

ORIGINAL ARTICLE

The voltage-gated sodium channel Na_v1.7 underlies endometriosis-associated chronic pelvic pain

Joel Castro^{1,2}  | Jessica Maddern^{1,2} | Chun Yuen Chow^{3,4}  | Poanna Tran³  |
Irina Vetter^{3,5} | Glenn F. King^{3,4} | Stuart M. Brierley^{1,2,6}

¹Visceral Pain Research Group, College of Medicine and Public Health, Flinders Health and Medical Research Institute (FHMRI), Flinders University, Bedford Park, South Australia, Australia

²Hopwood Centre for Neurobiology, Lifelong Health Theme, South Australian Health and Medical Research Institute (SAHMRI), Adelaide, South Australia, Australia

³Institute for Molecular Bioscience, The University of Queensland, St Lucia, Queensland, Australia

⁴Australian Research Council Centre of Excellence for Innovations in Peptide and Protein Science, The University of Queensland, St Lucia, Queensland, Australia

⁵School of Pharmacy, The University of Queensland, Brisbane, Australia

⁶Discipline of Medicine, University of Adelaide, Adelaide, South Australia, Australia

Correspondence

Joel Castro, Visceral Pain Research Group, Level 7, South Australian Health and Medical Research Institute (SAHMRI), North Terrace, Adelaide, SA 5000, Australia.
Email: joel.castro@sahmri.com

Funding information

Australia Research Council, Grant/Award Number: Centre of Excellence grant CE200100012 and Discovery Project DP220101269; Hospital Research Foundation, Grant/Award Number: PhD Scholarship SAPHD000242018; National Health and Medical Research Council, Grant/Award Number: Career Development Fellowship APP1162503, Development Grant APP2014250, Ideas Grant APP1181448, Investigator Leadership Grant APP2008727 and Principal Research Fellowship APP1136889

Abstract

Chronic pelvic pain (CPP) is the primary symptom of endometriosis patients, but adequate treatments are lacking. Modulation of ion channels expressed by sensory nerves innervating the viscera has shown promise for the treatment of irritable bowel syndrome and overactive bladder. However, similar approaches for endometriosis-associated CPP remain underdeveloped. Here, we examined the role of the voltage-gated sodium (Na_v) channel Na_v1.7 in (i) the sensitivity of vagina-innervating sensory afferents and investigated whether (ii) Na_v1.7 inhibition reduces nociceptive signals from the vagina and (iii) ameliorates endometriosis-associated CPP. The mechanical responsiveness of vagina-innervating sensory afferents was assessed with ex vivo single-unit recording preparations. Pain evoked by vaginal distension (VD) was quantified by the visceromotor response (VMR) in vivo. In control mice, pharmacological activation of Na_v1.7 with OD1 sensitised vagina-innervating pelvic afferents to mechanical stimuli. Using a syngeneic mouse model of endometriosis, we established that endometriosis sensitised vagina-innervating pelvic afferents to mechanical stimuli. The highly selective Na_v1.7 inhibitor Tsp1a revealed that this afferent hypersensitivity occurred in a Na_v1.7-dependent manner. Moreover, in vivo intra-vaginal treatment with Tsp1a reduced the exaggerated VMRs to VD

Abbreviations: ANOVA, analysis of variance; AP, action potential; AUC, area under the curve; CNS, central nervous system; CPP, chronic pelvic pain; DRG, dorsal root ganglia; EMG, electromyography; GEE, generalised estimating equations; GPCR, G-protein coupled receptor; IBS, irritable bowel syndrome; IGF, insulin-like growth factor; IVC, individually ventilated cages; LSD, least significant difference; Kv, voltage-gated potassium channel; Na_v, voltage gated sodium channel; OAB, overactive bladder syndrome; RRID, research Resource Identifier; SPPS, solid-phase peptide synthesis; SEM, standard error of the mean; TRPV, transient receptor potential vanilloid; TTX, tetrodotoxin; VD, vaginal distension; VFH, von Frey hair; VMR, visceromotor response.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2023 The Authors. *Journal of Neurochemistry* published by John Wiley & Sons Ltd on behalf of International Society for Neurochemistry.



which is characteristic of mice with endometriosis. Conversely, Tsp1a did not alter ex vivo afferent mechanosensitivity nor in vivo VMRs to VD in Sham control mice. Collectively, these findings suggest that Na_v1.7 plays a crucial role in endometriosis-induced vaginal hyperalgesia. Importantly, Na_v1.7 inhibition selectively alleviated endometriosis-associated CPP without the loss of normal sensation, suggesting that selective targeting of Na_v1.7 could improve the quality of life of women with endometriosis.

KEYWORDS

chronic pelvic pain, endometriosis, mechanosensitivity, vagina-innervating afferents, visceromotor response, voltage-gated sodium channels

1 | INTRODUCTION

Endometriosis is a debilitating condition characterised by infertility and chronic pelvic pain (CPP) that affects approximately 11% of women worldwide (Becker et al., 2020; Eisenberg et al., 2018). Vaginal hyperalgesia, also known as dyspareunia, or painful intercourse, is one of the most debilitating symptoms affecting women with endometriosis. Current treatments aimed at relieving the CPP associated with endometriosis include hormonal suppression of ovarian function or surgery. Unfortunately, both interventions have variable success and negatively impact fertility (Zito et al., 2014), reflecting a clear need for alternative treatment options.

Physiological and painful stimuli are detected and transmitted by ion channels and receptors expressed within the terminals of sensory afferent nerve fibres projecting from the periphery to the central nervous system (CNS). Modulation of ion channels expressed within sensory neurons innervating the colon and the bladder has proved to be a promising approach for the treatment of chronic pain associated with irritable bowel syndrome (IBS) and overactive bladder syndrome (Broad et al., 2009; Grundy, Erickson, et al., 2018; Israel et al., 2019; Jiang et al., 2021; Michel & Igawa, 2015; Sadeghi et al., 2018; Salvatierra et al., 2018). A similar approach directed to relieve endometriosis-associated CPP remains underdeveloped. This is in part because of the lack of knowledge regarding the specific ion channels and receptors expressed within sensory neurons projecting to the female reproductive tract in healthy states. To date, the transient receptor potential vanilloid 1 channel TRPV1 (Chaban, 2008), purinergic receptor P2X₃ (Wang et al., 2015), voltage-gated sodium (Na_v) channels (Erickson et al., 2018; Lee et al., 2020), and the voltage-gated potassium channels (K_v) K_v6.4 and K_v2.1 (Lee et al., 2020) have been reported to be expressed in these sensory afferents. Whilst tetrodotoxin (TTX)-sensitive Na_v channels have a functional role in pain sensation from the female reproductive tract of healthy mice (Castro, Maddern, Erickson, et al., 2021), the specific identity of Na_v channel(s) contributing to this pain signalling pathway, and whether they contribute to endometriosis-associated CPP, remains unknown.

The Na_v channel family consists of nine isoforms (Na_v1.1–Na_v1.9) that are characterised as either TTX-sensitive

(Na_v1.1–Na_v1.4, Na_v1.6, and Na_v1.7) or TTX-resistant (Na_v1.5, Na_v1.8, and Na_v1.9) (Catterall, 2012; Catterall et al., 2005). Na_v1.7 is a therapeutic target of interest as loss-of-function mutations in the gene encoding Na_v1.7 (*SCN9A*) lead to a congenital inability to sense pain, whilst gain-of-function mutations lead to increased pain perception (Bennett & Woods, 2014; King & Vetter, 2014). Some Na_v1.7 inhibitors have been tested in clinical trials for various types of pain (Alexandrou et al., 2016; Bagal et al., 2014; Goldberg et al., 2012; Kotecha et al., 2020; Zakrzewska et al., 2017). Despite the abundant expression of Na_v1.7 within visceral-innervating neurons (98–100% expression in colon-, bladder-, and vagina-innervating lumbosacral DRG neurons [Castro, Maddern, Erickson, et al., 2021; Grundy, Erickson, et al., 2018; Insera et al., 2017]), the contribution of Na_v1.7 to visceral sensation and pain is controversial (Erickson et al., 2018). Some studies suggest that Na_v1.7 is involved in somatic, but not visceral, pathways in acute pain (Hockley et al., 2017), whilst others have shown that it plays an important role in the chronic abdominal pain models of IBS (Jiang et al., 2021).

In this study, we used a highly selective inhibitor of Na_v1.7 (Jiang et al., 2021) to investigate the role of this channel in vaginal sensation and evoked pain. Additionally, we examined whether Na_v1.7 contributes to the allodynia and hyperalgesia experienced by mice with endometriosis. We show that Na_v1.7 contributes to endometriosis-associated vaginal hyperalgesia, which can be ameliorated by treatment with a Na_v1.7 inhibitor. Our data suggest that inhibition of Na_v1.7 may be a viable option to treat endometriosis-associated CPP.

2 | MATERIALS AND METHODS

2.1 | Animals

All experiments involving animals were approved by the Animal Ethics Committee of the South Australian Health and Medical Research Institute (SAHMRI; ethics number SAM342) and conformed to the relevant regulatory standards and ARRIVE guidelines. Female C57BL/6J mice at 8–13 weeks of age were used and acquired

from an in-house C57BL/6J breeding program (JAX strain #000664 (RRID:IMSR_JAX:000664); originally purchased from The Jackson Laboratory (breeding barn MP14; Bar Harbor)) within SAHMRI's specific and opportunistic pathogen-free animal care facility. Mice were group-housed (maximum five mice per cage) within individually ventilated cages (IVC), which were filled with coarse chip dust-free aspen bedding (PuraChips Aspen coarse 63L; Cat# ASPJMAEB-CA, Able Scientific). These cages were stored on ventilated IVC racks in specific housing rooms within a temperature-controlled environment of 22°C and a 12h light/12h dark cycle. Mice had free access to LabDiet® JL Rat and Mouse/Auto6F chow (Cat# 5 K52, Speciality Feeds, Australia) and autoclaved reverse osmosis purified water. Female mice were group housed in IVC cages and the littermate male mice were separated at weaning. All female mice use in this study were unmated virgins.

2.2 | Study design

The animals used in this study were arbitrarily assigned to each group or treatment. Blinding was achieved by each experimenter only knowing the animal's ID (blinded to the treatment group), or by data analysis being performed by an independent researcher. The sample size was determined using power calculations based on previously published studies (Castro et al., 2022; Castro, Maddern, Grundy, et al., 2021; Jiang et al., 2021), using a power value of 0.80 and an alpha error probability of 0.05.

A total of 75 female mice were used in this study, with 30 mice allocated to the 'Sham' group, 30 mice allocated to the 'Endo' group and 15 mice used for donor tissue collection to generate the Endo model. Exclusion criteria included mice that were not able to complete all data points in experimental protocols because of technical issues. For this study, the final number of animals used for data collection was 59 mice, including 30 Sham and 29 Endo mice. One Endo mouse was excluded because of wire displacement during behavioural studies causing an incomplete VMR recording set and was not replaced. The total number of mice used

in each individual data set is specified on each figure legend (identified as N).

2.3 | Study timeline

Endometriosis (or Sham) was surgically induced in ovariectomised mice at 7 weeks of age and allowed to develop for 8–10 weeks before mice were used for either ex vivo afferent recordings from the pelvic nerve or in vivo assessment of vaginal pain sensitivity to vaginal distension (VD). A schematic representation demonstrating the timeline for surgical interventions and the timing of ex vivo and in vivo experimental methods is shown in Figure 1.

2.4 | Pharmacological modulators

The α -scorpion toxin OD1, a selective agonist of $\text{Na}_v1.7$, was produced via solid-phase peptide synthesis (SPPS) as previously described (Durek et al., 2013). The spider-venom peptide Tsp1a, a selective inhibitor of $\text{Na}_v1.7$, was also produced via SPPS as previously described (Jiang et al., 2021).

The concentration of OD1 used in this study was 100nM, which has been shown to selectively activate $\text{Na}_v1.7$ channels over other Na_v channel isoforms (Maertens et al., 2006). As previously described, OD1 potently activates $\text{Na}_v1.7$ ($\text{EC}_{50} = 4.5$ nM, exerting its effect by impairing the fast inactivation of the channel). OD1 affects $\text{Na}_v1.3$ and $\text{Na}_v1.8$ at concentrations higher than 1–2 μM (Maertens et al., 2006), and only weakly affects $\text{Na}_v1.5$ and $\text{Na}_v1.6$ ($\text{EC}_{50} > 1$ μM), with no demonstrated effect on $\text{Na}_v1.2$ (Durek et al., 2013). OD1 can activate $\text{Na}_v1.4$ in the nanomolar range (Durek et al., 2013), but $\text{Na}_v1.4$ channels are poorly expressed in DRGs sensory neurons so we can rule out any off-target effect on $\text{Na}_v1.4$ (Castro, Maddern, Erickson, et al., 2021).

The concentration of Tsp1a used in this study was 200nM, which has been shown to selectively inhibit $\text{Na}_v1.7$ channels over other Na_v channel isoforms (Jiang et al., 2021). As previously described,

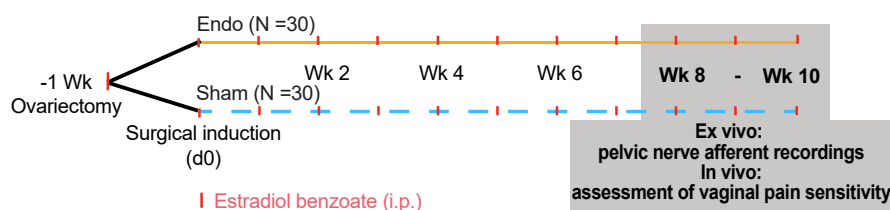


FIGURE 1 Schematic diagram showing the timeline for surgical interventions and experimental procedures performed in this study. Schematic representation of timelines for both Sham and Endo induction procedures. Mice were ovariectomised 1 week (–1 Wk) prior to either Sham (N = 30) or Endo (N = 30) induction surgery at day 0 (d0). All mice were injected weekly with estradiol benzoate (i.p., red marks) to maintain steady levels of circulating oestrogen. Ex vivo pelvic nerve afferent recordings and in vivo assessment of vaginal pain sensitivity to vaginal distension experiments were performed between Week 8 and Week 10 of model development. The final number of mice used for data collection in this study was 59 mice, including 30 Sham and 29 Endo mice. One Endo mouse was excluded because of wire displacement during behavioural studies causing an incomplete VMR recording set and was not replaced. The number of mice used for each experiment can be found within individual data sets, represented by N.

Tsp1a potently inhibits $\text{Na}_v1.7$ ($\text{IC}_{50} = 10 \text{ nM}$) with 100-fold selectivity over $\text{Na}_v1.3$ – $\text{Na}_v1.6$ and $\text{Na}_v1.8$, and 24-fold selectivity over $\text{Na}_v1.2$. Therefore, we can rule out action on $\text{Na}_v1.2$ – $\text{Na}_v1.6$ and $\text{Na}_v1.8$. $\text{Na}_v1.1$ remains a potential off-target Na_v channel, however, Tsp1a has 45-fold selectivity towards $\text{Na}_v1.7$ over $\text{Na}_v1.1$ (IC_{50} on $\text{Na}_v1.1} = 452 \text{ nM}$). Therefore, Tsp1a will have minimal effect on $\text{Na}_v1.1$ at the concentration used in this study (200 nM) (Jiang et al., 2021).

When dosed intravenously, Tsp1a is well tolerated in vivo, with no adverse side effects reported (Jiang et al., 2021). We have previously shown that mice treated intravenously with Tsp1a maintained normal oxygen saturation levels, heart rate, core temperature, and electrocardiogram recordings (Jiang et al., 2021).

2.5 | Syngeneic inoculation mouse model of endometriosis

In this study, we used a syngeneic mouse model of endometriosis, previously established and characterised by our group (Maddern et al., 2022). Briefly, female mice were ovariectomised under isoflurane (initially induced at 5%/0.5 L O_2 and maintained at 2.5%/0.5 L O_2) and received, prophylactic antibiotics (Baytril® 5 mg/kg s.c.), a low dose (0.05 mg/kg) of analgesic buprenorphine, and oestrogen (100 µg/kg β -estradiol 3-benzoate, Sigma Cat# E515-200MG) intraperitoneally (i.p.). Following ovariectomy recovery (minimum of 5 days), endometriosis (Endo) or Sham inductions were performed as previously described (Maddern et al., 2022). For this, recipient mice were anaesthetised under isoflurane (initially induced at 5%/0.5 L O_2 and maintained at 2.5%/0.5 L O_2), and a small (0.5 cm) incision was made into the peritoneal space just below the umbilicus. A 250 µL of minced endometrial tissue suspension (PBS plus penicillin: 100 U/mL and streptomycin: 100 µg/mL) obtained from donor mice (humanely culled via cervical dislocation immediately prior to tissue collection) was then inoculated into the peritoneal cavity using a 1-ml pipette, as described previously (Maddern et al., 2022). A ratio of 1 donor mouse per 2 recipient mice (Endo) was used. The incision was then closed using 6.0 Prolene sutures, and a gentle massaging of the abdominal cavity was performed to help disperse the inoculated endometrial fragments. Sham surgeries were performed by inoculating 250 µL of sterile PBS plus penicillin (100 U/mL) and streptomycin (100 µg/mL) in the absence of any tissue. All recipient mice received a low dose (0.05 mg/kg) of analgesic buprenorphine prior to the commencement of surgery.

Throughout the surgery and during the recovery period, animals were kept on a heating pad to maintain body temperature and monitored daily (for 5 consecutive days) for post-surgical complications. To maintain steady levels of circulating oestrogen and minimise any difference related to the stage of the oestrous cycle, both Endo mice and Sham control mice were given i.p. injections of oestrogen (100 µg/kg estradiol benzoate) immediately after surgery. All mice continued to receive oestrogen i.p. once a week

until full development of endometriosis (up to 8–10 weeks). Both ex vivo and in vivo recordings have performed a minimum of 24 h following i.p. oestrogen treatment to minimise the acute effects of oestrogen.

2.6 | Ex vivo afferent recording from pelvic nerves innervating the female reproductive tract

Single-unit afferents recordings from the pelvic nerve innervating the vagina of Sham control mice and mice with endometriosis were performed using an ex vivo afferent recording preparation as previously described (Castro, Maddern, Erickson, et al., 2021). Briefly, on the day of the experiment, mice were humanely culled via asphyxiation with CO_2 . The intact female reproductive tract was then removed along with the attached neurovascular bundle containing the pelvic nerve and transferred to ice-cold Krebs solution (in mM: 117.9 NaCl, 4.7 KCl, 25 NaHCO_3 , 1.3 NaH_2PO_4 , 1.2 $\text{MgSO}_4 \cdot (\text{H}_2\text{O})_7$, 2.5 CaCl_2 , 11.1 D-glucose). Tissue was opened longitudinally and pinned flat, mucosal side up, in a specialised organ bath consisting of two adjacent compartments generated from clear acrylic (Danz Instrument Service, Adelaide, South Australia, Australia), the floors of which were lined with Sylgard (Dow Corning Corp.). The neurovascular bundle containing the pelvic nerve was extended from the tissue compartment into the parafin oil filled recording compartment where they were laid onto a mirror. The organ compartment was perfused with Krebs solution and bubbled with carbogen (95% O_2 , 5% CO_2) at a temperature of 34°C. The pelvic nerve was dissected away from the neurovascular tissue and divided into 6–10 bundles. One of the bundles was placed onto a platinum recording electrode. A separate platinum reference electrode rested on the mirror in a small pool of Krebs solution adjacent to the recording electrode. Action potentials, generated by mechanical stimuli applied to the afferent's receptive field, were recorded using a differential amplifier, and filtered and sampled (20 kHz) using a 1401 interface (Cambridge Electronic Design).

2.6.1 | Mechanosensory profile of pelvic afferents innervating the vagina

Receptive fields tested in this study were limited to the vagina (above the vaginal opening and below the cervix) and were identified by systematically stroking the mucosal surface of the vagina with a stiff brush to activate all subtypes of vaginal mechanoreceptors. Mechanosensory properties of the pelvic afferents innervating a particular receptive field within the vagina were assessed by three distinct mechanical stimuli as previously described (Castro, Maddern, Erickson, et al., 2021). These included: (i) static probing with calibrated von Frey hairs (vfh; 2 g force; applied 3 times for a period of 3 s); (ii) mucosal stroking of the vaginal surface with calibrated vfh (10–1000 mg force; applied 10 times each); and (iii) circular



stretch (5g; applied for a period of 30 s). Once baseline mechanosensitivity was tested, a small chamber was then placed onto the mucosal surface of the vagina, surrounding the afferent receptive field. Residual Krebs solution within the chamber was aspirated and the Na_v channel modulators OD1 (100 nM) and Tsp1a (200 nM) were applied in separate experimental preparations for 5 min each. The afferent receptive field was then re-tested using the same three mechanical stimuli.

2.6.2 | Statistical analysis of afferent recording data

Action potentials were analysed offline using Spike 2 (version 5.21) software (RRID: SCR_000903, Cambridge Electronic Design) and discriminated as single units based on distinguishable waveforms, amplitudes, and durations. Data are expressed as mean \pm SEM. n = the number of afferents recorded, N = the number of animals used for those specific experiments. The normality of data was assessed using the Shapiro–Wilk test for normal distribution; no test for outliers was conducted. Data were statistically compared using Prism 9 software (RRID: SCR_002798, GraphPad Software). Paired data (Baseline (veh) vs. Tsp1a) were analysed using a paired Student's t test for two groups of equal variances or Wilcoxon matched-pairs signed rank test for two groups with unequal variances. Unpaired data (Sham vs. Endo) was statistically analysed using the Mann–Whitney non-parametric test for two groups with unequal variances or an unpaired Student's t test with two tails for two groups of equal and one- or two-way analysis of variance (ANOVA) with Bonferroni post-hoc tests. Differences were considered statistically significant at $p < 0.05$.

2.7 | Assessment of vaginal pain sensitivity in vivo

The visceromotor response (VMR) is a nociceptive brainstem reflex eliciting contraction of the abdominal muscles in response to noxious distension of hollow organs such as the vagina (Castro, Maddern, Erickson, et al., 2021; Castro, Maddern, Grundy, et al., 2021; Maddern et al., 2022). We quantified the VMR to VD as an objective measurement of vaginal sensitivity to pain in fully awake mice as below described and previously reported (Castro, Maddern, Erickson, et al., 2021; Castro, Maddern, Grundy, et al., 2021; Maddern et al., 2022).

2.7.1 | Surgical implantation of electromyography electrodes and VMR assessment

Electromyography (EMG) electrodes were implanted in the abdominal musculature. All mice received prophylactic antibiotics (Baytril® 5 mg/kg s.c.) and low-dose analgesic (buprenorphine 0.05 mg/kg s.c.). After surgery, animals were single housed to protect the EMG electrodes. Animals were allowed to recover from

surgery for at least 3 days before VMR assessment. On the day of the VMR assessment, animals were briefly sedated with isoflurane (initially induced at 5% / 0.5 L O_2 and maintained at 2.5% / 0.5 L O_2) and 50 mL vehicle (saline) were administered intravaginally via a small cannula inserted into the vaginal canal for baseline VMR measurements. Immediately after, a lubricated 3 mm length latex balloon was gently passed through the vagina and inserted up to 1 mm proximal to the vaginal verge and secured to the base of the tail. The balloon was then connected to a barostat (Isobar 3 Barostat, G&J Electronics) for pressure-controlled rapid inflation of air. Animals were transferred to a restrainer with dorsal access and the EMG electrodes were relayed to a data acquisition system. VDs were applied by the barostat, ranging from the non-noxious to the noxious range (20–30–40–60–70 mm Hg, 30 s duration, 3 min interval between distensions), after animals regained consciousness (~10 min drug administration and balloon insertion). The corresponding EMG signal was recorded (NL100AK headstage), amplified (NL104), filtered (NL 125/126, Neurolog, Digitimer Ltd, bandpass 50–5000 Hz), and digitised (CED 1401, Cambridge Electronic Design) to a PC for off-line analysis using Spike2 (RRID: SCR_000903, Cambridge Electronic Design). Following initial baseline measurements, mice were allowed to recover in their home cage for a minimum of 2 h. Subsequently, the same cohort of mice were treated with intravaginal Tsp1a (200 nM) and the VD protocol was repeated as above to determine the effect of $\text{Na}_v 1.7$ inhibition on baseline VMR responses. Following the final VD, all mice were humanely culled via cervical dislocation.

2.7.2 | Statistical analysis of VMR data

To quantify the magnitude of the VMR at each distension pressure, the area under the curve (AUC) during the distension (30 s) was corrected for the baseline activity (AUC pre-distension, 30 s). The normality of data was assessed using the Shapiro–Wilk test for normal distribution; no test for outliers was conducted. AUC data were statistically analysed by generalised estimating equations followed by Fisher's least significant difference (LSD) post-hoc test when appropriate using SPSS 23.0 (RRID: SCR_002865). Total AUC was quantified by adding the individual AUC at each distension pressure. Paired total AUC (baseline vehicle vs. subsequent Tsp1a treatment) was statistically analysed using a paired Student's t test for two groups of equal variances or Wilcoxon matched-pairs signed rank test for two groups with unequal variances. Unpaired data (Sham vs. Endo) was statistically analysed using the Mann–Whitney non-parametric test for two groups with unequal variances or an unpaired Student's t test with two tails for two groups of equal variances. VMR data are presented as mean \pm SEM. N represents the number of animals. Analysis and figures were prepared using Prism 9 (RRID: SCR_002798, GraphPad Software). Differences were considered statistically significant at $p < 0.05$.

3 | RESULTS

3.1 | Activation of Na_v1.7 increases vaginal afferent sensitivity to mechanical stimuli

Relatively little is known about how mechanical stimuli are sensed by female reproductive organs such as the vagina. In the current study, we investigated the specific contribution of Na_v1.7, which is expressed by 100% of vagina-innervating neurons (Castro, Maddern, Erickson, et al., 2021).

Compared to baseline responses, application of the α -scorpion toxin OD1, a selective agonist of Na_v1.7 (Durek et al., 2013), significantly increased pelvic vaginal afferent responses to stroking of the vaginal surface with calibrated von Frey hairs (vhf) (Figure 2a(i,ii); for full details, see Figure 1a(ii)), Bonferroni's multiple comparison tests, $p < 0.0001$ – 0.6384), focal compression of the receptive field with 2g vfh (Figure 2b(i,ii); $t_{(5)} = 3.791$, $p = 0.0127$, Baseline: 19.97 ± 2.473 vs. OD1: 27.80 ± 3.330 , paired t test), and circular stretch of the vaginal tissue (Figure 2c(i,ii); $t_{(5)} = 3.733$, $p = 0.0135$, Baseline: 7.165 ± 0.9399 vs. OD1: 12.26 ± 1.414 , paired t test). In addition to an increased number of action potentials (AP) generated by circular stretch, OD1 significantly reduced the latency of the response (time for the first AP to be generated) to circular stretch (Figure 2c(iii); $p = 0.0312$, Baseline: 3.500 ± 0.6708 vs. OD1: 1.533 ± 0.5426 , Wilcoxon matched-pairs signed rank test). Interestingly, more than 50% of the vaginal afferents studied continued to fire APs after cessation of the mechanical stimuli (Figure 2c(i)). Overall, these results indicate that the mechanical responsiveness of vaginal afferents can be augmented by the activation of Na_v1.7 expressed within these fibres.

3.2 | Inhibition of Na_v1.7 does not alter the mechanosensitivity of vaginal afferents, nor pain to VD in Sham control mice

We next used a Tsp1a (Jiang et al., 2021), a selective inhibitor of Na_v1.7, to determine whether this channel contributes to vaginal afferent responsiveness to mechanical stimuli, ex vivo. In Sham control mice, exposure of vaginal afferent endings to Tsp1a did not affect vaginal afferent responses to stroking of the vaginal surface (Figure 3a(i,ii); for full details, see Figure 2a(ii)), Bonferroni's multiple comparison tests, $p = 0.4156$ – >0.9999), focal compression (Figure 3b(i,ii); $t_{(12)} = 0.5769$, $p = 0.5747$, Baseline: 18.97 ± 1.036 vs. Tsp1a: 18.63 ± 0.9794 , paired t test), and circular stretch (Figure 3c(i,ii); $p = 0.6848$, Baseline: 6.098 ± 1.036 vs. Tsp1a: 5.615 ± 0.9166 , Wilcoxon matched-pairs signed rank test and Figure 3c(iii); $p = 0.3750$, Baseline: 2.538 ± 0.4139 vs. Tsp1a: 2.308 ± 0.3985 , Wilcoxon matched-pairs signed rank test).

We then investigated whether Tsp1a could modulate pain sensitivity evoked by VD in conscious mice. In Sham control mice treated intravaginally with vehicle (veh), we found that VD evoked an increase in the VMR, with the degree of VMR related to the amount of pressure applied to the vagina (Figure 4a,b). When Tsp1a

was administered intravaginally, in vivo sensitivity to pain evoked by VD in Sham control mice was equal in magnitude to recordings obtained in the same mice intravaginally treated with vehicle (Figure 4b; for full details, see Figure 4b, general estimating equations, $p = 0.2440$ – 0.9170 and Figure 4c(i); $t_{(8)} = 0.5694$, $p = 0.5847$, Veh: 373.9 ± 55.94 vs. Tsp1a: 420.1 ± 97.19 , paired t test). This effect was observed across both non-noxious (Figure 4c(ii); $p = 0.3594$, Veh: 42.33 ± 17.37 vs. Tsp1a: 66.78 ± 14.64 , Wilcoxon matched-pairs signed rank test) and noxious distension pressures (Figure 4c(iii); $t_{(8)} = 0.2824$, $p = 0.7848$, Veh: 331.6 ± 54.23 vs. Tsp1a: 353.3 ± 98.25 , paired t test). Collectively, these results suggest that whilst activation of Na_v1.7 can enhance afferent hypersensitivity in healthy control states, inhibition of Na_v1.7 does not alter the capacity of vaginal afferents to sense non-noxious and noxious stimuli.

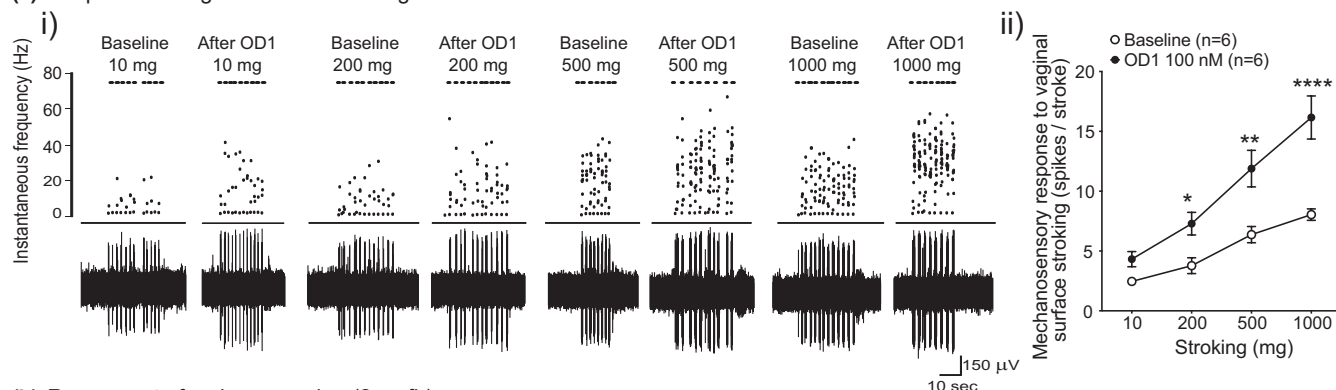
3.3 | Vaginal afferents from mice with endometriosis display hypersensitivity to mechanical stimuli

We next investigated whether Na_v1.7 plays a functional role in nociceptive signalling associated with endometriosis. Using our ex vivo vaginal afferent recordings, we characterised the response of pelvic vagina-innervating afferents to three different mechanical stimuli, in both Sham control mice and mice with fully developed endometriosis (Endo) (Figure 5). Compared to Sham control mice, vaginal afferents from Endo mice fired significantly more APs in response to vaginal surface stroking (Figure 5a(i–iii); for full details, see Figure 4a(iii)), Bonferroni's multiple comparison tests, $p < 0.0001$ – 0.3393), focal compression of the afferent receptive field (Figure 5b(i–iii); $t_{(54)} = 2.386$, $p = 0.0206$, Sham: 16.88 ± 0.7454 vs. Endo: 19.79 ± 0.9307 , un-paired t test), and circular stretch (Figure 5c(i–iii); $p = 0.0006$, Sham: 5.757 ± 0.5538 vs. Endo: 8.100 ± 0.4596 , Mann–Whitney test). Moreover, AP firing evoked by circular stretch commenced more rapidly within afferents from Endo mice (Figure 5c(iv); $p = 0.0322$, Sham: 2.808 ± 0.2816 vs. Endo: 2.143 ± 0.2470 , Mann–Whitney test). Overall, these data indicate that the development of endometriosis enhances the sensitivity of pelvic vaginal afferents to mechanical stimuli.

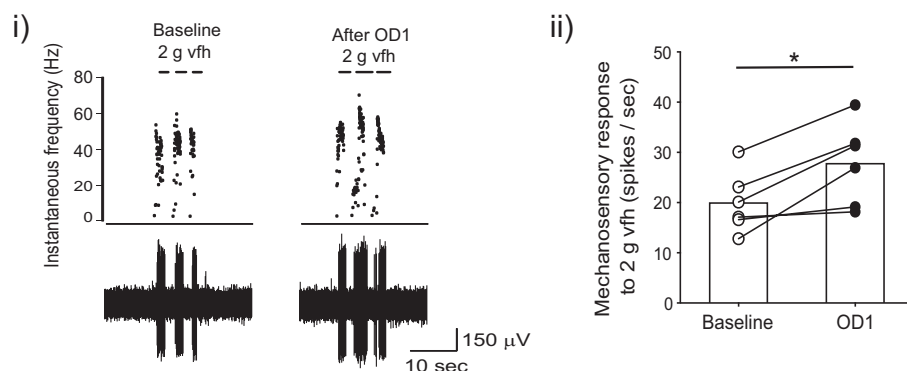
3.4 | Inhibition of Na_v1.7 reduces endometriosis-associated vaginal afferent hypersensitivity

We then examined whether selective inhibition of Na_v1.7 with Tsp1a (Jiang et al., 2021) was able to reverse endometriosis-associated vaginal afferent hypersensitivity. We found that exposure of vaginal afferent endings to Tsp1a significantly reduced vaginal afferent firing to vagina surface stroking (Figure 6a(i–iii); for full details, see Figure 4a(iii)), Bonferroni's multiple comparison tests, $p < 0.0001$ – 0.9971), focal compression (Figure 6b(i–iii); $p = 0.0005$, Baseline: 20.41 ± 1.302 vs. Tsp1a: 14.40 ± 1.015 , Wilcoxon matched-pairs signed rank test), and circular stretch

(a) Response to vaginal surface stroking



(b) Response to focal compression (2 g vfh)



(c) Response to circular stretch (5 g vfh)

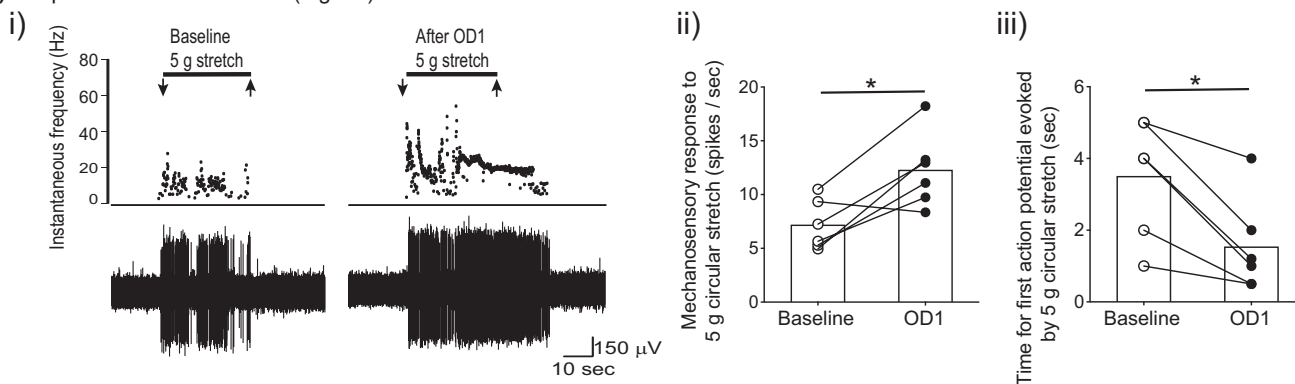
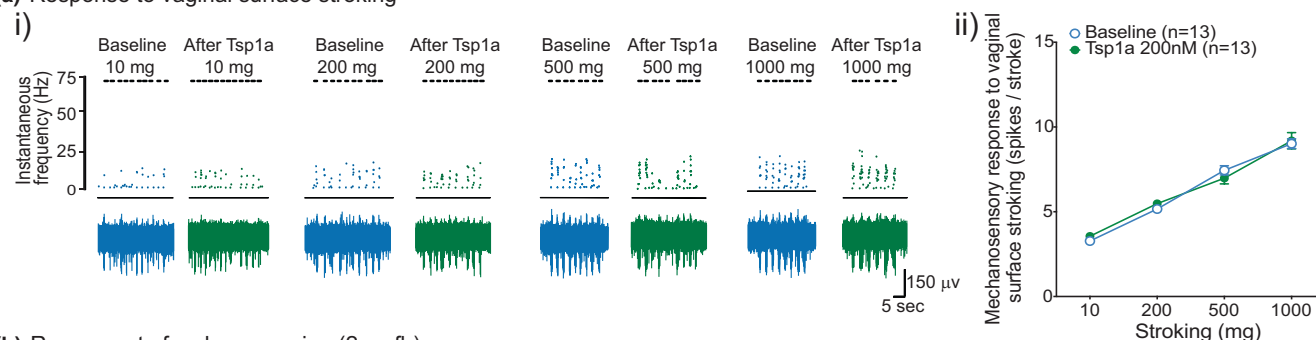


FIGURE 2 Pelvic vaginal afferents from Sham control mice display enhanced responses to a variety of mechanical stimuli following activation of $\text{Na}_v1.7$ with OD1. (a) (i) Representative traces obtained with an ex vivo single-unit nerve recording preparation, showing AP discharge of vaginal afferents in response to graded stroking of the vagina surface at baseline and following incubation of the receptive field with OD1 (100 nM) for 5 min. (ii) Group data showing that OD1 significantly increased AP discharge elicited by stroking of the vagina surface ($p = 0.6384$ at 10 mg, $*p = 0.049$ at 200 mg, $**p = 0.0013$ at 500 mg, and $***p < 0.0001$ at 1000 mg, two-way repeated-measures ANOVA followed by Bonferroni post-hoc comparison tests). (b) (i) Representative traces of vaginal afferent responses to focal compression with a 2 g vfh filament at baseline and following OD1 application (100 nM) for 5 min. (ii) Group data showing that OD1 (100 nM) sensitised vaginal afferents to focal compression ($*p = 0.0127$, paired Student's t test). (c) (i) Representative traces showing the response to circular stretch (5 g) of a vaginal afferent before and after OD1 (100 nM). Note that incubation with OD1 caused the afferent to continue to fire APs after cessation of the mechanical stimuli. (ii, iii) Grouped data showing OD1-induced sensitisation of vaginal afferents to circular stretch (5 g). OD1 induced increases in vaginal afferent firing to circular stretch were evident by (ii) an increased number of APs fired ($*p = 0.0135$, paired Student's t test), and (iii) reduced time to elicit AP firing ($*p = 0.0312$, Wilcoxon matched-pairs signed rank test) generated after OD1. Grouped data are from $n = 6$ afferents from $N = 3$ Sham control mice. Data are mean \pm SEM.

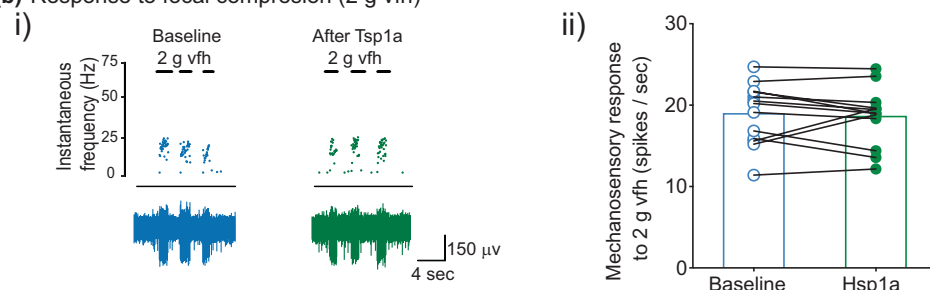
(Figure 6 c(i–iii); $t(12) = 7.869$, $p < 0.0001$, Baseline: 8.594 ± 0.5444 vs. Tsp1a 5.469 ± 0.6260 , paired t test) in Endo mice. Moreover, the time taken for these afferents to fire the first AP was significantly

longer after incubation with Tsp1a (Figure 6c(iv); $p = 0.0005$, Baseline: 1.446 ± 0.1870 vs. Tsp1a 4.154 ± 0.3729 , Wilcoxon matched-pairs signed rank test). Overall, these data indicate that

(a) Response to vaginal surface stroking



(b) Response to focal compression (2 g vfh)



(c) Response to circular stretch (5 g vfh)

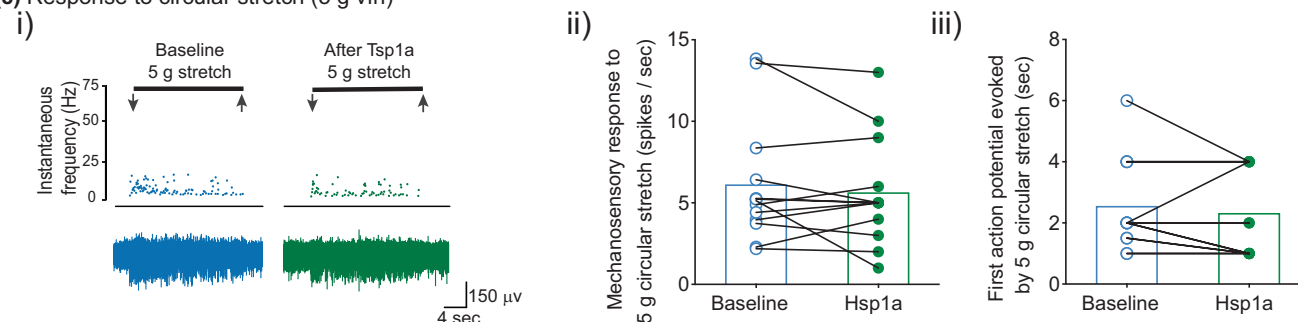


FIGURE 3 Inhibition of $\text{Na}_v1.7$ with Tsp1a does not alter the responsiveness of pelvic vaginal afferents from Sham control mice to mechanical stimuli. (a) (i) Representative traces from ex vivo single-unit recordings of vaginal afferent from Sham control mice showing responses to vaginal stroking at baseline and after incubation with the $\text{Na}_v1.7$ inhibitor Tsp1a (200 nM). (ii) Group data showing a lack of effect of Tsp1a (200 nM) on vaginal afferent sensitivity to receptive field stroking ($p > 0.9999$ at 10 mg, $p > 0.9999$ at 200 mg, $p = 0.4156$ at 500 mg, and $p > 0.9999$ at 1000 mg, two-way repeated-measures ANOVA followed by Bonferroni post-hoc comparison tests). (b) (i) Representative traces of vaginal afferent responses to focal compression with a 2 g vfh at baseline and in the presence of Tsp1a (200 nM). (ii) Group data showing a lack of effect of Tsp1a (200 nM) on vaginal afferent sensitivity to focal compression of the receptive field with 2 g vfh filament ($p = 0.5747$, paired Student's t test). (c) (i) Representative traces of vaginal afferent responses to circular stretch (5 g) at baseline and in the presence of Tsp1a (200 nM). (ii) Group data showing a lack of effect of Tsp1a (200 nM) on vaginal afferent sensitivity to the circular stretch of the vagina ($p = 0.6848$, Wilcoxon matched-pairs signed rank test). (iii) Additionally, Tsp1a did not change the time taken by vaginal afferents to fire their first AP in response to circular stretch ($p = 0.3750$, Wilcoxon matched-pairs signed rank test). Grouped data are from $n = 13$ afferents from $N = 4$ Sham control mice. Data are mean \pm SEM.

$\text{Na}_v1.7$ activity contributes to the enhanced sensitivity developed in vaginal afferents from Endo mice.

3.5 | Endometriosis induces hypersensitivity to VD in conscious mice

Compared to Sham control mice, Endo mice had elevated VMR responses to VD distension at all pressures tested (20–70 mmHg) (Figure 7a–c; for full details, see Figure 6b, general estimating equations, $p = 0.0003$ – <0.0001 and Figure 7c(i); $t_{(37)} = 0.4632$,

$p < 0.0001$, Sham: 349.8 ± 43.35 vs. Endo: 1027 ± 136.4 , unpaired t test). Further analysis revealed the VMR to non-noxious (20–30 mmHg) (Figure 7c(ii); $p < 0.0001$, Sham: 37.32 ± 10.21 vs. Endo: 158.8 ± 20.75 , Mann–Whitney test) and noxious (40–70 mmHg) (Figure 7c(iii); $t_{(37)} = 3.941$, $p = 0.0003$, Sham: 312.5 ± 40.96 vs. Endo: 868.7 ± 131.9 , unpaired t test) VD pressures were significantly enhanced in Endo mice. These results suggest that Endo mice display enhanced pain sensitivity to VD manifested as both allodynia and hyperalgesia, which are hallmark signs of CPP in a wide range of inflammatory diseases, including endometriosis (Berkley et al., 2001, 2007; Castro, Maddern, Grundy, et al., 2021; Maddern et al., 2020,

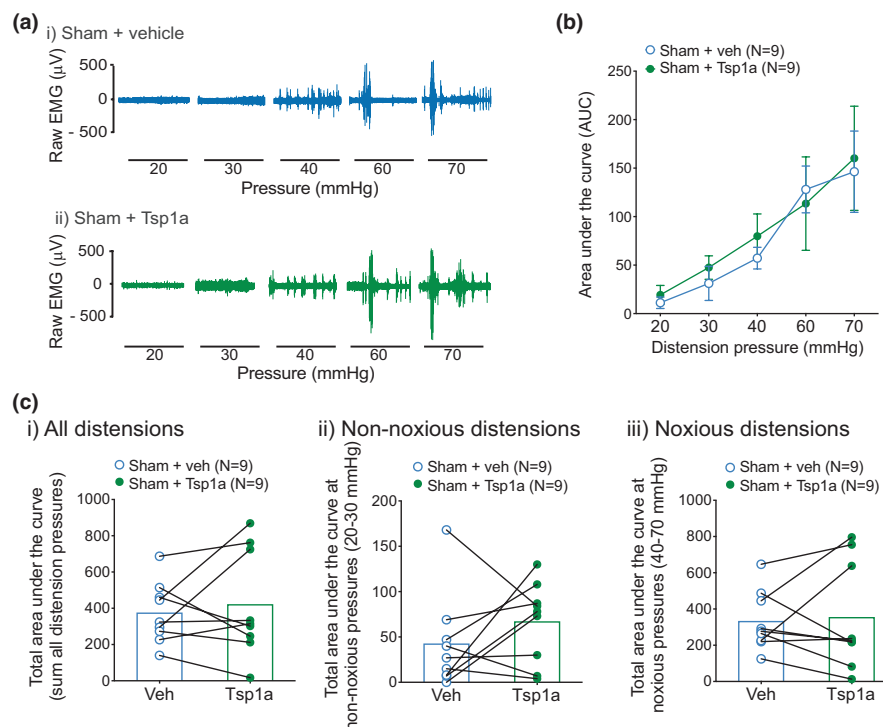


FIGURE 4 In vivo inhibition of $\text{Na}_v1.7$ with Tsp1a does not alter pain evoked by vaginal distension in Sham control mice. (a) Representative EMG recordings at increasing VD pressures (horizontal bars represent 30 s VD at pressures of 20–70 mmHg) in conscious Sham control mice at (i) baseline (intravaginal administration of vehicle (saline)) and (ii) following intravaginal treatment with Tsp1a (200 nM). (b) Grouped data showing that in Sham control mice, no significant difference was observed in VMRs between intravaginal vehicle treatment (baseline) or subsequent intravaginal treatment with Tsp1a ($p = 0.379$ at 20 mmHg, $p = 0.555$ at 30 mmHg, $p = 0.244$ at 40 mmHg, $p = 0.917$ at 60 mmHg and $p = 0.840$ at 70 mmHg, generalised estimating equations followed by LSD post-hoc test). (c) Grouped data showing no change from baseline VMRs following intravaginal treatment with Tsp1a, evidenced by no change in the total AUC of the VMRs (i) at all distensions pressures (sum of the AUCs obtained at each distension pressure, $p = 0.5847$, paired Student's *t* test), and the AUC of the VMRs at (ii) non-noxious distension pressures (20–30 mmHg, $p = 0.3594$, Wilcoxon matched-pairs signed rank test), and (iii) noxious distension pressures (40–70 mmHg, $p = 0.7848$, paired Student's *t* test). Group data are from $N = 9$ Sham control mice treated with vehicle (baseline) and subsequently treated Tsp1a. Data are mean \pm SEM.

2022; McAllister et al., 2009; Stratton & Berkley, 2011; Zondervan et al., 2018).

3.6 | Inhibition of $\text{Na}_v1.7$ reverses endometriosis-associated chronic pelvic pain to VD

Finally, we examined whether selective inhibition of $\text{Na}_v1.7$ with the intravaginal treatment of Tsp1a (Jiang et al., 2021) was able to reverse the elevated baseline vaginal sensitivity seen in mice with endometriosis. We found that intravaginal treatment with Tsp1a was able to significantly reduce the elevated VMRs evoked by VD previously seen in the vehicle (saline) treated Endo mice (Figure 8a–c; for full details, see Figure 8b; general estimating equations, $p = 0.012$ – 0.138 and Figure 8 c(i); $p = 0.0059$, Veh: 1176 ± 216.8 vs. Tsp1a: 532.6 ± 188.4 , Wilcoxon matched-pairs signed rank test). This effect was observed at non-noxious (20–30 mmHg) (Figure 8c(ii); $p = 0.0137$, Veh: 196.8 ± 30.46 vs. Tsp1a: 68.30 ± 33.95 , Wilcoxon matched-pairs signed rank test) and noxious (40–70 mmHg) (Figure 8c(iii); $p = 0.0137$, Veh: 979.2 ± 222.5 vs. Tsp1a: 464.3 ± 163.9 ,

Wilcoxon matched-pairs signed rank test) distension pressures tested. Interestingly, as illustrated in Figure 8b, Tsp1a reduced the VMRs in Endo mice to similar levels observed in Sham control mice, suggesting that pharmacological inhibition of $\text{Na}_v1.7$ on vaginal afferents is an attractive target to reverse vaginal allodynia and hyperalgesia associated with endometriosis (for visual comparison we included the VMR data (dotted line) from vehicle-treated Sham mice is shown in Figure 8b).

4 | DISCUSSION

Endometriosis is a chronic disorder characterised by infertility and CPP. Currently, there is a lack of effective analgesic treatments for endometriosis-associated CPP mainly because of our lack of knowledge about its aetiology and pathogenesis (Koninckx et al., 2019; Zito et al., 2014). How sensory neurons innervating the female reproductive tract detect and transmit pain and how they are altered in endometriosis remains poorly understood. We recently showed that all nine members of the Na_v channel family are expressed in

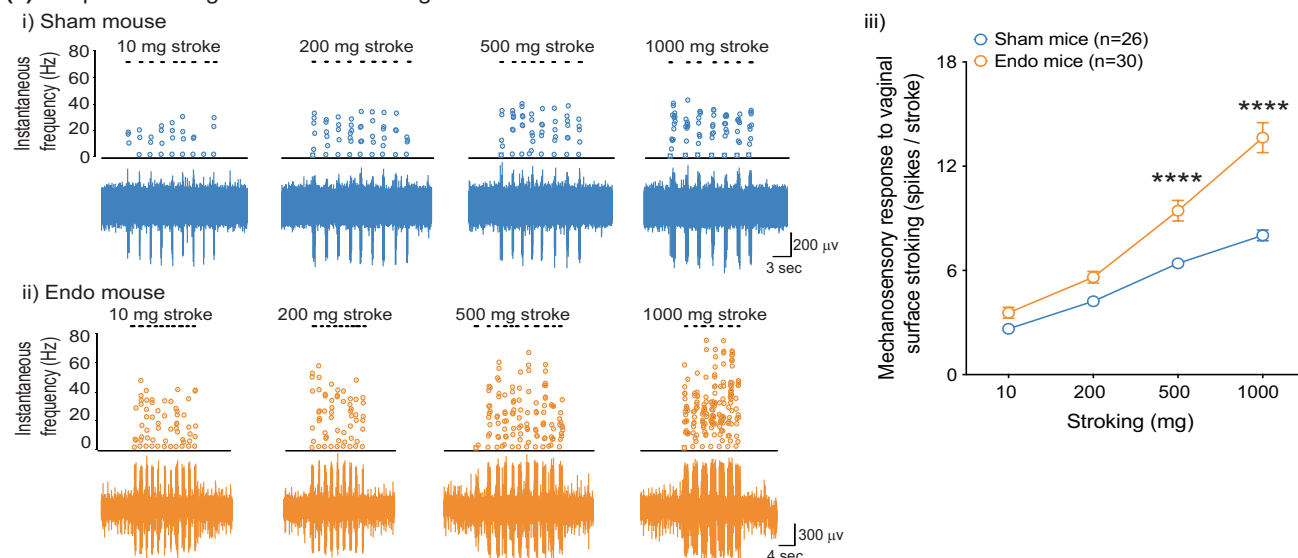
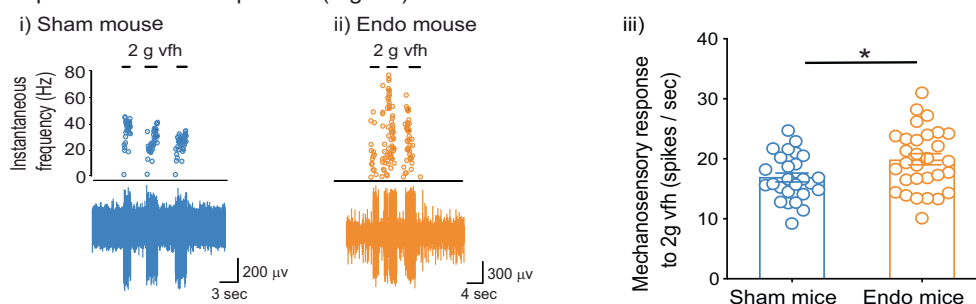
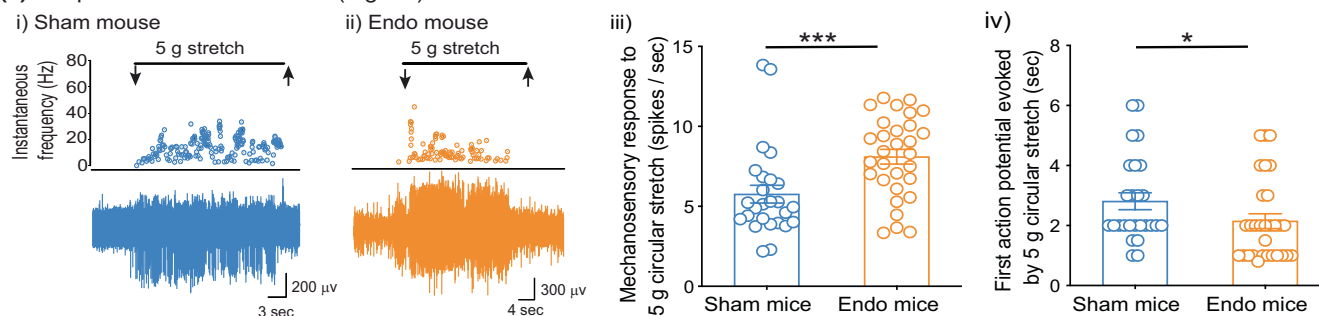
(a) Response to vaginal surface stroking**(b) Response to focal compression (2 g vfh)****(c) Response to circular stretch (5 g vfh)**

FIGURE 5 Pelvic vaginal afferents from endometriosis mice display hypersensitivity to mechanical stimuli. (a) Representative traces of vaginal afferents from (i) Sham control mice and (ii) endometriosis (Endo) mice showing baseline AP discharge in response to graded stroking of the vaginal surface. (iii) Group data showing that vaginal afferents from Endo mice display increased sensitivity to graded stroking of the vaginal surface ($p = 0.3393$ at 10 mg, $p = 0.0515$ at 200 mg, **** $p < 0.0001$ at 500 and 1000 mg, two-way ANOVA followed by Bonferroni post-hoc comparison tests). (b) Representative traces of vaginal afferents from (i) Sham control mice and (ii) Endo mice showing baseline responses to focal compression with a 2 g vfh. (iii) Group data showing hypersensitivity to focal compression of the vaginal afferent receptive field (* $p = 0.0206$, two-tailed unpaired Student's t test). (c) Representative traces of vaginal afferents from (i) Sham control mice and (ii) Endo mice showing baseline responses to circular stretch (5 g). Compared with Sham controls, vaginal afferents from Endo mice showed hypersensitivity to circular stretch, as determined by (iii) an increased number of APs generated by circular stretch (*** $p = 0.006$, Mann-Whitney non-parametric test), and (iv) reduced latency required for AP firing (* $p = 0.0322$, Mann-Whitney non-parametric test). Grouped data are from $n = 26$ afferents from $N = 8$ Sham control mice, and $n = 30$ afferents from $N = 9$ Endo mice. Data are mean \pm SEM.

pelvic vaginal afferents, although their relative expression in these DRG neurons varies greatly. The pan- Na_v channel agonist, veratridine, can drive vaginal afferent hypersensitivity, whilst the Na_v channel blocker TTX (which blocks $\text{Na}_v1.1$ – $\text{Na}_v1.4$, $\text{Na}_v1.6$, and

$\text{Na}_v1.7$) reduces vaginal afferent sensitivity. These findings demonstrate that Na_v channels play an important role in regulating vaginal sensation in healthy animals and point towards a key role of TTX-sensitive Na_v channels (Castro, Maddern, Erickson, et al., 2021).

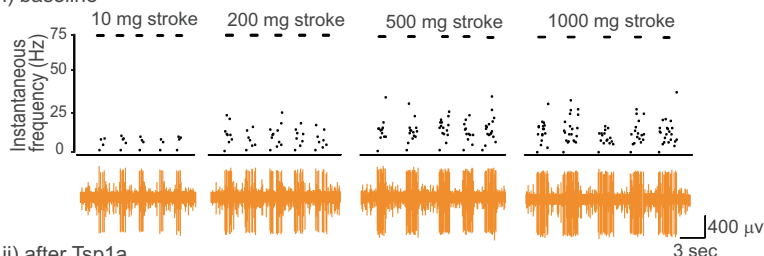
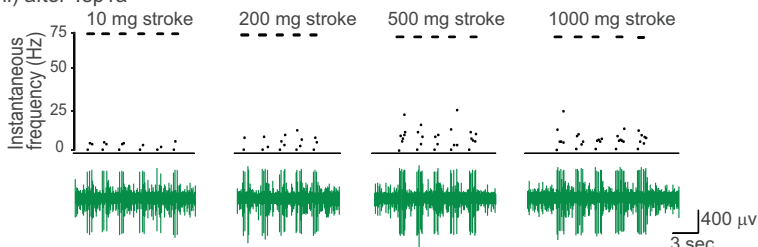
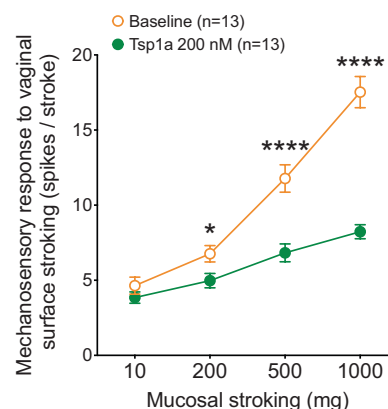
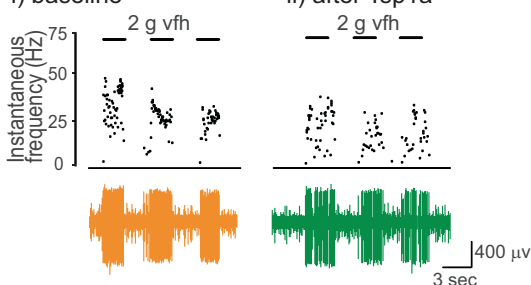
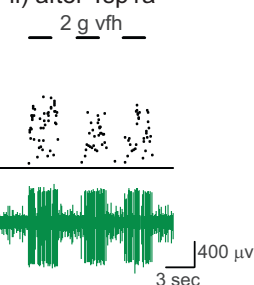
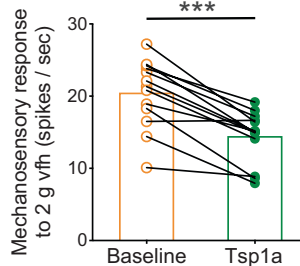
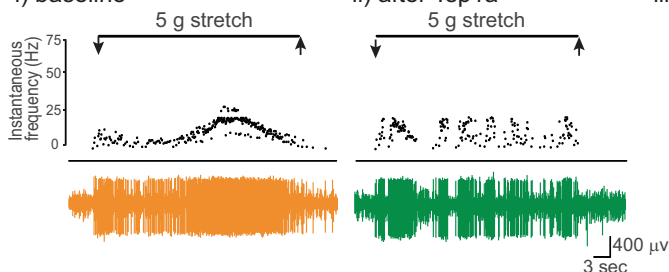
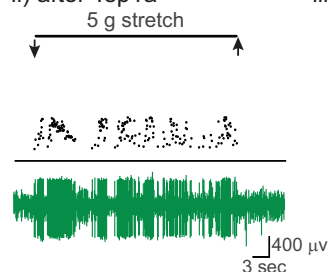
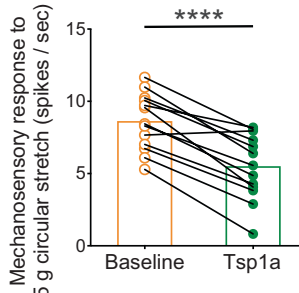
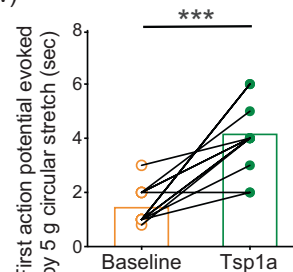
(a) Response to vaginal surface stroking**i) baseline****ii) after Tsp1a****iii)****(b) Response to focal compression (2 g vfh)****i) baseline****ii) after Tsp1a****iii)****(c) Response to circular stretch (5 g)****i) baseline****ii) after Tsp1a****iii)****iv)**

FIGURE 6 Vaginal afferents from endometriosis mice display reduced responsiveness to mechanical stimuli following inhibition of $\text{Na}_v1.7$ with Tsp1a. (a) Representative traces of vaginal afferents from endometriosis mice showing responses to vaginal stroking at (i) baseline and (ii) after incubation with the $\text{Na}_v1.7$ inhibitor Tsp1a (200 nM). (iii) Grouped data showing Tsp1a significantly reduces vaginal afferent sensitivity to stroking ($p = 0.9971$ at 10 mg, $*p = 0.0454$ at 200 mg, $****p < 0.0001$ at 500 mg and 1000 mg, two-way ANOVA followed by Bonferroni post-hoc comparison tests). (b) Representative traces of vaginal afferents from endometriosis mice showing responses to focal compression at (i) baseline and (ii) after incubation with the $\text{Na}_v1.7$ inhibitor Tsp1a (200 nM). (iii) Group data showing Tsp1a significantly reduces vaginal afferent sensitivity to probing ($***p = 0.0005$, Wilcoxon matched-pairs signed rank test). (c) Representative traces of vaginal afferents from endometriosis mice showing responses to circular stretch at (i) baseline and (ii) after incubation with the $\text{Na}_v1.7$ inhibitor Tsp1a (200 nM). (iii) Group data showing Tsp1a significantly reduces vaginal afferent sensitivity to the circular stretch of the vagina ($****p < 0.0001$, paired Student's t test). (iv) Additionally, Tsp1a significantly increased the time taken by vaginal afferents to fire their first AP in response to circular stretch (Civ) ($***p = 0.0005$, Wilcoxon matched-pairs signed rank test). Grouped data are from $n = 13$ afferents from $N = 4$ Endo mice. Data are mean \pm SEM.

However, the specific identity of the TTX-sensitive Na_v channels contributing to vaginal nociceptive signalling and their contribution to endometriosis-associated CPP has remained unclear.

In this study, we addressed this by investigating the role of $\text{Na}_v1.7$ in normal vaginal mechanosensitivity and endometriosis-associated vaginal mechanical hypersensitivity. We decided to target this

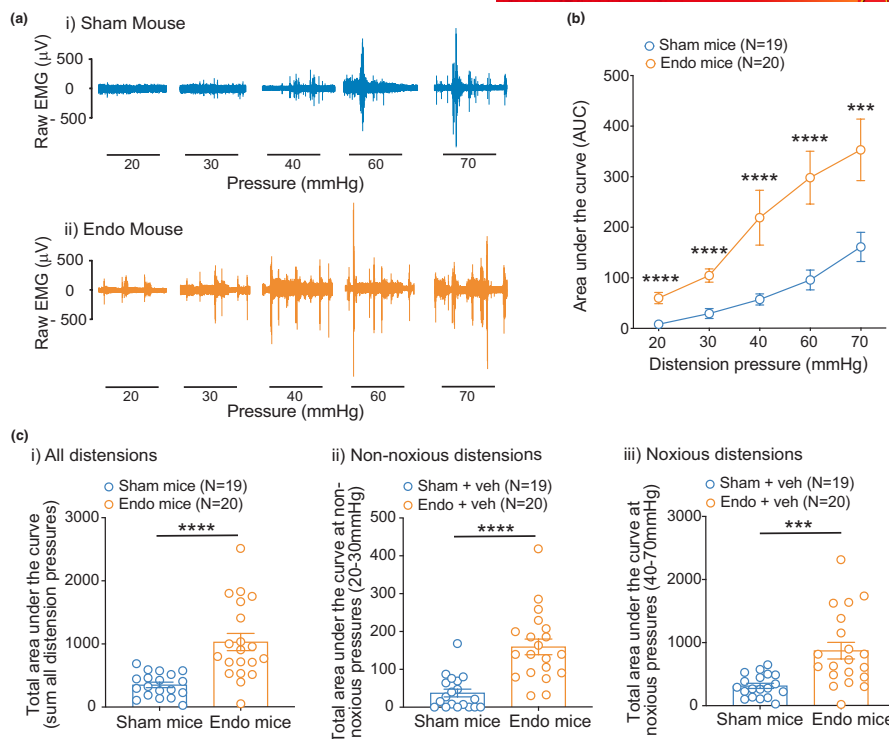


FIGURE 7 Mice with endometriosis display increased pain (allodynia and hyperalgesia) evoked by vaginal distension in vivo. (a) Representative EMG recordings showing the VMR to vaginal distension in (i) a Sham control mouse and (ii) a mouse with endometriosis. (b) Grouped AUC data showing that Endo mice displayed significantly enhanced VMRs compared to their Sham counterparts ($****p < 0.0001$ at 20, 30, 40, and 60 mmHg and $***p = 0.0003$ at 70 mmHg, generalised estimating equations followed by LSD post-hoc test). (c) (i) Grouped data showing that Endo mice have significantly enhanced total AUC compared with Sham control mice. Total AUC was obtained by combining the AUCs of the VMRs obtained at each distension pressure ($****p < 0.0001$, two-tailed unpaired Student's *t* test). Further analysis reveals that mice with endometriosis have increased VMRs to both (ii) non-noxious (20–30 mmHg, $****p < 0.0001$, Mann-Whitney non-parametric test), and (iii) noxious (40–70 mmHg, $***p = 0.0003$, two-tailed unpaired Student's *t* test) distension pressures. Grouped data are from $N = 19$ Sham control mice and $N = 20$ Endo mice. Data are mean \pm SEM.

channel because: (i) $\text{Na}_v1.7$ is TTX-sensitive; (ii) it is abundantly expressed (98–100%) in sensory neurons that innervate pelvic organs affected by endometriosis, including the vagina (Castro, Maddern, Erickson, et al., 2021), colon (Inserra et al., 2017), and bladder (Grundy, Erickson, et al., 2018); (iii) we have unique access to selective modulators of $\text{Na}_v1.7$ (Durek et al., 2013; Jiang et al., 2021); (iv) we recently demonstrated a role for $\text{Na}_v1.7$ in chronic visceral pain (Jiang et al., 2021); and (v) various $\text{Na}_v1.7$ -selective inhibitors are in clinical trials for various types of pain (Alexandrou et al., 2016; Bagal et al., 2014; Goldberg et al., 2012; Kotecha et al., 2020; Zakrzewska et al., 2017).

First, we demonstrated that activation of $\text{Na}_v1.7$ with OD1, a highly selective $\text{Na}_v1.7$ agonist (Durek et al., 2013), dramatically enhanced the responsiveness of vaginal afferents from Sham control mice to three different types of mechanical stimuli. Interestingly, we observed that whilst OD1 failed to elicit AP discharge in the absence of mechanical stimuli (direct activation of afferents), half of the afferents incubated with OD1 continued to fire APs after cessation of the mechanical stimulus. This observation could be explained by the mechanism by which OD1 alters $\text{Na}_v1.7$ channel function. Kinetically, $\text{Na}_v1.7$ is slow to close and subsequently inactivates following its activation (Hameed, 2019; King & Vetter, 2014). OD1

inhibits the fast inactivation of $\text{Na}_v1.7$, which ultimately disrupts the resting membrane potential, maintaining hyperexcitable sensory neurons in the absence of further stimuli (Durek et al., 2013; King & Vetter, 2014; Maertens et al., 2006). This is particularly relevant in genetic gain-of-function mutations, where the enhanced role of $\text{Na}_v1.7$ in sensory neurons maintains them in a hyperexcitable state, increasing AP firing and ultimately driving sensory signalling in the absence of stimuli (Dib-Hajj et al., 2005; King & Vetter, 2014). Overall, our findings indicate that vaginal afferent responses to mechanical stimuli in control conditions can be enhanced by pharmacological activation of $\text{Na}_v1.7$.

We next examined whether selective inhibition of $\text{Na}_v1.7$ could reduce the ability of vaginal afferents to sense baseline mechanical stimuli in Sham control mice. For this we used the peptide Tsp1a, a selective inhibitor of $\text{Nav}1.7$ derived from the venom of the Peruvian tarantula *Thrixopelma spec.* (Jiang et al., 2021). We found that Tsp1a does not alter the ability of vaginal afferents to respond to mechanical stimuli ex vivo. These findings were further supported by in vivo studies, whereby intravaginal application of Tsp1a failed to alter vaginal sensitivity to distension in conscious Sham control mice. Overall, our results are consistent with previous reports indicating that $\text{Na}_v1.7$ does not appear to contribute to baseline

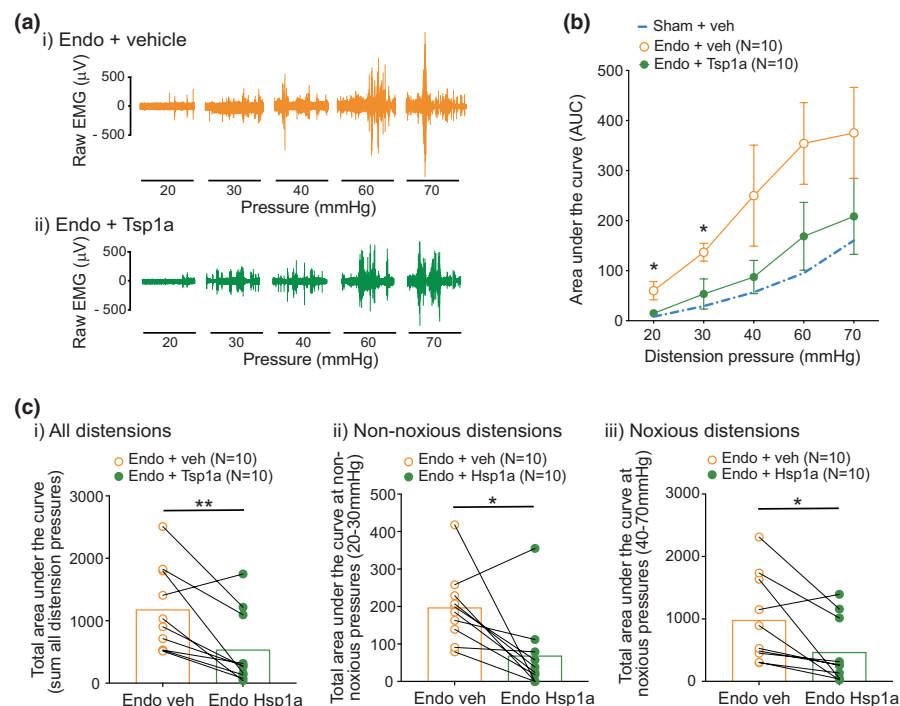


FIGURE 8 In endometriosis mice inhibition of $\text{Na}_v1.7$ with Tsp1a reduces allodynia and hyperalgesia to vaginal distension.

(a) Representative examples of EMG responses to VD in Endo mice intravaginally treated with (i) saline (vehicle) or (ii) Tsp1a. (b) Grouped data showing that the enhanced VMRs developed in the Endo mice treated with vehicle (Endo + veh, orange) are significantly reduced after intravaginal treatment with 200 nM Tsp1a (Endo + Tsp1a, green) ($*p = 0.014$ at 20 mmHg, $*p = 0.012$ at 30 mmHg, $p = 0.107$ at 40 mmHg, $p = 0.065$ at 60 mmHg and $p = 0.138$ at 70 mmHg, generalised estimating equations followed by LSD post-hoc test). Note: the VMRs of Endo mice treated with Tsp1a are comparable to those displayed by Sham control mice (blue dotted line, mean representative data from Figure 6b shown for visual comparison). (c) Grouped data showing intra-vaginal treatment of Endo mice with Tsp1a significantly reduces (i) the elevated total AUC (sum of the AUCs obtained at each distension pressure). ($**p = 0.0059$, Wilcoxon matched-pairs signed rank test). Further analysis of the AUC data shows that Tsp1a was effective in reducing VMRs at both (ii) non-noxious (20–30 mmHg, $*p = 0.0137$, Wilcoxon matched-pairs signed rank test); and (iii) noxious distension pressures (40–70 mmHg, $*p = 0.0137$, Wilcoxon matched-pairs signed rank test). Grouped data are from $N = 10$ Endo mice treated with saline (vehicle) and subsequently treated Tsp1a. Data are mean \pm SEM.

visceral nociception in healthy conditions (Hockley et al., 2017; Jiang et al., 2021).

However, a possible role for $\text{Na}_v1.7$ in endometriosis-associated CPP has previously been suggested. There is evidence of *SCN9A* (the gene encoding $\text{Na}_v1.7$) overexpression in endometrial lesions collected from women with endometriosis and pain, compared to women without endometriosis/without pain (Greaves et al., 2014). Notably, human embryonic stem cells with a nociceptive phenotype treated with the oestrogen receptor β agonist 2,3-bis(4-hydroxy-phenyl)-propionitrile display enhanced expression of *SCN9A* (Greaves et al., 2014). Furthermore, macrophages activated with peritoneal fluid from women with endometriosis can also enhance the expression of *SCN9A* in cultured human sensory neurons in vitro, via a macrophage-derived insulin-like growth factor 1 (IGF-1) mechanism (Forster et al., 2019). Whilst changes in the expression profile of $\text{Na}_v1.7$ have been associated with endometriosis in these studies, the functional role of $\text{Na}_v1.7$ in sensing pain from pelvic organs affected by endometriosis has not yet been explored. Therefore, we investigated the role of $\text{Na}_v1.7$ in our established syngeneic mouse model of endometriosis (Maddern et al., 2022). We show here, for the first time, that pelvic afferents innervating the mouse vagina

become hypersensitive to mechanical stimuli in endometriosis. This hypersensitivity was also displayed in vivo, with conscious Endo mice displaying both allodynia and hyperalgesia to VD compared to their Sham control counterparts. These findings demonstrate that increased nociceptive signalling from the vagina results in increased pain sensitivity in vivo, which closely corresponds with nociceptive mechanisms observed in other visceral organs (Castro et al., 2013, 2017a; Grundy et al., 2019; Grundy, Harrington, et al., 2018; Harrington et al., 2012).

Importantly, inhibition of $\text{Na}_v1.7$ with Tsp1a was effective in reducing ex vivo vaginal afferent hypersensitivity in mice with endometriosis. This attenuation was also apparent in vivo; the VMR evoked by VD in conscious Endo mice was reversed with a single intravaginal dose of Tsp1a, with sensitivity restored to the levels seen in Sham control animals. Notably, this effect was apparent at non-noxious and noxious distension pressures, suggesting that Tsp1a is able to alleviate vaginal allodynia and hyperalgesia in mice with endometriosis. Overall, our findings are consistent with the notion that enhanced activity of $\text{Na}_v1.7$ is able to drive, and contribute to, heightened pain and nociception in chronic disease states (Hameed, 2019; Jiang et al., 2021; Siebenga et al., 2020). However,

Na_v1.7 does not appear to contribute to visceral nociception in healthy conditions (Hockley et al., 2017; Jiang et al., 2021).

Whilst our study did not further examine the role of Na_v1.7 in healthy conditions and baseline sensation, we found that Na_v1.7 inhibition did not affect baseline responses in Sham control mice in our experiments. A possible explanation for this lack of effect could be attributed to the low Tsp1a concentration used in our study (200 nM), as it has been shown that a higher degree of channel blockade is required for analgesia to occur in otherwise healthy subjects (King & Vetter, 2014; Vetter et al., 2017). This is an important differentiation because an ideal analgesic should target the key mechanisms responsible for the pathological pain underlying the condition and provide pain relief without the loss of baseline sensory functions, which are essential for host responses to the external and internal environments. Further supporting our findings, the notion that Na_v1.7-mediated analgesia is only achieved during pathological pain has also been demonstrated in an animal model of post-surgical pain (Mueller et al., 2019). In this study, the degree of Na_v1.7-mediated analgesia was suggested to be driven by the interaction with endogenous opioid signalling pathways (Mueller et al., 2019). This study suggests that during pathological pain, Na_v1.7 may act as a signal amplifier, where enhanced excitability arises from elevated inflammatory mechanisms that contribute to abnormal pain in disease states (Mueller et al., 2019). This is especially relevant to endometriosis, which is known to be an inflammatory disease, and may explain the clear effect of Na_v1.7-mediated analgesia in the endometriosis model alone. Whilst the precise mechanism(s) explaining the selective effect of Tsp1a on endometriosis-associated chronic pain, versus healthy states, was beyond the scope of the current study, it is an important next step in understanding Na_v1.7-mediated analgesia in endometriosis.

Although Na_v1.7 is widely accepted as a pain target, the translation to clinical significance as a treatment for chronic pain has been lacking (Eagles et al., 2022). This is likely because of a multiplicity of factors including (i) genetic mutations impacting pain sensitivity in humans; (ii) the lack of selectivity and tissue penetration of some Na_v1.7 inhibitors, and (iii) the underestimated impact of the effect of the auxiliary β subunits on Na_v1.7 pharmacology (Eagles et al., 2022). Our findings showing different roles for Na_v1.7 in nociceptive signalling in health and endometriosis states are in keeping with recent studies. For example, inhibition of Na_v1.7 does not alter colonic afferent signalling in healthy states (Hockley et al., 2017; Jiang et al., 2021), whereas Na_v1.7 inhibition in a mouse model of IBS reverses mechanical hypersensitivity *ex vivo* and *in vivo* (Jiang et al., 2021). Other studies show that indirect targeting of Na_v1.7 activity in sensory neurons by specifically inhibiting Na_v1.7 trafficking and surface expression with compound 194, is effective at reversing thermal and mechanical hypersensitivity in a rodent model of neuropathic pain (Francois-Moutal et al., 2018; Li et al., 2022). Overall, the role of Na_v1.7 in pain is complex, with somatosensory studies

suggesting that Na_v1.7 blockers alone may not replicate the analgesic phenotype of Na_v1.7^{-/-} mutant mice and humans with congenital insensitivity to pain because of loss-of-function mutations in SCN9A. However, their effects may be potentiated with exogenous opioids (Minett et al., 2015) and G-protein coupled receptors (GPCRs) (Isensee et al., 2017; Mueller et al., 2019). For example, some studies have shown that Na_v1.7 regulates the efficacy and balance of GPCR-mediated pro- and anti-nociceptive intracellular signalling, so that without Na_v1.7 the balance of these processes is shifted towards anti-nociception (Isensee et al., 2017). Other animal studies show that post-surgical pain can be successfully treated with Na_v1.7 inhibitors alone or at subtherapeutic doses in combination with baclofen or opioids, suggesting super-additive anti-nociceptive effects when Na_v1.7 inhibitors are applied in combination with baclofen or opioids (Mueller et al., 2019). Whether opioidergic and GPCR mechanisms contribute to Na_v1.7's role in endometriosis-associated CPP will be the subject of future studies. However, it is interesting to note that several GPCR and opioidergic mechanisms are up-regulated in colonic nociceptors (Brust et al., 2016; Castro et al., 2017b, 2018, 2022; de Araujo et al., 2014; Hughes et al., 2014; Muratspahic et al., 2021; Sadeghi et al., 2018) in IBS models and that inhibition of Na_v1.7 also reverses pathological pain in these IBS models (Jiang et al., 2021).

In conclusion, our study demonstrates that Na_v1.7 contributes to the vaginal hyperalgesia associated with endometriosis. Moreover, it reveals that pharmacological inhibition Na_v1.7 with Tsp1a could be a viable therapeutic option to treat endometriosis-associated CPP. Together with our recently published findings showing that Tsp1a could reverse colonic hypersensitivity in a mouse model of IBS (Jiang et al., 2021), these results suggest that pharmacological targeting of Na_v1.7 is a valid approach to treat CPP associated with visceral pain disorders including endometriosis.

AUTHOR CONTRIBUTIONS

J.C., J.M., and S.M.B. contributed to experimental design, analysis, and intellectual input. J.C. and J.M. performed the experiments described in this paper. I.V. and G.F.K. provided peptides targeting Na_v channels and intellectual input. J.C. made the figures and wrote the manuscript with contributions from all authors.

ACKNOWLEDGMENTS

We acknowledge funding from the National Health and Medical Research Council of Australia (NHMRC Ideas Grant APP1181448 to J.C.; Investigator Leadership Grant APP2008727 to S.M.B.; Principal Research Fellowship APP1136889 to G.F.K.; Career Development Fellowship APP1162503 to I.V.; Development Grant APP2014250 to S.M.B. and G.F.K.), the Australian Research Council (Discovery Project DP220101269 to S.M.B.; Centre of Excellence grant CE200100012 to G.F.K.) and The Hospital Research Foundation (PhD Scholarship SAPHD000242018 to J.M.). Open access publishing facilitated by Flinders University, as part of the Wiley - Flinders University agreement via the Council of Australian University Librarians.



All experiments were conducted in compliance with the ARRIVE guidelines.

CONFLICT OF INTEREST STATEMENT

Stuart Brierley is a Guest Editor for the Pain special issue. The other authors have declared no potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

PRE-REGISTRATION

This study was not pre-registered.

PREVIOUS PRESENTATION OF RESEARCH

This preprint was posted on bioRxiv on October 07, 2022 (doi: [10.1101/2022.10.06.511228](https://doi.org/10.1101/2022.10.06.511228)). also found at: <https://www.biorxiv.org/content/10.1101/2022.10.06.511228v1>.

ORCID

Joel Castro <https://orcid.org/0000-0002-5781-2224>

Chun Yuen Chow <https://orcid.org/0000-0003-2861-5843>

Poanna Tran <https://orcid.org/0000-0003-3196-9896>

REFERENCES

- Alexandrou, A. J., Brown, A. R., Chapman, M. L., Estacion, M., Turner, J., Mis, M. A., Wilbrey, A., Payne, E. C., Gutteridge, A., Cox, P. J., Doyle, R., Printzenhoff, D., Lin, Z., Marron, B. E., West, C., Swain, N. A., Storer, R. I., Stupple, P. A., Castle, N. A., ... Stevens, E. B. (2016). Subtype-selective small molecule inhibitors reveal a fundamental role for Nav1.7 in nociceptor electrogenesis, axonal conduction and presynaptic release. *PLoS One*, 11(4), e0152405.
- Bagal, S. K., Chapman, M. L., Marron, B. E., Prime, R., Storer, R. I., & Swain, N. A. (2014). Recent progress in sodium channel modulators for pain. *Bioorganic & Medicinal Chemistry Letters*, 24(16), 3690–3699.
- Becker, C. M., Missmer, S. A., & Zondervan, K. T. (2020). Endometriosis. *The New England Journal of Medicine*, 383(2), 194.
- Bennett, D. L., & Woods, C. G. (2014). Painful and painless channelopathies. *Lancet Neurology*, 13(6), 587–599.
- Berkley, K. J., Cason, A., Jacobs, H., Bradshaw, H., & Wood, E. (2001). Vaginal hyperalgesia in a rat model of endometriosis. *Neuroscience Letters*, 306(3), 185–188.
- Berkley, K. J., McAllister, S. L., Accius, B. E., & Winnard, K. P. (2007). Endometriosis-induced vaginal hyperalgesia in the rat: Effect of oestropause, ovariectomy, and estradiol replacement. *Pain*, 132(Suppl 1), S150–S159.
- Broad, L. M., Mogg, A. J., Beattie, R. E., Ogden, A. M., Blanco, M. J., & Bleakman, D. (2009). TRP channels as emerging targets for pain therapeutics. *Expert Opinion on Therapeutic Targets*, 13(1), 69–81.
- Brust, A., Croker, D. E., Colless, B., Ragnarsson, L., Andersson, Å., Jain, K., Garcia-Caraballo, S., Castro, J., Brierley, S. M., Alewood, P. F., & Lewis, R. J. (2016). Conopeptide-derived κ -opioid agonists (Conorphins): Potent, selective, and metabolic stable dynorphin a mimetics with antinociceptive properties. *Journal of Medicinal Chemistry*, 59(6), 2381–2395.
- Castro, J., Garcia-Caraballo, S., Maddern, J., Schober, G., Lumsden, A., Harrington, A., Schmiel, S., Lindstrom, B., Adams, J., & Brierley, S. M. (2022). Olorinab (APD371), a peripherally acting, highly selective, full agonist of the cannabinoid receptor 2, reduces colitis-induced acute and chronic visceral hypersensitivity in rodents. *Pain*, 163(1), e72–e86.
- Castro, J., Grundy, L., Deiteren, A., Harrington, A. M., O'Donnell, T., Maddern, J., Moore, J., Garcia-Caraballo, S., Rychkov, G. Y., Yu, R., Kaas, Q., Craik, D. J., Adams, D. J., & Brierley, S. M. (2018). Cyclic analogues of α -conotoxin Vc1.1 inhibit colonic nociceptors and provide analgesia in a mouse model of chronic abdominal pain. *British Journal of Pharmacology*, 175(12), 2384–2398.
- Castro, J., Harrington, A. M., Garcia-Caraballo, S., Maddern, J., Grundy, L., Zhang, J., Page, G., Miller, P. E., Craik, D. J., Adams, D. J., & Brierley, S. M. (2017a). Alpha-conotoxin Vc1.1 inhibits human dorsal root ganglion neuroexcitability and mouse colonic nociception via GABAB receptors. *Gut*, 66(6), 1083–1094.
- Castro, J., Harrington, A. M., Garcia-Caraballo, S., Maddern, J., Grundy, L., Zhang, J., Page, G., Miller, P. E., Craik, D. J., Adams, D. J., & Brierley, S. M. (2017b). α -Conotoxin Vc1.1 inhibits human dorsal root ganglion neuroexcitability and mouse colonic nociception via GABA(B) receptors. *Gut*, 66(6), 1083–1094.
- Castro, J., Harrington, A. M., Hughes, P. A., Martin, C. M., Ge, P., Shea, C. M., Jin, H., Jacobson, S., Hannig, G., Mann, E., Cohen, M. B., MacDougall, J. E., Lavins, B. J., Kurtz, C. B., Silos-Santiago, I., Johnston, J. M., Currie, M. G., Blackshaw, L. A., & Brierley, S. M. (2013). Linacotide inhibits colonic nociceptors and relieves abdominal pain via guanylate cyclase-C and extracellular cyclic guanosine 3',5'-monophosphate. *Gastroenterology*, 145(6), 1334–1346.e11.
- Castro, J., Maddern, J., Erickson, A., Caldwell, A., Grundy, L., Harrington, A. M., & Brierley, S. M. (2021). Pharmacological modulation of voltage-gated sodium (Nav) channels alters nociception arising from the female reproductive tract. *Pain*, 162(1), 227–242.
- Castro, J., Maddern, J., Grundy, L., Manavis, J., Harrington, A. M., Schober, G., & Brierley, S. M. (2021). A mouse model of endometriosis that displays vaginal, colon, cutaneous, and bladder sensory comorbidities. *The FASEB Journal*, 35(4), e21430.
- Catterall, W. A. (2012). Voltage-gated sodium channels at 60: Structure, function and pathophysiology. *The Journal of Physiology*, 590(11), 2577–2589.
- Catterall, W. A., Goldin, A. L., & Waxman, S. G. (2005). International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels. *Pharmacological Reviews*, 57(4), 397–409.
- Chaban, V. V. (2008). Visceral sensory neurons that innervate both uterus and colon express nociceptive TRPV1 and P2X3 receptors in rats. *Ethnicity & Disease*, 18(2 Suppl 2), 20–24.
- de Araujo, A. D., Mobli, M., Castro, J., Harrington, A. M., Vetter, I., Dekan, Z., Muttenthaler, M., Wan, J. J., Lewis, R. J., King, G. F., Brierley, S. M., & Alewood, P. F. (2014). Selenoether oxytocin analogues have analgesic properties in a mouse model of chronic abdominal pain. *Nature Communications*, 5, 3165.
- Dib-Hajj, S. D., Rush, A. M., Cummins, T. R., Hisama, F. M., Novella, S., Tyrrell, L., Marshall, L., & Waxman, S. G. (2005). Gain-of-function mutation in Nav1.7 in familial erythromelalgia induces bursting of sensory neurons. *Brain*, 128(Pt 8), 1847–1854.
- Durek, T., Vetter, I., Wang, C. I., Motin, L., Knapp, O., Adams, D. J., Lewis, R. J., & Alewood, P. F. (2013). Chemical engineering and structural and pharmacological characterization of the alpha-scorpion toxin OD1. *ACS Chemical Biology*, 8(6), 1215–1222.
- Eagles, D. A., Chow, C. Y., & King, G. F. (2022). Fifteen years of Nav 1.7 channels as an analgesic target: Why has excellent in vitro pharmacology not translated into in vivo analgesic efficacy? *British Journal of Pharmacology*, 179(14), 3592–3611.
- Eisenberg, V. H., Weil, C., Chodick, G., & Shalev, V. (2018). Epidemiology of endometriosis: A large population-based database study from a healthcare provider with 2 million members. *BJOG: An International Journal of Obstetrics and Gynaecology*, 125(1), 55–62.
- Erickson, A., Deiteren, A., Harrington, A. M., Garcia-Caraballo, S., Castro, J., Caldwell, A., Grundy, L., & Brierley, S. M. (2018). Voltage-gated

- sodium channels: (NaV)igating the field to determine their contribution to visceral nociception. *The Journal of Physiology*, 596(5), 785–807.
- Forster, R., Sarginson, A., Velichkova, A., Hogg, C., Dorning, A., Horne, A. W., Saunders, P. T. K., & Greaves, E. (2019). Macrophage-derived insulin-like growth factor-1 is a key neurotrophic and nerve-sensitizing factor in pain associated with endometriosis. *The FASEB Journal*, 33(10), 11210–11222.
- Francois-Moutal, L., Dustrude, E. T., Wang, Y., Brustovetsky, T., Dorame, A., Ju, W., Moutal, A., Perez-Miller, S., Brustovetsky, N., Gokhale, V., Khanna, M., & Khanna, R. (2018). Inhibition of the Ubc9 E2 SUMO-conjugating enzyme-CRMP2 interaction decreases Nav1.7 currents and reverses experimental neuropathic pain. *Pain*, 159(10), 2115–2127.
- Goldberg, Y. P., Price, N., Namdari, R., Cohen, C. J., Lamers, M. H., Winters, C., Price, J., Young, C. E., Verschoof, H., Sherrington, R., Pimstone, S. N., & Hayden, M. R. (2012). Treatment of Nav1.7-mediated pain in inherited erythromelalgia using a novel sodium channel blocker. *Pain*, 153(1), 80–85.
- Greaves, E., Grieve, K., Horne, A. W., & Saunders, P. T. (2014). Elevated peritoneal expression and estrogen regulation of nociceptive ion channels in endometriosis. *The Journal of Clinical Endocrinology and Metabolism*, 99(9), E1738–E1743.
- Grundy, L., Erickson, A., & Brierley, S. M. (2019). Visceral pain. *Annual Review of Physiology*, 81, 261–284.
- Grundy, L., Erickson, A., Caldwell, A., Garcia-Caraballo, S., Rychkov, G., Harrington, A., & Brierley, S. M. (2018). Tetrodotoxin-sensitive voltage-gated sodium channels regulate bladder afferent responses to distension. *Pain*, 159(12), 2573–2584.
- Grundy, L., Harrington, A. M., Castro, J., Garcia-Caraballo, S., Deiteren, A., Madder, J., Rychkov, G. Y., Ge, P., Peters, S., Feil, R., Miller, P., Ghatti, A., Hannig, G., Kurtz, C. B., Silos-Santiago, I., & Brierley, S. M. (2018). Chronic linacotide treatment reduces colitis-induced neuroplasticity and reverses persistent bladder dysfunction. *JCI Insight*, 3(19), e121841.
- Hameed, S. (2019). Nav1.7 and Nav1.8: Role in the pathophysiology of pain. *Molecular Pain*, 15, 1744806919858801.
- Harrington, A. M., Brierley, S. M., Isaacs, N., Hughes, P. A., Castro, J., & Blackshaw, L. A. (2012). Sprouting of colonic afferent central terminals and increased spinal mitogen-activated protein kinase expression in a mouse model of chronic visceral hypersensitivity. *The Journal of Comparative Neurology*, 520(10), 2241–2255.
- Hockley, J. R., Gonzalez-Cano, R., McMurray, S., Tejada-Giraldez, M. A., McGuire, C., Torres, A., Wilbrey, A. L., Cibert-Goton, V., Nieto, F. R., Pitcher, T., Knowles, C. H., Baeyens, J. M., Wood, J. N., Winchester, W. J., Bulmer, D. C., Cendán, C. M., & McMurray, G. (2017). Visceral and somatic pain modalities reveal Nav1.7-independent visceral nociceptive pathways. *The Journal of Physiology*, 595(8), 2661–2679.
- Hughes, P. A., Castro, J., Harrington, A. M., Isaacs, N., Moretta, M., Hicks, G. A., Urso, D. M., & Brierley, S. M. (2014). Increased κ -opioid receptor expression and function during chronic visceral hypersensitivity. *Gut*, 63(7), 1199–1200.
- Insera, M. C., Israel, M. R., Caldwell, A., Castro, J., Deuis, J. R., Harrington, A. M., Karamidas, A., Garcia-Caraballo, S., Madder, J., Erickson, A., Grundy, L., Rychkov, G. Y., Zimmermann, K., Lewis, R. J., Brierley, S. M., & Vetter, I. (2017). Multiple sodium channel isoforms mediate the pathological effects of Pacific ciguatera-1. *Scientific Reports*, 7, 42810.
- Isensee, J., Krahe, L., Moeller, K., Pereira, V., Sexton, J. E., Sun, X., Emery, E., Wood, J. N., & Hucho, T. (2017). Synergistic regulation of serotonin and opioid signaling contributes to pain insensitivity in Nav1.7 knockout mice. *Science Signaling*, 10(461), eaah4874.
- Israel, M. R., Tanaka, B. S., Castro, J., Thongyoo, P., Robinson, S. D., Zhao, P., Deuis, J. R., Craik, D. J., Durek, T., Brierley, S. M., Waxman, S. G., Dib-Hajj, S. D., & Vetter, I. (2019). Nav 1.6 regulates excitability of mechanosensitive sensory neurons. *The Journal of Physiology*, 597(14), 3751–3768.
- Jiang, Y., Castro, J., Blomster, L. V., Agwa, A. J., Madder, J., Schober, G., Herzig, V., Chow, C. Y., Cardoso, F. C., Demétrio de Souza França, P., Gonzales, J., Schroeder, C. I., Esche, S., Reiner, T., Brierley, S. M., & King, G. F. (2021). Pharmacological inhibition of the voltage-gated Sodium Channel Nav1.7 alleviates chronic visceral pain in a rodent model of irritable bowel syndrome. *ACS Pharmacology and Translational Science*, 4(4), 1362–1378.
- King, G. F., & Vetter, I. (2014). No gain, no pain: Nav1.7 as an analgesic target. *ACS Chemical Neuroscience*, 5(9), 749–751.
- Koninckx, P. R., Ussia, A., Adamyan, L., Wattiez, A., Gomel, V., & Martin, D. C. (2019). Pathogenesis of endometriosis: The genetic/epigenetic theory. *Fertility and Sterility*, 111(2), 327–340.
- Kotecha, M., Cheshire, W. P., Finnigan, H., Giblin, K., Naik, H., Palmer, J., Tate, S., & Zakrzewska, J. (2020). Design of phase 3 studies evaluating vixotrigine for treatment of trigeminal neuralgia. *Journal of Pain Research*, 13, 1601–1609.
- Lee, M. C., Nahorski, M. S., Hockley, J. R. F., Lu, V. B., Ison, G., Pattison, L. A., Callejo, G., Stouffer, K., Fletcher, E., Brown, C., Drissi, I., Wheeler, D., Ernfors, P., Mef, D., Reimann, F., Smith, E. S. J., & Woods, C. G. (2020). Human labor pain is influenced by the voltage-gated Potassium Channel K(V)6.4 subunit. *Cell Reports*, 32(3), 107941.
- Li, J., Stratton, H. J., Lorca, S. A., Grace, P. M., & Khanna, R. (2022). Small molecule targeting Nav1.7 via inhibition of the CRMP2-Ubc9 interaction reduces pain in chronic constriction injury (CCI) rats. *Channels (Austin, Tex.)*, 16(1), 1–8.
- Madder, J., Grundy, L., Castro, J., & Brierley, S. M. (2020). Pain in endometriosis. *Front Cellular Neuroscience*, 14, 590823.
- Madder, J., Grundy, L., Harrington, A., Schober, G., Castro, J., & Brierley, S. M. (2022). A syngeneic inoculation mouse model of endometriosis that develops multiple comorbid visceral and cutaneous pain like behaviours. *Pain*, 163(8), 1622–1635.
- Maertens, C., Cuypers, E., Amininasab, M., Jalali, A., Vatanpour, H., & Tytgat, J. (2006). Potent modulation of the voltage-gated sodium channel Nav1.7 by OD1, a toxin from the scorpion *Odonthobuthus doriae*. *Molecular Pharmacology*, 70(1), 405–414.
- McAllister, S. L., McGinty, K. A., Resuehr, D., & Berkley, K. J. (2009). Endometriosis-induced vaginal hyperalgesia in the rat: Role of the ectopic growths and their innervation. *Pain*, 147(1–3), 255–264.
- Michel, M. C., & Igawa, Y. (2015). Therapeutic targets for overactive bladder other than smooth muscle. *Expert Opinion on Therapeutic Targets*, 19(5), 687–705.
- Minett, M. S., Pereira, V., Sikandar, S., Matsuyama, A., Lollignier, S., Kanellopoulos, A. H., Mancini, F., Iannetti, G. D., Bogdanov, Y. D., Santana-Varela, S., Millet, Q., Baskozos, G., MacAllister, R., Cox, J. J., Zhao, J., & Wood, J. N. (2015). Endogenous opioids contribute to insensitivity to pain in humans and mice lacking sodium channel Nav1.7. *Nature Communications*, 6, 8967.
- Mueller, A., Starobova, H., Morgan, M., Dekan, Z., Cheneval, O., Schroeder, C. I., Alewood, P. F., Deuis, J. R., & Vetter, I. (2019). Antiallodynic effects of the selective Nav1.7 inhibitor Pn3a in a mouse model of acute postsurgical pain: Evidence for analgesic synergy with opioids and baclofen. *Pain*, 160(8), 1766–1780.
- Muratspahic, E., Tomasevic, N., Koehbach, J., Duerrauer, L., Hadzic, S., Castro, J., Schober, G., Sideromenos, S., Clark, R. J., Brierley, S. M., Craik, D. J., & Gruber, C. W. (2021). Design of a stable cyclic peptide analgesic derived from sunflower seeds that targets the kappa-opioid receptor for the treatment of chronic abdominal pain. *Journal of Medicinal Chemistry*, 64(13), 9042–9055.
- Sadeghi, M., Erickson, A., Castro, J., Deiteren, A., Harrington, A. M., Grundy, L., Adams, D. J., & Brierley, S. M. (2018). Contribution of membrane receptor signalling to chronic visceral pain. *The International Journal of Biochemistry & Cell Biology*, 98, 10–23.
- Salvatierra, J., Castro, J., Erickson, A., Li, Q., Braz, J., Gilchrist, J., Grundy, L., Rychkov, G. Y., Deiteren, A., Rais, R., King, G. F., Slusher, B. S., Basbaum, A., Pasricha, P. J., Brierley, S. M., & Bosmans, F. (2018).



- NaV1.1 inhibition can reduce visceral hypersensitivity. *JCI. Insight*, 3(11), e121000.
- Siebenga, P., van Amerongen, G., Hay, J. L., McDonnell, A., Gorman, D., Butt, R., & Groeneveld, G. J. (2020). Lack of detection of the analgesic properties of PF-05089771, a selective nav 1.7 inhibitor, using a battery of pain models in healthy subjects. *Clinical and Translational Science*, 13(2), 318–324.
- Stratton, P., & Berkley, K. J. (2011). Chronic pelvic pain and endometriosis: Translational evidence of the relationship and implications. *Human Reproduction Update*, 17(3), 327–346.
- Vetter, I., Deuis, J. R., Mueller, A., Israel, M. R., Starobova, H., Zhang, A., Rash, L. D., & Mobli, M. (2017). Na(V)1.7 as a pain target—from gene to pharmacology. *Pharmacology & Therapeutics*, 172, 73–100.
- Wang, C., Wang, Z., Yang, Y., Zhu, C., Wu, G., Yu, G., Jian, T., Yang, N., Shi, H., Tang, M., He, Q., Lan, L., Liu, Q., Guan, Y., Dong, X., Duan, J., & Tang, Z. (2015). Pirt contributes to uterine contraction-induced pain in mice. *Molecular Pain*, 11, 57.
- Zakrzewska, J. M., Palmer, J., Morisset, V., Giblin, G. M., Obermann, M., Ettlin, D. A., Cruccu, G., Bendtsen, L., Estacion, M., Derjean, D., Waxman, S. G., Layton, G., Gunn, K., Tate, S., & study investigators. (2017). Safety and efficacy of a Nav1.7 selective sodium channel blocker in patients with trigeminal neuralgia: A double-blind, placebo-controlled, randomised withdrawal phase 2a trial. *Lancet Neurology*, 16(4), 291–300.
- Zito, G., Luppi, S., Giolo, E., Martinelli, M., Venturin, I., Di Lorenzo, G., & Ricci, G. (2014). Medical treatments for endometriosis-associated pelvic pain. *BioMed Research International*, 2014:191967, 1–12.
- Zondervan, K. T., Becker, C. M., Koga, K., Missmer, S. A., Taylor, R. N., & Vigano, P. (2018). Endometriosis. *Nature Reviews Disease Primers*, 4(1), 9.

How to cite this article: Castro, J., Maddern, J., Chow, C. Y., Tran, P., Vetter, I., King, G. F., & Brierley, S. M. (2024). The voltage-gated sodium channel Na_v1.7 underlies endometriosis-associated chronic pelvic pain. *Journal of Neurochemistry*, 168, 3760–3776. <https://doi.org/10.1111/jnc.15795>