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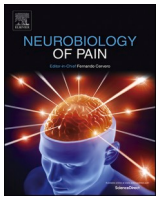


Original Research

# Delta opioid receptors in Nav1.8 expressing peripheral neurons partially regulate the effect of delta agonist in models of migraine and opioid-induced hyperalgesia

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ABSTRACT

Migraine is one of the most common pain disorders and causes disability in millions of people every year. Delta opioid receptors (DOR) have been identified as a novel therapeutic target for migraine and other headache disorders. DORs are present in both peripheral and central regions and it is unclear which receptor populations regulate migraine-associated effects. The aim of this study was to determine if DOR expressed in peripheral nociceptors regulates headache associated endpoints and the effect of delta agonists within these mouse models. We used a conditional knockout, in which DOR was selectively deleted from Nav1.8 expressing cells. Nav1.8-DOR mice and loxP control littermates were tested in models of chronic migraine-associated allodynia, opioid-induced hyperalgesia, migraine-associated negative affect, and aura. Nav1.8-DOR and loxP mice had comparable effect sizes in all of these models. The anti-allodynic effect of the DOR agonist, SNC80, was slightly diminished in the nitroglycerin model of migraine. Intriguingly, in the OIH model the peripheral effects of SNC80 were completely lost in Nav1.8-DOR mice while the cephalic effects remained intact. Regardless of genotype, SNC80 continued to inhibit conditioned place aversion associated with nitroglycerin and decreased cortical spreading depression events associated with migraine aura. These results suggest that DOR in Nav1.8-expressing nociceptors do not critically regulate the anti-migraine effects of delta agonist; and that brain-penetrant delta agonists would be a more effective drug development strategy.

## Introduction

Migraine is one of most prevalent neurological disorders and a leading cause of disability worldwide (Steiner et al., 2020; Vos et al., 2020). While there have been many recent breakthroughs in new migraine treatments (Ashina et al., 2021), many migraine patients find current pharmacotherapies ineffective or poorly tolerated (Lipton et al., 2019). The delta opioid receptor (DOR) has emerged as a promising therapeutic target for migraine (Pradhan, 2014). Delta agonists are effective in acute and chronic models of migraine-associated allodynia (Pradhan, 2014; Moye et al., 2019; Dripps et al., 2020; Moye et al., 2021; Bertels et al., 2021) and in models of other headache disorder including medication overuse headache (MOH) and post-traumatic headache (PTH) (Moye et al., 2019; Moye et al., 2019). In addition, delta agonists significantly inhibit cortical spreading depression (CSD) (Pradhan, 2014; Dripps et al., 2020; Bertels et al., 2021), considered to be a physiological correlate of migraine aura. Interestingly, repeated treatment with the commonly used delta agonist, SNC80, produced limited MOH or opioid induced hyperalgesia (OIH) relative to morphine or the acute migraine therapy, sumatriptan (Moye et al., 2019). These studies have resulted in the development of a delta agonist for migraine, TRV250, which completed a Phase I clinical trial and showed high safety and tolerability (Fossler et al., 2020).

How delta agonists produce anti-migraine effects is not entirely clear. DORs are highly expressed in both the peripheral and central nervous systems (Pradhan et al., 2011; Mansour et al., 1988). The trigeminovascular system plays a critical role in the regulation of migraine (Goadsby et al., 2017). Neurons within the trigeminal ganglia innervate the cerebral vasculature, dura, and facial skin (Edvinsson et al., 2020). These neurons terminate in the trigeminal nucleus caudalis (TNC) which sends projections to the thalamus, cortex, and reticular sites (Edvinsson et al., 2020). A recent study examining the expression of DOR in the

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trigeminal complex showed that delta receptors were expressed in neurons within trigeminal ganglia and TNC, and that these receptors were upregulated in a model of chronic migraine-associated pain (Moye et al., 2021). Rat and non-human primates also shows DOR expression within neurons innervating the dura (Rice et al., 2017), and DOR is also expressed in the TG and TNC of human post-mortem tissue (Mennicken et al., 2003). However, DORs are also highly expressed in central regions that regulate symptoms of headache/pain, including the somatosensory cortex, hippocampus, and striatum (Pradhan et al., 2011; Mansour et al., 1988). In one study, ablation of DOR from forebrain GABAergic neurons resulted in the complete loss of anti-migraine effects of the delta agonis, SNC80 (Dripps et al., 2020). It is therefore unclear what the specific contribution of peripherally restricted delta receptors are in migraine.

The aim of this study was to investigate the role of delta opioid receptors in peripheral ganglia on migraine-associated behaviors. We used a conditional knockout mouse in which delta opioid receptors were selectively deleted from Nav1.8 expressing cells (Gaveriaux-Ruff et al., 2011). Nav1.8 is a voltage-gated sodium channel, that was previously found to be expressed in $>$ 90 % of peripheral nociceptors as well as a number of mechanosensors (Shields et al., 2012). Initial characterization showed that Nav1.8-DOR conditional knockout mice expressed $\sim$ 50–70 % less DOR in small to medium diameter neurons of dorsal root ganglia compared to floxed controls (Gaveriaux-Ruff et al., 2011). In the periphery, this knockout results in enhanced mechanical and cold allodynia in response to inflammatory or neuropathic pain conditions. Importantly, in peripheral models of pain induced by Complete Freund's Adjuvant (CFA) or spared nerve ligation (SNL) the anti-allodynic effects of SNC80 are lost in Nav1.8-DOR mice (Gaveriaux-Ruff et al., 2011; Nozaki, 2012). In the current study, we tested Nav1.8-DOR in models of chronic migraine associated-pain, -negative affect, and -aura; as well as in a model of opioid induced hyperalgesia (OIH). These models are mechanistically distinct from the peripheral pain models previously tested, and the results suggest that peripheral delta opioid receptors play a less crucial role in headache disorders.

## Methods

### *Animals*

Mice, aged 8–12 weeks, were housed in a temperature and humidity-controlled colony room with a 12 h light/dark cycle. Groups of mice were housed together with a maximum of 5 animals/cage. Mouse cages were made of clear polypropylene with corn cob bedding and food and water were available *ad libitum*. A mixture of male and female mice were used in all experiments. Nav1.8-DOR mice were provided by B. Kieffer (McGill University). Littermate DOR loxP mice were used as controls. All animal procedures were previously approved by the guidelines of the University of Illinois at Chicago Animal Care Committee, which is AAALAC approved. Behavioral tests were done in a randomized fashion and all experimenters were blinded to the groups.

### *Materials*

Drugs were administered in a volume of 10 mL/kg. SNC80 (Tocris Bioscience, Pittsburgh, PA) was dissolved in 0.9 % saline with 2 μl of 1 M HCl per mg SNC80. Nitroglycerin (NTG) was obtained as a 5 mg/mL stock solution (30 % ethanol, 30 % propylene glycol in water; American Regent, Shirley, NY) and diluted to 1 mg/mL in 0.9 % saline both drugs were administered intraperitoneally (IP). Morphine (Hospira Inc, Lakeforest, IL) was diluted in 0.9 % saline and administered subcutaneously (SC).

### *Quantitative RT-PCR*

Procedures used in this study follow those described previously (Dripps et al., 2020; Ben Aissa et al., 2018). Briefly, RNA was isolated from flash frozen tissue brain punches or whole trigeminal ganglia. A RNeasy Mini kit from Qiagen was used throughout this procedure and the accompanying protocol was followed. RNA samples were reverse transcribed to single-stranded cDNA. cDNA transcription was used following the protocol from Superscript III (Life Technologies) and the TaqMan Gene Expression Assay System (Applied Biosystems). To confirm quality of the RNA sample, we always ran a qbit assay to determine the amount and quality of RNA and monitored the melt curves of the qPCR to ensure regular PCR events. Glyceraldehyde-3-phosphate dehydrogenase was used as the control housekeeping gene (GAPDH-5′-CAA TGT GTC CGT CGT GGA TCT-3′ 5′-GTC CTC AGT GTA GCC CAA GATG-3′). ORPD1 was the gene of interest (′-GCT CGT CAT GTT TGG CAT C-3′ 5′-AAG TAC TTG GCG CTC TGG AA-3′). Threshold cycle (CT) of the ORPD1 gene was determined and compared to the housekeeping gene of the same sample to calculate difference (ΔCT). The fold change (2-ΔΔCT) for each was calculated relative to the median ΔCT from the loxP control littermates. We did not observe any difference in GAPDH cycle number between loxP and Nav1.8-DOR mice in any of the regions tested (TG: loxP 19.27 $\pm$ 0.64, Nav1.8 18.59 $\pm$ 0.31; TNC: loxP 18.02 $\pm$ 0.5, Nav1.8 18.51 $\pm$ 0.41; STR loxP 19.66 $\pm$ 033, Nav1.8 19.63 $\pm$ 0.2).

### *Mechanical sensitivity testing*

Mechanical responses were determined as described previously (Dripps et al., 2020; Pradhan et al., 2014). Mice were habituated to the testing rack 1–2 h a day for 2 days prior to testing. For cephalic measurements, mice were also habituated in a 4 oz paper cup placed in the testing box. The up-and-down method was utilized to determine response to acute mechanical stimuli. For cephalic measurements, the periorbital region caudal to the eyes near the midline of the head was stimulated, while the plantar surface of the left hindpaw was tested for all peripheral measurements. A series of 8 manual von Frey filaments ranging from 0.008 to 2 g were used in these experiments. Cephalic responses were characterized as repeated shaking or ducking of the head, vigorous grooming, or biting at the instrument. Hindpaw responses were characterized as lifting, shaking, or biting of the stimulated paw following bending of the filament.

### *NTG induced migraine model*

NTG was administered as previously described (Pradhan et al., 2014). NTG was administered every other day for 9 days (days 1,3,5,7, and 9). Basal mechanical thresholds were assessed prior to NTG injection, and post-treatment responses 2 h post-NTG. For hindpaw responses mice were tested every day of NTG/VEH treatment. For cephalic responses they were only tested on days 1,5, and 9.

### *Opioid induced hyperalgesia*

To establish OIH mice were injected SC twice daily with vehicle or morphine (20 mg/kg/injection days 1–3 and 40 mg/kg/injection day 4) (Dripps et al., 2020; Moye et al., 2019). Injections occurred at 9AM and 5PM. Periorbital mechanical thresholds were assessed prior to the first morphine injection on days 1 and 3. On day 5, basal thresholds were measured in the AM prior to injection of SNC80 or vehicle. Mechanical thresholds were assessed again 45 min post-SNC80. On day 8, hindpaw hypersensitivity was measured according to the same protocol as day 5. Individual mice received the same drug regimen on days 5 and 8, and no drug was given days 6 and 7.

### *Conditioned place aversion (CPA)*

We used a 2-chamber conditioning paradigm. The conditioning apparatus was comprised of two equal dimensioned compartments with a guillotine door separating the two halves. The floor textures and wall

designs of each compartment were distinct to differentiate between the two sides. Live video tracking was collected using a DMK 22AUC03 USB 2.0 monochrome industrial camera (The Imaging Source, Charlotte, NC), and analyzed using ANY-Maze software (Stoelting, Wood Dale, IL). CPA experiments were conducted over 4 days. Day one was the pre-conditioning day in which mice were randomly placed in one of the two compartments and allowed to freely explore for 20 min. A biased design was used such that the test compounds were given in the preferred compartments. Day two was the conditioning day which consisted of two sessions. In the first session, mice received saline injections 1h15min apart. Thirty minutes after the second saline injection mice were confined to the non-preferred chamber for 30 min. In the second session, mice received injections of NTG (10 mg/kg, IP) or vehicle and 1h15min after administration they received SNC80 (5 mg/kg, IP) or vehicle. Thirty minutes following SNC80 mice were placed in their preferred chamber for 30 min (1:45–2:15 time post NTG). On day 3 a state independent test was conducted in which mice were allowed to freely move around both compartments for 20 min drug/treatment free. Preference scores were calculated through time spent in the conditioned chamber on the test day minus the time spent in the same chamber on the pre-conditioning day.

### *Cortical spreading depression*

The procedure we used in this study was based on previous work (Dripps et al., 2020; Bertels et al., 2021; Bertels et al., 2021). Mice were anesthetized with isoflurane (induction 3–4 %; maintenance 0.75 to 1.25 %; in 67 % $N_2$ / 33 % $O_2$) and placed in a stereotaxic frame on a homeothermic heating pad. Core temperature, non-peripheral oxygen saturation, heart rate, and respiratory rate were continually monitored (PhysioSuite; Kent Scientific Instrument's, Torrington, CT, USA). To ensure proper anesthetic depth mice were regularly given tail and hindpaw pinches. CSD events were verified through both optical intrinsic imaging (OIS) and local field potential recordings (Pradhan, 2014; Dripps et al., 2020). Following induction the scalp was opened, a small area of the skull was thinned to make a cortical window (~0.5 mm from sagittal, and ~ 1.4 from coronal and lambdoid sutures), and mineral oil was applied to allow for greater visualization. A green LED (530 nm) light was used to illuminate the skull throughout the CSD experiment. For imaging, a camera and lens (HR Plan Apo 0.5 Å~ WD 136) through a 515LP emission filter on a Nikon SMZ 1500 stereomicroscope (Nikon Instruments, Melville, NY, USA) were used. Images were acquired at 1–5 Hz using a high-sensitivity USB monochrome CCD camera (CCE-B013-U; Mightex, Pleasanton, CA, USA) with 4.65-μm square pixels and 1392–1040 pixel resolution. Two burr holes were drilled lateral to the window. These burr holes were deeper than the previously thinned skull region, such that the dura was exposed but was not damaged. In the caudal burr hole a pulled glass pipette filled with saline was inserted to record local field potentials (LFP). This electrode was connected to a ground wire placed underneath the skin, caudal to the skull, which was then connected to an amplifier. Following insertion of the electrode an hour of background activity was recorded and monitored. This allowed stabilization in the event that a CSD occurred during surgery. Baselines were recorded for an hour after which a glass pipette filled with 1 M KCl was placed into the rostral burr hole such that it was not in direct contact with the skull or brain tissue. An initial drop of KCl was formed and then an even flow of KCl was maintained, but closely monitored to prevent overflow. After 400 s from the initial KCl drip mice were injected with vehicle or SNC80 (10 mk/kg, IP) and recorded for 1 h. Only mice that showed at least 2 CSDs in the first 400 s were included in the analysis. Video and subsequent LFP recordings were used to confirm CSD counts.

### *Data analysis*

All values in the text are reported as mean ± SEM. All data and statistical analysis were performed using GraphPad Prism. The level of significance (α) for all tests was set to 0.05. Post-hoc analysis was conducted using the Holm Sidak post-hoc analysis to correct for multiple comparisons. Post-hoc analysis was only performed when a significant interaction occurred (F values achieved $p < 0.05$). Experiments were designed to have an equal n/group. However, groups were sometimes slightly unequal to accommodate for using all animals in a litter. Statistical analysis for each figure is reported in the figure legend. The following number of mice were used in each experiment: qPCR: 32, NTG: 79, OIH: 66, CPA: 85, CSD: 25.

## Results

### *Nav1.8 conditional knockout reduces delta receptor expression in trigeminal ganglia*

Conditional knockout of DOR from peripheral nociceptors was achieved by crossing floxed DOR mice with a Nav1.8-Cre line, as was previously described (Gaveriaux-Ruff et al., 2011). As we were investigating the role of delta receptors in headache disorders, we examined the effect of Nav1.8-DOR on delta receptors in trigeminal ganglia (TG), trigeminal nucleus caudalis (TNC), and we used the striatum as a control region. We compared Nav1.8-DOR and littermate floxed controls. We observed a ~ 50 % decrease in *Oprd1* mRNA in peripheral trigeminal ganglia of Nav1.8-DOR mice relative to controls (Fig. 1A). In contrast, there was no significant difference in *Oprd1* expression in the TNC or striatum of Nav1.8-DOR mice compared to loxP (Fig. 1 B,C). These results confirm that Nav1.8-DOR showed a conditional knockout of delta receptors from primarily peripheral sensory neurons, similar to a previously published report (Gaveriaux-Ruff et al., 2011).

### *Nav1.8-DOR knockout does not alter responding in a model of chronic migraine-associated pain*

We have previously shown that in wildtype and in DOR loxp mice, SNC80 effectively inhibited acute and chronic allodynia induced by NTG (Dripps et al., 2020; Moye et al., 2019; Pradhan et al., 2009; Pradhan et al., 2014; Moye, 2021). We next determined if knockout of delta receptors from Nav1.8 expressing cells affected NTG-induced allodynia and the anti-allodynic effects of the delta agonist, SNC80, in this model. Mice were treated in an established model of chronic migraine (Pradhan et al., 2014) which uses the known human migraine trigger nitroglycerin (NTG) (Ashina et al., 2017). Mice were injected with vehicle or NTG (10 mg/kg IP) every other day for 9 days (Fig. 2A). Separate groups of mice were tested for cephalic (periorbital) and peripheral (hindpaw) responses. On test days mice were assessed before NTG administration (basal responses) and 2 h post-injection (post-treatment responses). Both Nav1.8-DOR mice and loxP littermate controls developed periorbital allodynia to similar degrees in response to acute and chronic administration of 10 mg/kg NTG (Fig. 2B, clear symbols). Treatment with 10 mg/kg SNC80 prevented the development of basal periorbital allodynia following chronic intermittent NTG in both genotypes (Fig. 2B, filled symbols). This cephalic effect of SNC80 appears to be partially diminished in the Nav1.8-DOR knockout mice, however this difference was not statistically significant. When measured 2 h after NTG administration, SNC80 inhibited acute NTG-induced allodynia similarly in both genotypes (Fig. 2C). In the hindpaw, chronic NTG also produced significant chronic allodynia (Fig. 2D, clear symbols), which was inhibited by SNC80 in both genotypes (Fig. 2D, filled symbols). At the 2 h timepoint post-NTG, SNC80 also inhibited acute peripheral allodynia induced by NTG (Fig. 2E), but statistical analysis revealed that this effect was significantly diminished in Nav1.8-DOR mice, relative to loxP controls. These data demonstrate that DOR in Nav1.8 cells do not affect the development or severity of NTG-induced allodynia; and that this conditional knockout results in a small decrease in the peripheral anti-

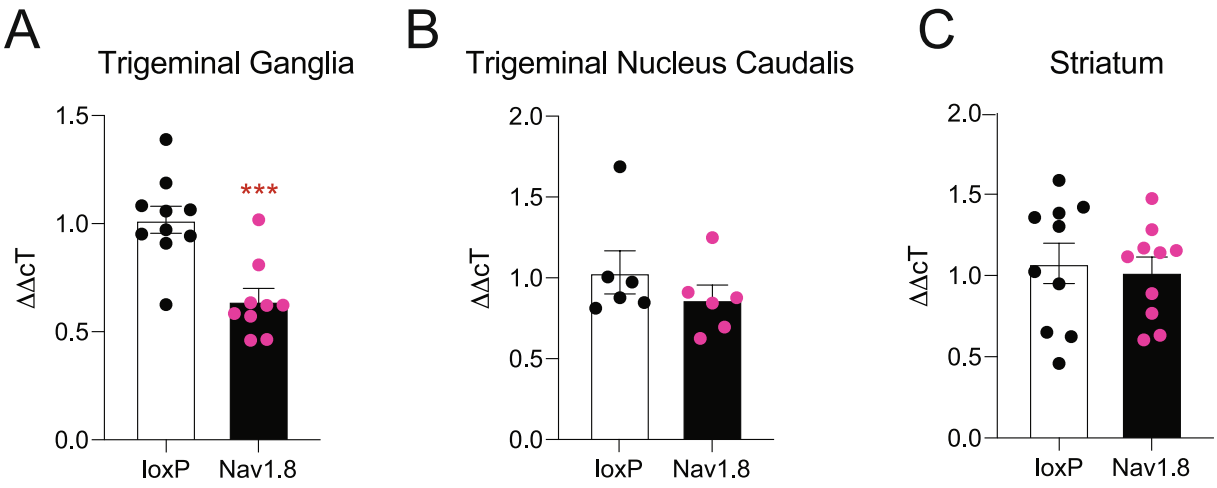


**Fig. 1.** Expression of delta receptors in Nav1.8-DOR conditional knockout mice. Quantitative RT-PCR was performed on tissue from the (A) trigeminal ganglia, (B) trigeminal nucleus caudalis, and (C) striatum. Tissue was taken from adult Nav1.8-DOR mice and loxP litter mate controls. Corrected t-tests showed a significant reduction of *Oprd1* only in trigeminal ganglia (***$p < 0.001$). n = 6–10/genotype.

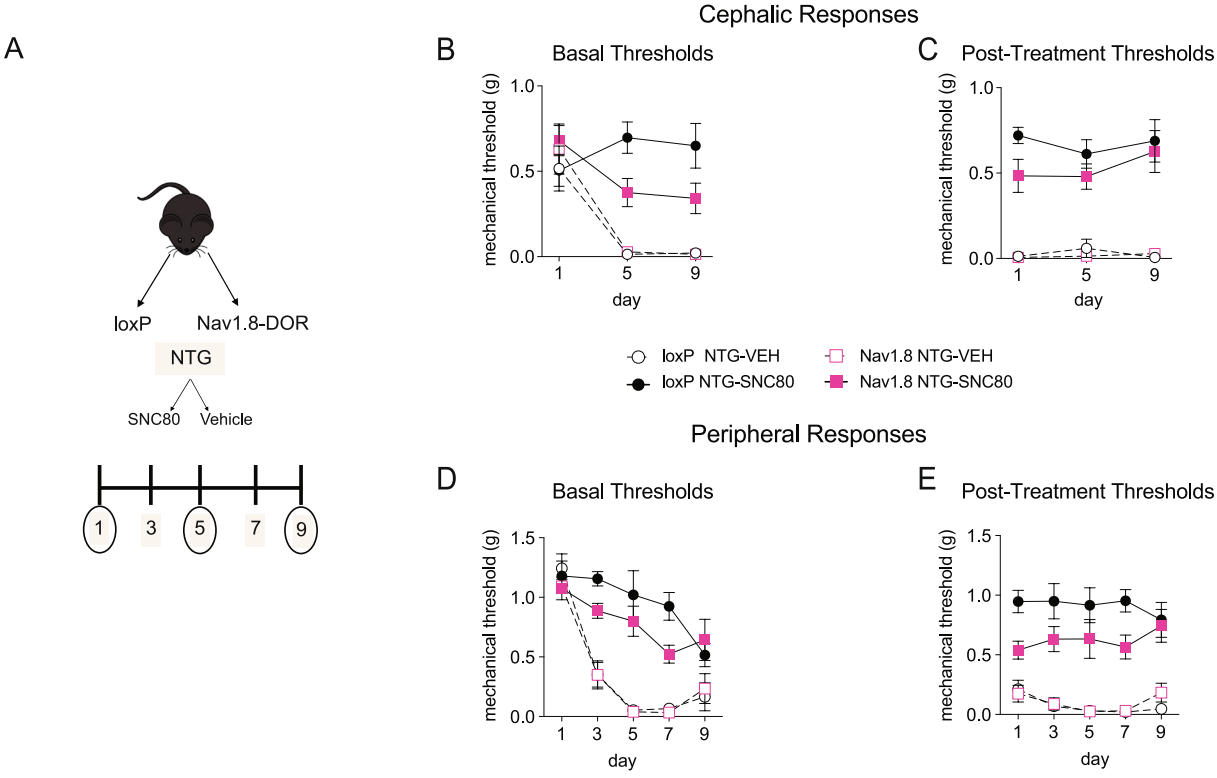


**Fig. 2.** Role of peripheral DOR in NTG-induced allodynia. (A) Experimental design – All mice were injected with NTG (10 mg/kg IP), and 1h15min later with vehicle or SNC80 (10 mg/kg IP), every other day for 9 days. Separate groups of mice were used to determine periorbital (B,C) or hindpaw (D,E) responses. Cephalic responses were tested on days 1,5, and 9 (ovals), and peripheral testing occurred on each treatment day (shading). Mechanical thresholds were determine before NTG administration (Basal Responses; B,D), and 2 h post-NTG (Post-treatment Responses; C,E). (B) Administration of NTG produced robust chronic periorbital allodynia by day 5 in the loxP group that was not present in the SNC80 treated group. Three-way ANOVA revealed significant effects of time ($p < 0.001$), treatment ($p < 0.001$), timeXtreatment ($p < 0.001$), and timeXgenotype ($p = 0.03$). There was also a trend for a genotypeXtreatment effect ($p = 0.069$). (C) Post-treatment measures 2 h after NTG/45 min after SNC80 revealed that SNC80 inhibited the acute cephalic allodynic effects of NTG in Nav1.8-DOR mice and loxP controls. Three-way repeated measured ANOVA, significant effect of treatment ($p < 0.001$), n = 6/group. (D) NTG produced chronic peripheral allodynia in both groups of mice despite genotype that was inhibited by administration of SNC80. Three-way repeated measures ANOVA, significant effects of time ($p < 0.001$), genotype ($p = 0.042$), treatment ($p < 0.001$), and interaction of timeXtreatment ($p < 0.001$) n = 7–9. (E) NTG produced significant acute hindpaw allodynia, and the inhibitory effects of SNC80 were partially lost in Nav1.8-DOR. Three-way repeated measures ANOVA, significant effect of genotype ($p = 0.004$), treatment ($p < 0.001$), and genotypeXtreatment ($p = 0.001$). n = 7–9/group.

allodynic effects of SNC80 in this migraine model.

*Nav1.8-DOR show altered responding in peripheral but not cephalic endpoints associated with opioid induced hyperalgesia.*

We have previously shown that SNC80 can block both peripheral and cephalic endpoints in a model of opioid induced hyperalgesia (OIH) (Moye et al., 2019). We next tested the effect of Nav1.8-DOR within this paradigm. Mice were treated SC twice daily with vehicle or 20 mg/kg/injection morphine on days 1–3 and 40 mg/kg/injection on day 4 (Fig. 3A). Mice were tested for cephalic responses on days 1 and 3 of the morphine paradigm treatment. On days 5 and 8 mice were administered SNC80 and had cephalic (Day 5) and hindpaw (Day 8) responses assessed. Chronic morphine produced significant cephalic allodynia in both Nav1.8-DOR and loxP controls (Fig. 3B, day 3), and this allodynia was still present 15–18 h following the final morphine injection (Fig. 3B, day 5). On day 5 mice were challenged with either vehicle or SNC80 (5 mg/kg, IP) and cephalic responses were determined 45 min later. SNC80 significantly reversed cephalic allodynia induced by chronic morphine, regardless of genotype (Fig. 3C). The same cohort of mice were challenged with vehicle or SNC80 on day 8 (4 days after the final morphine treatment), and peripheral/hindpaw responses were determined. At this time point mice previously treated with morphine continued to show allodynia (Fig. 3D, morphine-vehicle). SNC80 significantly reversed this peripheral allodynia in loxp mice. However, SNC80 had no effect in the Nav1.8-DOR mice when tested in the hindpaw. These data suggest that in a model of OIH, peripheral DOR are necessary for the peripheral antiallodynic effects of DOR agonist but not the cephalic effects.

*Nav1.8-DOR do not show altered responses in a model of migraine-associated negative affect*

SNC80 was previously found to block conditioned place aversion (CPA) induced by NTG (Pradhan, 2014; Dripps et al., 2020). We sought to determine if this effect was affected by knockout of delta receptors from Nav1.8 expressing cells (Fig. 4A). In both loxP controls and Nav1.8-DOR, conditioning with 10 mg/kg NTG produced a robust place aversion that was blocked by treatment with 5 mg/kg SNC80 (Fig. 4B). In addition, SNC80 in the absence of NTG did not cause any preference in either genotype. These results demonstrate that DOR in peripheral Nav1.8 expressing cells are not necessary for regulating the negative affective state induced by NTG, or the alleviating effects of delta agonist in this model.

*Nav1.8-DOR do not show altered responses in cortical spreading depression, a model of migraine aura.*

The ability for substances to reduce the number of cortical spreading depression (CSD) events has been used as an effective method for predicting migraine preventive medications (Ayata et al., 2006; Bogdanov et al., 2016). DOR agonists, including SNC80, have been shown to reduce CSD events (Pradhan, 2014; Bertels et al., 2021). We next determined if knockout of DOR from Nav1.8 expressing cells altered CSD. We triggered CSD events by applying a constant amount of

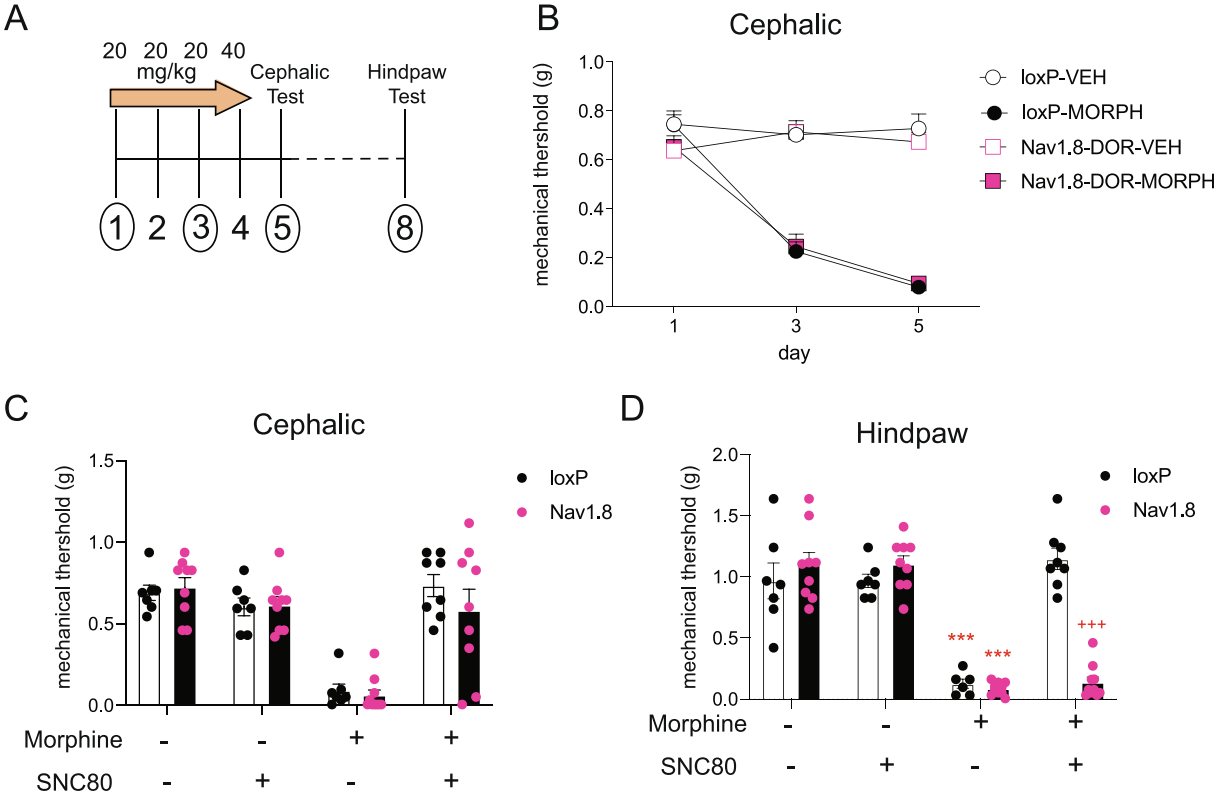


**Fig. 3.** Chronic opioid administration produced allodynia that was reversed by SNC80 in cephalic but not hindpaw regions in Nav1.8-DOR mice. (A) Animals were treated twice daily for 4 days with Vehicle or Morphine (20 mg/kg SC/injection on days 1–3 and 40 mg/kg SC /injection on day 4). Mice were subsequently tested for mechanical allodynia on days 1, 3, and 5 for cephalic responses and on day 8 for hindpaw responses. (B) Morphine induced cephalic allodynia in both genotypes. Three-way repeated measures ANOVA, significant effect of time ($p < 0.001$), drug ($p < 0.001$), and timeXdrug interaction ($p < 0.001$). (C) Morphine produced significant cephalic allodynia that was reversed by treatment with SNC80 in both genotypes. Three-way ANOVA, significant effect of treatment ($p < 0.001$), drug ($p < 0.001$), and treatmentXdrug interaction ($p < 0.001$). (D) Morphine-induced allodynia was still observed in both genotypes on day 8 in the hindpaw, and this allodynia was reversed by SNC80 in only the loxP mice. Three-way ANOVA, significant effect of genotype ($p = 0.002$), treatment ($p < 0.001$), drug ($p < 0.001$), genotypeXdrug ($p < 0.001$), treatmentxdrug ($p < 0.001$), and genotypextreatmentxdrug ($p < 0.001$). ***$p < 0.001$ relative to mice treated with vehicle for 4 days; +++ relative to loxP (morphine-SNC80 groups). n = 7–10 mice/group.

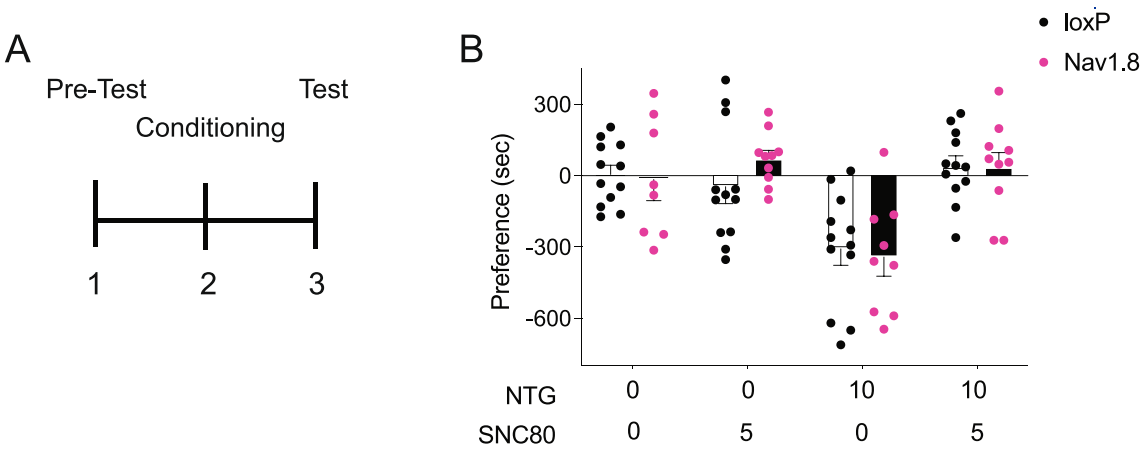


**Fig. 4.** Role of peripheral delta receptors in NTG-induced CPA. (A) Mice freely explored both chambers during the pre-conditioning phase (day 1). On day 2, mice were conditioned to the preferred side with NTG or Vehicle and SNC80 or Vehicle. On day 3 mice were tested and allowed to freely explore both chambers. (B) Conditioning with 10 mg/kg NTG (IP) produced CPA in all genotypes. Conditioning with SNC80 (5 mg/kg IP) alone produced no significant preference or aversion, but when paired with NTG, SNC80 inhibited NTG induced CPA. Three-way ANOVA, significant effect of treatment (NTG/VEH; $p < 0.01$), drug ($p < 0.001$) and treatmentxdrug interaction ($p < 0.001$). n = 8–12/group.

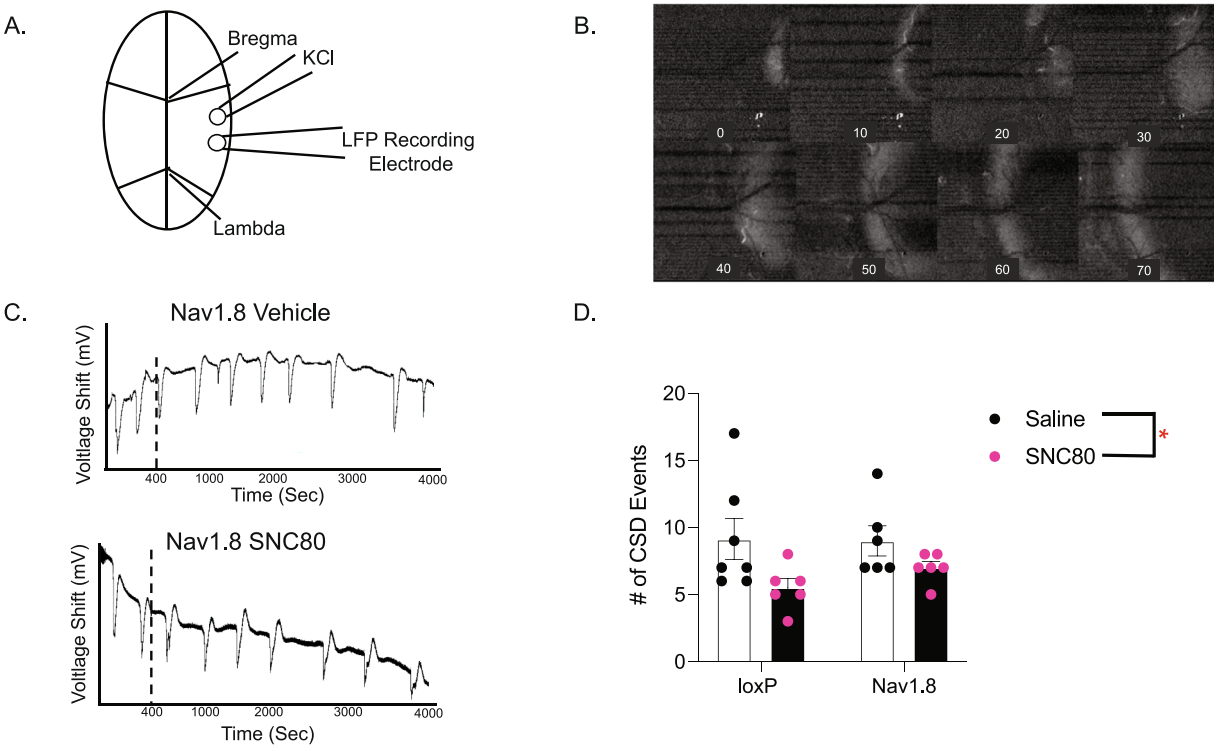


**Fig. 5.** SNC80 maintains its ability to reduce CSD events despite knockout of delta receptors from Nav1.8 cells. (A) Schematic of CSD induction. A thinned skull preparation was used. Two burr holes were drilled for KCl administration or local field potential (LFP) recording . (B) Schematic montage of what a CSD wave looks like as it passes across the cortex (C) Representative line tracing showing CSD events over an hour recording with Vehicle or SNC80 administration at 400 s. (D) SNC80 significantly reduced the number of CSD events, 2-way ANOVA, significant effect of drug ($p = 0.018$). n = 6–7.

suprathreshold KCl onto the dura while optical intrinsic signals and local field potentials (LFP) were recorded (Fig. 5A,B). Saline or SNC80 (10 mg/kg IP) was injected 400 s after initial KCl application and CSD events were recorded for 1 h following administration (Fig. 5C). LoxP controls and Nav1.8-DOR mice showed a comparable number of CSD events in response to KCl (Fig. 5D, clear bars). SNC80 significantly decreased the number of CSD events in both genotypes (Fig. 5D, dark bars). These findings demonstrate that delta receptors in Nav1.8 cells do not affect cortical excitability associated with migraine, or the inhibitory effects of delta agonist in this model.

## Discussion

In this study, we investigated the relative contributions of DORs expressed in peripheral nociceptors to the regulation of various headache-associated endpoints. Specifically, we evaluated how this receptor population affects the ability of SNC80 to inhibit allodynia in models of chronic migraine and OIH/medication overuse headache, NTG-induced negative affect, and CSD. Nav1.8-DOR mice showed a ~ 50 % decrease in *Oprd1* transcript in trigeminal ganglia, which is in keeping with the high expression of Nav1.8 in this region (Agarwal

et al., 2004). In addition, we did not observe a decrease in DOR expression in central regions such as the trigeminal nucleus caudalis or striatum. Knockout of DOR from peripheral nociceptors did not significantly impact allodynia induced by chronic NTG or morphine. In the NTG model of chronic migraine, SNC80 continued to be effective regardless of region tested, although there was a small decrease in its effect peripherally in Nav1.8-DOR. In contrast, the anti-allodynic effects of SNC80 were completely lost in the periphery in the OIH model, but cephalic responses remained intact. Nav1.8-DOR and loxP controls responded similarly in a model of migraine-associated negative affect, and in response to CSD. The effect of delta agonist was not altered in these more centrally-mediated behaviors in Nav1.8-DOR mice. Collectively, these data suggest that peripheral DOR alone only partially regulate the anti-migraine properties of delta agonists.

In inflammatory and neuropathic peripheral pain models knockout of DOR from Nav1.8 expressing cells results in enhanced allodynia (Gaveriaux-Ruff et al., 2011). In a model of chronic migraine-associated pain or after chronic morphine, Nav1.8-DOR and loxP control mice developed comparable mechanical allodynia in cephalic and peripheral regions. This allodynia was similar to that observed in wildtype C57BL6 mice in response to these agents (Pradhan, 2014; Moye et al., 2019; Pradhan et al., 2014). These data suggest that unlike inflammatory or neuropathic pain, NTG and morphine-induced allodynia is not heavily regulated by a peripheral tone of endogenous opioids acting at delta receptors. NTG and OIH is mechanistically distinct from CFA or SNL models. For example, mice do not easily recover from CFA or SNL lesion, while their mechanical responses will return to naïve baselines within 7–10 days following cessation of NTG or morphine. It is also possible that a difference could be detected between genotypes if a less severe/lower doses of NTG or morphine were tested, and this is an active area of study. In addition, Nav1.8-DOR developed conditioned place aversion to NTG and were comparable to loxP animals in response to KCl-induced CSD events. Together these data indicate that DOR in Nav1.8 cells do not regulate outcomes in these migraine related models.

The anti-allodynic effects of delta agonists were substantially reduced or lost in Nav1.8-DOR in inflammatory and neuropathic models of pain (Gaveriaux-Ruff et al., 2011; Nozaki, 2012). In the current study, SNC80 effectively inhibited acute and chronic allodynia in the NTG model in both Nav1.8-DOR and loxP controls. Further, an anti-allodynic effect of SNC80 was still observed in both hindpaw and periorbital regions, although there was a slight decrease in effect in Nav1.8-DOR. It is possible that DOR in non-Nav1.8 expressing peripheral neurons contribute to the anti-allodynic effect of SNC80 in this model. We observed a ~ 50 % reduction in *Oprd1* gene expression in TGs of Nav1.8-DOR mice. Nav1.8 is primarily expressed on nociceptors (Stirling et al., 2005), and has also been shown on some but not all mechanosensors (Shields et al., 2012). Delta receptors are expressed on medium to large diameter neurons in the DRG (Bardoni et al., 2014) and TG (Moye, 2021). Approximately 40 % of DOR positive TGs, express the pro-migraine neuropeptide (Edvinsson and Goadsby, 2019), calcitonin gene related peptide (CGRP) (Moye, 2021). Not all CGRP expressing cells in the TG express Nav1.8 (Lindquist et al., 2021), and it is possible that these cells may also express DOR. In this case, activation of delta receptors by SNC80 could inhibit NTG-induced allodynia by decreasing CGRP release. Another possibility is that the anti-allodynic effects of SNC80 in this migraine model occurs through delta receptors in the central nervous system. To support this idea, we have previously shown that knockout of DOR from forebrain GABAergic neurons results in a complete loss of the effects of SNC80 within the NTG model (Dripps et al., 2020). Nevertheless, this study shows a clear distinction from inflammatory and neuropathic pain states in which SNC80 effects were lost in Nav1.8-DOR mice.

Chronic opioid use can result in OIH which has been observed in peripheral pain patients as well as in individuals suffering from opioid use disorder (Yi and Pryzbylkowski, 2015). Opioids are still commonly prescribed for migraine (Lipton et al., 2020), and can result in increased migraine severity and frequency (Bigal and Lipton, 2009). This form of OIH is known as medication overuse headache (MOH) (HIS, 2018), and is very difficult to treat. We have previously shown that a well characterized OIH paradigm can cause allodynia in both hindpaw and cephalic regions (Moye et al., 2019). The current study revealed that there is a mechanistic distinction between these two regional endpoints. In Nav1.8-DOR, SNC80 effectively reversed cephalic allodynia induced by chronic morphine, similar to loxP controls. Contrastingly, the effects of SNC80 were completely lost in this model when the hindpaw was tested. These results indicate that chronic opioid use could result in distinct adaptations at peripheral and central pain processing sites, which may be differentially regulated by delta receptors. Cephalic OIH may be regulated by sensitization of sites within the central nervous system, including the trigeminal nucleus caudalis. In contrast, peripheral OIH may be more strongly governed by changes in peripheral ganglia, especially dorsal root ganglia to spinal cord processing. Opioids have been shown to induce nociceptor neuroplasticity (Khomula et al., 2019), which may be more important for peripheral versus cephalic OIH. Future studies will focus on delineating these differential mechanisms.

The conditioned place aversion paradigm is used to reflect the negative affective state induced by the human migraine trigger, NTG (Pradhan, 2014; Dripps et al., 2020). Nav1.8-DOR and loxP control mice developed comparable levels of aversion to NTG. We have previously shown that knockout of delta receptors from forebrain GABAergic neurons (Dlx-DOR) also does not affect the development of CPA (Dripps et al., 2020). These results suggest that endogenous signaling at delta receptors does not regulate this negative affective state, nor does knockout of this receptor alter memory associated with aversion learning. We also observed that knockout of DOR from Nav1.8 expressing neurons did not alter the anti-aversive effects of SNC80, and both loxP and Nav1.8-DOR mice failed to produce CPA when conditioned with both NTG in combination with SNC80. These results contrast with Dlx-DOR, where deletion of forebrain delta receptors resulted in a complete loss of the effects of SNC80 in this assay. These data show that delta receptors in peripheral nociceptors are largely unnecessary for regulating the affective components of migraine, while those in the forebrain play a more crucial role.

Approximately-one third of migraine patients experience migraine aura symptoms (Charles and Baca, 2013). Cortical spreading depression is widely viewed as the electrophysiological correlate of migraine aura (Charles and Baca, 2013; Brennan and Pietrobon, 2018). Previously, drugs that can reduce the number of CSD events were found to be an indicator of effectiveness as a migraine preventive (Ayata et al., 2006). Along this line SNC80 and a G protein biased delta agonist, KNT-127, were both found to effectively reduce CSD events (Pradhan, 2014; Bertels et al., 2021). Here we find that despite peripheral knockout of delta receptors, SNC80 continued to inhibit CSD. Although SNC80 was still effective in this model, it is possible that peripheral delta receptors could regulate behavioral endpoints induced by CSD. For example, CSD can activate neurons throughout the migraine pathway, including in peripheral trigeminal ganglia (Zhang et al., 2011; Zhang et al., 2010). Accordingly, CSD events can result in photophobia, cephalic allodynia, and anxiety (Tang et al., 2020; Harriott et al., 2021). Future studies will determine the effect of peripheral delta receptor knockout on behavioral outcomes evoked by CSD.

Together, our data show that delta receptors in Nav1.8 expressing neurons are not crucial in the regulation of migraine-associated symptoms or for the anti-migraine effects of delta agonist. Intriguingly, our data also reveal that opioid induced hyperalgesia (peripheral) and opioid-induced medication overuse headache (cephalic) are regulated by different sub-populations of delta receptors. Delta agonists are currently being developed for the treatment of migraine (Fossler et al., 2020) and other pain conditions (Conibear et al., 2020). Our data suggest that targeting of peripheral delta receptors would be insufficient for the treatment of migraine; and that brain-penetrant delta agonists would be a more successful drug development strategy.

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The authors declare no conflicts of interest.

## Author contributions

ZB, ID, and AAP designed experiments, ZB, ID, LM, AT, PS, KS performed experiments, and ZB, ID, and AAP analyzed data and wrote the manuscript.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References

Agarwal, N., Offermanns, S., Kuner, R., 2004. Conditional gene deletion in primary nociceptive neurons of trigeminal ganglia and dorsal root ganglia. Genesis 38 (3), 122–129.

Ashina, M., Hansen, J.M., á Dunga, B.O., Olesen, J., 2017. Human models of migraine - short-term pain for long-term gain. Nat. Rev. Neurol. 13 (12), 713–724.

Ashina, M., Buse, D.C., Ashina, H., Pozo-Rosich, P., Peres, M.F.P., Lee, M.J., Terwindt, G. M., Halker Singh, R., Tassorelli, C., Do, T.P., Mitsikostas, D.D., Dodick, D.W., 2021. Migraine: integrated approaches to clinical management and emerging treatments. Lancet 397 (10283), 1505–1518.

Ayata, C., Jin, H., Kudo, C., Dalkara, T., Moskowitz, M.A., 2006. Suppression of Cortical Spreading Depression in Migraine Prophylaxis. Ann. Neurol. 59 (4), 652–661.

Bardoni, R., Tawfik, V., Wang, D., François, A., Solorzano, C., Shuster, S., Choudhury, P., Betelli, C., Cassidy, C., Smith, K., de Nooij, J., Mennicken, F., O'Donnell, D., Kieffer, B., Woodbury, C.J., Basbaum, A., MacDermott, A., Scherrer, G., 2014. Delta opioid receptors presynaptically regulate cutaneous mechanosensory neuron input to the spinal cord dorsal horn. Neuron 81 (6), 1312–1327.

Ben Aissa, M., *Soluble guanylyl cyclase is a critical regulator of migraine-associated pain.* 2018. **38**(8): p. 1471-1484.

Bertels, Z., Singh, H., Dripps, I., Siegersma, K., Tipton, A.F., Witkowski, W.D., Sheets, Z., Shah, P., Conway, C., Mangutov, E., Ao, M., Petukhova, V., Karumudi, B., Petukhov, P.A., Baca, S.M., Rasenick, M.M., Pradhan, A.A., 2021. Neuronal complexity is attenuated in preclinical models of migraine and restored by HDAC6 inhibition. eLife 10.

Bertels, Z., Witkowski, W.D., Asif, S., Siegersma, K., Rijn, R.M., Pradhan, A.A., 2021. A non-convulsant delta-opioid receptor agonist, KNT-127, reduces cortical spreading depression and nitroglycerin-induced allodynia. Headache 61 (1), 170–178.

Bigal, M.E., Lipton, R.B., 2009. Excessive opioid use and the development of chronic migraine. Pain 142 (3), 179–182.

Bogdanov, V.B., Middleton, N.A., Theriot, J.J., Parker, P.D., Abdullah, O.M., Ju, Y.S., Hartings, J.A., Brennan, K.C., 2016. Susceptibility of Primary Sensory Cortex to Spreading Depolarizations. J. Neurosci. 36 (17), 4733–4743.

Brennan, K.C., Pietrobon, D., 2018. A Systems Neuroscience Approach to Migraine. Neuron 97 (5), 1004–1021.

Charles, A.C., Baca, S.M., 2013. Cortical spreading depression and migraine. Nat. Rev. Neurol. 9 (11), 637–644.

Conibear, A.E., Asghar, J., Hill, R., Henderson, G., Borbely, E., Tekus, V., Helyes, Z., Palandri, J., Bailey, C., Starke, I., von Mentzer, B., Kendall, D., Kelly, E., 2020. A Novel G Protein-Biased Agonist at the δ Opioid Receptor with Analgesic Efficacy in Models of Chronic Pain. J. Pharmacol. Exp. Ther. 372 (2), 224–236.

Dripps, I.J., Bertels, Z., Moye, L.S., Tipton, A.F., Siegersma, K., Baca, S.M., Kieffer, B.L., Pradhan, A.A., 2020. Forebrain delta opioid receptors regulate the response of delta agonist in models of migraine and opioid-induced hyperalgesia. Sci. Rep. 10 (1).

Edvinsson, L., Goadsby, P.J., 2019. Discovery of CGRP in relation to migraine. Cephalalgia 39 (3), 331–332.

Edvinsson, J.C.A., Viganò, A., Alekseeva, A., Alieva, E., Arruda, R., De Luca, C., D'Ettore, N., Frattale, I., Kurnukhina, M., Macerola, N., Malenkova, E., Maiorova, M., Novikova, A., Řehulka, P., Rapaccini, V., Roshchina, O., Vanderschueren, G., Zvaune, L., Andreou, A.P., Haanes, K.A., 2020. The fifth cranial nerve in headaches. J Headache Pain 21 (1).

Fossler, M.J., Schmith, V., Greene, S.A., Lohmer, L., Kramer, M.S., Arscott, K., James, I. E., Demitrack, M.A., 2020. A Phase I, Randomized, Single-Blind, Placebo-Controlled, Single Ascending Dose Study of the Safety, Tolerability, and Pharmacokinetics of Subcutaneous and Oral TRV250, a G Protein-Selective Delta Receptor Agonist, in Healthy Subjects. CNS Drugs 34 (8), 853–865.

Gaveriaux-Ruff, C., Nozaki, C., Nadal, X., Hever, X.C., Weibel, R., Matifas, A., Reiss, D., Filliol, D., Nassar, M.A., Wood, J.N., Maldonado, R., Kieffer, B.L., 2011. Genetic ablation of delta opioid receptors in nociceptive sensory neurons increases chronic pain and abolishes opioid analgesia. Pain 152 (6), 1238–1248.

Goadsby, P.J., Holland, P.R., Martins-Oliveira, M., Hoffmann, J., Schankin, C., Akerman, S., 2017. Pathophysiology of Migraine: A Disorder of Sensory Processing. Physiol. Rev. 97 (2), 553–622.

Harriott, A.M., Chung, D.Y., Uner, A., Bozdayi, R.O., Morais, A., Takizawa, T., Qin, T., Ayata, C., 2021. Optogenetic Spreading Depression Elicits Trigeminal Pain and Anxiety Behavior. Ann. Neurol. 89 (1), 99–110.

Headache Classification Committee of the International Headache Society (IHS) The International Classification of Headache Disorders, 3rd ed. Cephalalgia, 2018. **38**(1): p. 1-211.

Khomula, E.V., Araldi, D., Levine, J.D., 2019. In Vitro Nociceptor Neuroplasticity Associated with In Vivo Opioid-Induced Hyperalgesia. J. Neurosci. 39 (36), 7061–7073.

Lindquist, K.A., Belugin, S., Hovhannisyan, A.H., Corey, T.M., Salmon, A., Akopian, A.N., 2021. Identification of Trigeminal Sensory Neuronal Types Innervating Masseter Muscle. eNeuro 8 (5).

Lipton, R.B., Munjal, S., Buse, D.C., Alam, A., Fanning, K.M., Reed, M.L., Schwedt, T.J., Dodick, D.W., 2019. Unmet Acute Treatment Needs From the 2017 Migraine in America Symptoms and Treatment Study. Headache 59 (8), 1310–1323.

Lipton, R.B., Buse, D.C., Friedman, B.W., Feder, L., Adams, A.M., Fanning, K.M., Reed, M. L., Schwedt, T.J., 2020. Characterizing opioid use in a US population with migraine: Results from the CaMEO study. Neurology 95 (5), e457–e468.

Mansour, A., Khachaturian, H., Lewis, M.E., Akil, H., Watson, S.J., 1988. Anatomy of CNS opioid receptors. Trends Neurosci. 11 (7), 308–314.

Mennicken, F., Zhang, J.i., Hoffert, C., Ahmad, S., Beaudet, A., O'Donnell, D., 2003. Phylogenetic changes in the expression of delta opioid receptors in spinal cord and dorsal root ganglia. J. Comp. Neurol. 465 (3), 349–360.

Moye, L.S., et al., 2021. Delta opioid receptor regulation of calcitonin gene-related peptide dynamics in the trigeminal complex. Pain 162 (8), 2297–2308.

Moye, L.S., et al., 2019. Delta opioid receptor agonists are effective for multiple types of headache disorders. Neuropharmacology 148, 77–86.

Moye, L.S., Novack, M.L., Tipton, A.F., Krishnan, H., Pandey, S.C., Pradhan, A.AA., 2019. The development of a mouse model of mTBI-induced post-traumatic migraine, and identification of the delta opioid receptor as a novel therapeutic target. Cephalalgia 39 (1), 77–90.

Moye, L.S., Siegersma, K., Dripps, I., Witkowski, W., Mangutov, E., Wang, D., Scherrer, G., Pradhan, A.A., 2021. Delta opioid receptor regulation of CGRP dynamics in the trigeminal complex. Publish Ahead of Print Pain.

Nozaki, C., et al., 2012. delta-Opioid mechanisms for ADL5747 and ADL5859 effects in mice: analgesia, locomotion, and receptor internalization. J. Pharmacol. Exp. Ther. 342 (3), 799–807.

Pradhan, A.A., et al., 2014. delta-Opioid receptor agonists inhibit migraine-related hyperalgesia, aversive state and cortical spreading depression in mice. Br. J. Pharmacol. 171 (9), 2375–2384.

Pradhan, A.A.A., Becker, J.A.J., Scherrer, G., Tryoen-Toth, P., Filliol, D., Matifas, A., Massotte, D., Gavériaux-Ruff, C., Kieffer, B.L., Steinhardt, R., 2009. In vivo delta opioid receptor internalization controls behavioral effects of agonists. PLoS One 4 (5), e5425.

Pradhan, A.A., Befort, K., Nozaki, C., Gavériaux-Ruff, C., Kieffer, B.L., 2011. The delta opioid receptor: an evolving target for the treatment of brain disorders. Trends Pharmacol. Sci. 32 (10), 581–590.

Pradhan, A.A., et al., 2014. Characterization of a novel model of chronic migraine. Pain 155 (2), 269–274.

Rice, F.L., Xie, J.Y., Albrecht, P.J., Acker, E., Bourgeois, J., Navratilova, E., Dodick, D.W., Porreca, F., 2017. Anatomy and immunochemical characterization of the non-arterial peptidergic diffuse dural innervation of the rat and Rhesus monkey: Implications for functional regulation and treatment in migraine. Cephalalgia 37 (14), 1350–1372.

Shields, S.D., Ahn, H.-S., Yang, Y., Han, C., Seal, R.P., Wood, J.N., Waxman, S.G., Dib-Hajj, S.D., 2012. Nav1.8 expression is not restricted to nociceptors in mouse peripheral nervous system. Pain 153 (10), 2017–2030.

Steiner, T.J., Stovner, L.J., Jensen, R., Uluduz, D., Katsarava, Z., 2020. Migraine remains second among the world's causes of disability, and first among young women: findings from GBD2019. J. Headache Pain 21 (1).

Stirling, C.L., Forlani, G., Baker, M.D., Wood, J.N., Matthews, E.A., Dickenson, A.H., Nassar, M.A., 2005. Nociceptor-specific gene deletion using heterozygous NaV1.8-Cre recombinase mice. Pain 113 (1), 27–36.

Tang, C., Unekawa, M., Kitagawa, S., Takizawa, T., Kayama, Y., Nakahara, J., Shibata, M., 2020. Cortical spreading depolarisation-induced facial hyperalgesia, photophobia and hypomotility are ameliorated by sumatriptan and olcegepant. Sci. Rep. 10 (1).

Vos, T., Lim, S.S., Abbafati, C., Abbas, K.M., Abbasi, M., Abbasifard, M., Abbasi-Kangevari, M., Abbastabar, H., Abd-Allah, F., Abdelalim, A., Abdollahi, M., Abdollahpour, I., Abolhassani, H., Aboyans, V., Abrams, E.M., Abreu, L.G., Abrigo, M.R.M., Abu-Raddad, L.J., Abushouk, A.I., Acebedo, A., Ackerman, I.N., Adabi, M., Adamu, A.A., Adebayo, O.M., Adekanmbi, V., Adelson, J.D., Adetokunboh, O.O., Adham, D., Afshari, M., Afshin, A., Agardh, E.E., Agarwal, G., Agesa, K.M., Aghaali, M., Aghamir, S.M.K., Agrawal, A., Ahmad, T., Ahmadi, A., Ahmadi, M., Ahmadieh, H., Ahmadpour, E., Akalu, T.Y., Akinyemi, R.O., Akinyemiju, T., Akombi, B., Al-Aly, Z., Alam, K., Alam, N., Alam, S., Alam, T., Alanzi, T.M., Albertson, S.B., Alcalde-Rabanal, J.E., Alema, N.M., Ali, M., Ali, S., Alicandro, G., Alijanzadeh, M., Alinia, C., Alipour, V., Aljunid, S.M., Alla, F., Allebeck, P., Almasi-Hashiani, A., Alonso, J., Al-Raddadi, R.M., Altirkawi, K.A., Alvis-Guzman, N., Alvis-Zakzuk, N.J., Amini, S., Amini-Rarani, M., Aminorroaya, A., Amiri, F., Amit, A.M.L., Amugsi, D.A., Amul, G.G.H., Anderlini, D., Andrei, C.L., Andrei, T., Anjomshoa, M., Ansari, F., Ansari, I., Ansari-Moghaddam, A., Antonio, C. A.T., Antony, C.M., Antriyandarti, E., Anvari, D., Anwer, R., Arabloo, J., Arab-

Zozani, M., Aravkin, A.Y., Ariani, F., Ärnlöv, J., Aryal, K.K., Arzani, A., Asadi-Aliabadi, M., Asadi-Pooya, A.A., Asghari, B., Ashbaugh, C., Atnafu, D.D., Atre, S.R., Ausloos, F., Ausloos, M., Ayala Quintanilla, B.P., Ayano, G., Ayanore, M.A., Aynalem, Y.A., Azari, S., Azarian, G., Azene, Z.N., Babaee, E., Badawi, A., Bagherzadeh, M., Bakhshaei, M.H., Bakhtiari, A., Balakrishnan, S., Balalla, S., Balassyano, S., Banach, M., Banik, P.C., Bannick, M.S., Bante, A.B., Baraki, A.G., Barboza, M.A., Barker-Collo, S.L., Barthelemy, C.M., Barua, L., Barzegar, A., Basu, S., Baune, B.T., Bayati, M., Bazmandegan, G., Bedi, N., Beghi, E., Béjot, Y., Bello, A.K., Bender, R.G., Bennett, D.A., Bennitt, F.B., Bensenor, I.M., Benziger, C.P., Berhe, K., Bernabe, E., Bertolacci, G.J., Bhageerathy, R., Bhala, N., Bhandari, D., Bhardwaj, P., Bhattacharyya, K., Bhutta, Z.A., Bibi, S., Biehl, M.H., Bikbov, B., Bin Sayeed, M.S., Biondi, A., Birihane, B.M., Bisanzio, D., Bisignano, C., Biswas, R.K., Bohlouli, S., Bohluli, M., Bolla, S.R.R., Boloor, A., Boon-Dooley, A.S., Borges, G., Borzì, A.M., Bourne, R., Brady, O.J., Brauer, M., Brayne, C., Breitborde, N.J.K., Brenner, H., Briant, P.S., Briggs, A.M., Briko, N.I., Britton, G.B., Bryazka, D., Buchbinder, R., Bumgarner, B.R., Busse, R., Butt, Z.A., Caetano dos Santos, F.L., Cámera, L.LA.A., Campos-Nonato, I.R., Car, J., Cárdenas, R., Carreras, G., Carrero, J.J., Carvalho, F., Castaldelli-Maia, J.M., Castañeda-Orjuela, C.A., Castelpietra, G., Castle, C.D., Castro, F., Catalá-López, F., Causey, K., Cederroth, C.R., Cercy, K.M., Cerin, E., Chandan, J.S., Chang, A.R., Charlson, F.J., Chattu, V.K., Chaturvedi, S., Chimed-Ochir, O., Chin, K.L., Cho, D.Y., Christensen, H., Chu, D.-T., Chung, M.T., Cicuttini, F.M., Ciobanu, L.G., Cirillo, M., Collins, E.L., Compton, K., Conti, S., Cortesi, P.A., Costa, V.M., Cousin, E., Cowden, R.G., Cowie, B.C., Cromwell, E.A., Cross, D.H., Crowe, C.S., Cruz, J.A., Cunningham, M., Dahlawi, S.M.A., Damiani, G., Dandona, L., Dandona, R., Darwesh, A.M., Daryani, A., Das, J.K., Das Gupta, R., das Neves, J., Dávila-Cervantes, C.A., Davletov, K., De Leo, D., Dean, F.E., DeCleene, N. K., Deen, A., Degenhardt, L., Dellavalle, R.P., Demeke, F.M., Demsie, D.G., Denova-Gutiérrez, E., Dereje, N.D., Dervenis, N., Desai, R., Desalew, A., Dessie, G.A., Dharmaratne, S.D., Dhungana, G.P., Dianatinasab, M., Diaz, D., Dibaji Forooshani, Z. S., Dingels, Z.V., Dirac, M.A., Djalalinia, S., Do, H.T., Dokova, K., Dorostkar, F., Doshi, C.P., Doshmangir, L., Douiri, A., Doxey, M.C., Driscoll, T.R., Dunachie, S.J., Duncan, B.B., Duraes, A.R., Eagan, A.W., Ebrahimi Kalan, M., Edvardsson, D., Ehrlich, J.R., El Nahas, N., El Sayed, I., El Tantawi, M., Elbarazi, I., Elgendy, I.Y., Elhabashy, H.R., El-Jaafary, S.I., Elyazar, I.RF., Emamian, M.H., Emmons-Bell, S., Erskine, H.E., Eshrati, B., Eskandarieh, S., Esmaeilnejad, S., Esmaeilzadeh, F., Esteghamati, A., Estep, K., Etemadi, A., Etisso, A.E., Farahmand, M., Faraj, A., Fareed, M., Faridnia, R., Farinha, C.S.e.S., Farioli, A., Faro, A., Faruque, M., Farzadfar, F., Fattahi, N., Fazlzadeh, M., Feigin, V.L., Feldman, R., Fereshtehnejad, S.-M., Fernandes, E., Ferrari, A.J., Ferreira, M.L., Filip, I., Fischer, F., Fisher, J.L., Fitzgerald, R., Flohr, C., Flor, L.S., Foigt, N.A., Folayan, M.O., Force, L. M., Fornari, C., Foroutan, M., Fox, J.T., Freitas, M., Fu, W., Fukumoto, T., Furtado, J. M., Gad, M.M., Gakidou, E., Galles, N.C., Gallus, S., Gamkrelidze, A., Garcia-Basteiro, A.L., Gardner, W.M., Geberemariyam, B.S., Gebrehiwot, A.M., Gebremedhin, K.B., Gebreslassie, A.A.A.A., Gershberg Hayoon, A., Gething, P.W., Ghadimi, M., Ghadiri, K., Ghafourifard, M., Ghajar, A., Ghamari, F., Ghashghaee, A., Ghiasvand, H., Ghith, N., Gholamian, A., Gilani, S.A., Gill, P.S., Gitimoghaddam, M., Giussani, G., Goli, S., Gomez, R.S., Gopalani, S.V., Gorini, G., Gorman, T.M., Gottlich, H.C., Goudarzi, H., Goulart, A.C., Goulart, B.N.G., Grada, A., Grivna, M., Grosso, G., Gubari, M.I.M., Gugnani, H.C., Guimaraes, A.L.S., Guimarães, R.A., Guled, R.A., Guo, G., Guo, Y., Gupta, R., Haagsma, J.A., Haddock, B., Hafezi-Nejad, N., Hafiz, A., Hagins, H., Haile, L.M., Hall, B.J., Halvaei, I., Hamadeh, R.R., Hamagharib Abdullah, K., Hamilton, E.B., Han, C., Han, H., Hankey, G.J., Haro, J. M., Harvey, J.D., Hasaballah, A.I., Hasanzadeh, A., Hashemian, M., Hassanipour, S., Hassankhani, H., Havmoeller, R.J., Hay, R.J., Hay, S.I., Hayat, K., Heidari, B., Heidari, G., Heidari-Soureshjani, R., Hendrie, D., Henrikson, H.J., Henry, N.J., Herteliu, C., Heydarpour, F., Hird, T.R., Hoek, H.W., Hole, M.K., Holla, R., Hoogar, P., Hosgood, H.D., Hosseinzadeh, M., Hostiuc, M., Hostiuc, S., Househ, M., Hoy, D.G., Hsairi, M., Hsieh, V.-R., Hu, G., Huda, T.M., Hugo, F.N., Huynh, C.K., Hwang, B.-F., Iannucci, V.C., Ibitoye, S.E., Ikuta, K.S., Ilesanmi, O.S., Ilic, I.M., Ilic, M.D., Inbaraj, L.R., Ippolito, H., Irvani, S.S.N., Islam, M.M., Islam, MdMohaimenul, Islam, S.M.S., Islami, F., Iso, H., Ivers, R.Q., Iwu, C.C.D., Iyamu, I.O., Jaafari, J., Jacobsen, K.H., Jadidi-Niaragh, F., Jafari, H., Jafarinia, M., Jahagirdar, D., Jahani, M.A., Jahanmehr, N., Jakovljevic, M., Jalali, A., Jalilian, F., James, S.L., Janjani, H., Janodia, M.D., Jayatilleke, A.U., Jeemon, P., Jenabi, E., Jha, R.P., Jha, V., Ji, J.S., Jia, P., John, O., John-Akinola, Y.O., Johnson, C.O., Johnson, S.C., Jonas, J.B., Joo, T., Joshi, A., Jozwiak, J.J., Jürisson, M., Kabir, A., Kabir, Z., Kalani, H., Kalani, R., Kalankesh, L.R., Kalhor, R., Kamiab, Z., Kanchan, T., Karami Matin, B., Karch, A., Karim, M.A., Karimi, S.E., Kassa, G.M., Kassebaum, N.J., Katikireddi, S.V., Kawakami, N., Kayode, G.A., Keddie, S.H., Keller, C., Kereselidze, M., Khafaie, M.A., Khalid, N., Khan, M., Khatab, K., Khater, M.M., Khatib, M.N., Khayamzadeh, M., Khodayari, M.T., Khundkar, R., Kianipour, N., Kieling, C., Kim, D., Kim, Y.-E., Kim, Y.J., Kimokoti, R.W., Kisa, A., Kisa, S., Kissimova-Skarbek, K., Kivimäki, M., Kneib, C.J., Knudsen, A.K.S., Kocarnik, J.M., Kolola, T., Kopec, J.A., Kosen, S., Koul, P.A., Koyanagi, A.i., Kravchenko, M.A., Krishan, K., Krohn, K.J., Kuate Defo, B., Kucuk Bicer, B., Kumar, G.A., Kumar, M., Kumar, P., Kumar, V., Kumaresh, G., Kurmi, O.P., Kusuma, D., Kyu, H.H., La Vecchia, C., Lacey, B., Lal, D.K., Lalloo, R., Lam, J.O., Lami, F.H., Landires, I., Lang, J.J., Lansingh, V.C., Larson, S.L., Larsson, A.O., Lasrado, S., Lassi, Z.S., Lau, K.-M., Lavados, P.M., Lazarus, J.V., Ledesma, J.R., Lee, P.H., Lee, S.W.H., LeGrand, K. E., Leigh, J., Leonardi, M., Lescinsky, H., Leung, J., Levi, M., Lewington, S., Li, S., Lim, L.-L., Lin, C., Lin, R.-T., Linehan, C., Linn, S., Liu, H.-C., Liu, S., Liu, Z., Looker, K.J., Lopez, A.D., Lopukhov, P.D., Lorkowski, S., Lotufo, P.A., Lucas, T.C.D., Lugo, A., Lunevicius, R., Lyons, R.A., Ma, J., MacLachlan, J.H., Maddison, E.R., Maddison, R., Madotto, F., Mahasha, P.W., Mai, H.T., Majeed, A., Maled, V., Maleki, S., Malekzadeh, R., Malta, D.C., Mamun, A.A., Manafi, A., Manafi, N., Manguerra, H., Mansouri, B., Mansournia, M.A., Mantilla Herrera, A.M., Maravilla, J.C., Marks, A., Martins-Melo, F.R., Martopullo, I., Masoumi, S.Z., Massano, J., Massenburg, B.B., Mathur, M.R., Maulik, P.K., McAlinden, C., McGrath, J.J., McKee, M., Mehndiratta, M.M., Mehri, F., Mehta, K.M., Meitei, W.B., Memiah, P.T.N., Mendoza, W., Menezes, R.G., Mengesha, E.W., Mengesha, M.B., Mereke, A., Meretoja, A., Meretoja, T.J., Mestrovic, T., Miazgowski, B., Miazgowski, T., Michalek, I.M., Mihretie, K.M., Miller, T.R., Mills, E.J., Mirica, A., Mirrakhimov, E.M., Mirzaei, H., Mirzaei, M., Mirzaei-Alavijeh, M., Misganaw, A.T., Mithra, P., Moazen, B., Moghadaszadeh, M., Mohamadi, E., Mohammad, D.K., Mohammad, Y., Mohammad Gholi Mezerji, N., Mohammadian-Hafshejani, A., Mohammadifard, N., Mohammadpourhodki, R., Mohammed, S., Mokdad, A.H., Molokhia, M., Momen, N.C., Monasta, L., Mondello, S., Mooney, M.D., Moosazadeh, M., Moradi, G., Moradi, M., Moradi-Lakeh, M., Moradzadeh, R., Moraga, P., Morales, L., Morawska, L., Moreno Velásquez, I., Morgado-da-Costa, J., Morrison, S.D., Mosser, J.F., Mouodi, S., Mousavi, S.M., Mousavi Khaneghah, A., Mueller, U.O., Munro, S.B., Muriithi, M.K., Musa, K.I., Muthupandian, S., Naderi, M., Nagarajan, A.J., Nagel, G., Naghshtabrizi, B., Nair, S., Nandi, A.K., Nangia, V., Nansseu, J.R., Nayak, V.C., Nazari, J., Negoi, I., Negoi, R.I., Netsere, H.B.N., Ngunjiri, J.W., Nguyen, C.T., Nguyen, J., Nguyen, M., Nguyen, M., Nichols, E., Nigatu, D., Nigatu, Y.T., Nikbakhsh, R., Nixon, M.R., Nnaji, C.A., Nomura, S., Norrving, B.o., Noubiap, J.J., Nowak, C., Nunez-Samudio, V., Oţoiu, A., Oancea, B., Odell, C.M., Ogbo, F.A., Oh, I.-H., Okunga, E.W., Oladnabi, M., Olagunju, A.T., Olusanya, B.O., Olusanya, J.O., Oluwasanu, M.M., Omar Bali, A., Omer, M.O., Ong, K.L., Onwujekwe, O.E., Orji, A.U., Orpana, H.M., Ortiz, A., Ostroff, S.M., Otstavnov, N., Otstavnov, S.S., Øverland, S., Owolabi, M.O., P a, M., Padubidri, J.R., Pakhare, A.P., Palladino, R., Pana, A., Panda-Jonas, S., Pandey, A., Park, E.-K., Parmar, P.G.K., Pasupula, D.K., Patel, S.K., Paternina-Caicedo, A.J., Pathak, A., Pathak, M., Patten, S.B., Patton, G.C., Paudel, D., Pazoki Toroudi, H., Peden, A.E., Pennini, A., Pepito, V.C.F., Peprah, E.K., Pereira, A., Pereira, D.M., Perico, N., Pham, H.Q., Phillips, M.R., Pigott, D.M., Pilgrim, T., Pilz, T.M., Pirsaheb, M., Plana-Ripoll, O., Plass, D., Pokhrel, K.N., Polibin, R.V., Polinder, S., Polkinghorne, K.R., Postma, M.J., Pourjafar, H., Pourmalek, F., Pourmirza Kalhori, R., Pourshams, A., Poznańska, A., Prada, S.I., Prakash, V., Pribadi, D.R.A., Pupillo, E., Quazi Syed, Z., Rabiee, M., Rabiee, N., Radfar, A., Rafiee, A., Rafiei, A., Raggi, A., Rahimi-Movaghar, A., Rahman, M.A., Rajabpour-Sanati, A., Rajati, F., Ramezanzadeh, K., Ranabhat, C.L., Rao, P.C., Rao, S.J., Rasella, D., Rastogi, P., Rathi, P., Rawaf, D.L., Rawaf, S., Rawal, L., Razo, C., Redford, S.B., Reiner, R.C., Reinig, N., Reitsma, M.B., Remuzzi, G., Renjith, V., Renzaho, A.M.N., Resnikoff, S., Rezaei, N., Rezai, M.S., Rezapour, A., Rhinehart, P.-A., Riahi, S.M., Ribeiro, A.L.P., Ribeiro, D.C., Ribeiro, D., Rickard, J., Roberts, N.L.S., Roberts, S., Robinson, S.R., Roever, L., Rolfe, S., Ronfani, L., Roshandel, G., Roth, G.A., Rubagotti, E., Rumisha, S.F., Sabour, S., Sachdev, P.S., Saddik, B., Sadeghi, E., Sadeghi, M., Saeidi, S., Safi, S., Safiri, S., Sagar, R., Sahebkar, A., Sahraian, M.A., Sajadi, S.M., Salahshoor, M.R., Salamati, P., Salehi Zahabi, S., Salem, H., Salem, M.R.R., Salimzadeh, H., Salomon, J.A., Salz, I., Samad, Z., Samy, A.M., Sanabria, J., Santomauro, D.F., Santos, I.S., Santos, J.V., Santric-Milicevic, M.M., Saraswathy, S.Y.I., Sarmiento-Suárez, R., Sarrafzadegan, N., Sartorius, B., Sarveazad, A., Sathian, B., Sathish, T., Sattin, D., Sbarra, A.N., Schaeffer, L.E., Schiavolin, S., Schmidt, M.I., Schutte, A.E., Schwebel, D.C., Schwendicke, F., Senbeta, A.M., Senthilkumaran, S., Sepanlou, S.G., Shackelford, K. A., Shadid, J., Shahabi, S., Shaheen, A.A., Shaikh, M.A., Shalash, A.S., Shams-Beyranvand, M., Shamsizadeh, M., Shannawaz, M., Sharafi, K., Sharara, F., Sheena, B.S., Sheikhtaheri, A., Shetty, R.S., Shibuya, K., Shiferaw, W.S., Shigematsu, M., Shin, J.I., Shiri, R., Shirkoohi, R., Shrime, M.G., Shuval, K., Siabani, S., Sigfusdottir, I.D., Sigurvinsdottir, R., Silva, J.P., Simpson, K.E., Singh, A., Singh, J.A., Skiadaresi, E., Skou, S.T.S., Skryabin, V.Y., Sobngwi, E., Sokhan, A., Soltani, S., Sorensen, R.J.D., Soriano, J.B., Sorrie, M.B., Soyiri, I.N., Sreeramareddy, C.T., Stanaway, J.D., Stark, B.A., Ştefan, S.C., Stein, C., Steiner, C., Steiner, T.J., Stokes, M.A., Stovner, L.J., Stubbs, J.L., Sudaryanto, A., Sufiyan, M.B., Sulo, G., Sultan, I., Sykes, B.L., Sylte, D.O., Szócska, M., Tabarés-Seisdedos, R., Tabb, K.M., Tadakamadla, S.K., Taherkhani, A., Tajdini, M., Takahashi, K., Taveira, N., Teagle, W.L., Teame, H., Tehrani-Banihashemi, A., Teklehaimanot, B.F., Terrason, S., Tessema, Z.T., Thankappan, K.R., Thomson, A.M., Tohidinik, H.R., Tonelli, M., Topor-Madry, R., Torre, A.E., Touvier, M., Tovani-Palone, M.R.R., Tran, B.X., Travillian, R., Troeger, C.E., Truelsen, T.C., Tsai, A.C., Tsatsakis, A., Tudor Car, L., Tyrovolas, S., Uddin, R., Ullah, S., Undurraga, E.A., Unnikrishnan, B., Vacante, M., Vakilian, A., Valdez, P.R., Varughese, S., Vasankari, T.J., Vasseghian, Y., Venketasubramanian, N., Violante, F.S., Vlassov, V., Vollset, S.E., Vongpradith, A., Vukovic, A., Vukovic, R., Waheed, Y., Walters, M.K., Wang, J., Wang, Y., Wang, Y.-P., Ward, J.L., Watson, A., Wei, J., Weintraub, R.G., Weiss, D.J., Weiss, J., Westerman, R., Whisnant, J.L., Whiteford, H.A., Wiangkham, T., Wiens, K. E., Wijeratne, T., Wilner, L.B., Wilson, S., Wojtyniak, B., Wolfe, C.D.A., Wool, E.E., Wu, A.-M., Wulf Hanson, S., Wunrow, H.Y., Xu, G., Xu, R., Yadgir, S., Yahyazadeh Jabbari, S.H., Yamagishi, K., Yaminfirooz, M., Yano, Y., Yaya, S., Yazdi-Feyzabadi, V., Yearwood, J.A., Yeheyis, T.Y., Yeshitila, Y.G., Yip, P., Yonemoto, N., Yoon, S.-J., Yoosefi Lebni, J., Younis, M.Z., Younker, T.P., Yousefi, Z., Yousefifard, M., Yousefinezhadi, T., Yousuf, A.Y., Yu, C., Yusefzadeh, H., Zahirian Moghadam, T., Zaki, L., Zaman, S.B., Zamani, M., Zamanian, M., Zandian, H., Zangeneh, A., Zastrozhin, M.S., Zewdie, K.A., Zhang, Y., Zhang, Z.-J., Zhao, J.T., Zhao, Y., Zheng, P., Zhou, M., Ziapour, A., Zimsen, S.R.M., Naghavi, M., Murray, C.J. L., 2020. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet 396 (10258), 1204–1222.

Yi, P., Pryzbylkowski, P., 2015. Opioid Induced Hyperalgesia. Pain Med. 16 (suppl_1), S32–S36.
Zhang, X., Levy, D., Noseda, R., Kainz, V., Jakubowski, M., Burstein, R., 2010. Activation of meningeal nociceptors by cortical spreading depression: implications for migraine with aura. J. Neurosci. 30 (26), 8807–8814.
Zhang, XiChun, Levy, D., Kainz, V., Noseda, R., Jakubowski, M., Burstein, R., 2011. Activation of central trigeminovascular neurons by cortical spreading depression. Ann. Neurol. 69 (5), 855–865.