Glutamatergic Signalling in the Spinal Cord

Nociceptive afferents fibres synapse with second-order neurons in the dorsal horn of the spinal cord using glutamate as the primary excitatory neurotransmitter. Therefore, we reasoned that changes observed in GLS1 expression at peripheral level might influence central pain-processing mechanisms related to glutamatergic signalling. To investigate this, we first performed Western blot analysis to assess GLS1 expression in the dorsal horn of L4-L5 spinal cord segment. Results showed that SNI did not affect GLS1 expression in either control littermates or GLS1 cKO mice (Figure 4b; 1.05 ± 0.04 and 1.28 ± 0.05 for naïve and SNI control mice, and 1.15 ± 0.04 and 1.35 ± 0.15 for naïve and SNI cKO mice; $n = 4$ and 3 mice per group; two-way ANOVA followed by Sidak's post hoc test, $p \geq 0.05$). This result strongly suggests that modulation of GLS1 occurs exclusively at peripheral level following nerve injury. Next, we evaluated the expression of glutamate receptors in the dorsal horn of control and cKO mice, both at baseline and 14 days after SNI. mRNA levels for *AMPA*, *mGlu1* and *mGlu5* receptors remained unchanged across groups (Figure 5a; $n = 4$ mice per group; two-way ANOVA followed by Sidak's post hoc test, $p \geq 0.05$). Protein levels of NMDA receptor were comparable between naïve control and GLS1 cKO mice. However, 14 days after SNI, NMDA protein levels decreased in both groups, although this reduction only reached statistical significance in control mice (Figure 5b; 0.78 ± 0.08 and 0.37 ± 0.06 for naïve and SNI control mice, and 0.70 ± 0.13 and 0.45 ± 0.08 for naïve and SNI cKO mice $n = 5$ mice per group; two-way ANOVA followed by Sidak's post hoc test, $p < 0.05$).

4 | Discussion and Conclusions

GLS1 is broadly expressed in both neuronal and non-neuronal tissues and, in addition to its critical role in glutamate generation, may be involved in other diverse functions (Márquez et al. 2006). Considering also that GLS1 null mutants die shortly after birth (Masson et al. 2006), we propose nociceptor-specific *Gls1* gene deletion as a useful approach to defining its role in pain pathways. Here, we generated a new transgenic mouse with a specific deletion of the *Gls1* gene in sensory neurons expressing Nav1.8. Specifically, GLS1$^{lox/lox}$ mice (Mingote et al. 2016) were crossed with mice expressing Cre recombinase under the control of the Nav1.8 promoter (Stirling et al. 2005) (Figure 1a). Nav1.8 is a voltage-gated sodium channel expressed in a subset of sensory neurons, over 85% of which are nociceptors (Djouhri et al. 2003; Stirling et al. 2005). Therefore, the Nav1.8-Cre mouse line is a valuable tool for analysing the effects of deleting floxed genes on pain behaviour and has, in fact, been successfully used for this purpose (Huang et al. 2017; Nassar et al. 2004, 2006). To the best of our knowledge, this is the first study specifically targeting GLS1 expression in nociceptors. Deletion of the *Gls1* gene within the DRG of GLS1 cKO mice was confirmed by the amplification of the Δ allele from genomic DNA (Figure 1b,c; Mingote et al. 2016), while the reduction in protein levels was validated through Western blotting and immunofluorescence. Consistent with previous reports, we find that in the DRG from control mice, GLS1 protein was present in sensory neurons of all sizes, with the highest levels observed in small- to medium-sized neurons (Cangro et al. 1985; Hoffman et al. 2010; Miller et al. 1993) (Figure 3a,c). However, in GLS1 cKO mice, immunofluorescence was abolished in small- to medium-sized neurons, in line with the expression pattern of Nav1.8 (Djouhri et al. 2003), while low levels were maintained in large-sized neurons (Figure 3b,d). Accordingly, GLS1 levels also decreased threefold in DRG protein extracts. Consequently, GLS1 cKO mice lose GLS1 activity primarily in nociceptors.

GLS1 cKO mice displayed a paw withdrawal threshold to mechanical stimulation similar to that of control littermates (Figure 2). This indicates that the glutamatergic circuitry mediating the nociceptive withdrawal reflex at the spinal level is not dependent on GLS1 activity. The role of glutamate in pain transmission at spinal level is crucial (Dickenson 1995), suggesting that GLS1-deficient nociceptors have adequate levels of glutamate and, hence, might obtain the neurotransmitter from other sources, such as the second glutaminase isoenzyme Glutaminase 2 (GLS2), other synthetic pathways, or simply by amino acid reuptake. GLS1 and GLS2 are co-expressed in many brain cells (Campos-Sandoval et al. 2015), and although mitochondrial GLS1 has been suggested as the major contributor to the glutamate pool in neurons (Hertz 2004), GLS2 may contribute to glutamate synthesis under certain conditions. This could be the case in our experimental setting, as we detected GLS2 mRNA (data not shown) and protein in the DRG of both control and GLS1 cKO mice. Additionally, sensory neurons import glutamate into their cell bodies and axons via the excitatory amino acid transporter 1 (EAAT1; Tao et al. 2004).

On the contrary, under neuropathic conditions, GLS1 deficiency affected nociceptive behaviours. Two weeks after peripheral nerve injury, control littermates developed mechanical allodynia, whilst GLS1 cKO maintained the normal paw withdrawal (Figure 2). Why does GLS1 deficiency have a different impact in physiological and neuropathic conditions? This could be explained by the findings observed in the GLS1 null mutant mice. Cultured GLS1$^{-/-}$ cortical neurons exhibited typical glutamatergic synaptic function under basal activity conditions; however, sustained synaptic stimulation led to rapid glutamate depletion. Consequently, more active glutamatergic circuits, such as those involved in respiratory function and goal-directed behaviours, were impaired in GLS1 null mutants, leading to death shortly after birth (Masson et al. 2006). Considering that SNI induces increased excitability in sensory neurons, which is associated with a sustained rise in glutamate release in the dorsal horn (Inquimbert et al. 2012), our findings suggest that GLS1 activity is not essential for the detection of acute nociceptive mechanical stimuli, which depend on basal glutamate levels. However, it becomes crucial for initiating or maintaining central sensitisation, which requires elevated and sustained levels of glutamate. On the other hand, our results may appear to contrast with those obtained from the GLS1$^{+/-}$ heterozygous mouse, which exhibits reduced responses to mechanical stimuli under physiological conditions (Kayser et al. 2015). However, it is important to note that in this mouse strain, GLS1 deficiency affects all cell types. Given that the paw withdrawal reflex involves not only nociceptor activation but also a complex network of interneurons that relay signals to motoneurons (Sandrini et al. 2005), it is plausible that the diminished mechanical sensitivity observed in GLS1$^{+/-}$ mice results from GLS1 loss throughout the entire neural network. In contrast, in our study, Gls1 gene expression is selectively disrupted in Nav1.8-expressing sensory neurons, while its expression is preserved in the rest of the nervous system and other body tissues. Therefore, it is possible that, under physiological conditions, other nociceptor populations are sufficient to elicit the reflex response, or that glutamate derived from glutaminase-independent sources within Nav1.8-expressing neurons is adequate to fulfil this role.

The role of GLS1 in the mechanisms of neuropathic pain at the peripheral level is further emphasised by its significant increase in the DRG, but not in the spinal cord, of control mice 2 weeks after SNI. In sharp contrast, GLS1 levels remained unchanged in the DRG of GLS1 cKO mice (Figure 4), suggesting that, although nerve injury affects all DRG neuron populations, GLS1 modulation specifically occurs in Nav1.8- expressing neurons. This neuronal population has been shown to play a significant role in the onset and maintenance of pain hypersensitivity. Previous studies have shown that silencing Nav1.8-positive afferents alleviates both inflammatory and neuropathic pain (Daou et al. 2016), and Nav1.8 expression is also essential for spontaneous activity in damaged sensory axons (Roza et al. 2003). Importantly, the Nav1.8 inhibitor suzetrigine has recently been approved by the FDA for managing acute post-surgical pain and are currently undergoing phase II clinical trials for neuropathic pain conditions like diabetic neuropathy (Hang Kong et al. 2024). Our results contribute to the growing evidence supporting the therapeutic potential of targeting primary nociceptors for pain management.

As far as we know, this is the first report demonstrating GLS1 modulation in sensory neurons under neuropathic conditions. However, substantial evidence shows that GLS1 protein levels

and activity increase in medium- to small-sized sensory neurons under inflammatory pain conditions, such as adjuvant-induced arthritis (Hoffman et al. 2016; Miller et al. 2012), and after surgical incision (Crosby et al. 2015). GLS1 elevation during inflammation depends on nerve growth factor (NGF) retrograde signalling (Gujar et al. 2024), a mechanism that could also operate after nerve injury, as NGF is a key component in the pathophysiology of neuropathic pain (García-Domínguez 2025).

Increased GLS1 levels may enhance glutamate production (Inagaki et al. 1987) and vesicular uptake via vesicular glutamate transporters (VGLUTs). The abundant expression of VGLUT2 in the DRG, along with its active transport to both central and peripheral axons, suggests its crucial role in glutamate release at these terminals (Brumovsky 2013; Brumovsky et al. 2007) and, consequently, in the sensitisation that occurs following nerve injury. Chronic pain may be influenced by the interaction between VGLUT2 and GLS1, since both are upregulated in DRG neurons and their projections within the sciatic nerve after adjuvant-induced arthritis (Malet and Brumovsky 2015). These changes could explain the observed increases in peripheral glutamate release in this scenario (Lawand et al. 2000).

Finally, the levels of *mGlu1*, *mGlu5*, *AMPA* and NMDA receptor subunit 1 were comparable between control and GLS1 cKO mice under naïve and neuropathic conditions (Figure 5). Thus, GLS1 deficiency did not affect the expression of metabotropic and ionotropic receptors in the dorsal horn of the spinal cord.

Unexpectedly, we did not detect modulation of *mGlu1*, *mGlu5* and *AMPA* receptors in control SNI mice. Previous studies have shown an increase in these receptors under inflammatory and neuropathic conditions. It should be noted that the neuropathic models used differed from SNI, including spinal cord injury, sciatic nerve ligation or complete transection, and diabetic neuropathy (Bleakman et al. 2006). Thus, a more detailed analysis of receptor expression following partial peripheral nerve injury is required. On the other hand, NMDA receptor 1 (NR1) decreased after SNI. This result is supported by previous reports showing a reduction of NR1 after chronic constriction injury (Garry et al. 2003; Wilson et al. 2005). NR2B increased, while NR2A remained unaltered in this context. Therefore, NMDA receptor subunits are important in the cellular mechanisms underlying neuropathic sensitisation, play an important role in the cellular mechanisms underlying neuropathic sensitisation, but the specific role of each subunit requires further clarification.

The main limitation of this study is that behavioural experiments were conducted exclusively in female mice. The literature provides strong evidence that females are more sensitive to pressure-induced pain and suggests that the mechanism underlying neuropathic chronic pain differs between sexes (DeLeo and Rutkowski 2000; Fillingim et al. 2009; Jeon et al. 2021; LaCroix-Fralish et al. 2006; Tall et al. 2001). Consequently, the antiallodynic effect of GLS1 deletion observed in females may not be replicated to the same extent in male mice and should be investigated in male cohorts.

In conclusion, neuropathic pain following peripheral nerve injury arises from the interplay between peripheral and central mechanisms. We propose that after nerve transection, nociceptors upregulate GLS1 expression, which is necessary and sufficient for sustained glutamate release into the dorsal horn of the spinal cord. This process leads to sensitisation and the development of mechanical allodynia. Therefore, reversing GLS1 upregulation or inhibiting GLS1 activity at the peripheral level may offer a promising analgesic strategy for neuropathic pain relief.

Author Contributions

Anabel Martínez-Padilla, Carolina Roza and **Elsa Cisneros:** conceptualisation, data curation, investigation, methodology, formal analysis, visualisation, review and editing. **Javier Márquez:** funding, review and editing. **Miguel Á. Huerta:** visualisation, review and editing. **Carolina Roza** and **Elsa Cisneros:** writing original draft. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Acknowledgements

This research was supported by grants from Universidad de Alcalá (CCG19/CCS-013) and Junta de Andalucía (UMA18-FEDERJA-082). During the preparation of this work, the authors used ChatGPT to enhance the clarity and quality of the writing. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

Conflicts of Interest

The authors declare no conflicts of interest.

References

Bleakman, D., A. Alt, and E. S. Nisenbaum. 2006. "Glutamate Receptors and Pain." *Seminars in Cell & Developmental Biology* 17: 592–604.

Bonin, R. P., C. Bories, and Y. D. Koninck. 2014. "A Simplified Up-Down Method (SUDO) for Measuring Mechanical Nociception in Rodents Using Von Frey Filaments." *Molecular Pain* 10: 26.

Brumovsky, P., M. Watanabe, and T. Hökfelt. 2007. "Expression of the Vesicular Glutamate Transporters-1 and -2 in Adult Mouse Dorsal Root Ganglia and Spinal Cord and Their Regulation by Nerve Injury." *Neuroscience* 147: 469–490.

Brumovsky, P. R. 2013. "VGLUTs in Peripheral Neurons and the Spinal Cord: Time for a Review." *International Scholarly Research Notices* 2013: 829753.

Campos-Sandoval, J. A., M. Martín-Rufián, C. Cardona, C. Lobo, A. Peñalver, and J. Márquez. 2015. "Glutaminases in Brain: Multiple Isoforms for Many Purposes." *Neurochemistry International* 88: 1–5.

Cangro, C. B., P. M. Sweetnam, J. R. Wrathall, W. B. Haser, N. P. Curthoys, and J. H. Neale. 1985. "Localization of Elevated Glutaminase Immunoreactivity in Small DRG Neurons." *Brain Research* 336: 158–161.

Crosby, H. A., M. Ihnat, D. Spencer, and K. E. Miller. 2015. "Expression of Glutaminase and Vesicular Glutamate Transporter Type 2 Immunoreactivity in Rat Sacral Dorsal Root Ganglia Following a Surgical Tail Incision." *Pharmacy & Pharmacology International Journal* 2: 00023.

Daou, I., H. Beaudry, A. R. Ase, et al. 2016. "Optogenetic Silencing of Nav1.8-Positive Afferents Alleviates Inflammatory and Neuropathic Pain." *ENeuro* 3: 1–12.

DeLeo, J. A., and M. D. Rutkowski. 2000. "Gender Differences in Rat Neuropathic Pain Sensitivity Is Dependent on Strain." *Neuroscience Letters* 282: 197–199.

Dickenson, A. H. 1995. "Spinal Cord Pharmacology of Pain." *British Journal of Anaesthesia* 75: 193–200.

Djouhri, L., X. Fang, K. Okuse, J. N. Wood, C. M. Berry, and S. N. Lawson. 2003. "The TTX-Resistant Sodium Channel Nav1.8 (SNS/PN3): Expression and Correlation With Membrane Properties in Rat Nociceptive Primary Afferent Neurons." *Journal of Physiology* 550: 739–752.

Fillingim, R. B., C. D. King, M. C. Ribeiro-Dasilva, B. Rahim-Williams, and J. L. Riley. 2009. "Sex, Gender, and Pain: A Review of Recent Clinical and Experimental Findings." *Journal of Pain* 10: 447–485.

García-Domínguez, M. 2025. "NGF in Neuropathic Pain: Understanding Its Role and Therapeutic Opportunities." *Current Issues in Molecular Biology* 47: 93.

Garry, E. M., A. Moss, A. Delaney, et al. 2003. "Neuropathic Sensitization of Behavioral Reflexes and Spinal NMDA Receptor/CaM Kinase II Interactions Are Disrupted in PSD-95 Mutant Mice." *Current Biology* 13: 321–328.

Gujar, V., R. D. Pande, B. M. Hardas, and S. Das. 2024. "Nerve Growth Factor Signaling Modulates the Expression of Glutaminase in Dorsal Root Ganglion Neurons During Peripheral Inflammation." *International Journal of Molecular Sciences* 25: 6053.

Hang Kong, A. Y., H. S. Tan, and A. S. Habib. 2024. "VX-548 in the Treatment of Acute Pain." *Pain Management* 14: 477–486.

Harrison, M., A. O'Brien, L. Adams, et al. 2013. "Vertebral Landmarks for the Identification of Spinal Cord Segments in the Mouse." *NeuroImage* 68: 22–29.

Hertz, L. 2004. "Intercellular Metabolic Compartmentation in the Brain: Past, Present and Future." *Neurochemistry International* 45: 285–296.

Hoffman, E. M., R. Schechter, and K. E. Miller. 2010. "Fixative Composition Alters Distributions of Immunoreactivity for Glutaminase and Two Markers of Nociceptive Neurons, Nav1.8 and TRPV1, in the Rat Dorsal Root Ganglion." *Journal of Histochemistry and Cytochemistry* 58: 329–344.

Hoffman, E. M., Z. Zhang, R. Schechter, and K. E. Miller. 2016. "Glutaminase Increases in Rat Dorsal Root Ganglion Neurons After Unilateral Adjuvant-Induced Hind Paw Inflammation." *Biomolecules* 6: 10.

Huang, C., F. Lu, P. Li, C. Cao, and Z. Liu. 2017. "Tlx3 Function in the Dorsal Root Ganglion Is Pivotal to Itch and Pain Sensations." *Frontiers in Molecular Neuroscience* 10: 205.

Inagaki, N., Y. Kamisaki, H. Kiyama, Y. Horio, M. Tohyama, and H. Wada. 1987. "Immunocytochemical Localizations of Cytosolic and Mitochondrial Glutamic Oxaloacetic Transaminase Isozymes in Rat Primary Sensory Neurons as a Marker for the Glutamate Neuronal System." *Brain Research* 402: 197–200.

Inquimbert, P., K. Bartels, O. B. Babaniyi, L. B. Barrett, I. Tegeder, and J. Scholz. 2012. "Peripheral Nerve Injury Produces a Sustained Shift in the Balance Between Glutamate Release and Uptake in the Dorsal Horn of the Spinal Cord." *Pain* 153: 2422–2431.

Jeon, S.-M., D. Chang, A. Geske, D. D. Ginty, and M. J. Caterina. 2021. "Sex-Dependent Reduction in Mechanical Allodynia in the Sural-Sparing Nerve Injury Model in Mice Lacking Merkel Cells." *Journal of Neuroscience* 41: 5595–5619.

Kayser, V., F. Viguier, M. Melfort, S. Bourgoin, M. Hamon, and J. Masson. 2015. "Altered Nociception in Mice With Genetically Induced Hypoglutamatergic Tone." *Neuroscience* 293: 80–91.

Kvamme, E., I. A. Torgner, and B. Roberg. 2001. "Kinetics and Localization of Brain Phosphate Activated Glutaminase." *Journal of Neuroscience Research* 66: 951–958.

LaCroix-Fralish, M. L., V. L. Tawfik, N. Nutile-McMenemy, and J. A. DeLeo. 2006. "Progesterone Mediates Gonadal Hormone Differences in Tactile and Thermal Hypersensitivity Following L5 Nerve Root Ligation in Female Rats." *Neuroscience* 138: 601–608.

Lawand, N. B., T. McNearney, and K. N. Westlund. 2000. "Amino Acid Release Into the Knee Joint: Key Role in Nociception and Inflammation." *Pain* 86: 69–74.

Malet, M., and P. R. Brumovsky. 2015. "VGLUTs and Glutamate Synthesis—Focus on DRG Neurons and Pain." *Biomolecules* 5: 3416–3437.

Márquez, J., C. Cardona, J. A. Campos-Sandoval, et al. 2013. "Mammalian Glutaminase Isozymes in Brain." *Metabolic Brain Disease* 28: 133–137.

Márquez, J., A. R. López de la Oliva, J. M. Matés, J. A. Segura, and F. J. Alonso. 2006. "Glutaminase: A Multifaceted Protein Not Only Involved in Generating Glutamate." *Neurochemistry International* 48: 465–471.

Masson, J., M. Darmon, A. Conjard, et al. 2006. "Mice Lacking Brain/Kidney Phosphate-Activated Glutaminase Have Impaired Glutamatergic Synaptic Transmission, Altered Breathing, Disorganized Goal-Directed Behavior and Die Shortly After Birth." *Journal of Neuroscience* 26: 4660–4671.

Miller, K. E., J. C. Balbás, R. L. Benton, et al. 2012. "Glutaminase Immunoreactivity and Enzyme Activity Is Increased in the Rat Dorsal Root Ganglion Following Peripheral Inflammation." *Pain Research and Treatment* 2012: 414697.

Miller, K. E., V. D. Douglas, and T. Kaneko. 1993. "Glutaminase Immunoreactive Neurons in the Rat Dorsal Root Ganglion Contain Calcitonin Gene-Related Peptide (CGRP)." *Neuroscience Letters* 160: 113–116.

Miller, K. E., E. M. Hoffman, M. Sutharshan, and R. Schechter. 2011. "Glutamate Pharmacology and Metabolism in Peripheral Primary Afferents: Physiological and Pathophysiological Mechanisms." *Pharmacology & Therapeutics* 130: 283–309.

Mingote, S., J. Masson, C. Gellman, et al. 2016. "Genetic Pharmacotherapy as an Early CNS Drug Development Strategy: Testing Glutaminase Inhibition for Schizophrenia Treatment in Adult Mice." *Frontiers in Systems Neuroscience* 9: 165.

Nassar, M. A., M. D. Baker, A. Levato, et al. 2006. "Nerve Injury Induces Robust Allodynia and Ectopic Discharges in Nav1.3 Null Mutant Mice." *Molecular Pain* 2: 33.

Nassar, M. A., L. C. Stirling, G. Forlani, et al. 2004. "Nociceptor-Specific Gene Deletion Reveals a Major Role for Nav1.7 (PN1) in Acute and Inflammatory Pain." *Proceedings of the National Academy of Sciences of the United States of America* 101: 12706.

Roza, C., J. M. A. Laird, V. Souslova, J. N. Wood, and F. Cervero. 2003. "The Tetrodotoxin-Resistant Na+ Channel Nav1.8 Is Essential for the Expression of Spontaneous Activity in Damaged Sensory Axons of Mice." *Journal of Physiology* 550: 921–926.

Sandrini, G., M. Serrao, P. Rossi, A. Romaniello, G. Cruccu, and J. C. Willer. 2005. "The Lower Limb Flexion Reflex in Humans." *Progress in Neurobiology* 77: 353–395.

Schindelin, J., I. Arganda-Carreras, E. Frise, et al. 2012. "Fiji: An Open-Source Platform for Biological-Image Analysis." *Nature Methods* 9: 676–682.

Shields, S. D., W. A. Eckert, and A. I. Basbaum. 2003. "Spared Nerve Injury Model of Neuropathic Pain in the Mouse: A Behavioral and Anatomic Analysis." *Journal of Pain* 4: 465–470.

Stirling, L. C., G. Forlani, M. D. Baker, et al. 2005. "Nociceptor-Specific Gene Deletion Using Heterozygous NaV1.8-Cre Recombinase Mice." *Pain* 113: 27–36.

Tall, J. M., S. L. Stuesse, W. L. R. Cruce, and T. Crisp. 2001. "Gender and the Behavioral Manifestations of Neuropathic Pain." *Pharmacology Biochemistry and Behavior* 68: 99–104.

Tao, F., W.-J. Liaw, B. Zhang, et al. 2004. "Evidence of Neuronal Excitatory Amino Acid Carrier 1 Expression in Rat Dorsal Root Ganglion Neurons and Their Central Terminals." *Neuroscience* 123: 1045–1051.

Temmermand, R., J. E. Barrett, and A. C. K. Fontana. 2022. "Glutamatergic Systems in Neuropathic Pain and Emerging Non-Opioid Therapies." *Pharmacological Research* 185: 106492.

Wilson, J. A., E. M. Garry, H. A. Anderson, et al. 2005. "NMDA Receptor Antagonist Treatment at the Time of Nerve Injury Prevents Injury-Induced Changes in Spinal NR1 and NR2B Subunit Expression and Increases the Sensitivity of Residual Pain Behaviours to Subsequently Administered NMDA Receptor Antagonists." *Pain* 117: 421–432.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.