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Age and sex differences in chronic neuropathic pain in humans and preclinical research with a focus on molecular mechanisms

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2. Abstract

Neuropathic pain as a chronic condition poses a challenge for both preclinical and clinical studies. Due to its heterogeneous and multidimensional characteristics, a clear definition of pathological molecular processes and treatments is hindered. It's believed that more women than men and more older people than younger people are affected by chronic neuropathy. Hence, new insights at the molecular and cellular levels should be a research focus. However, the confrontation with all the obstacles spanning all levels is undeniable. This includes questions about the plausibility and validity of translating the obtained results from preclinical mouse models to humans. Further barriers to consider would be differences between the respective animal models, including sex as a biological variable, the adequate and representative numbers of animals and patients used, and the uniform age classification. Considering these aspects, the recommendation of individualized support and treatment of the patient is justified. It should be carried out regardless of the patient's socioeconomic status, associated costs, and available medical facilities time.

This work aims to illustrate the most important, though not all (as it would exceed the scope of this thesis), preclinical and clinical sex- and age-specific differences at the molecular level in chronic neuropathic pain. I will also address the current limitations, challenges, and difficulties, as well as the importance of including sex and age in all future research and interpreting findings based on these variables.

3. Kurzfassung

Neuropathischer Schmerz als chronischer Zustand stellt sowohl für präklinische, als auch für klinische Untersuchungen eine Herausforderung dar. Durch seine heterogenen und multidimensionalen Eigenschaften ist eine eindeutige Definition der pathologischen molekularen Prozesse dieses Krankheitsbildes und dessen Behandlung erschwert. Es wird vermutet, dass mehr Frauen als Männer, aber auch mehr Ältere als Jüngere von der chronischen Neuropathie betroffen sind, weshalb neue Erkenntnisse, sowohl auf molekularer, als auch auf zellulärer Ebene im Fokus der Forschung liegen sollten. Dennoch ist man immer wieder mit Hürden, die sich über alle Ebenen erstrecken, konfrontiert. Diese betreffen unter anderem die Frage der tatsächlichen Plausibilität und Validität der Übertragung der Forschungsergebnisse vom präklinischen Mausmodell auf den Menschen. Weitere Barrieren sind die Differenzen zwischen den einzelnen Modellen, die Einbeziehung des Geschlechts als biologische Variable, die adäquate und repräsentative Anzahl von den verwendeten Tieren und Patienten, sowie die einheitliche Altersklassifizierung. Unter der Berücksichtigung dieser Aspekte, ist die Empfehlung einer individualisierten Patientenbetreuung-, und Behandlung gerechtfertigt, die unabhängig vom sozioökonomischen Status der leidenden Person und der damit verbundenen Kosten und verfügbaren Zeit der medizinischen Einrichtungen sein sollte.

Mit dieser Arbeit sollen die wichtigsten, wenn auch nicht alle (da es den Rahmen dieser Masterarbeit sprengen würde), präklinischen und klinischen geschlechts- und altersspezifischen Unterschiede auf molekularer Ebene beim chronischen neuropathischen Schmerz veranschaulicht werden. Ebenso möchte ich auf die derzeitigen Limitationen, Probleme und Schwierigkeit eingehen und wieso es für die Gesellschaft essentiell ist, das Geschlecht und das Alter in jede zukünftige Forschung miteinzubeziehen und diese anhand dieser Variablen entsprechend zu interpretieren.

4. Introduction

Neuropathic pain (NP) represents a “severe form of chronic pain” (Baskozos et al., 2023). While the global prevalence of chronic pain involves approximately 20% of the population (Ghazisaeidi et al., 2023), “chronic neuropathic pain” occurs in approximately “7-10% of the” world’s population (Rodrigues et al., 2022). NP is defined by the International Association for the Study of Pain (IASP) as “pain that arises as a direct consequence of a lesion or diseases affecting the somatosensory system” (International Association for the Study of Pain [IASP], n.d.). Usually, signals propagate from the periphery to central areas, such as the brain (Fiore et al., 2023). Therefore, injuries “span the entire pain neuro-axis” (Meacham et al., 2017). The expanding nature of signals, starting at primary sensory neurons, can be seen in Figure 1, in which step-by-step signal transmission is shown (Esposito et al., 2019).

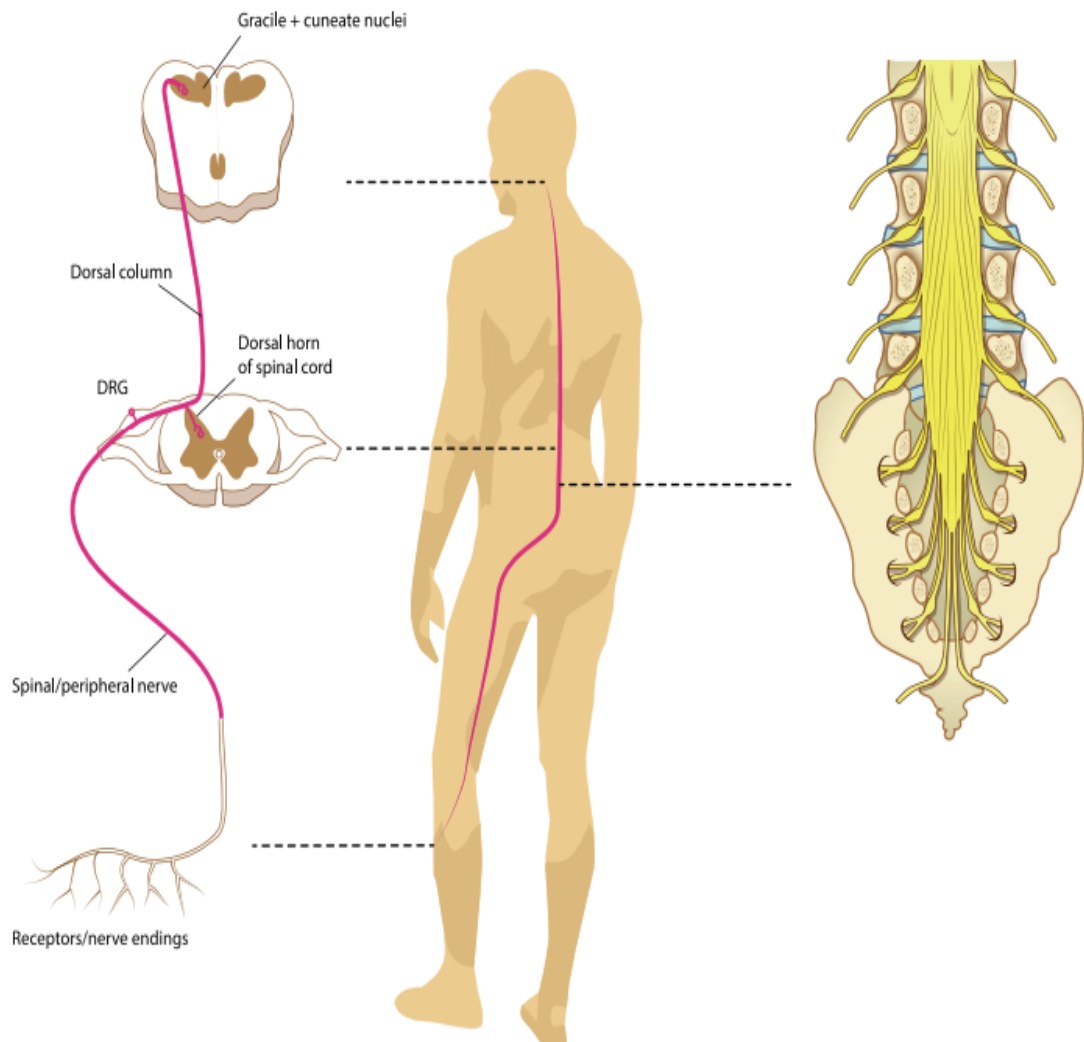


Figure 1. A schematic diagram showing the expanse of the primary sensory neuron (middle) and its terminations (left). The locations of dorsal root ganglia (DRGs) in the lumbar and sacral spine are illustrated (right); note the utility of vertebral pedicles as landmarks at lumbar levels and the greater variability of DRG location at sacral levels. Reprinted from *Unique Characteristics of the Dorsal Root Ganglion as a Target for Neuromodulation* by Esposito et al. (2019), *Pain Med.* 2019 Jun; 20(Suppl 1):

Apart from sensory ongoings, neuropathic pain is accompanied by the involvement of the immune system, which results in a bidirectional microglia-neuronal interaction (Machelska & Celik, 2016). However, it is not always caused by direct nerve damage (Finnerup et al., 2021). Still, in some cases, another underlying disease (Meulen et al., 2021), such as diabetes, human immunodeficiency virus (HIV), chemotherapy, autoimmune diseases, and more (Finnerup et al., 2021), is the culprit. Another established and significant reason for the development and occurrence of chronic neuropathic pain is that it can be driven by sex itself (J. S. Mogil, 2020). Studies have already demonstrated the differentially experienced pain between the sexes and their response to analgesics (Noya-Riobo et al., 2022). This includes sexually dimorphic central pain- processing in which women experience pain stronger compared to men (Abraham et al., 2018). Even though women are more affected by this condition (J. S. Mogil, 2020), showing more comorbidities and a lower quality of life (QoL) (Meulen et al., 2021), more than 80% of studies exclude females as subjects (Boullon et al., 2021). Significantly, sex-based differences also depend on the model itself or the type of pain (Al-HadlaQ & Masocha, 2023). The legitimacy of using such models when portraying human mechanisms was confirmed in a translational fashion several times. It was stated that pain- neurophysiology is largely “conserved across” species and sexes but isn’t fully understood yet (ACC. Williams, 2019). Unfortunately, a precise disclosure of existing sexual differences in neuropathy is quite tricky to manage, as neuropathic pain studies are often challenged by the limited availability of participants (Ghafouri et al., 2022).

Additionally, age presents a further factor for the development and persistence of chronic pain and is connected with alterations in neuronal anatomy and pain perception (Giovannini et al., 2021). Even though NP can occur in infants, it is more likely to develop in late childhood and adolescence, with a median of 13 years, marking the beginning of pediatric neuropathic pain (Fitzgerald & McKelvey, 2015). Chronic NP also affects 3 out of 10 patients over the age of 70 who are often undiagnosed (Giovannini et al., 2021), while in children, the prevalence of chronic NP is unknown (S. Walker, 2020).

When diagnosing chronic NP in a clinical setting, pain anamnesis, clinical examinations, including neurological testing, diagnostic tests, and pain reports (Ghafouri et al., 2022) are used. Preclinical studies, on the other hand, use peripheral nerve injury (PNI) pain models (Jaggi et al., 2011) to “mimic injury-induced neuropathic pain” (Yeh et al., 2020).

Individuals with chronic NP experience symptoms such as allodynia, hyperalgesia (Li et al., 2023), burning, and electrical-like sensations (Ghafouri et al., 2022). Subsequently, increased pain sensitivity and spontaneous pain exhibit persistence and/or episodic recurrence, leading to chronification (Scholz et al., 2019). In addition, patients are more likely to suffer from depression, anxiety, and sleeping disorders compared to non-NP

patients (Ghafouri et al., 2022). Conversely, individuals with “mental stress, depression and anxiety” show a higher prevalence of chronic pain than those without (Salduker et al., 2019). This also explains pharmacological off-label treatments (Serrano et al., 2022). However, only 50% of patients experience a 30-50% pain reduction, and the other 50% are refractory to available therapies (Hange et al., 2022). Pain is undeniably a “multidimensional” experience (Abboud et al., 2021) with heterogeneous characteristics (Finnerup et al., 2021), which might explain the unsatisfactory results in pain treatment. Nevertheless, obstacles in pain management on all levels can contribute to failing patients, resulting in dissatisfaction from all parties involved. One of such difficulties can be attributed to drug discovery and development itself, which takes 10-15 years (Sun et al., 2022), with more than 50% of drugs failing “to advance from phase 2 to phase 3” (Langford et al., 2023). The journey from the “pre-discovery stage” to clinical trials and the subsequent “post-market monitoring stage” (Singh et al., 2023), as well as the high failure rate, highlight the challenging character of pain- research and- management. Also, the fact that between 1996 and 2005, most preclinical studies had been conducted using male rodents only resulted in a male-biased pain literature. Only recently, it has been urged to include sex as a biological variable (SABV) (J. S. Mogil, 2020). Even though “in 1993, National Institutes of Health (NIH) Revitalization Act in the United States” commanded “the inclusion of” females “in NIH-funded clinical trials”, many investigators ignored this order or just ignored the sex-based interpretation of the results (Mauvais-Jarvis et al., 2021). These factors contribute to the high pain management expense, estimated to be more than \$600 billion in the US (Zah et al., 2019) and around €300 billion in total cost in Europe (J. Adams, 2023).

By analyzing neuropathic pains’ complexity and being a lot of researchers’ focus, there are still a lot of unanswered questions. This work aims to highlight the most recent findings in clinical and preclinical studies on sex and age differences in chronic neuropathic pain. It also explores molecular differences and discusses current pharmacological and non-pharmacological strategies for both sexes in an understandable and accessible manner.

5. Neuroanatomy

Pain or “nociception” (Khalid & Tubbs, 2017) is “self-limiting” (Verma et al., 2015). It acts as an adequate physiological response to painful and possibly harmful stimuli, posing as an early protection (Marwaha et al., 2016). Significantly, pain as chronic NP is location-based (Finnerup et al., 2021) and classified as peripheral or central pain (Rodrigues et al., 2022). Peripheral pain is marked by nerve damage due to trauma or an already ongoing disease, while central pain is the result of a lesion (Machelska & Celik, 2016).

First and foremost, pain-sensing neurons, termed nociceptors (Marwaha et al., 2016), are pivotal. They act as sensory receptors in the peripheral somatosensory system (Fiore et al., 2023). and their nociceptive afferents (Meacham et al., 2017) pose as axons of such nociceptors (Dubin & Patapoutian, 2010). Under healthy circumstances and when the threshold is reached, received stimuli are converted into action potentials (AP), propagating along primary afferent fibers (Khalid & Tubbs, 2017). These signals are transmitted to the dorsal root ganglion (DRG) and subsequently to the brain (Fiore et al., 2023), resulting in the sensation of pain (Khalid & Tubbs, 2017). Under pathological conditions, an inappropriate response to those signals occurs (Meacham et al., 2017), with afferents contributing to the maintenance of chronic pain and clinical allodynia (Guha & Shamji, 2016). This pathological pain sensation can be ascribed to both malfunctions of nociceptors and changes in signal processing (Marwaha et al., 2016), linking “long-term pain” (Maguire et al., 2022) to maladaptive plasticity in CNS and increased excitatory signals (Swanson et al., 2023). The signal-misreading of the CNS to a usually temporarily painful input (Meacham et al., 2017) characterizes the underlying mechanisms of NP (Yi Dai, 2015). Sometimes, the threshold for transmitting peripheral signals to the CNS increases, weakening the signal (Johnson et al., 2021). However, in NP conditions, nociceptive and afferent thresholds often decrease, amplifying the sensory response and causing central amplification (Guha & Shamji, 2016). In turn, the signal becomes compensated as the spinal cord amplifies the received signal by increasing the synaptic efficacy of somatosensory neurons on the DRG (Johnson et al., 2021). The dynamic reflects the adaptive yet maladaptive processes contributing to NP. Notably, peripheral sensitization of nociceptors (Maguire et al., 2022) is caused by repetitive stimulation (Meacham et al., 2017). However, signals reaching specific brain areas (Fiore et al., 2023) include transmitting pain-related information from the thalamus, cortical areas, and the parabrachial nucleus and subsequently projecting to the amygdala. In turn, the amygdala inhabits interconnected nuclei like the basolateral (BLA) and central amygdala (CeA) nuclei and intercalated cells (ITS) (Pereira et al., 2022). Figure 2 demonstrates the journey of nociceptive information and its’ arrival in different brain areas (Chen & Tang, 2024).

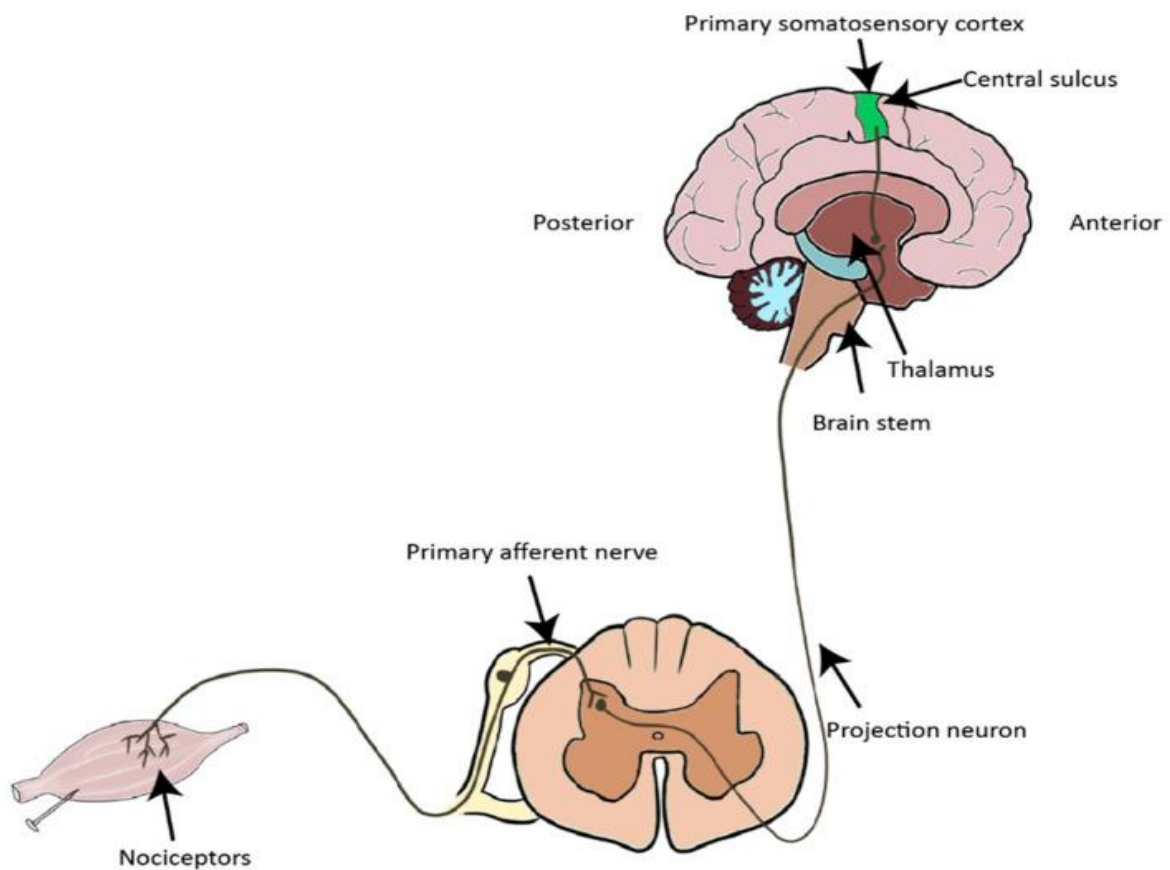


Figure 2. Diagrammatic outline of the ascending pain pathways. Noxious stimuli are transformed into electrical signals in nociceptors and then transmitted to the spinal cord, where the signals are decoded and processed. The nociceptive signals are converged onto projection neurons, which relay the nociceptive information to the brain stem and the thalamus. Reprinted from *Neural Circuitry Polarization in the Spinal Dorsal Horn (SDH): A Novel Form of Dysregulated Circuitry Plasticity during Pain Pathogenesis* by Chen & Tang (2024), *Cells* 2024 Feb 25;13(5):398. Copyright © 2024 by the authors. This article is licensed under the Creative Commons Attribution (CC BY) license.

5.1. DRG- spine- brain circuit

Dorsal root ganglion (DRG) poses as an enlargement of the dorsal root (Esposito et al., 2019). It presents a heterogeneous structure that houses a diversity of cells, of which 15% are primary sensory neurons (PSN). The DRG sits close to the neuroforamen, and its neurons' somata are surrounded by Schwann cells and satellite glial cells (SGC) (Guha & Shamji, 2016). SGC wrap around neuronal cell bodies and communicate in a "bidirectional" manner. It is suspected that these glial cells in the spinal cord are vital for generating and maintaining pain, resulting in hyperalgesia (Hanani et al., 2020). DRG's distal axons represent primary afferent sensory nerves while the proximal axons, even though absent of synapses within the DRG, synapse onto dorsal horn cells via dorsal spinal roots. Therefore, after a peripheral nerve injury or a DRG injury itself, spontaneous action potential (AP) in DRG and an increase in the number of A β - fibers is characteristic, but not when the injury is proximal (Esposito et al., 2019). Cohesively, a chronic constriction injury- (CCI) injured

somata displays a higher glutamate release in DRG, as well as an increased receptor expression on glial cells and sensory neurons. Further, the proliferation of Schwann and glial cells within DRG contributes to the onset of chronic pain. It responds to an autoimmune reaction or an inflammatory process, while astrocytes and microglia proliferation could be seen within DRG when peripherally injured (Guha & Shamji, 2016). Again, this produces central sensitization by pathologically amplifying pain signals in the brain (Johnson et al., 2021). At this point, it's important to point to differences in male versus female brain anatomy. This would include a larger brain and total gray matter volume, as well as larger volume in regions, such as the visual cortex, the medial prefrontal cortex (mPFC), hippocampus, hypothalamus, and amygdala (including the medial nucleus of the amygdala) in males. Females, however, have significantly larger regional volumes in the frontal, cingulate, and somatosensory cortices, among others (Guma et al., 2024). The “mPFC and the anterior cingulate cortex (ACC)” play crucial roles “in regulating” emotional “symptoms of neuropathic pain”, with long-term changes in their structure, function, and biochemistry (Dai et al., 2022). The ACC, a part of the limbic system (Shen et al., 2023), shows sexually dimorphic functional connectivity (FC), with subgenual ACC “(sgACC) FC being abnormal in women but not in men”. This highlights the importance of sex differences in the development of pain treatment (Osborne et al., 2021). For illustration purposes, different brain areas can be seen in Figure 3 and the brain’s involvement in modulating pain (Caston et al., 2020). Of importance is periaqueductal gray (PAG), which interacts with “ascending and descending projections from numerous sites” as it “receives inputs from higher brain centers,” as well as from SDH (Ossipov et al., 2014). PAG also accounts as an anatomical structure for the primary site for opiate action (Averitt et al., 2019) and produces “opioid mediated” analgesia. The rostroventromedial medulla (RVM) can inhibit or elicit pain via a “bidirectional pain modulatory effect” (Ossipov et al., 2014).

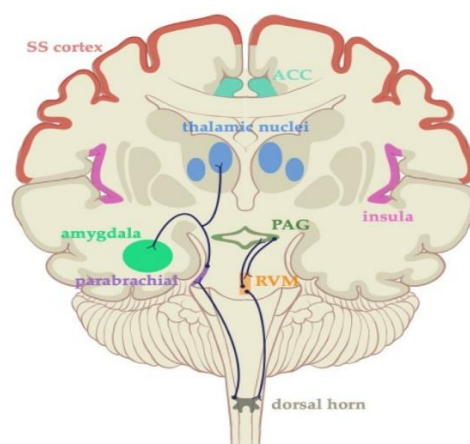


Figure 3. Descending pain modulatory pathway. The pathway originates in the cerebrum and descends to the periaqueductal gray (PAG) matter, rostral ventromedial medulla, and projects to the dorsal horn of the spinal cord. Bidirectional nociceptive pathways through the medullary nuclei are shown. The efferent pathway from the dorsal horn goes through the parabrachial nucleus, amygdala, and thalamic nuclei. Reprinted from *The Cerebral Localization of Pain: Anatomical and Functional Considerations for Targeted Electrical Therapies* by Caston et

Besides structural sex differences, preclinical studies highlight the importance of DRG and its involvement in neuronal hyperexcitability under pathological conditions (Guha & Shamji, 2016). The following findings demonstrate the importance of including SABV in studies and why NP- treatment would profit from a sex-based approach. In addition, the most critical findings are compiled in Table 3, which involve the targeted molecule, the performed procedure, the underlying mechanism, the pain model used, age, and species.

Preclinical

For example, the maintenance of pain can be ascribed to the chemokine ligand CXCL10, which is responsible for neuronal hyperexcitability and the activation of p38 and ERK in DRG. This spinal nerve ligation (SNL)- induced neuronal hyperexcitability can be attributed to the permanent increasement of this ligand (Kong et al., 2021). Even though no sex differences were mentioned, another study found that “mRNA gene expression of CXCL10 showed a stronger response in males compared to females” after polyinosinic: polycytidylic acid (poly I: C) injection (Posillico et al. 2021). Additional information is stated in table 3.

In another SNL model, Presto and Neugebauer (2022) found that calcitonin-gene-related polypeptide (CGRP) affects amygdala function and behaviour in a sex- and time-specific manner. They observed increased CGRP mRNA in the central amygdala (CeA) at various times, depending on the sex. An administration of the CGRP receptor antagonist CGRP 8-37 to the amygdala reduced the duration of ultrasonic vocalizations in females (Presto & Neugebauer, 2022).

Also, neuronal plasticity, to which brain-derived neurotrophic factor (BDNF) contributes by binding to tyrosine kinase receptor (TrkB) and subsequently interacting with NMDAR (Kohno & Tsuda, 2021), was discussed in an EAE model. This model is often used to replicate inflammation, maladies, and pain that come with it. In contrast to other PNI-induced animal pain models focusing on the spinal cord, the EAE model concentrates on DRG. It was found that a possible reason for females displaying higher hyperexcitability is due to higher levels of plasticity- proteins, such as activating transcription factor 3 (ATF3) (NCBI, 2024, August 21) and pCREB. Regarding males, their DRG had a higher cytochrome c release and more caspase 3 cleavage. This increases the risk for apoptosis and neuropathy as male sensory neurons are more prone to neuronal damage (Maguire et al., 2022). Only recently, it was suggested that Atf3 might be a major contributor to NP (Deng et al., 2023), and an “ATF3 knockdown may be useful where immune responses are inadequate” (Thompson et al., 2010).

In a protein expression profiling and subsequent DRG comparison of mice, Vradenburgh et al. (2023) found that females had a larger relative abundance in (Trk) B⁺ and TrkC⁺ neurons, as well as in the subset of neuronal and putative phagocytic glia cells. Males had a larger

relative abundance in TrkA⁺ peptidergic nociceptors and showed more mature neuronal populations than females (Vradenburgh et al., 2023).

Another preclinical hDRG-based study found that females had an increased expression of CCL1, CCL21, proenkephalin (PENK), toll-like receptor (TLR) 3, and centromere protein-coding genes. Conversely, males showed a higher expression of IL-1 β , TNF, CXCL14, OSM, and members of JUN-FOS signaling (Ray et al., 2023).

More recently, Li and Ward (2024) demonstrated sex- and location-specific differences after paclitaxel-induced neuropathy. While some pain and inflammation markers, such as TLR4 and IL-6, were increased in the male DRG, they were increased in both the female DRG and PAG. Additionally, a prophylactic administration of the anti-inflammatory cannabinoid cannabigerol (CBG) prevented the “increased expression of inflammation and pain markers”, especially Tlr4, in the female PAG (Li & Ward, 2024).

Older PAG-oriented studies using chemotherapy-induced peripheral neuropathy (CIPN) and a “complete Freund’s adjuvant (CFA)-induced inflammatory pain model” demonstrated that only male mice displayed decreased GPR171 protein levels in the PAG during NP. When treated with its agonist MS15203, only males showed a reduction in chronic NP as the protein levels could be restored (Ram et al., 2021).

Clinical

In a clinical setting, Meyer- Frießem and team (2020) revealed significant sex differences. Regarding the perception pain threshold (PPT) in complex regional pain syndrome (CRSP), the wind-up ratio (WUR), and mechanical detection threshold, females showed lower pain thresholds in general (Meyer-Frießem et al., 2020).

Then, Fauchon et al. (2021) demonstrated sex differences in alpha brain activity in a magnetoencephalography (MEG)- based study. These activities stand in connection with the modulation and processing of pain. A comparison of the peak alpha frequency (PAF) in patients with different maladies brought the following results: Neuropathic males had an increased PAF in ascending antinociceptive pathway (ANP) mainly. Meanwhile, neuropathic females had faster PAF in ANP, salience network (SN), and dynamic pain connectome (DPC) in general. Additionally, females had lower PAF in the subgenual anterior cingulate cortex (sgACC), indicating abnormal functional connectivity (FC) of sgACC, which wasn’t present in men. Regarding age differences, healthy women had a lower PAF in DPC and a subsequent decrease over time. In males, a PAF power decrease could be seen until 20 years old and an increase until 80 years of age (Fauchon et al., 2021).

5.2. From signal to neuropathic pain: the duality of interneurons

Lamina I-VI of DRG (Harding et al., 2020) in the spinal dorsal horn (SDH) (Inoue & Tsuda, 2018) “receive input from peripheral sensory fiber” (Takazawa et al., 2017). Depending on the type of afferents, they terminate in their respective lamina (A. J. Todd, 2010). A balance

between excitation and inhibition in SDH depends (Kohno & Tsuda, 2021) on interneurons via gate control, which decides if NP will occur (Inoue & Tsuda, 2018). Mainly, lamina I contains projection neurons, which are “responsible for nociception” (Krotov et al., 2023). While in normal conditions, inhibitory interneurons which contain γ -aminobutyric acid (Doyle et al., 2017) (GABA) and/ or Glycine (Inoue & Tsuda, 2018) “continuously release GABA to decrease the excitability of lamina I output neuron” (Yin et al., 2018), in pathological state, this system is damaged, mainly affecting lamina I (Inoue & Tsuda, 2018). Lamina I, the farthest layer, also contains excitatory synaptic NMDARs and neurons responsible for the output projection (Dedek et al., 2022). Notably, NMDAR is a glutamate receptor (Barcelon et al., 2023), and nociceptors release glutamate as their primary neurotransmitter, which acts post-synaptically (Dubin & Patapoutian, 2010). In connection, nerve injury-induced physiological changes in the spinal cord and brain could be linked to increased neuronal activity by various researchers (Meacham et al., 2017). For instance, in the spinal cord, postsynaptic NMDAR activation (Barcelon et al., 2023), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (Doyle et al., 2017) (AMPA) receptor recruitment to the postsynaptic site and altered expression of potassium chloride cotransporter 2 (KCC2) was observed. Additionally, activated spinal cord microglia show altered secretion of glutamate, substance P, CGRP, BDNF, IL-1 β , and tumor necrosis factor (TNF) α (Barcelon et al., 2023). Figure 4 illustrates the transmission of pain signals in dependency of such inhibitory glycin- and/or GABAergic interneurons. While in (A), received signals are balanced out by the activated inhibitory interneurons, which results in no pain during healthy conditions, in (B), a “disinhibition” of this circuit leads to pain, as inhibitory interneurons are inactivated (Breitinger & Breitinger, 2023).

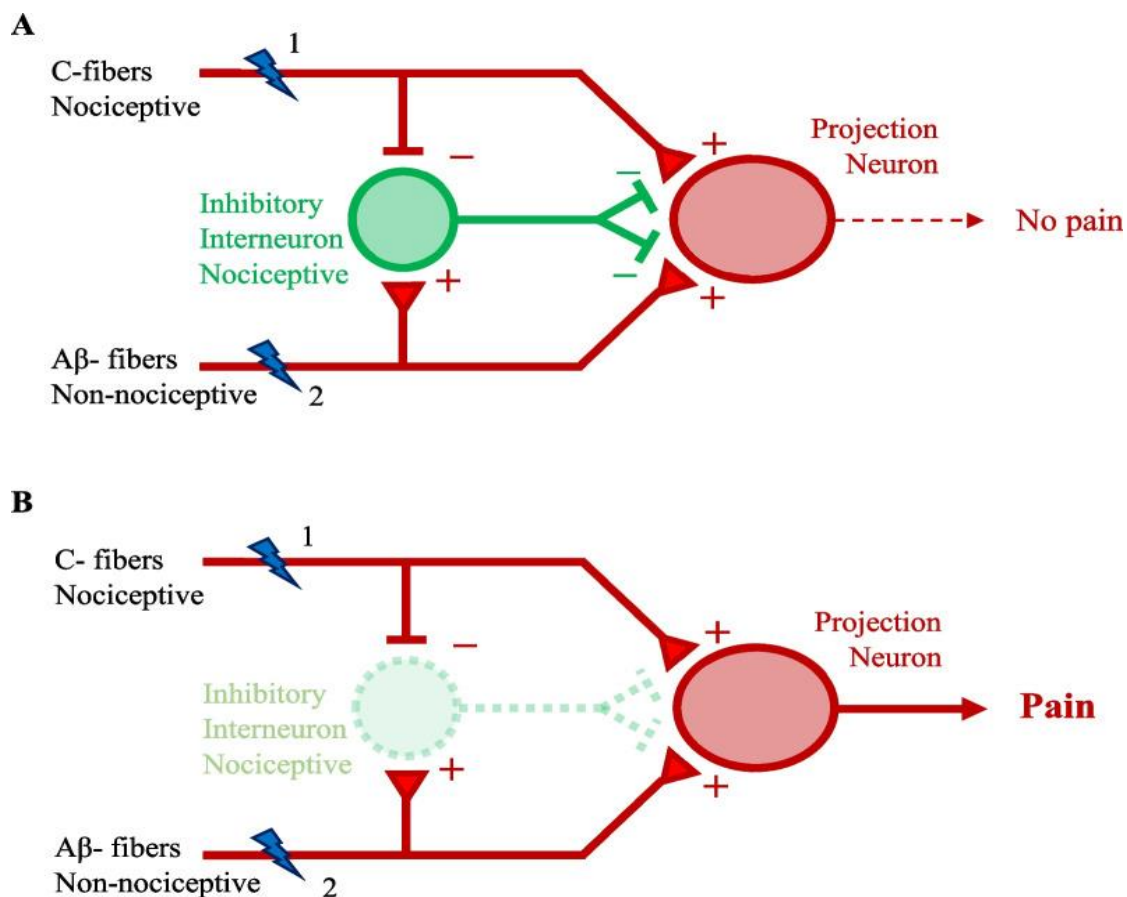


Figure 4. Forward inhibition and allodynia. Nociceptive and non-nociceptive signals are summated in nociceptive projection neurons, providing a control gate (Wall 1978) defining the input of signals to the brain. Transmission of pain signals is modulated by inhibitory glycinergic and/or GABAergic interneurons. **A** Under normal conditions, simultaneous activation of pain (1) and touch (2) receptors does not lead to pain sensation. **B** In case of inactivation of inhibitory interneurons (disinhibition), even tactile stimuli alone (2) lead to sensation of pain. Reprinted from *Excitatory and inhibitory neuronal signaling in inflammatory and diabetic neuropathic pain* by Breitingner & Breitingner (2023), Mol Med 29, 53 (2023). Copyright 2023 by the authors. This article is licensed under the Creative Commons Attribution (CC BY) license.

Furthermore, neurons are surrounded by different cell types, such as glial cells (e.g., astrocytes), vascular cells (e.g., endothelial cells), and fibroblasts (Zavvarian et al., 2020), which is partially illustrated in Figure 5. This image shows that a release of various molecules (e.g., chemokines, cytokines, and growth factors) takes place after PNI. This causes structural and functional changes (e.g., sensitization, gene expression). This triggers spontaneous activity in primary afferents and their release of 2nd mediators, such as colony-stimulating factor (CSF) 1. Also, activating glial cells and releasing other molecules (e.g., BDNF, IL-1 β) causes nociceptive transmission to spinal-, thalamic- and cortical regions (P. Smith, 2023). It is apparent that “the transition from acute to chronic pain” and the persistence of allodynia and hyperalgesia involve CNS activation of microglia (Gregus et al., 2021), the myelin-synthesizing (Ghafouri et al., 2022) oligodendrocytes and astrocytes. This activation triggers surrounding glial cells and neurons, resulting in the

maintenance of pain (Gregus et al., 2021). The sex-dependent variety in activating those cells and the respective molecules will be discussed later.

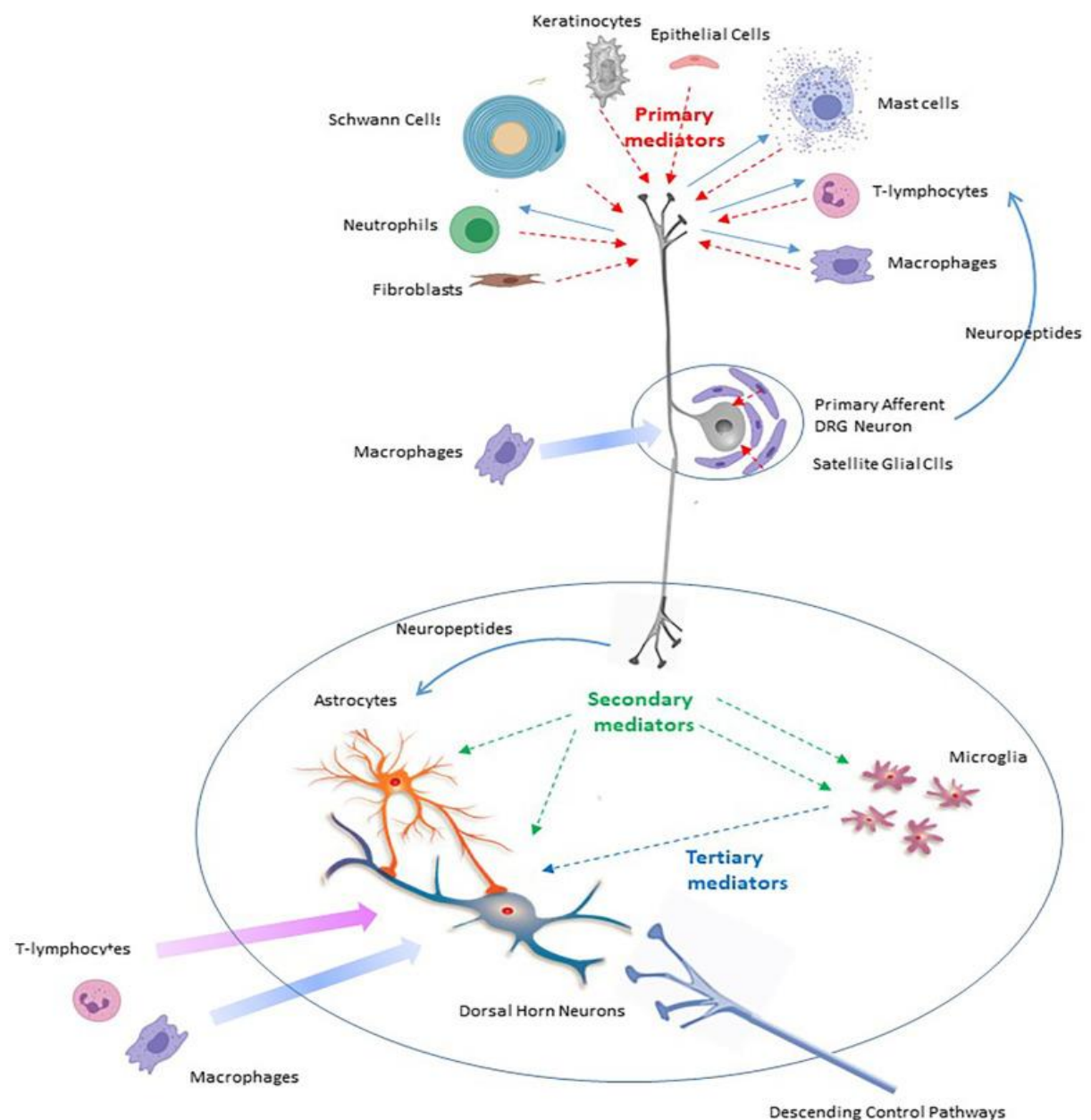


Figure 5. Scheme to illustrate the roles of primary, secondary and tertiary mediators in the onset and maintenance of neuropathic pain in response to peripheral nerve injury. Reprinted from *Neuropathic pain; what we know and what we should do about it* by P. A. Smith (2023), *Front Pain Res (Lausanne)*. 2023; 4: 1220034. Copyright © 2023 Smith. This article is licensed under the Creative Commons Attribution (CC BY) license

5.3. Fibers

Fibers, as extensions of nociceptors (Dubin & Patapoutian, 2010), are classified in $A\alpha$, $A\beta$, $A\delta$, and C-fibers and their somata houses in the DRG, where they connect with their respective lamina (Harding et al., 2020), which can be seen in Table 1. Additionally, Figure

6 provides an overview of the different fiber types, summarizing their characteristics and roles in sensory processing.

FIBER type:	A α	A β	A δ	C-fibers
LAMINA termination:	V-VI	III-V	V-VI	I-II
INFO type:	Proprioceptive	- Antinociceptive - Innocuous	- Noxious - innocuous	Nociceptive (including noxious)

Table 1. Overview of (pain-related) fiber types (Harding et al., 2020)

As stated in Table 1, noxious stimuli are transferred through A δ -fibers (Inoue & Tsuda, 2018), which terminate in laminae V-VI. It is also transferred through C- fibers, which inhabit lamina I and II (Harding et al., 2020). In case of degeneration and malfunction of A δ - and C-fibers, small fiber neuropathy (SFN) symptoms can occur (Scheliga et al., 2024). A β -fibers, as antinociceptive afference, transmit information to deeper regions of the dorsal horn (Inoue & Tsuda, 2018), primarily terminating in laminae III-V (Harding et al., 2020). They connect with nociceptive neurons in lamina I via inhibitory interneurons (Inoue & Tsuda, 2018). Typically, A β -fibers transmit touch and vibrations. They are also responsible for the majority of ectopic discharges after a nerve injury due to electrophysiological changes. This results in their capability to transmit pain and likely contributes to central sensitization (Esposito et al., 2019).

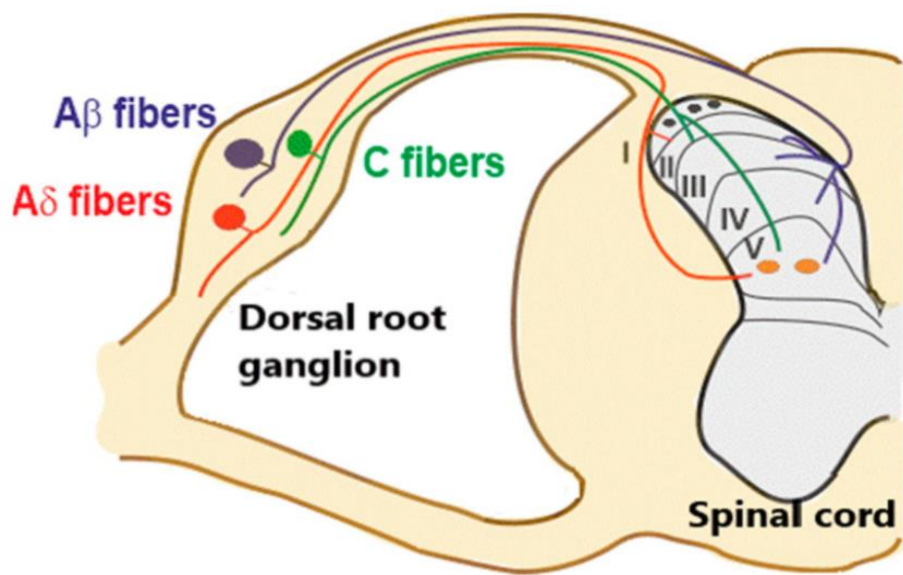


Figure 6. Organization of primary afferent fibers projecting to spinal cord dorsal horn. Nociceptive fibers terminate mainly in superficial dorsal horn (laminae I–II), while tactile afferents (A β and LT (low threshold) A δ) project mainly to deep dorsal horn (laminae III–V). Nociceptive projection neurons are located in lamina I (black circles), while wide dynamic range neurons (orange circles), activated by both tactile and nociceptive stimuli, are localized in lamina V. Muscle afferent fibers (A α) are not considered in this diagram. Reprinted from *Presynaptic Inhibition of Pain and Touch in the Spinal Cord: From Receptors to Circuits* by Comitato & Bardoni, 2021. International journal of molecular sciences, 22(1), 414. Copyright 2021 by the authors. . This article is licensed under the Creative Commons Attribution (CC BY) license

Regarding fibers and chronic pain, neuropathic pain onset begins with damaged nociceptive afferents (Meacham et al., 2017) and changes on a molecular and cellular level (Esposito et al., 2019), subsequently impacting the transduction and transmission of information (Meacham et al., 2017). Such changes include alterations in fiber- density and - hyperexcitability, altered synaptic functions in DRG, trigeminal ganglia (TG), and spinal- and supraspinal-nociceptive pathways (Meacham et al., 2017). This is important as axons' diameter and myelination determine conduction speed and sensitivity, affecting synaptic signaling in the neuropathic state (Dubin & Patapoutian, 2010). Also, errors in expression and function in receptors, ion channels (e.g., Nav⁺, Kv⁺, Ca²⁺, and TRP), and immune cells (Esposito et al., 2019) are present.

Preclinical, fiber-oriented studies' primary focus is understanding neuropathic pain by finding peripheral- and DRG-sensitization among those fibers (Meacham et al., 2017). On the other hand, clinical assessment of fibers' functionality and the corresponding CNS pathways can be conducted in quantitative sensory testing (QST) (Meyer-Frießman et al., 2020). Importantly, preclinical data shows that nociceptors are sexually dimorphic and age-dependent. However, a link between these differences and nociceptors has still to be made in humans. At this point, it's also essential to acknowledge molecular and functional differences between human and animal nociceptors (Middleton et al., 2021).

Preclinical, Translational, and Clinical

Species-based differences could be shown in a translational study by Tavares-Ferreira et al. (2022). They used human lumbar DRG tissues to characterize human DRG (hDRG) sensory neurons. Besides the differences, this study highlighted the conserved aspects of these nociceptors between humans, mice, and non-human primates, such as macaques. Macaques- human differences could be obtained in parvalbumin "(PVALB) gene expression". It "was enriched" in human "proprioceptors and A β " slowly adapting (SA) low threshold mechanoreceptor (LMTR). While in macaques, it was enriched in the peptidergic PEP2 population (Tavares-Ferreira et al., 2022). The Ca²⁺-binding protein PVALB is vital in developing mechanical allodynia by controlling firing patterns in parvalbumin-expressing neurons, also called inhibitory neurons. After nerve injury, these patterns change from tonic to adaptive or initial firing, and PVALB expression is decreased, triggering pain (Qui et al., 2024).

Another Ca²⁺-binding protein, hippocalcin (HPCA), which mediates neurogenesis and "inhibits gliogenesis" in neuronal stem cells (Park et al., 2016), was found to be enriched in pruritogen receptors, silent nociceptors, and putative C-LTMRs in humans. In macaques, it was additionally enriched in TRPM8⁺ and PEP1. Further, differences between human- and mouse nociceptors were made. For example, a broader expression in the voltage-gated sodium channel (VGNaC)- gene SCN4B and in anti-inflammatory interleukin- (IL) receptors was found in humans. Also, GPCR was more expressed in humans, while it was restricted

to non-peptidergic neurons in mice. The results of this study indicate “that peptidergic and nonpeptidergic subsets of sensory neurons do not” directly map to human biology, with the most prominent evolutionary mouse-human divergence in neuropeptides, TRPV1 and NTRK1 (Tavares-Ferreira et al., 2022). Table 2 demonstrates hDRG subtypes concerning the respective fiber.

Group	<i>A-fiber</i>	<i>C-fiber</i>
Subtypes	1. Proprioceptors 2. A β SA LTMRs 3. A β RA LTMRs 4. A δ 5. putative A β nociceptors 6. HTMRs	1. cold nociceptors 2. PENK-enriched nociceptors 3. TRPA1- enriched nociceptors 4. putative silent nociceptors 5. puritogen receptor- enriched

Table 2. Demonstration of hDRG subtypes according to their group. HTMR, high threshold mechanoreceptor; LTMR, low threshold mechanoreceptors; PENK, proenkephalin; RA, rapidly adapting; SA, slowly adapting; TRP, Transient receptor potential (Tavares-Ferreira et al., 2022)

Regarding sex differences, expression of calcitonin-related polypeptide alpha (CALCA) (Tavares-Ferreira et al., 2022), which encodes the CGRP protein (Li and Ward, 2024), was found to be higher in female pruritogen receptor enriched neurons (Tavares-Ferreira et al., 2022) in this preclinical and translational setting. This subsequently higher CGRP-expression could explain more intense pain in female mice. Such was suggested when researching sex-related explanations for migraine. CGRP was administered in picogram dose and produced hypersensitivity in females, while male mice stayed “unaffected” by even higher doses (Avona et al., 2019).

Clinical

Meanwhile, clinically based evidence for sexual dimorphism in connection to fibers and women being more affected by chronic NP was presented by Abraham and his colleagues (2018). They compared type II diabetes patients with either suspected polyneuropathy or an established diabetic sensorimotor neuropathy (DSP). After large- and small nerve fiber tests, women had more NP effects, such as higher frequency and intensity of pain. These results were interesting as men displayed higher large- and small nerve fiber impairment, which suggests different central pain processing in females (Abraham et al., 2018).

Later, a sex-based comparison in pain modulation using a conditioned pain modulation (CPM) approach in patients undergoing surgical nerve repair was conducted. Initially, females reported higher pain ratings and greater clinical pain, regardless of chronic NP. However, after adjustments, no statistically significant sex differences were found. Despite that, it was noted that females returned to their baseline quicker than men, suggesting better adaptation to stimuli (Miclescu et al., 2022).

Earlier in the previous study, Birnbaum et al. (2019) published their results with Sjögren’s Syndrome (SS) patients. They distinguished between DRG and axonal degeneration in

connection with length-dependent (LD) and non-length-dependent (NLD) pain distribution. Patients with a pure form of small fiber neuropathy (SFN) were younger at the onset of NP, and men with SS SNF had a higher prevalence (Birnbbaum et al., 2019).

Further clinical investigations of these fibers' function, morphology, and impact on NP were examined in another clinical small-fiber neuropathy (SFN) study. It was disclosed that SFN-patients display a reduced grey matter volume (GMV) and cortical thickness (CT). Additionally, a connection between SFN and “gain-of-function” mutations in Na_v^+ could be made (Scheliga et al., 2024). Unfortunately, most current clinical studies still do not incorporate a sex-based approach, so sex-specific differences can't be determined here. The same issue can be seen in preclinical studies, still dominated by male subjects.

5.4. Nociceptive channels and receptors

Transient receptor potential (TRP) channels are transmembrane, ligand-gated cation channels that express and act as mechano- thermo- and chemoreceptors (Marwaha et al., 2016). They are “densely expressed” (Tajti et al., 2023) on (primary afferent) nociceptors. When activated, TRPs are responsible for “an increase in the intracellular” ion concentration and the transduction of the received stimuli (Marwaha et al., 2016). Meanwhile, centrally expressed TRPs are still “under-studied” (Hu et al., 2023). Among various effects (e.g., neurotransmitter release and muscle contraction), activation of TRP channels can be observed in neuropathic pain conditions. The six TRPs, with TRPV1-4, TRPA1, and TRPM8 being the most important, differ in their activation, regulation, function, distribution, and ion selectivity (Marwaha et al., 2016). However, TRPV1, a channel expressed in small DRG and trigeminal (TG) neurons (Takayama et al., 2019) and on cells like CD4^+ and microglia (Linher-Melville et al., 2020), might be the most mentionable one, as various studies found significant sex-based differences. Also, receptors such as the IL-17A receptor and estrogen receptor subunit α ($\text{ER}\alpha$) are co-expressed in TRPV1^+ neurons (Luo et al., 2021). Many studies mention IL-17A and its receptor as essential in developing chronic pain as various cells produce this interleukin “in response to pathogens or tissue damage” (Jiang et al., 2022). Specifically, TRPV1 is responsible for more pain in females, as estrogen increases this channel's expression and sensitivity (Seol & Chung, 2022). Hence, when binding an agonist to $\text{ER}\alpha$, an intense pain response can be expected, mediated by estrogen itself (Luo et al., 2021). These female-specific factors were confirmed when the TRPV1 agonist capsaicin elicited clinical pain in females while suppressed in males (Linher-Melville et al., 2020). Paradoxically, capsaicin can decrease pain intensity by modulating neuropeptide release from the nociceptors when applied in higher concentrations (Tajti et al., 2023). To elaborate on the “pro-nociceptive effect on TRPV1” (Seol & Chung, 2022), Luo and team (2021) attributed sexual dimorphism to the “IL-23/ IL-17A/ TRPV1” pathway, with female macrophages as antigen-presenting cells (APC), releasing more of these cytokines (e.g. IL-

23 and IL-17) after CIPN, compared to males. Females were also more sensitive to IL-17A, while males required higher doses for similar pain responses. Firstly, they demonstrated in a spared nerve injury (SNI) model that capsaicin-evoked pain was caused by estrogen in females while being suppressed by androgens in males. In their CIPN-based research, mechanical allodynia was reduced in orchiectomized males by administering an IL-23R antagonist, P2305, and in females, by IL-23 and MPP, an ER α antagonist. Lastly, they showed that inhibition of IL-23-induced mechanical pain was achieved in males by administering alioanthone, an androgen receptor antagonist, in combination with IL-23 and PPT, an ER α agonist. In females, testosterone inhibited pain (Luo et al., 2021), confirming the sexually dimorphic and female-specific nociceptive pathway.

The following important channel is TRPM8. It is a cold-sensitive receptor responsible for allodynia in NP (Meacham et al., 2017). It is located in the peripheral nervous system (PNS) and CNS. It has a testosterone-binding site and could be used for paclitaxel-induced peripheral neuropathy in men (Cabanero et al., 2022). A preclinical study showed that after an oxaliplatin administration, female rats displayed higher TRPM8-mRNA- levels in ganglia. This could explain the more severe cold allodynia, while in the spinal cord (SC), no sex differences could be observed (Miguel et al., 2022).

Recent studies have found that TRPA1 and TRPV4, despite being significant in chronic neuropathic pain, do not exhibit sexual dimorphism (Hu et al., 2023). However, for completeness of this work, their role in pain will be briefly discussed. For example, TRPA1 is activated during neuroinflammation and induces hyperalgesia when sensitized in DRG neurons (Takayama et al., 2019). Their NP- function is associated with their down- and upregulation in their respective location. The administration of an antagonist reduces allodynia and sensory hypersensitivity in general (Monteiro de Araujo et al., 2020). Clinically, phenotypical classifications of neuropathic patients' genomic DNA showed that TRPA1 variants couldn't be associated with this condition. Instead, it could be found in chronic widespread pain and fibromyalgia. (Marchi et al., 2023).

Lastly, TRPV4, as a thermosensitive channel (Yi Dai, 2015), is associated with inflammatory diseases and is expressed in different cells, such as macrophages (Rodrigues et al., 2022). Agonists induce the release of various molecules, such as substance P, in the spinal cord (Marwaha et al., 2016). For instance, in an SNI-induced pain model, spinal microglial TRPV4 expression was elicited by the agonist GSK101, while the antagonist GSK219 prevented mechanical pain development (Hu et al., 2023). The following Figure 7 illustrates different types of TRPs on mammalian sensory neurons and their corresponding pain type (Mickle et al., 2016).

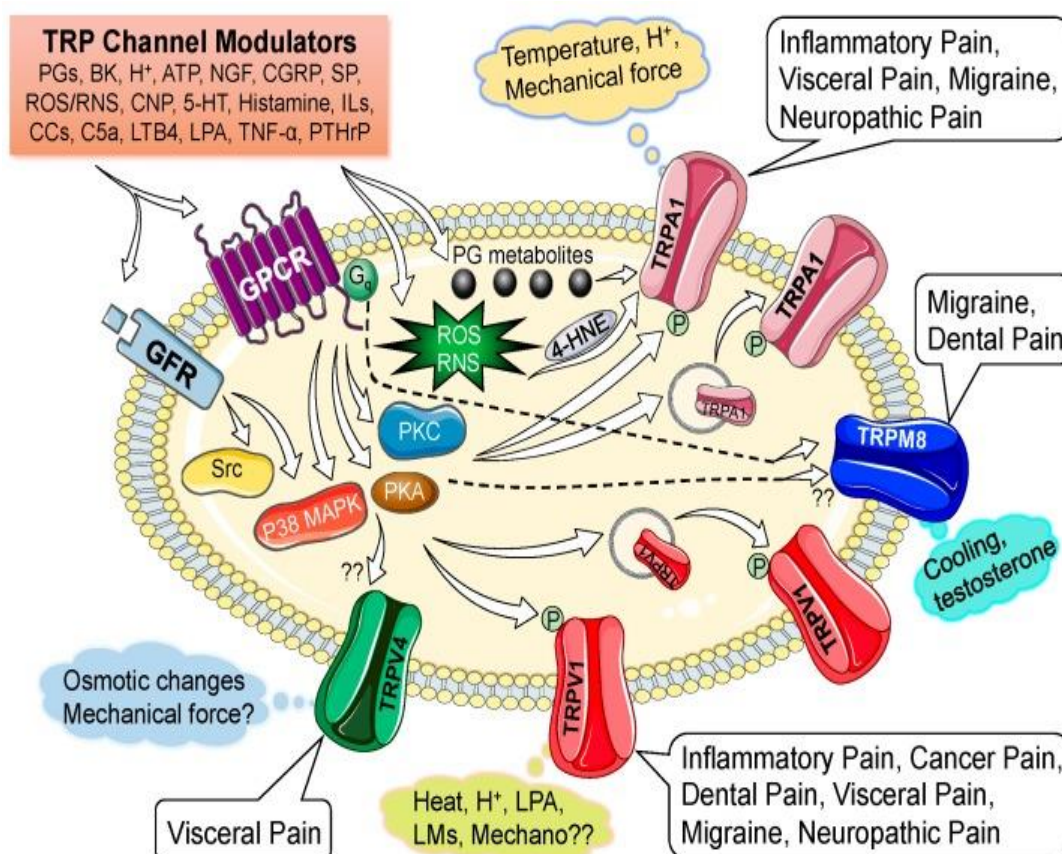


Figure 7. Scheme depicting specific activators and modulators of various nociceptive transient receptor potential (TRP) channels on mammalian sensory neurons. Involvement of individual TRP channels in specific pain-related pathologies are indicated/listed within call-out boxes. Abbreviations: 5-HT, serotonin; ATP, adenosine triphosphate; BK, bradykinin; CCs, chemokines; CGRP, calcitonin gene-related peptide; CNP, C-type natriuretic peptide; GFR, growth factor receptor; GPCR, G protein-coupled receptor; H^+ , protons; ILs, interleukins; LMs, lipid mediators; LPA, lysophosphatidic acid; LTB4, leukotriene B4; NGF, nerve growth factor; p38 MAPK, p38 mitogen-activated protein kinase; PGs, prostaglandins; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PTHrP, parathyroid hormone-related peptide; ROS, reactive oxygen species; RNS, reactive nitrogen species; SP, substance P or neurokinin; Src, src-type protein kinase; TRPA1, transient receptor potential sub-family ankyrin, member-1; TRPM8, transient receptor potential sub-family melastatin, member-8; TRPV1, transient receptor potential sub-family vanilloid, member-1; TRPV4, transient receptor potential sub-family vanilloid, member-4. Reprinted from *Nociceptive TRP Channels: Sensory Detectors and Transducers in Multiple Pain Pathologies* by Mickle et al. (2016), Pharmaceuticals (Basel) 2016 Nov 14;9(4):72, Copyright © 2016 by the authors; licensee MDPI, Basel, Switzerland. This article is licensed under the Creative Commons Attribution (CC BY) license.

Nociceptors also express prolactin receptors (Prlr), which suggest the female-specific pain drive (Luo et al., 2021). Its ligand, prolactin (PRL), is regulated by estrogen, pointing to Prlrs' connection to estrogen-driven pathology (Patil et al., 2019). Such findings were already recognized by Diogenes et al. (2006), stating that PRL augmentation of capsaicin-evoked response, followed by a TRPV1 phosphorylation, is estrogen-dependent (Diogenes et al., 2006). When Prlr is activated (e.g., by PGE2), a more persistent mechanical hypersensitivity in females occurs, as estrogen prolongs the pain. In the absence of the hormone,

hypersensitivity is reduced. When Prlrs are ablated or antagonized, hypersensitivity is reduced in females only (Paige et al., 2020). More specifically, the “female-selective” (Chen et al., 2020) involvement of Prlr in sensory neurons was demonstrated with prostaglandin (PG) E₂. The induced mechanical hypersensitivity was more persistent in females compared to males. The researchers attributed this to estrogen, which prolonged hypersensitivity in females and reduced it when estrogen was absent, hence in ovariectomized (OVX) mice. In males, testosterone regulated pain, prolonging it when testosterone was lacking; thus, it was in gonadectomized (GdX) mice. Conversely, ablation of Prlr in sensory neurons and administration of the PRL antagonist, Δ PRL, reduced hypersensitivity in females only (Paige et al., 2020). In this regard, Stratton and colleagues (2024) confirmed the sex-specific role of PRL in neurons more recently. They demonstrated that PRL increased the action potential frequency in female mice and human donors only. In the DRG, Prlr expression was higher in female mice and human donors than in males. In macaques, PRL showed higher expression in female neurons compared to males. Interestingly, while PRL is female-selective, the researchers found orexin B (ORXB) to be male-specific (Stratton et al., 2024). Orexin B, termed hypocretin-2 (Rainero et al., 2007), is a male-specific molecule that binds to Hcrtr (Stratton et al., 2024) (hypocretin receptor 2) (Fan et al., 2018), which only exists in the male hDRG, having the same effects as PRL in females (Stratton et al., 2024). New preclinical evidence points to the effectiveness of orexin B treatment as it prevents microglia activation (Zhu et al., 2024). In addition, male pain-suppression is due to testosterone, which has a suppressive effect on the immune system in general and causes quicker resolution of pain (V. Taneja, 2018). Testosterone impacts microglial activity, such as the testosterone-dependent TLR4 receptors (Lee et al., 2023). These microglial receptors elicit pain in males only, with male microglia preferentially expressing the M1 phenotype post-injury (O’Connor & Nissen, 2023). Also, TLR9 receptors resolve allodynia in males, as post-CIPN, it was gone in males but persisted in females (Luo et al., 2019). More details regarding such male-specific findings in pain research will be discussed later in the respective chapters.

6. Sex- related factors

Estrogens and androgens play an important role during puberty, where an activational sex difference in the immune system is induced (Rainville & Hodes, 2018). The onset of puberty in humans marks “10 and 11 years for females and 11 and 12 years for males”, with girls undergoing pubertal development 2-3 years longer than their opposite sex. Interestingly, mice develop similarly to humans (Smith et al., 2023). When the same age as their opposite sex, females show more innate and adaptive leucocytes except for Treg- and innate lymphoid- cells, like natural killer (NK) cells. Additionally, hormones influence leucocyte behavior. For instance, estrogen can inhibit the innate immune response while facilitating a humoral immune response. Testosterone can inhibit humoral immune activity while facilitating innate immune response (Meester et al., 2020). Furthermore, estrogen and progesterone are responsible for the suppression of the Th1 response while increasing the Th2 response. Conversely, testosterone “suppresses Th2 responses with contradictory effects on Th1 responses (Mogil et al., 2024). Estradiol and progesterone especially play an important role in the perception of female pain (Shen et al., 2023). With estrogen and progesterone being very low in postmenopausal women, some studies suggest that sex differences in pain seem to be less pronounced with higher age (Vincent & Tracey, 2008). Such diminishing sex differences “with advancing age” were later mentioned by Girotti and colleagues (2019) when measuring pressure pain thresholds (PPT). The researchers attributed this to the reduced “androgen concentrations” in males and other factors, indicating that lowered male hormone concentrations reduce the hormone-driven sex differences in pain. However, PPT is generally lower in females and older individuals than men and younger individuals. (Girotti et al., 2019). Hormones, such as estradiol, progesterone, and testosterone, are also responsible for an adequate TRP expression in nociceptors and act in a sex-specific manner (Miguel et al., 2022). In general, a suppressive effect of testosterone on the immune system and an enhancing effect of estrogen have been discovered (V. Taneja, 2018). However, the actual effect of estrogen is highly dependent on its levels, acting pro-inflammatory when fluctuating and protective when stable (Athnaiel et al., 2023). Additionally, estrogen can increase pain when its levels decrease (Strand et al., 2024). Interestingly, it’s also been reported that experimental “gender-role manipulation” influenced the sex-related differences in pain response in connection with gender-based expectation, with women showing higher pain tolerance (Kim & Kim, 2020). Such expectations include males being less willing to report pain, enduring more pain, and being less sensitive to pain (Robinson et al., 2001). Cohesively, women reported being more sensitive and willing to report pain. This only confirmed the “classical gender pain theorem where masculinity is associated with stoicism and less willingness to express pain and femininity associated with sensitivity to pain and a willingness to express pain” (Alabas et al., 2013). Men are also less willing to seek health care and are seen as

“the norm” to which women are compared and, importantly, “being deviant from the norm”, going as far as female pain being “psychologized” (Samulowitz et al., 2018). Notably, such sex, or rather gender differences also being less pronounced when older, which suggests male indifference to masculine stereotypes (Girotti et al., 2019).

6.1. Estrogen

Estrogen is known to protect from harmful stimuli, reduce neutrophil infiltrates, suppress innate immune response, increase humoral response, and reduce central tolerance (Meester et al., 2020). It also regulates various genes, such as MUSTN1 (Ray et al., 2019), which stands for musculoskeletal, embryonic nuclear protein 1 (NCBI, 2024, August 17). Among others, estrogen was found to correlate with age inversely and is associated with chronic pain (Ray et al., 2019). Microglial receptors, like ER α and β , bind estrogen, inhibiting their activation, proliferation, and production of pro-inflammatory cytokines (Linher-Melville et al., 2020). Additionally, it can modulate and eventually inhibit P2X3 receptors and attenuate female pain (Seol & Chung, 2021). On the other hand, estrogen can also cause a pro-inflammatory response and increase pain sensitivity in females, depending on its concentration (Athnail et al., 2023). To emphasize estrogens' effect in connection to its levels and pain, Figure 8 (A) demonstrates hormonal changes during a “normal” menstrual cycle (Chidi-Ogbolu & Baar, 2019). Dependent on these levels, clinical studies have shown that estrogen stands in connection with vulnerability to migraines. For example, low estrogen is present in the “early” and “late luteal phase” and the early follicular phase, increasing the risk for migraine, while in the “midluteal” phase, higher estrogen is responsible for less intense headache (M. de Tommaso, 2011). Further investigations revealed that pain scores are higher in the luteal phase than in the follicular phase. However, pain perception varies between individuals (Wang et al., 2019). Image 8 (C) reflects estrogen levels 5 years before and after menopause (Chidi-Ogbolu & Baar, 2019). Perimenopause occurs 2-8 years before menopause (Godley et al., 2024) and is associated with musculoskeletal pain (Strand et al., 2024). The postmenopausal stage is marked by low estrogen and progesterone, which is associated with increased pain, such as bone pain (Strand et al., 2024).

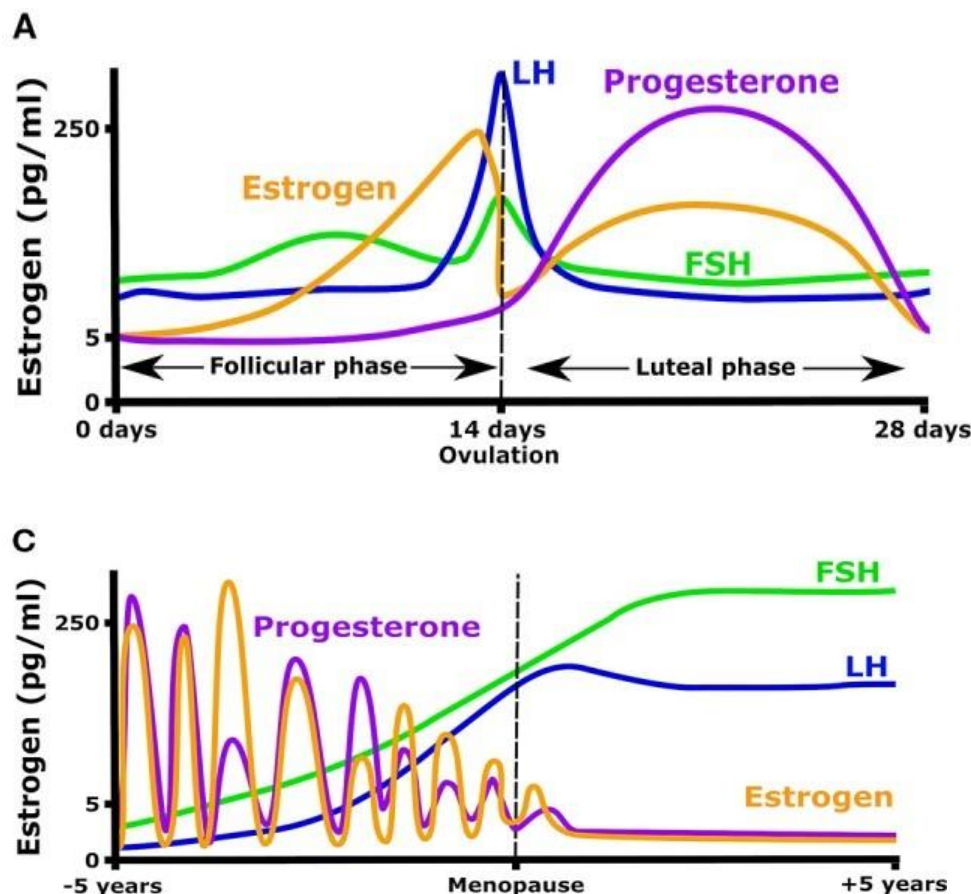


Figure 8: Hormonal fluctuation during (A) a normal menstrual cycle (C) in the years before and after menopause. Adapted from: *Effects of Estrogen on Musculoskeletal Performance and Injury Risk* by Chidi-Ogbolu & Baar. (2019), *Frontiers in Physiology*. 2019 Jan 15;9:1834. Copyright © 2019 by Chidi-Ogbolu and Baar. This article is licensed under the Creative Commons Attribution (CC BY) license.

Preclinical

The estrogen-protective function in females and the importance of estrogen (E₂)- mediated sexual dimorphism was presented by Lee et al. (2023), using intrathecal ganglioside GT1b-administration. GT1b acts as an endogenous microglial TLR2 agonist, which activates spinal cord microglia, expressing similar transcript levels of TLR2 in both sexes. However, it induced central pain sensitization in male mice only, while in females, it is mediated by recruited T-cells to the spinal cord. While females had a reduced microglial process length, males displayed an increased one on day 1 post-injection. Female resistance to GT1b-induced mechanical allodynia was confirmed when 2 weeks post ovariectomy (OVX), estrogen levels decreased and, in return, made female mice susceptible to GT1b- induced pain (Lee et al., 2023).

Pro-inflammatory estrogen properties can occur when they are low in concentration. Only recently, Athaniel et al. (2023) mentioned that estrogen contributes to a higher prevalence of various pain-related conditions, such as migraine in females. The pro-inflammatory effects of estrogen are linked to its fluctuations, making females more prone to decreased pain thresholds, while stable levels act as protectors. As mentioned, low estrogen is

responsible for a greater pro-inflammatory response, while increased levels cause higher pain sensitivity. Considering the dual nature of estrogen, different age- and cycle-dependent concentrations (Athnaiel et al., 2023) highlight the complexity of pain and its treatment in females.

Clinical

When it comes to clinical sex-based evidence, this pro-inflammatory, hormone (or sex)-dependent aspects could already be seen in a 2007 cross-sex hormone therapy study, in which “male to female transsexuals (MtF)” received estrogens and anti-androgens, while “female to male transsexuals (FtM)” received androgens. Besides the desired outcomes in the feminization or masculinization of the opposite sex, respectively, hormone-dependent pain development was reported. Approximately one-third of MtF participants developed chronic pain, such as headache, and their pain sensitivity increased. On the other hand, approximately half of the FtM candidates experienced relief in their chronic pain, while FtM participants who didn’t suffer from chronic pain before didn’t show any change in their sensitivity (Aloisi et al., 2007). Later, in an age-based study with both sexes, oxaliplatin-induced peripheral neuropathy (OIPN) was examined by Wang and team (2022). They evaluated in a meta- analysis of observational studies the risk factors for CIPN. Results showed that older females are more likely to develop OIPN in general. This implicates a connection to the decrease of the protective estrogen on peripheral nerves (Wang et al., 2022), again marking the importance of stable and adequate estrogen levels in order to alleviate pain in females (Athnaiel et al., 2023).

6.2. Progesterone

Progesterone, which dominates the luteal phase in the menstrual cycle, as demonstrated in Figure 8 (Chidi-Ogbolu & Baar, 2019), exhibits sedative, anxiolytic, analgesic and anticonvulsant effects via the interaction with GABA_A receptor (Filipescu & Stefan, 2020). More specifically, its’ metabolite, allopregnanolone accounts for analgesia in rodent models (Choi et al., 2023), as it can “potentiate the inhibitory effects of GABA” (Nasirzadeh et al., 2024). Progesterone also inhibits the upregulation and phosphorylation of NMDAR subunits (Coronel et al., 2017). This “neurosteroid” (Choi et al., 2023) can be neuro-protective, and it can sustain its effects even after treatment. The reason for this is that it modulates pain by reducing inflammatory response via microglia and astrocytes post-SCI (González & Coronel, 2016). Hence, progesterone prevents the production of pro-inflammatory mediators, such as IL-6 and TNF α (Coronel et al., 2017). Furthermore, progesterone, like testosterone, is responsible for the downregulation of peripheral adaptive immunity, promotes peripheral innate immunity and the recruitment of monocytes (Meester et al., 2022). Due to generating its own “molecular microenvironment”, the activation of protective neuropeptides might be redundant when progesterone is administered chronically (Coronel

et al., 2017). Interestingly, progesterone and testosterone and pain exhibit an “inverse relationship” in females suffering from fibromyalgia (Strand et al., 2024). These patients reported lower pain when progesterone levels were high (mid-luteal phase) and greater pain when all hormones were low (menstrual phase) (Schertzinger et al., 2018). Additionally, progesterone “mediates antinociception” during pregnancy (Strand et al., 2024). Also noteworthy are galanin and tyrosine, known as neuropeptide Y (NPY), whose expressions are modulated by progesterone (Coronel et al., 2017). NPY binds to its receptor, Y1R, and plays an important role in pain. In this regard, a dose-dependent reduction of mechanical and cold allodynia could be observed when a Y1R agonist was administered (Malet et al., 2017). The alleviating effect of Y1R agonists in neuropathic pain was confirmed, even though no significant sex difference could be obtained (Nelson et al., 2023).

Preclinical

Pain-alleviating effects have already been demonstrated by Dableh & Henry (2011) when administering progesterone to rats. They found that progesterone reduces the development of pain and causes recovery from pain when given “immediately after nerve injury” and over a certain “period of time” (Dableh & Henry, 2011).

A more recent study by Abtin and team (2023) demonstrated the pain-dependent interaction between progesterone, ephrin-B2, and NMDAR (Abtin et al., 2023). Notably, Ephrin-B/EphB is responsible for NMDAR phosphorylation and activation (Kaur et al., 2024). In the study, a CCI-induced upregulation of ephrin-B2 expression, a ligand of the Eph- receptor, could be blocked by progesterone administration, reducing pain behavior in rats, suggesting an alleviating effect in NP (Abtin et al., 2023). The injury-increased activity of postsynaptic NMDAR and the development of chronic pain were already mentioned by Coronel et al. (2011). They found that NMDAR effects could be prevented by progesterone in rats. Specifically, it prevented the increase of the number of NMDAR subunit 1, NR1, which accounts for the receptor activity. Furthermore, PKC γ was also inhibited in its number-increasement (Coronel et al., 2011).

However, sex differences related to progesterone and chronic NP were found by Ungard and team (2020) in a cancer-induced bone pain (CIBP) model and a NP model when examining progesterone and pregabalin treatment. According to the findings, progesterone and pregabalin administration was able to delay mechanical hypersensitivity in females, compared to their control group. Males, on the other hand, showed no significant reduction in pain sensitivity post-treatment. Next, in a NP model, males returned to their baseline 2 weeks after treatment, recovering from mechanical hypersensitivity and having a generally increased excitation threshold, while female pain thresholds remained increased (Ungard et al., 2020).

Also sexually dimorphic is the expression of “PNS-myelin proteins”, such as “peripheral myelin protein 22 (PMP22)”, stimulated by progesterone, and secondly, “glycoprotein zero

(P0)", being stimulated by progesterone and dihydroprogesterone (DHP) in male Schwann cells only. Meanwhile, tetrahydroprogesterone (THP) stimulates PMP22 and P0 in female cultures only (Magnaghi et al., 2006).

Clinical

From a clinical perspective, high-level progesterone is responsible for the reduction of how pain is perceived by disconnecting it from emotional pain-aspects, highlighting the connection between sensory and emotional perception, known as "luteal analgesia" (Vincent et al., 2018).

In a case-control study, menopausal women with trigeminal neuralgia (TN) had generally lower estrogen and higher progesterone levels when compared to healthy female controls, but the results weren't significant. On the other hand, testosterone was significantly higher and prolactin lower in TN patients. However, "abnormal" progesterone and estrogen levels are more common in the TN group, suggesting that hormonal imbalance increases the risk for PNP in menopausal women (Lavaee et al., 2022).

6.3. Testosterone

Testosterone is synthesized from cholesterol and its precursors, such as pregnenolone (PREG), dehydroepiandrosterone (DHEA), androstenedione, with dihydrotestosterone (DHT) being its active form (Falvo et al., 2020). As mentioned before, testosterone shares the same immune system- characteristics as progesterone but it additionally reduces the production of immunoglobulin G (IgG) and IgM (Meester et al., 2022). Even though sharing the same intracellular molecule known as myeloid differentiation primary response (MyD) 88, there's a difference between the testosterone-independent TLR2-mediated pain and TLR4-induced pain. TLR4-induced pain is testosterone-dependent, as TLR4 includes an additional interferon regulatory factor (IRF) 3- signaling (Lee et al., 2023). In this regard, testosterone points to male pain- suppression by having a suppressive effect on the immune system in general and causing quicker resolution of pain (V. Taneja, 2018). Testosterone impacts microglial activity, such as the testosterone-dependent TLR4 receptors (Lee et al., 2023). These microglial receptors elicit pain in males only, with male microglia preferentially expressing the M1 phenotype post-injury (O'Connor & Nissen, 2023). Also, TLR9 receptors resolve allodynia in males, as post-CIPN, it was gone in males but persisted in females (Luo et al., 2019).

Preclinical

Preclinical evidence shows that microglia are activated in certain pain conditions. However, it was discovered that male mice develop less hypersensitivity if they lack adaptive immune cells. Females on the other hand, switch to T-cell activation and show higher T-cell levels overall. Hyperexcitability- and sensitivity in females are suggested to rely on/be caused by

different expressional states in T-cells, which present a testosterone-dependent sexual dimorphism in the expression of peroxisome proliferator-activated receptors (PPARs). Testosterone increases the expression of PPAR α but decreases PPAR γ -expression and interferon gamma (INF γ)-production. This observation may explain the relatively diminished abundance of pro-inflammatory mediators in male mice (Mappleback et al., 2017).

Another preclinical study also mentioned a testosterone-dependent pain mechanism, where an administration of minocycline blocked the microglial purinergic receptor P2X₄ and decreased mechanical allodynia in male mice only (Sorge et al., 2015). However, Ciapała and Mika (2023) attribute the “analgesic effect of minocycline” to a “direct inhibition” of matrix metalloproteinase (MMP) and p38 mitogen-activated protein kinase (MAPK), which in turn affects spinal pronociceptive factor levels indirectly (Ciapała & Mika, 2023). This example shows how much further work is required to unambiguously be able to pinpoint the mechanistic underpinnings of certain treatments (e.g., minocycline) in the context of sex differences.

Sexual dimorphism can also occur at different time points, which was demonstrated in an analysis of metabolic processes in adipose tissue (AT) post-injury. Results showed that insulin levels were upregulated in both sexes 6h post-PNI, returning to their base levels (BL) 7 days post-injury (dpi), but being generally higher in females at the latter time point. Glucagon levels were decreased in females and increased in males 6h post-PNI, but were decreased in both sexes 7 dpi. Triglyceride levels were downregulated in females and upregulated in males 24h post-injury, with a downregulation in males 3 dpi. 48h and 7 dpi, only females had a lower respiratory exchange ratio (RER), while in males, such was observed at the latter time point. Additionally, females had higher energy expenditure (EE) and resting energy expenditure (REE) 48h and 7 dpi, with a decrease in both sexes 7 dpi. Lastly, males exhibited an upregulation in PPAR γ and adiponectin, while in females, a higher sex hormone secretion was found (Vacca et al., 2023).

Further differences could be shown in an older preclinical study by Vacca and team (2021) when analyzing expressional patterns post-CCI. While testosterone has an important role in leptin secretion in males, a dysregulation of leptin itself is associated with pain in females. In this regard, an upregulation of leptin was found in female lysates while being downregulated in serum. FasL followed the same pattern. However, 17 β - estradiol administration caused an upregulation of both molecules and decreased allodynia in females. Generally, CCI elicited higher macrophage infiltration in male mice. On the other hand, female sciatic nerve lysates and serum samples contained upregulated interleukins, such as IL-10 and CXC motif chemokine ligand (CXCL) 11, among others. Also, here, 17 β -estradiol administration resulted in CXCL11 downregulation in females (Vacca et al., 2021).

Considering the appetite-regulating and pro-inflammatory effects of leptin (Younger et al., 2016) and its' inverse relationship with testosterone (e.g., high testosterone, low leptin) (Lima et al., 2020), leptin-dependent pain in females seems plausible.

7. Neuroimmunology

The immune response is coded by genes located on the X chromosome, and its chromosomal inactivation is associated with neural disease (Linher-Melville et al., 2020). It has more immune-related genes compared to the Y chromosome. Meanwhile, the Y chromosome inhabits genes that regulate immune system gene transcription (Rainville & Hodes, 2018). Notably, sex chromosomes mark the immunological differences between sexes, while sex hormones are responsible for dynamic regulations of the immune system (Lakshmikanth et al., 2024). Additionally, different pain- pathways describe the complexity of this pain-immology and will be discussed later on. It is evident that the immune system and neuroinflammation contribute to chronic neuropathic pain by activating nociceptors and central sensitization via different cells (Basu et al., 2023). Glia cells in the nervous system are crucial in neuropathic pain pathogenesis and occur in CNS in the form of “microglia, astrocytes, and oligodendrocytes”. In the peripheral nervous system, “Schwann cells, satellite glial cells (SGC), as well as enteric glia” are present (McGinnis & Ji, 2023). Astrocytes, which can be pro-inflammatory A1 or anti-inflammatory A2 (Mao et al., 2024), and microglia liberate BDNF and IL-1 β among other molecules, transmitting information to the brain (P. Smith, 2023). Notably, IL-1 β causes NMDAR phosphorylation, subsequently modulating synaptic excitation. Also, BDNF binds to its receptor, tyrosine receptor kinase (Trk) B (Bathina & Das, 2015) and attributes to the phosphorylation of the NMDAR Subunit (Kohno & Tsuda, 2021). Its activation is also associated with sexually dimorphic hyperexcitability (Linher-Melville et al., 2020) which will be discussed later on. In both cases, BDNF and IL- 1 β cause a GABA- and glycine-mediated synaptic inhibition in SDH in a “BDNF-TRKB-KCC2 pathway”- manner (Kohno & Tsuda., 2021). The release of BDNF and its binding to TrkB causes pro-nociceptive effects, which subsequently contribute to neuropathic pain (Ismail et al., 2024). By inhibiting the TrkB receptor, PNI- induced mechanical allodynia could be reversed in males only (Ghazisaedi et al., 2023). Also, SNI- induced mechanical hypersensitivity is dependent on DRG- macrophages. They contribute to the initiation and maintenance of pain and their expansion can be inhibited by the deletion of colony-stimulating factor-1 (CSF-1) from sensory neurons in male mice only (Yu et al., 2020). The literature suggests that females display a stronger immune response, which can be attributed to a larger immune cell population, higher immunoglobulin (Ig)- levels, increased cytokine production and T-cell response (Rainville & Hodes, 2018). It is suspected that the reason for this sex- biased immunological differences is evolutionally ingrained in us, as it’s crucial during the reproductive years, hence for the preservation of mankind (V. Taneja, 2018). In this regard, Figure 9 illustrates preclinical male and female CSF-1 pathway- differences in dorsal horn excitation. In males, CSF-1 causes the expression of microglial P2X4, an ATP receptor and a subsequent BDNF release, while in

females, CSF-1 activates (regulatory) T-lymphocytes, leaving microglia unaffected (P. Smith, 2023).

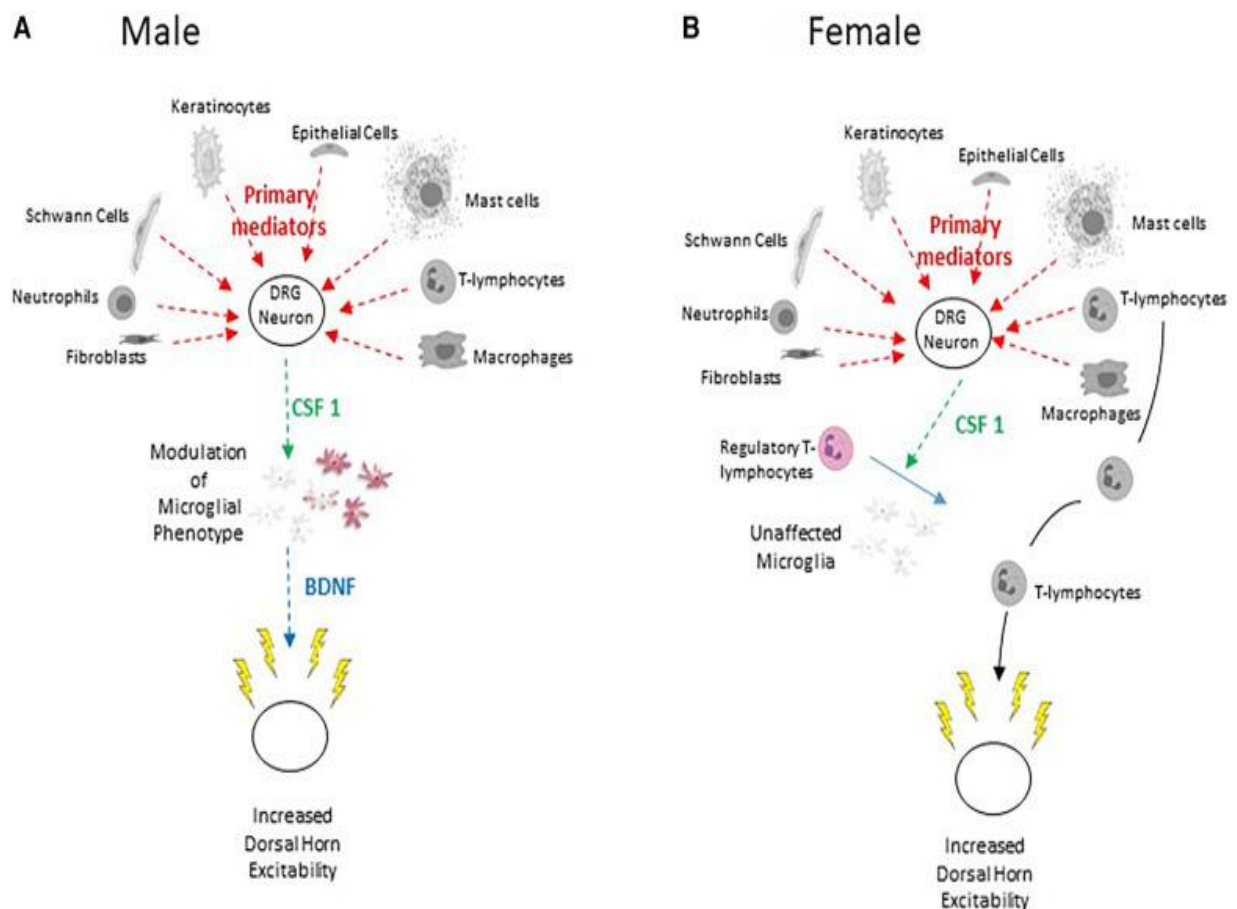


Figure 9. **(A)** Scheme to illustrate the sensory neuron – CSF-1 – microglia – BDNF pathway characterized in male rodents. Lightly coloured microglia represent those in the resting state and the purple colour represents their transformation to the P2X4 expressing phenotype **(B)** Scheme to illustrate train of events leading to injury-induced increased dorsal horn excitability in females. Reprinted from *Neuropathic pain; what we know and what we should do about it* by P. A. Smith (2023), *Front Pain Res (Lausanne)*, 2023; 4: 1220034. Copyright © 2023 Smith. This article is licensed under the Creative Commons Attribution (CC BY) license.

To further highlight the importance of sex and their respective immune (e.g., infectious, inflammatory, autoimmune) response, a study focusing on testosterone's impact was conducted. In this study, “trans men, who were assigned female sex at birth” underwent “gender-affirming hormone therapy (GAHT)” with testosterone. Administering the hormone into biological females attenuated their IFN-I response, resulting in a decreased IFN α production in dendritic cells (DC). Further, IFN γ production in NK cells was increased, resulting in higher immune response. Lastly, SLAMF7, a receptor potentiating the TNF response, was upregulated in T-cells and monocytes, resulting in a potentiated TNF activation in monocytes. TNF and CSF-1 “induce NF κ B- regulated genes” and inhibit IFN-I. These outcomes led the researchers to the conclusion that trans-male patients exhibit similar immune responses regarding TNF and IFN-I when compared to male cisgender cohorts (Lakshmikanth et al., 2024). Considering the antiviral properties of IFN-I and the generally stronger response of this molecule in females (Pujantell & Altfeld, 2022), it seems

plausible that testosterone would reduce IFN- γ response in transmen during GAHT. Also, males are known to have more NK cells and express more pro-inflammatory cytokines (e.g., IL-6, TNF α) compared to females (Ruggieri et al., 2016). This could also explain the higher production of IFN γ in NK cells when testosterone is applied in biological females (or transmen). These results accentuate testosterone's impact on immunological events between biological men and women. Individuals born females and receiving this masculinizing hormone exhibit similar properties as biological men. This could suggest an adaptation to the respective immunological processes when the respective hormone is present.

Noteworthy are the results of a clinical data analysis by Togioka et al. (2023) regarding a gender-affirming hormone (GAH) therapy. After gender-affirming surgery (GAS), the incidence of postoperative neuropathic pain was 3.9%, which meant that for every 26th GAS, one neuropathy case occurred. Additionally, the occurrence of neuropathy in transgender patients could be associated with longer procedure duration and an increased number of pressure point assessments (Togioka et al., 2023). Unfortunately, from this study, it is unknown if the incidence of chronic NP was higher in trans males or trans females, as in this case, NP is the result of an operation, leaving room for further sex-based investigations.

7.1. Microglia

Having previously mentioned the significance of microglia, it's inevitable to delve deeper into their critical role in chronic neuropathic pain. Microglia, also known as macrophages, are a non-neuronal part of the CNS and a part of the immune system (Prinz et al., 2019). They contribute to neurogenesis, synaptogenesis, and synaptic plasticity (Vandermeulen et al., 2024). They express microglial receptors, like the P2XR (Machelska & Celik, 2016), the cannabinoid receptor 2 (CB2) (Fiore et al., 2023), and myeloid-like receptors, such as TLRs. Further microglial receptors include the triggering receptor expressed on myeloid cells (TREM) and CD36. Trem2 is a DNAX-activating protein of 12 kDa (DAP12) associated receptor, and in case of nerve injury, the latter is phosphorylated in active microglia. Their co-dependency was evident when, in DAP12 deficient mice, Trem2-dependent allodynia was inhibited (Kobayashi et al., 2016). Microglia generally occur in their pro-inflammatory and neurodegenerative M1 phenotype and anti-inflammatory and healing M2 phenotype (O'Connor & Nissen, 2023). While microglia play an essential role in the progression of neuropathic pain in male rodents via the P2X4/p38/BDNF pathway (Machelska & Celik, 2016), it seems that females, dependent on the pain model, likely rely on a T-cell interaction (Mappleback et al., 2017) or NF κ B-signaling (Maguire et al., 2022). Figure 10 illustrates microglia and the upregulation of its receptors as a response to nerve injury (Jo et al., 2009).

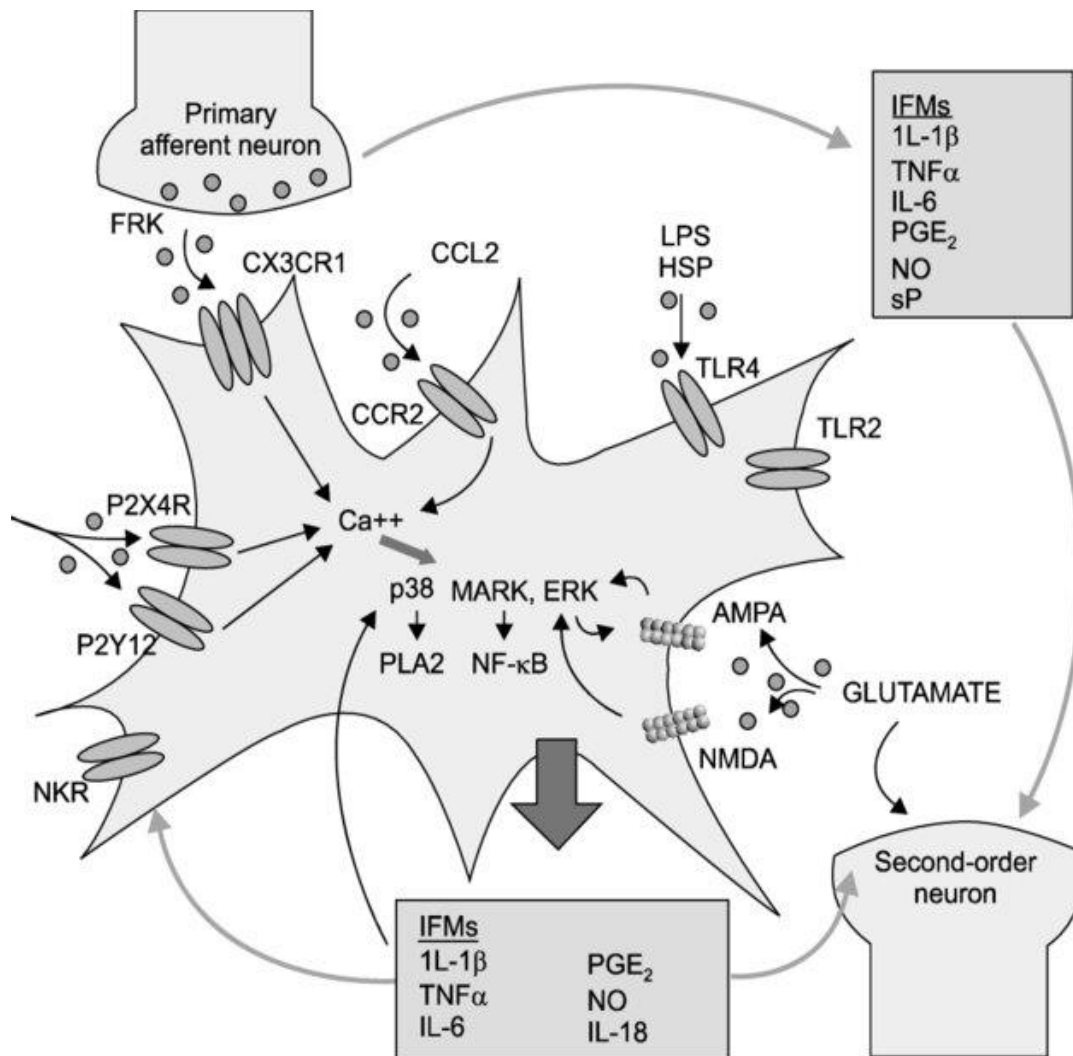


Figure 10. Activation and response of microglia in neuropathic pain. Nerve injury upregulates multiple receptors, including the chemokine receptors CX3CR1, P2X/Y, CCR2 and TRL4 in spinal microglia, and it induces the release of fractalkine and neurotransmitters from the primary afferent neuron that also activate microglia. Activation causes microglial cell increase intracellular calcium, and initiates the p38 MAPK/ERK pathway, which is necessary for the release of pre-inflammatory substances. The activated microglia release several pro-inflammatory cytokines, chemokines and other agents that modulate neural transmission by affecting presynaptic release of neurotransmitters and/or postsynaptic excitability. AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid, ATP: adenosine triphosphate, CCL2: cystein chemokine ligand 2, CCR2: chemokine ligand 2 receptor, ERK: extracellular signal regulated kinase, FRK: fractalkine, HSP: heat shock protein, IFMs: inflammatory mediators, IL-1β: interleukin-1beta, IL-6: interleukin-6, IL-18: interleukin-18, LPS: lipopolysaccharide, MAPK: mitogen activated protein kinase, NF-κB: nuclear factor-κB, NKR: neurokinin receptor, NMDA: N-terminalD-aspartate, NO: nitric oxide, PGE₂: prostaglandin E₂, PLA2: phospholipase A₂, TLR: toll like receptor, TNFα: tumor necrosis factor alpha. Reprinted from *Glial Mechanisms of Neuropathic Pain and Emerging Interventions* by Jo et al. (2009), *The Korean journal of pain* 22(1). CC BY-NC 3.0.

Without adaptive immune cells, preclinical evidence shows that female mice could switch to a male-specific microglial pathway to produce allodynia (Sorge et al., 2015). In this connection, it is believed that while pain chronification in males seems to be mediated by the innate immune system, in females, it is thought to be connected to the adaptive immune system (Gregus et al., 2021). The microbiome also seems to be an important indicator of

microglial vulnerability, as in males, it is more sensitive in early life stages, while in females, it's in their adulthood (Halievski et al., 2020). These and other sexually dimorphic microglia-dependent factors have been observed in preclinical studies using various pain models.

Preclinical

For example, in a CCI pain model, sexual dimorphism in microglia was linked to differences in gene expression. There, males exhibited an overall shift in gene transcription, with increased ribosomal genes, higher microglial activation, and a stronger inflammatory response. They displayed an upregulation of different microglial genes associated with demyelination and myelination. Male spinal microglia were also more likely “to develop a reactive phenotype and phagocytose debris after” injury. In contrast, females showed increased other molecules, such as Trem2 (Fiore et al., 2022). However, sex differences in pain depend on various factors, which Huck et al. (2022) mentioned among other researchers. They implicated the importance of sex-specific timing in glial interventions. Therefore, in a complex regional pain syndrome (CRPS) model, microglial activity- markers, such as Ionized calcium-binding adapter molecule 1 (Iba1) and TMEM119, were increased in both sexes at different time points. For example, males had increased Iba1 activity starting on day 1 post fracture, peaking on week 1, while females had a delayed increase on week 1. TMEM119 increase was reported on day 1 in males and week 3 in females. Additionally, females had a higher percentage of IL-6 mRNA expression in TMEM119- microglial cells, compared to no expression in males. The study also found that males had more myeloid lineage cells infiltrating the injury site in the first week, while females showed this increase in the 7th week (Huck et al., 2022). However, “TMEM119 is neither a specific microglia marker protein nor does it label all microglia” (Vankriekelsvenne et al., 2022). More recent evidence regarding microglial activity was conducted in an experimental autoimmune encephalomyelitis (EAE) model. An intraperitoneal (i.p.) administration of the TNFR2 agonist, p53-sc-mTNF_{R2}, at different time points reduced cortical Iba1 and mechanical hypersensitivity in both sexes. Additionally, reduced motor symptoms were seen in females only, while at later time points, the agonists’ efficacy for mechanical hypersensitivity was gone in males only (Nguyen et al., 2024). These results stand in contrast to previous findings where it has been indicated that pharmacological activation of TNFR2 works in a sex- independent manner using the CCI model (Fischer et al., 2019), again highlighting the importance of the used pain model. Figure 11 demonstrates the pro-inflammatory pathways of TNFR1 and the anti-inflammatory ones of TNFR2.

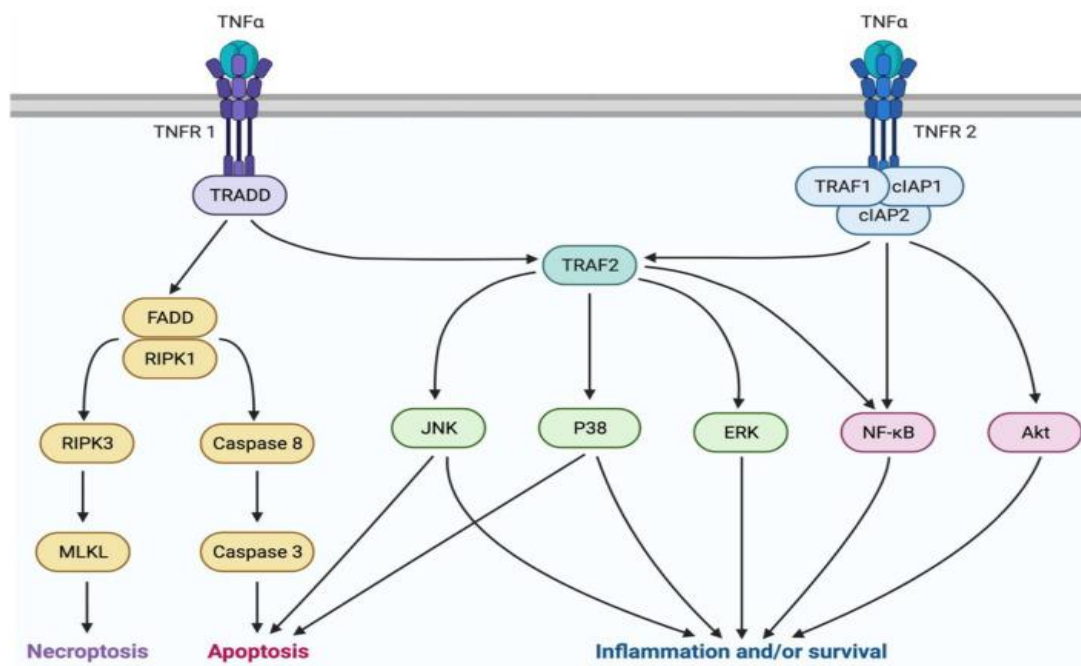


Figure 11. TNF α signaling is mediated by two isoforms of the cytokine and two receptor subtypes. sTNF α can interact with TNFR1 to mediate either pro-inflammatory or pro-death signaling. Pro-death signaling occurs through recruitment of FADD and RIPK1, which can either lead to necroptosis through RIPK3 and MLKL activation, or apoptosis through caspase8 and caspase 3 activation. Pro-inflammatory signaling occurs through TRAF2 activation of P38, JNK, or ERK MAPKs, or NF κ B. Alternatively, tmTNF α can interact with TNFR2 to mediate pro-homeostatic or pro-inflammatory signaling through TRAF1, cIAPs 1 and 2, and TRAF2. This pathway can be mediated by activation of MAPKs, NF κ B, or Akt. Reprinted from *TNF α in MS and Its Animal Models: Implications for Chronic Pain in the Disease* by Maguire et al., 2021, Front Neurol. 2021 Dec 6;12:780876. Copyright © 2021 by the authors. This article is licensed under the Creative Commons Attribution (CC BY) license.

Regarding Iba1 expression and hypersensitivity, it points to the age-dependent activity of microglia post-PNI. For example, Zeinali et al. (2016) reported that Iba1 expression was significantly lower in juvenile rats (6 weeks old) compared to pups (4 weeks old) and mature rats (10-12 weeks old) (Zeinali et al., 2016). Later, Iba1 expression was lower in neonatal rodents (10 days old) than in adults due to increased anti-inflammatory cytokines, like IL-4 and IL-10, in SDH (Inoue & Tsuda, 2018). The results highlight the importance of integrating all age categories, ideally standardized and unified, to provide a more coherent insight into microglial activity with their markers.

Recent evidence for another sexually dimorphic immunological activation was documented by Al-HadlaQ and Masocha (2023) in an antiretroviral-induced pain model. They used the antiretroviral drug “2’-3’- dideoxycytidine (ddC), a nucleoside reverse transcriptase inhibitor (NRTI)”. Elevated mRNA transcript levels of microglial markers Cd11b and Iba1, T-cells, pro-inflammatory cytokines IL-1 β and TNF, and anti-inflammatory cytokines like IL-10 were observed in the spinal cord. Females showed higher protein levels of Cd11b and phosphorylated p38 MAPK, while males had higher Iba1 protein levels, indicating greater sensitivity to mechanical stimuli in females. However, the development of “ddC-induced

mechanical allodynia” occurred “to the same extent in both” sexes. These findings suggest a higher susceptibility to drug-induced neuroinflammation in females (Al-HadlaQ and Masocha, 2023).

Regarding interleukins, their importance has already been addressed, and the differences in the respective sexes will be elucidated here. For instance, sex differences in IL-1 β production and expression were observed in a CCI model on days 7 and 14. The injury caused an increase in IL-1 β mRNA levels in both sexes, but it was generally higher in females at both time points. IL-1 β protein levels were only present at the later time point in both sexes, being typically higher in males. Blocking IL-1 β with IL-1RA reversed allodynia in both sexes at both measured time points. These results indicate that gene and protein expression can vary between sexes. Additionally, different inflammasomes, such as NLRP3 and AIM2, were elevated depending on the measured time points and the respective sex (Green-Fulgham et al., 2023). Inflammasomes, such as NLRP3, known as “nucleotide-binding oligomerization domain-like receptors family pyrin domain containing 3,” can be found in chronic pain conditions. Activating TLR or TNFR activates the NF κ B pathway, which triggers “inflammasome-related compounds,” subsequently resulting in IL-1 β release (Basu et al., 2023).

7.2. Selected sexually dimorphic pathways

While females depend on the female-specific T-cell pathway (Mappleback et al., 2017) and the Prolactin- pathway (Luo et al., 2021), male-specific pathways include the STEP₆₁-pFyn-pGluN2B pathway (Dedek et al., 2022) and the P2X4/p38/BDNF pathway (Machelska & Celik, 2016). In the last one, the inhibition of spinal P2X4R reverses hypersensitivity in males only, while females lack a spinal microglial P2X4R upregulation (Kim & Kim, 2020). Sexually unspecific pathways involve the BDNF-TrkB-KCC2 pathway, IL- 23/ IL- 17A/ TRPV1 pathway, BH4&KYN pathway (Ghafouri et al., 2022), LKB1/AMPK pathway (Garner & Burton, 2022) and Tiam1/Rac1 pathway (Li et al., 2023).

7.2.1. TLR- Pathway

As already mentioned, in preclinical literature, it's been implicated that microglial activation via toll-like receptor 4 (TLR4) induces pain in males only (Taves et al., 2016). In turn, their activation triggers NF κ B signaling and the production of inflammatory cytokines in myeloid cells and is also expressed in neurons (Li & Ward, 2024). TLR9 has also been indicated to play a sexually dimorphic role in CIPN (Luo et al., 2019). Interestingly, TLR7 is known to be x-gene linked, which tends to be expressed at higher levels in females, but has been found to help resolve pain in both acute and chronic inflammation models for both sexes (Friedman et al., 2023).

Preclinical

For example, in a CIPN model with TLR9 deficient mice and other mouse strains, the role of TLR9 was examined. TLR9-deficient and wild-type (WT) mice developed mechanical allodynia after intraperitoneal paclitaxel injection. The pain was fully resolved within 4 weeks in male wild-type (WT) mice while failing to do so in WT females. In TLR9 mutants, the onset of mechanical allodynia was delayed in males, whereas females experienced it earlier.

Post PTX treatment, the administration of TLR9 antagonist, ODN2088, reduced pain in males only. This study also found sex-specific regulation in cytokine and chemokine release from macrophages and DRG and highlighted the additional involvement of T-cells in females (Luo et al., 2019).

In other preclinical evidence, sex-dependent hypersensitivity, induced by “disulfide high mobility group box 1 (HMGB1)-protein” and its activation of spinal glia via TLR4, was examined. While an i.t. disulfide HMGB1 administration resulted in microglial activation in both sexes, cultured male microglia stimulation showed a higher cytokine and chemokine expression. Correspondingly, these findings, a TLR4 ablation, and minocycline administration caused downregulation of protein-activated receptor 2 (PAR2) expression in both sexes. Meanwhile, an increasement in anti-inflammatory and anti-nociceptive associated proteins could be observed in males only. This resulted in male-specific inhibition of HMGB1-induced mechanical hypersensitivity (Agalave et al., 2021).

7.2.2. P2X4/ p38/ BDNF- Pathway

As previously mentioned, microglia play a crucial role in developing peripheral neuropathic pain, mainly through the expression of purinergic receptors like P2X4R. ATP activates them, and following a PNI, there is an upregulation of P2X4R. These receptors can also be expressed by Schwann cells, dorsal root ganglia (DRG), and peripheral macrophages (Kohno & Tsuda, 2021). Increased microglial P2X4R expression results in the phosphorylation of p38 mitogen-activating kinase (MAPK) and causes transcription, translation, and microglial BDNF release (Halievski et al., 2020). Thus, when present at increased levels, BDNF is involved in chronic pain and is responsible for neuronal hyperexcitation. This produces hypersensitivity, predominantly when synthesized by spinal dorsal horn neurons (Ismail et al., 2024). Additionally, interferon regulatory factor (IRF)- 1, - 5, - 8, and MAFB, known as transcriptional factors, are also responsible for microglial P2X4R expression. Interestingly, IRF-1 is controlled by IRF- 8 (Kohno et al., 2021), exclusively upregulated in microglia after PNI (Inoue & Tsuda, 2018) directly and regulates IL- 1 β expression, which contributes to pain hypersensitivity (Kohno et al., 2021). It also regulates the IRF- 5 transcription, which is transported by fibronectin to the P2X4R promoter (Inoue & Tsuda, 2018). P2X4R-dependent pain-modulating mechanisms and their elevated expression in activated microglia post-PNI can be seen in Figure 12. Also, the role of IRF,

as well as other molecules, such as BDNF, can be derived from the image. More details will be discussed in the following sections.

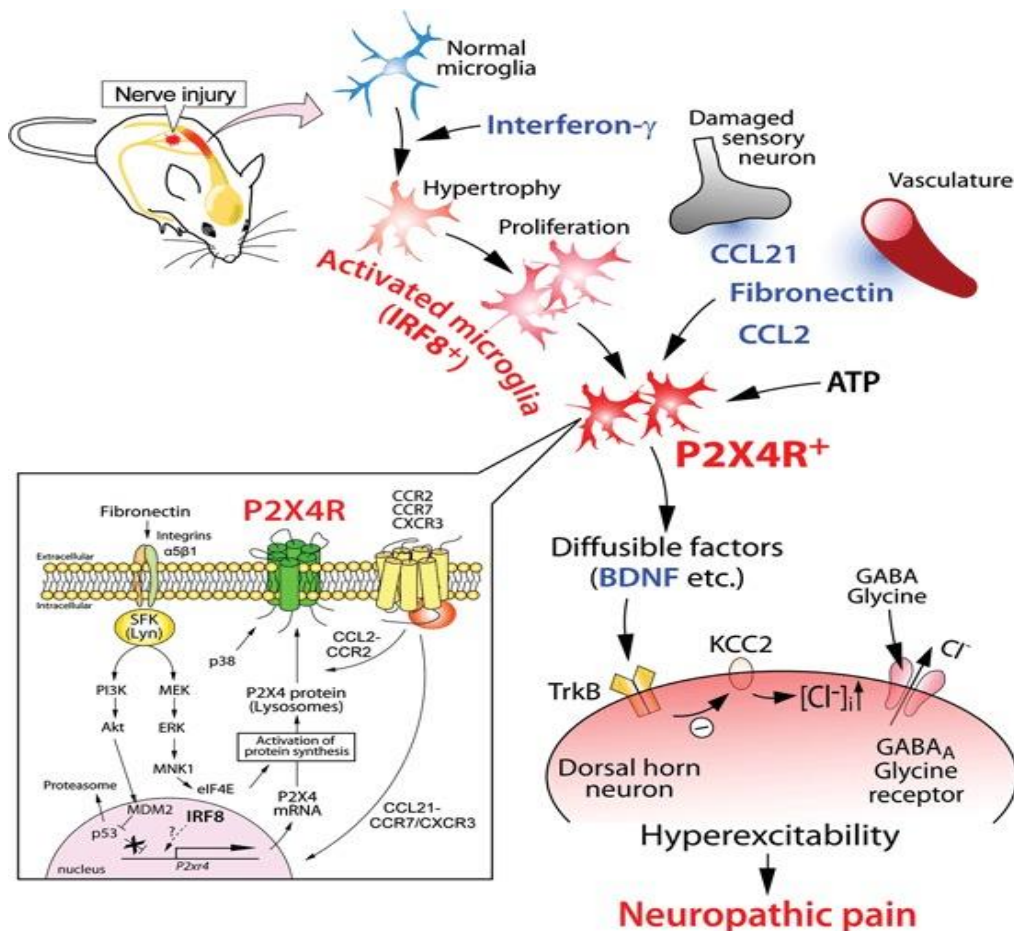


Figure 12. Schematic illustration of the potential mechanisms by which P2X4R in activated microglia modulate pain signaling in the dorsal horn after PNI. Nerve injury activates microglia in the dorsal horn of the spinal cord. Activated microglia show increased expression of P2X4R. The upregulation of microglial P2X4R expression involves signaling by fibronectin and chemokine (C-C motif) ligand 21 (CCL21). CCL2 signaling promotes P2X4R trafficking to cell surface of microglia. P2X4R is activated by ATP and, in turn, release bioactive diffusable factors, such as BDNF. BDNF downregulates the potassium-chloride transporter KCC2 via TrkB, causes an increase in intracellular $[Cl^-]_i$, and leads to the collapse of the transmembrane anion gradient in dorsal horn neurons which in turn induces depolarization of these neurons following stimulation by GABA and glycine. The resultant hyperexcitability in the dorsal horn pain network induced by factors from activated microglia may be responsible for neuropathic pain. Reprinted from *P2X4 receptors and neuropathic pain* by Tsuda et al. (2013), Front Cell Neurosci 2013 Oct 28:7:191. Copyright © 2013 Tsuda, Masuda, Tozaki-Saitoh and Inoue. This article is licensed under the Creative Commons Attribution (CC BY) license.

Preclinical

Evidence indicates a sex-specific pain pathway involving the inhibition of microglial receptors and signaling molecules. For instance, minocycline was able to inhibit microglial P2X4R and microglia itself in males only. Similarly, BDNF and p38/MAPK inhibitors could suppress these signaling pathways, but again, only in males. As a result, SNI-induced allodynia could be reversed (Sorge et al., 2015).

Consistent with these findings, Taves and team (2016) confirmed the male-specific pain pathway, but this time, it was in a CCI-induced pain model. The antagonist of p38/MAPK, skepinone prevented formalin-induced inflammatory pain and a reduction of mechanical allodynia only in males and early time points. Again, no such effects were present in females. Notably, these results refer to the inhibition of spinal microglia via intrathecal injection of the antagonist (Taves et al., 2016). Additionally, upregulation of P2X4 and p38/MAPK activation was seen less in females than in male rodents, as reported in several preclinical studies (Inoue & Tsuda, 2018).

Another evidence for sex differences in microglial-mediated neuroinflammation and hypersensitivity was shown by examining the Ca^{2+} channel, Orai1. This channel is responsible for microglial reactivity. By conducting SNI, the levels of IL-6, $\text{TNF}\alpha$, IL-1 β , and BDNF were comparably elevated in both sexes. Deleting Orai1 reduced inflammation and pain in males only. This effect was not seen in females, suggesting that males rely on Orai1-dependent microglial pathways for pain, while females might use different mechanisms involving T-cell signaling (Tsujikawa et al., 2023). Again, this only validates previous findings for the male-specific microglial pathway, which doesn't play such an important role in females.

7.3. T-cells

As I already mentioned, in literature, sexual dimorphism in neuropathic pain is linked to different pain pathways between the sexes, with women showing a T-cell specificity (Mappleback et al., 2017). Here, I would like to discuss the role of T-cells and their attribution to sex-specific formation and alleviation of chronic neuropathic pain. T-cell activation can be mediated by myelin basic protein (MBP), which causes female hypersensitivity in PNI. Additionally, T-cell-dependent secretion of pro-inflammatory cytokines mediates neuronal changes like amplification of NMDA function (Mappleback et al., 2017).

Preclinical

Sexual dimorphism, regarding T-cell- (Tregs) effects in pain resolution, was seen when Fiore and team (2023) initiated mechanical allodynia in both sexes via CCI, followed by EAE. After treatment with Tregs, pain reduction occurred in both sexes but differed in their cell profiles, respectively. Males had a decrease in total leukocyte level, microglial reactivity, and neurotoxic astrocytes. Females displayed higher levels of regulatory and effector memory T-cells and neuroprotective astrocytes (Fiore et al., 2023). These findings suggest a less pronounced immune response in males compared to females.

On a molecular level, it's also important to mention that the protein T-cell lymphoma invasion and metastasis 1 (Tiam1) plays an important role in the development of chronic NP. It acts as a guanine exchange factor (GEF), which activates Rac1 and has been studied by Li et al. (2023). Preclinically, the deletion of Tiam1 in spinal dorsal horn neurons

prevented the development of CIPN and diabetes-induced neuropathic pain (DIPN), attenuated actin polymerization, and prevented the development of NP. They found that using Rac1 inhibitor, NSC23766, and Tiam1-antisense oligonucleotide (ASO) prevented the transition from acute to chronic pain (Li et al., 2023). Here, sex wasn't used as a variable.

Clinical

Rac1 and its polymorphic role in neuropathic pain were also revealed in a clinical study. There, the importance of genetic factors in NP in a study involving Russian patients over 35 years old with type 2 diabetes (T2D) who were suffering from diabetic neuropathy (DN) was demonstrated. After examining their genomic DNA samples for six common SNPs of the Rac1 gene, they found a specific allele, rs7784465-C, to be associated with DN in females. An increased risk for DN selectively found in males, along with different haplotypes. Female-associated and DN-protective haplotypes were also identified (Azarova et al., 2023).

8. Age-related factors in chronic neuropathic pain

Clinical studies show that the prevalence of chronic pain increases with age in both sexes (Linher-Melville et al., 2020). Further, age-dependent response to analgesics and pain-sensitivity stands in connection with sex hormones, differences in body composition, and metabolic ability (Packiasabapathy & Sadhasivam, 2018). When considering clinical pain states, women report more severe, frequent, and longer-lasting pain, where these differences get additionally complicated by age (E. Keogh, 2008). These sex-based differences in pain reporting may be rooted in the overall higher T-lymphocyte number in the periphery in women, as already mentioned in previous chapters. Even though females seem to be more sensitive to mechanical and thermal pain (Linher-Melville et al., 2020), they are under-represented in drug trials and are more often not analyzed with regard to the sex-related drug response when included in large numbers (S. Lee, 2018). Another interesting age- aspect is the fact that older adults are likely to underreport their pain for reasons like being labeled as complainers, weaklings, or because of the fear of dismissal (Singh et al., 2023). Taking into account the number of undiagnosed elderly, it's important to include the patient's family members and evaluate behavioral changes, functional status, and their life quality generally (Giovannini et al., 2021). Additionally, in the context of the clinical situation, comorbidities, drug metabolism, other medications, including polypharmacy, as well as cognitive status, geriatric patients require individualized treatment to prevent possible iatrogenic diseases and drug-drug interactions (Pedowitz et al., 2021). In regards to children, the prevalence of chronic NP is unknown, and their presentation of PNP can be influenced by age-related factors, such as the challenging articulation of altered sensitivity in young and pre-verbal children. Thus, it's crucial for all involved parties to focus on behavioral observations (S. Walker, 2020).

The following preclinical and clinical findings shall demonstrate the age-dependent NP characteristics. Unfortunately, in the context of chronic neuropathic pain, often only one variable, such as age or sex is investigated, while neglecting to mention their interdependence. At this point, it would also be beneficial to mention at what age range the elderly fall into, as the categorization isn't always clear, and it's inconsistent in the preclinical and clinical data, respectively.

Preclinical

In preclinical settings, the development of pain- chronification and central sensitization could be demonstrated in an age-dependent manner (Crespo et al., 2023), which was already shown by different preclinical experiments.

For example, young (6-9 months) and adult (>22 months) mice were compared to determine if eukaryotic translation initiation factor (eIF4E) phosphorylation has an effect on pro-inflammatory cytokines and the mice's behavior. After a CFA injection, it was observed that

all adults had less thermal hyperalgesia, spontaneous pain, and mechanical allodynia. These parameters were generally lower in adult mutants, who lack phosphorylated eIF4E, indicating the inflammatory- modulation by eIF4E phosphorylation. Hence, a blockage of eIF4E phosphorylation could attenuate and resolve inflammatory pain in adults faster. Such effects couldn't be observed in young mice, despite elevated IL- 1 β and TNF α levels in adults (Mody et al., 2020).

In another preclinical study by dos Santos et al. (2023), age- and sex-dependent neuroimmunological differences between young and adult mice were demonstrated. These two variables influence the microglial response and nerve fiber density in the context of surgical pain, as the plantar incision model was used. The results were amplified by several findings. For example, both sexes and ages showed robust thermal hypersensitivity post-injury. However, aged males had delayed mechanical hypersensitivity and faster recovery, but not females. While all adults had less intraepidermal nerve fiber (IENF) density, only adult males had less projection in nerve endings to the epidermis. Hence, older females are more prone to altered pain sensitivity due to hormonal changes. Aged rats (>22 months old) had a higher average microglial density, which was more flattened and higher CD11b-expression. Young rats (3-6 months old), on the other hand, had an increase in Iba1-expression (Dos Santos et al., 2023).

Again, it's important to mention that at an older age, it's difficult to communicate pain due to age-related cognitive decline. This could be explained by the decrease in genes related to energy metabolism and stands in connection with astrocytes (Krawczyk et al., 2021). Considering the age-related metabolic changes, a preclinical study examined sex-related effects in hypersensitivity and energy use in neurons due to metabolic stress. The researchers used lumbar DRG from mice where the liver kinase B1 (LKB1) gene was removed from certain sensory neurons, such as Na v 1.8 $^{+}$. Results showed that both sexes with missing LKB1 had lower energy capacity when in fasted state. Fasted females who had LKB1 had higher energy capacity. Fasted males showed greater pain sensitivity, regardless of LKB1. The results suggest that after experiencing metabolic stress, such as fasting, LKB1 in females, but not in males, is needed for the regulation of neuronal energy use (Garner & Burton, 2022).

Clinical

While the prevalence of chronic neuropathic pain can reach 10% of the global population (Vieira et al., 2024), in the elderly the data is scarce (Giovannini et al., 2021). Some even report a prevalence of up to 39% (Pedowitz et al., 2021). The following clinical data demonstrates the importance of understanding NP in older populations. In some cases, sex-dependent age factors are considered, but also these findings should be viewed with caution. The reason for that is that even though both sexes might be included, the uneven representation of males and females might be responsible for the sex-specific findings. Also,

age and the resulting outcomes should be viewed critically, as the sample sizes may vary significantly. Taking into account that advanced age is a risk factor for DIPN (Bodman et al., 2024) and the prevalence of NP can increase up to 30% in diabetes patients (Baskozos et al., 2023), aging type 1 diabetes (T1D) individuals are more likely to experience increased neuropathic symptoms (Cardinez et al., 2019)

For instance, when including SABV, patients with type 1 diabetes displayed sex differences in the severity of cardiovascular autonomic neuropathy. This cross-sectional study classified patients by age and their menopausal status. Women had more severe cardio-autonomic NP compared to men. Additionally, women had increased prevalence when being menopausal and above 50 years of age in comparison to their younger counterparts. However, no significant sex differences regarding the prevalence were found (Nattero-Chávez et al., 2023).

In contrast to the recent findings in T1D patients, a female-biased occurrence could be observed in T2D patients. Results of neuropathic phenotyping and nerve conduction study (NCS) showed that females had a higher prevalence of NP than males. Notably, the majority of patients were female and above the age of 50 (Cardinez et al., 2019).

More recently, Elliott and team (2023) were interested in the predictors for painful diabetic peripheral neuropathy (DPN). Again, they found women to be more likely to develop painful DPN. The same findings were shown in other studies as well, but it's still unclear if there's really a sex-specific pathophysiology (Elliott et al., 2023).

Furthermore and independently of DIPN, a positive correlation between higher age and NP was already demonstrated by Antunes and his team (2017). "Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) scale" with sickle-cell disease patients between the ages of 14 and 32 showed higher NP rates among adults. No correlation between sex and NP could be drawn (Antunes et al., 2017).

More recently, Baskozos et al. (2023) presented clinical results in which they differentiated "between neuropathic pain (NeuP)", "non-neuropathic pain (non-NeuP)", and nociceptive pain (NoCP). The prevalence of NeuP was 9,2% overall. It was clear that the most affected ones were middle- to older-aged people (Baskozos et al., 2023).

This result wasn't very surprising, considering that with advanced age, different illnesses and general health conditions start to make their appearance.

In this regard, Roghani et al. (2019) demonstrated a 13.7% chronic neuropathic pain prevalence in the elderly who were Iranian (Roghani et al., 2019).

Also important to mention is the role of age in the topical treatment of NP, as Pickering et al. (2024) presented in their analysis of 18 clinical trials. They separated the <73 and < 75-year-olds and ≥73 and ≥ 75-year-old patients, according to their numeric rating pain scale (NRPS) scores, into efficacy and safety groups, respectively. As a result, the <73 year old patch and control group patients had slightly lower baseline pain scores and generally

higher responder rates compared to 73 year and older ones. Patients in the patch treatment group older than 73 years showed higher improvement and greater chances for a responder status compared to the control group. Regarding the safety of this treatment, 78.1% of the patch group patients younger than 75 years had treatment-emergent adverse events (TEAEs). 62.0% had application-site reactions, and 1.0% discontinued the treatment for this reason. Then, 81.6% of patients older than 75 years experienced TEAE, and 0.9% discontinued the treatment. Overall, the study found the capsaicin 179 mg patch effective and well-tolerated in both younger and older patients (Pickering et al., 2024).

In contrast to some recent findings regarding sex differences, Blankenburg et al. (2011) didn't report such when conducting quantitative sensory testing (QST) on 7 to 14-year-olds from Germany. The main focus was to find age- and sex-related differences in the perception of somatosensory stimuli, hence focusing on detection- and pain- thresholds. While an insignificantly higher sensitivity to warm stimuli and, therefore, a lower detection-threshold in females was reported, no other significant differences were observed. Nevertheless, age-related differences were obtained when 7-year-olds reported the most differences in mechanical pain. This stands in connection with lower pain- thresholds, while similar detection- thresholds, with the exception of cold stimuli were reported (Blankenburg et al., 2011).

9. Neuropathic pain treatment: the present & the future

Due to the overall unsatisfying pharmacological treatment of NP and its long-term side effects (Bonomo et al., 2024) has resulted in patients' unmet needs (Hange et al., 2022). Additionally, problematic is the drugs' unpredictable effectiveness (Basu et al., 2023). The necessity to identify new targets and the development of more effective therapies (Bernatoniene et al., 2023) has to be prioritized by healthcare professionals and researchers to find better strategies for managing (Shkodina et al., 2024) chronic neuropathic pain. This altogether unsatisfactory efficacy of pain medication brought the use of off-label drugs into the therapeutic focus (Serrano et al., 2022). Nevertheless, the line between first-and second-line treatments can be blurry sometimes. Known targets in NP are NMDA,- GABA,- serotonin,- purinergic,- and opioid receptors, the endocannabinoid,- noradrenergic,- and neuroinflammation system, TRP,- voltage-gated sodium and potassium channels, as well as potassium,- and acid-sensing ion channels (ASICs) and epigenetic modifications (Bernatoniene et al., 2023). Commonly used drugs include first-line treatment-drugs, such as anticonvulsants gabapentin and pregabalin, the antidepressants serotonin- and norepinephrine reuptake inhibitor (SNRI), and tricyclics (TCAs) (Imkamp et al., 2024) and topical agents, such as capsaicin (Bernatoniene et al., 2023). Second-line treatments include "non-steroidal anti-inflammatory drugs (NSAIDs)" and "NMDA-receptor antagonists" (Bernatoniene et al., 2023). Here, opioids can also be used if the patient has cancer, as well as third-line treatment in non-cancer patients. It is to highlight that the opioid morphine shouldn't be understood as the best solution regarding clinical treatment (Imkamp et al., 2024). With this said, "patients who are refractory to treatment with morphine" (J. G. McGivern, 2007) can receive pain-relieving benefits with ziconotide (Shao et al., 2020), called Prialt. It is used to treat severe, long-term pain in adults who need painkillers by intrathecal injection (European Medicines Agency [EMA], 2014, March 12). Ziconotide doesn't require an interaction with opioid receptors and has no life-threatening effects, such as respiratory depression. However, it was responsible for an 88% high incidence of side effects, with dizziness being the most common one (Shao et al., 2020). Although no sex and age comparisons were considered in this recent study, significant differences could still be obtained post-ziconotide treatment. For instance, neuropathic patients showed altered levels of histidine, lysine, and N-acetyl aspartic acid (NAA) among others, compared to healthy participants (Ghafouri et al., 2022).

Nevertheless, our society is faced with some challenges in clinical trials. Despite the improved representation of women (S. Lee, 2018), who have a higher prevalence of pain disorders and report pain more frequently, the inclusion of SABV in an independent manner is still limited (Averitt et al., 2019). Considering all of these aspects, it isn't surprising that "there has been little progress in NP pharmacotherapy in the last decade". Additionally, there's a realization that the efficacy of currently used pharmacological approaches is less

than expected (Serrano et al., 2022). With that said, new pharmacological approaches are on the horizon, such as ambroxol, cannabidiol, bromelain, melatonin, N- acetyl- L- cysteine (NAC), acetyl- L- carnitine, α - lipoic acid, spermidine, resveratrol, curcumin and non-coding RNA molecules (Bernatoniene et al., 2023).

In clinical settings, NP questionnaires as screening tools, including tools like the neuropathic pain scale, Leeds assessment of neuropathic symptoms and signs (LANSS), a neuropathic pain questionnaire, painDETECT, ID- pain and the Douleur neuropathique (DN4) (Bernatoniene et al., 2023) can be used. Nevertheless, regarding the diagnostic-, treatment-, and organizational aspects, the availability of local and national protocols is marginal, and the use of international guidelines is dimly used (Haren et al., 2024). This approach is apparent when viewing some of the clinical evidence.

Clinical

For example, this was the case when de Leeuw et al. (2020) published their study in which “the international practice amongst practitioners for the diagnosis and treatment of chronic, neuropathic” and mixed pain “in children and adolescents” (de Leeuw et al., 2020) was investigated. According to the survey, nearly half of 117 pediatric pain specialists and nurses “spent less than 30% of their” time “treating children with chronic” neuropathic pain. Only 45.3% used the IASP criteria for diagnosing NP and 38.5% didn’t use any specific questionnaires. For NP treatment and management, just 9.4% followed a standard protocol, while 35.9% didn’t use any at all. Gabapentin was the most common first-line treatment for moderate and severe NP, followed by amitriptyline and pregabalin. Only 44% use non-pharmacologic approaches (de Leeuw et al., 2020). At this point, it’s important to mention that not all questions have been answered.

From another perspective, Hopkins et al. (2022) were interested in the “use of pharmacological and non-pharmacological treatments” in Australian chronic non-cancer patients (CNCP) aged 18 and over. This telephone interview-based study included 57.2% female participants. In a follow-up, 97.6% of participants reported using paracetamol as the most common non-opioid drug in the last 12 months and antidepressants as the 2nd most used non-opioid. In the last 30 days, 96.8% reported paracetamol as their first choice, followed by antidepressants, with women more likely to use both. Non-pharmacological treatments were also common, with 91.8% contacting specialists or physiotherapists in the last year and 66.1% doing so in the last month. Patients with private health insurance or severe pain were more likely to use both pharmacological and non-pharmacological treatments (Hopkins et al., 2022).

9.1. Opioids

Many chronic pain patients rely on opioids, which are commonly used for acute,- and cancer pain. In this way, they try to manage their unrelieved and under-treated pain, as the opioid receptors, to which the opioids bind, are distributed in brain areas, such as the PAG, which is responsible for pain signaling (Dagher et al., 2024). Among the prescribed opioids, morphine is one of the most widely prescribed ones to alleviate persistent or severe pain (Averitt et al., 2019). Additionally, the treatment effectiveness of opioids is influenced by p38 (Ciapala & Mika, 2023), as they “bind to the glycoprotein myeloid differentiation factor-2 (MD-2) on TLR4” which results in “an inflammatory response through” the activation of NFκB and phosphorylation of p38MAPK (Averitt et al., 2019). Morphine’s ability to bind to microglial TLR4 causes a reduction in the analgesic effect, especially in women (Linher-Melville et al., 2020). In support of this, the inhibition of TLR4 “in the PAG enhanced morphine efficacy in females but not in males” (Halievski et al., 2020). In this regard, opioid receptors, μ (MOR), δ (DOR), κ (KOR) play an essential role in analgesia, as well as in other functions. They are “G-protein coupled receptors that are” distributed in different regions of the body and count as one of the most widely expressed ones in the nervous system. Apart from their analgesic function that can be reached through either the endogenous system via β -endorphins, enkephaline, and dynorphin or external applied substances, they display a widely used target for pain relief, even though its many side effects, including tolerance, addiction, and hyperalgesia (Li et al., 2023). It is still considered a second or third-line treatment for chronic NP with a pain-relieving effect in some patients (Smith, 2012). In Figure 13, areas that opioids target and the associated mechanisms are demonstrated.

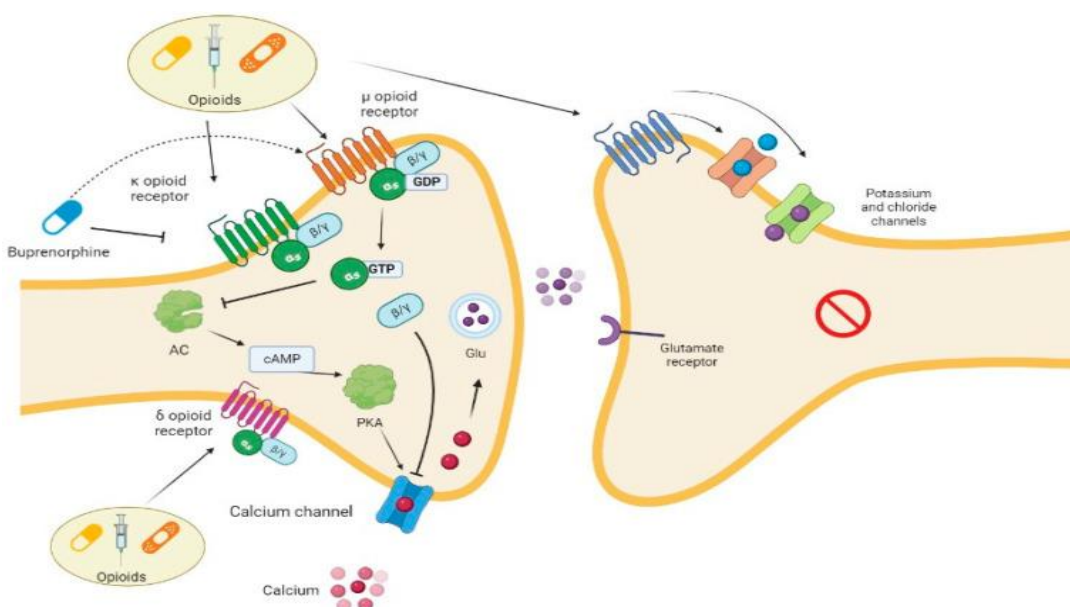


Figure 13: Opioid mechanism of action. Opioids bind to their μ , κ , and δ receptors at presynaptic level, carrying out different actions. After the interaction with a receptor, the α subunit of protein G inhibits the pathway of AC, resulting in a reduction in calcium channel activity and then the release of glutamate.

The same channel is inhibited via the $\beta\gamma$ subunit. Buprenorphine is a particular drug since it has partial agonist activity on μ receptor and antagonist activity on κ receptors. Opioids also exert stimulating activity on calcium and chloride channels, resulting in hyperpolarization at postsynaptic level. AC, adenylate cyclase; cAMP, cyclic adenosine monophosphate; GDP, guanosine diphosphate; Glu, glutamate; GTP, Guanosine-5'-triphosphate; PKA, protein kinase A. Reprinted from: *The Pharmacological Treatment of Chronic Pain: From Guidelines to Daily Clinical Practice* by Marcianò et al., 2023, *Pharmaceutics* 2023, 15(4), 1165, Copyright © 2023 by the authors. This article is licensed under the Creative Commons Attribution (CC BY) license.

Clinically, patients with NP of both genders, displaying a sexually dimorphic response to externally delivered analgesics like opioids, as well as to endogenous opioids, report poor pain control and low opioid efficacy. This can be explained by the downregulation of the receptors in PNS and CNS caused by the prolonged and repeated use of opioids. Increased expression of proinflammatory modulators, such as cytokines, pronociceptive neurotransmitters, lower levels of β -endorphins, higher levels of dynorphin expression, and higher nociception could be observed in NP conditions (Li et al., 2023). Paradoxically, long-term use of opioids can lead to “opioid-induced hyperalgesia (OIH)”, causing central sensitization (Stoicea et al., 2015). Preclinical evidence shows that morphine prolongs post-surgical nociceptive response in male rats when administered before surgery or for 7 days after laparotomy. Additionally, opioid-induced analgesia in males requires μ -OR, and in females, κ -OR, which dimerizes with μ -OR and recruits dynorphin (Linher-Melville et al., 2020). Although most preclinical studies overlook sex as a factor and its' possible impact on therapeutic effectiveness, the pain-relieving effects of some analgesics differ between males and females (Gensel et al., 2019). Such differences include women generally experiencing a greater opioid-induced analgesic effect while also having greater sedative effects and respiratory depression from these drugs (Whitley & Lindsey, 2009). This correlates with women being more likely to report opioid-related side effects, such as gastrointestinal (e.g., vomiting), skin and subcutaneous tissue (e.g., itching), and nervous system (e.g., dizziness)- related effects being the most common ones. This occurred especially when using tramadol or oxycodone (Lopes et al., 2021). Because of a higher likelihood among women to experience various chronic pain syndromes, reports of greater pain in more locations than men are the results. Additionally, women display a higher abuse of prescribed opioids, primarily to self-medicate, while men usually misuse non-prescribed opioids (Templeton et al., 2020). While men are more likely to overdose on opioids, the risk for overdosing has increased faster in women since 2000, with women experiencing a greater opioid craving when stressed (Sharp et al., 2022). These sex-based differences undeniably arise from general differences in pharmacokinetics- and dynamics between men and women (Whitley & Lindsey, 2009). For example, opioids are differently metabolized, which in turn can be influenced by sex hormones and, again, is cycle-dependent in women. Also, the lipophilic character of opioids plays into these differences, as men and women

differ in body composition- and weight (e.g., women have more body fat and weigh less) (Pisanu et al., 2019). Noteworthy is that opioid sensitivity between sexes is dependent on which opioid is used.

Preclinical

In this regard, sex-specific responses to opioid analgesia have already been demonstrated by Dahan et al. (2008). They found higher morphine levels in males regarding the morphine disposition but not in serum concentration. Further, it had been observed that male mice showed an increased sensitivity to morphine. It is suggested that such sex differences in morphine analgesia are due to differences in the “endogenous neurochemical system,” which includes “endogenous opioid peptides,” like enkephalins. This suggestion was because blocking enkephalinase had a greater antinociceptive effect in males, and inhibiting NMDA-sensitive receptors reduced morphine’s analgesic effects in male rodents (Dahan et al., 2008).

Despite no evident sex differences “in the number of microglia in PAG” (Halievski et al., 2020), their sexually dimorphic activation in PAG was demonstrated in a preclinical study. For example, when injecting mice with morphine and examining the caudal ventrolateral midbrain periaqueductal gray (vlPAG) microglia, males had more ramified microglia and showed a correlation between morphine sensitivity and microglia activation. In contrast, females showed no effect on morphine while having a higher percentage of non-ramified and intermediate-activated microglia and higher ED₅₀ values. Females also had a stronger PAG response to TLR4 activation (e.g., LPS), higher IL-1 β levels, and lower IL-10 levels compared to males. Administering naloxone, a TLR4 antagonist, increased the antinociceptive effect in females (Doyle et al., 2017).

Later, Averitt et al. (2019) found that males have stronger analgesia than females despite females having more output neurons. The reason might be that males show higher MOR protein levels and binding within PAG. Further, PAG- rostral ventromedial medulla (RVM) is more active during pain and morphine administration in males. Interestingly, after a site-specific lesion, males switch to a “female-specific” morphine- response (Averitt et al., 2019). On the contrary, just recently, Brazile and team (2024) reported that the initial female response to morphine is stronger, resulting in a more pronounced pain relief. Also, their tolerance to morphine is slower than that of male mice (Brazile et al., 2024). Once again, this highlights the complexity of sex-based pain literature and the dependency on the focus of the respective study.

More recently, it was shown that the KOR agonist U50,488 was more effective in males in a CIPN model (Patron et al., 2022). The naturally occurring hallucinogen 16-ethynyl Salvinorin A (Han et al., 2023) reduced pain more in males for cold allodynia and in females for mechanical allodynia. Morphine also reduced allodynia in both sexes but at different time points (Patron et al., 2022).

As mentioned before, preclinical literature indicates that opioids have a more potent analgesic effect in males compared to females. Consistent with this, Gabel et al. (2022) showed that males had a higher morphine mean residence time (MRT) and half-life, indicating a slower morphine metabolism. On the other hand, females had higher levels of morphine's metabolite morphine-3-glucuronide (M3G) and faster peripheral metabolism. Female mice needed higher doses of morphine for the same pain relief and developed tolerance faster. In the brain, females had lower morphine levels but higher M3G in the amygdala, more morphine in the lumbar spinal cord, and less in the olfactory bulbs (OB). Males had the opposite pattern. Both sexes differ in morphine metabolism and distribution, depending on the measured area (Gabel et al., 2022).

Further, Boorman & Keay (2022) showed in a CCI study that after morphine administration in the commissural nuclei of the solitary tract (cNTS), males showed higher neuronal activity. A higher density of astrocytes was found in males, as the researchers showed greater glial fibrillary acidic protein (GFAP) expression (Boorman & Keay, 2022). GFAP is an intermediate filament and the key component of the astrocytic cytoskeleton (Liang & Shen, 2023). Females, on the other hand, showed a greater GFAP expression in the postrema area, the only region investigated that didn't show any significant expression in male rats (Boorman & Keay, 2022).

Generally, opioids can activate glial cells and release pro-inflammatory mediators, which in turn can affect synaptic plasticity and withdrawal-long-term potentiation (LTP). Sex differences can be seen in these processes. For instance, male rats require the activation of spinal astrocytes and the release of TNF and IL-1 to induce morphine-withdrawal- LTP. In females, morphine- withdrawal-LTP is independent of spinal glial cells (Hadschieff et al., 2022).

Similarly, the role of astrocytes in neuropathic pain seems to vary depending on the specific conditions and treatments. For example, by blocking the astrocytic sphingosine-1-phosphate receptor 1 (S1PR1), the results for anti-allodynia were male-specific for bortezomib-induced hypersensitivity but not for paclitaxel-induced mechanical hypersensitivity, which showed a sex independent mechanism (Ghazisaeidi et al., 2023), again implicating the importance of the type of injury, the used drug and the involvement of glia cells.

Clinical

While the relative opioid efficacy and potency in males and females point to controversial clinical data (Hadschieff et al., 2022), it's relatively common that men require higher opioid doses than females (Dahan et al., 2008). It is worth noting that a couple of studies suggested that women depend on a higher morphine concentration than men to achieve similar levels of opioid-induced analgesia (Linher-Melville et al., 2020). One such study in which higher doses in females were mentioned was conducted by Aubrun et al. (2005) via

a visual analog pain scale (VAS). They reported that immediately post-operation, women required more morphine than men but that those sex differences in opioid administration disappeared in the elderly. The researchers mentioned that studies that report exact opposite effects, meaning that men require higher doses, are such with patient-controlled analgesia (PCA). Those have “major limitations” as they didn’t measure the analgesic effect at the given time but rather the drug consumption by the patient itself (Aubrun et al., 2005). Also, Dahan et al. (2008) shared their view on the problematic use of PCA, as opioid consumption can “be affected by other factors” (e.g., fear of addiction) (Dahan et al., 2008). Contrary to that, more recent studies support the view that males depend on higher opioid doses. For example, Zin et al. (2019) demonstrated that “the risk of high opioid dose escalation was higher in men than in women” despite women being more likely to use opioids long-term (Zin et al., 2019). Meanwhile, the escalation to higher doses in females is faster than in men (Sharp et al., 2022). Furthermore, a meta-analysis conducted by Pisanu and the team (2019) included VAS and PCA. They reported that women self-administer lower doses of opioids, assuming a better response to these drugs, and therefore generally require a lower therapeutic dose than men. This points to higher sensitivity to opioids in females, which would also explain the higher vulnerability to “opioid-induced side effects and mortality” (Pisanu et al., 2019). However, it’s also important to mention that, again, PCA “experiments are confounded” as women report more gastrointestinal (e.g., vomiting) side effects (Sharp et al., 2022), influencing their use of opioids.

Nevertheless, such results could point to sex differences in the neuroanatomy and neurophysiology “of the descending inhibitory circuit” (Averitt et al., 2019). For example, sex-specific PAG- characteristics have already been recognized by Linnman et al. (2011) when demonstrating their findings regarding high pain levels. Men had higher functional connectivity between their PAG, amygdala, and putamen. The male amygdala shows lower μ -opioid availability and more significant changes in their availability. In contrast, women’s midbrain white matter tracts were less pronounced (Linnman et al., 2011).

Moisset et al. (2021) compared the recommended treatments to show the response to NP-medications. Results showed that before visiting a pain clinic, 62.0% of patients had used at least one first-line treatment (e.g., antidepressants, antiepileptics), with 21.5% using both. Additionally, 37.2% were on potent opioids that are usually recommended as third-line treatment. After visiting a clinic and receiving treatment for six months, strong opioids were more likely to be discontinued, as they weren’t needed anymore, rather than because of their side effects. Six months post-visit, 79.7% of patients were on at least one first-line treatment, 34.6% on both, and 36.9% on strong opioids. Lastly, pain intensity decreased by 30% in 23.0% of patients but only 13.9% of opioid users. A 50% pain reduction was achieved by 13.6% of patients and by 5.6% on strong opioids, indicating a lower response in opioid users (Moisset et al., 2021).

9.2. NSAIDs

“Non-steroidal anti-inflammatory drugs are the most frequently used anti-inflammatory drugs” (Farkouh et al., 2021). Due to the limited pain management in NP, these drugs are often used as a second-line treatment when NP is accompanied by inflammation (Bernatoniene et al., 2023). Their ability to inhibit cyclooxygenase (COX) 1 and 2 results in the suppression of binding pro-inflammatory molecules. While females experience inflammatory diseases more frequently and, therefore, use anti-inflammatory drugs more often, males tend to suffer from serious gastrointestinal effects more commonly (Farkouh et al., 2021).

Preclinical

Preclinical evidence shows that NSAIDs' effect (e.g., ketoprofen, ibuprofen) is time-dependent, as in females, it brought pain relief with a more substantial antinociceptive effect shortly after inducing inflammation. Meanwhile, “anti-inflammatory effects tend to favor males” (Craft et al., 2021).

Further study demonstrated that equivalent withdrawal thresholds and number of infiltrating macrophages occur in both sexes after CCI. In response to a COX-2 inhibitor, celecoxib, both male and female rats showed peak pain relief at 4 days, with maximum relief at 12 days. However, females had a weaker response, with significant pain relief lasting only 1 day compared to 5 days in males. Additionally, females had more macrophages and CD8+ cells at the injury site after 12 days (Deal et al., 2022).

Another research team, led by Deal (2023), explored inflammatory “sex differences in gene expression during” neuropathic pain in a CCI model. The rats were treated with either celecoxib theranostic nanoemulsion (10X CXB-NE) or a drug-free nanoemulsion (DF-NE). Post-injury and during inflammation, the sexes differed in the number of differentially expressed genes (DEGs) as well as in the types of genes expressed. After 10X CXB-NE treatment, both sexes shared only one common DEG and differed in the downregulation of some genes. 10X CXB-NE resulted in similar levels of pain relief from hypersensitivity in both sexes. On the contrary, DF-NE did not provide pain relief in either sex (Deal et al., 2023).

Clinical

In a clinical setting, studies on experimental pain and the analgesic effects of ibuprofen showed that men had higher pain tolerance and pain detection thresholds and that only men had an analgesic response to this drug. In females, however, the analgesic effect of ibuprofen was absent despite a drug distribution volume that was twice as high as that of their male counterparts (Walker & Carmody, 1998). However, later this claim was contradicted by Barden et al. (2002), stating that no sex differences in pain relief of ibuprofen 400mg could be obtained (Barden et al., 2002). More conclusive information regarding

NSAID efficacy would be necessary, considering the results of a recent study on analgesic and NSAID “use before and after total hip arthroplasty (THA)”, in which women and older patients use this kind of medication more than men and younger patients. Specifically, “women used more NSAIDs and opioids”, while “older patients used more opioids compared to younger” ones (Lind et al., 2023). Additionally, women use more over-the-counter analgesics compared to men (Packiasabapathy & Sadhasivam, 2018). Again, this only confirms previously described pharmacological habits for treating pain.

9.3. Off-labels

9.3.1. Antidepressants, antipsychotics

The most effective ones in this category are tricyclic antidepressants (TCAs), such as Amitriptyline. This is because they suppress mechanical allodynia, while the selective serotonin reuptake inhibitor (SSRI) and SNRI duloxetine are known for reducing hyperalgesia (Coelho et al., 2024). Both Amitriptyline and duloxetine are subject to a recommendation for first-line treatment for chronic neuropathic pain (Petroianu et al., 2023). Even though duloxetine is recommended and approved for diabetic neuropathy patients, the implication of its efficacy in CIPN has also been known in the literature (Serrano et al., 2022).

Xu et al. (2017) demonstrated the relationship between neurotransmitters serotonin and dopamine and pain. It was evident that major depression disorder (MDD)-related conditions, such as pain, were differently affected by duloxetine administration, depending on the respective sex. In females, the 5-HT metabolism was more affected than the dopamine (DA) system, while affecting both in males. The results indicated an estrogen-independent duloxetine effect in females, whereas testosterone can enhance the antidepressive effect in males (Xu et al., 2017).

Based on the examples, the reduction of pain can be attributed to the enhancement of the inhibitory pain pathways, which are mediated by serotonin and norepinephrine (Smith & Nicholson, 2007), the role of P2X4 receptor blockade has been implicated in the alleviation of chronic pain (Petroianu et al., 2023).

Again, it is important to keep in mind that sexual dimorphism can be regional-dependent, like Murata et al. (2023) demonstrated in their study with non-human primates, *Macaca fascicularis*. Both sexes underwent SNL or sham surgery, followed by a straight leg rise (SLR) test. Males exhibited decreased SLR thresholds for a shorter period of time compared to females. Duloxetine and pregabalin did not significantly alter SLR thresholds, but morphine increased thresholds and reduced brain activation in both sexes. Post SNL, SLR activated males' contralateral insular and somatosensory cortices and thalamus. In females, the cingulate cortex and contralateral insular/somatosensory cortex were activated.

Duloxetine and pregabalin decreased cingulate cortex activation in females, while in males, they reduced thalamic activation (Murata et al., 2023).

Another example of sexual dimorphism in the preclinical activity of an antidepressant (fluoxetine) was demonstrated after inducing pain with formalin. Researchers mentioned a reduction in the second phase (flinching, lifting, and licking) in females, as well as decreased HDAC2 levels and increased expression of mGlu2 receptors after fluoxetine treatment (Zammataro et al., 2017).

9.3.2. Anticonvulsants

Even though not all antiepileptic drugs (AED) cause satisfactory relief from pain, some of them are used to treat chronic NP (Sidhu & Sadhotra, 2016). For example, the GABA analogues (Coelho et al., 2024), gabapentin, which is often used as first-line treatment (Bernationiene et al., 2023), and pregabalin (Petroianu et al., 2023), are known to reduce mechanical and cold hypersensitivity in CCI models. It can be attributed to the binding to specific sites of the CNS, causing a modulation of pain transmission. On the other hand, they possibly regulate Ca^{2+} signaling, hence reducing neuronal hyperexcitability. This results in reduced pain- pain-transmitting neurotransmitters and increased levels of GABA (Coelho et al., 2024). Nevertheless, these off-label drugs are known for their frequently reported adverse effects, such as dizziness and somnolence (Petroianu et al., 2023). Pregabalin was approved for NP by the United States Food and Drug Administration (FDA) in 2004, while in chronic pain conditions, it's marked as an off-label drug (Cross et al., 2024), as already mentioned. Just recently, Smith et al. (2023) reported in the Journal of Pain that pregabalin fully attenuated mechanical hypersensitivity in males only (Smith et al., 2023). On the contrary, only in females, a greater antiallodynic effect of positive allosteric modulators of the α_6 containing GABA_A receptors could be seen. Notably, The GABA_A receptor with α_6 subunit is present in CGRP⁺ and P2X3⁺ primary afferent fibers in the spinal dorsal horn. When these receptors are blocked or knocked down, only female mice experience hypersensitivity and spontaneous pain (Rodriguez-Palma et al., 2023). What's also interesting is that female mice exhibit a dose-dependent "pregabalin-seeking behavior," which researchers attribute to estrogen and its influence on the dopamine- system as a reward- system. This indicates that females are more likely to be addicted to pregabalin (Alsaab et al., 2020).

Further analgesic sex differences of the anticonvulsant carbamazepine (CBZ) were reported by Baggio et al. (2024), with the drug generally being more effective in female rats. For example, after constriction of the infraorbital nerve (CCI-ION), CBZ was more effective in alleviating pain in females and reduced facial grooming in males (Baggio et al., 2024). However, the effectiveness of anticonvulsants (e.g., gabapentin, pregabalin) in NP can't be "conclusively determined" and needs further investigation (Ayub et al., 2024).

9.3.3. Anesthetics

Evidence points to the therapeutic effects of local anesthetics in chronic pain, such as neuralgia, as “they effectively block the conduction of peripheral nociceptive nerve fibers, thereby attenuating the activity of the entire nociceptive system” (Vinyes et al., 2023). Anesthetics can be applied locally, where the injury originates, as well as intravenously or regionally near nerves and distant from the injury (Weinstein et al., 2018). Therefore, local anesthetics can be used when a specific part or region of the body is affected (Bagshaw et al., 2015). Because of the increased risk for “perioperative morbidity and mortality” in elderly patients, local anesthesia gains importance, as it doesn’t affect vital organs and prevents side effects (e.g., nausea) (Cuvillon et al., 2022). By blocking peripheral nerves and subsequently inhibiting CNS activation, regional anesthesia reduces post-operative chronic pain (Chen et al., 2021) and prolongs pain relief. However, dose- standardization still has to be established (Cassai & Geraldini, 2023). First-line topical treatments for localized NP can be a lidocaine plaster of 5% and a capsaicin cutaneous patch of 179 mg, with capsaicin treatment also counting as a 2nd- line treatment (Pickering et al., 2024). The sodium channel-blocking and NMDA receptor-modulating lidocaine can be used to inhibit “the transmission of pain signals” and “reduce central sensitization” (Bernatoniene et al., 2023). About capsaicin and its sexual dimorphism, it has been elucidated in the previous chapter that it elicits more pain in females than males (Linher-Melville et al., 2020), and its efficacy can be estrogen-dependent (Luo et al., 2021). When administering lidocaine and its oral analogues (e.g., mexiletine) (Challapalli et al., 2005) intravenously, it “may slightly reduce NP compared to placebo, but the efficacy of these interventions is comparable to anticonvulsants or morphine” (G. Iolascon, 2020).

Even though it’s been reported that women suffer from adverse effects more frequently than men, no evidence for sex-based differences “in the efficacy and safety of lidocaine to treat neuropathic pain were found” (Salis et al., 2024).

9.3.4. Cannabinoids

Cannabinoids gained attention after the opioid epidemic (Petroianu et al., 2023). They are known for modulating pain perception and transmission through cannabinoid receptors CB1 in the CNS and CB2 in peripheral immune cells. After receptor activation, 5HT1A heteroceptors inhibit the release of pain-processing neurotransmitters, such as substance P and glutamate, while 5HT1A autoceptors on serotonin-releasing neurons regulate serotonin synthesis and release (Bernatoniene et al., 2023). For instance, Anandamide is an endocannabinoid that activates cannabinoid receptors (W. Masocha, 2018) but can also bind to TRV receptors, resulting in an anxiolytic and hypoalgesic effect (Petroianu et al., 2023). Also, in this treatment category, expressional and antinociceptive sex differences have been reported (W. Masocha, 2018). For instance, in a paclitaxel-induced neuropathic

pain (PINP) model, CB2 agonists, such as LY2828360, display sex differences in the treatment of pain. As already known, when treated with morphine, both males and females develop tolerance to this drug. When co-administering CB2 agonist and morphine, morphine-induced tolerance could be prevented in males only. Also, by administering the agonist after a tolerance has already occurred, drug tolerance could be reversed only in males (Guenther et al., 2024).

9.4. Pain management and recommendations

Generally, neuropathic pain treatment differs between the sexes, as Oliva et al. (2015) demonstrated in their analysis of the guideline-recommended care from the Veterans Health Administration (VHA). They found that women are more likely to receive more of the guideline-recommended practices and are also more likely to be treated with sedative prescriptions at the same time (Oliva et al., 2015). This sex-biased approach possibly stems from the fact that pain sensitivity can be seen in women more frequently and that they also report more significant pain in comparison to the opposite sex (Zammataro et al., 2017).

However, to identify the plausibility and efficiency of the present “recommendations for the use of screening questionnaires and diagnostic tests” (Truini et al., 2022), also known as clinical markers (Bouhassira & Attal, 2023) in neuropathic pain patients, Truini et al. (2022) conducted a systematic review of studies with the intention to generate evidence-based recommendations. As a result, they declare in the “screening questionnaire” category strong recommendations for “DN4, I-DN4, and LANSS” and “a weak recommendation” for “S-LANSS and PainDETECT”. In the “quantitative sensory” testing category, they give “weak recommendations” for using QST. Regarding clinical neurophysiology, they declare weak recommendations for using nociceptive evoked potentials, while trigeminal reflex tests received a strong recommendation. Skin biopsy was also strongly recommended, especially when small-fibre neuropathy was suspected. On the other hand, there was limited evidence for using peripheral nerve block. At the same time, functional neuroimaging, even though it’s not a diagnostic tool, is responsible for a better understanding of NP in humans and its underlying mechanism (Truini et al., 2022).

Also, since drugs do not work the same for all patients, using biological biomarkers as quantifiable indicators can help personalize treatment. Clinical markers and patient-reported outcomes (PROs) can help determine sensory profiles. Genetic and genomic biomarkers can additionally reveal genetic variations. Functional (central and peripheral) biomarkers can identify nerve and brain activity, while structural biomarkers can measure nerve fiber density. These approaches can facilitate more personalized treatment strategies (Bouhassira & Attal, 2023).

At this point, it's important to mention that genetic testing isn't currently standard for assessing neuropathic pain. However, ongoing research with large samples and clear phenotyping may change this in the future (Truini et al., 2022).

Regarding pharmacological neuropathic pain management in children, first-line treatment recommendations, such as the off-label use of anticonvulsants and tricyclic antidepressants, can be found. This derives from evidence-based guidelines for adults, as very few controlled trials have been performed in children (Walker et al., 2020).

Nevertheless, it's important to consider that present recommendations can quickly become outdated; hence, regular updates are necessary (X. Moisset, 2024).

10. From rodents to humans

PNI-rodent pain models were established (Jaggi et al., 2011). Even though no animal model can fully replicate all aspects of human neuropathic pain, it still can mimic it (Bouali-Benazzouz et al., 2021). Ray et al. (2018) confirmed the reliability of using such models by showing that gene expression in adult humans and mice is conserved through evolution. They mentioned the PR domain zinc finger protein 12 (PRDM12) being crucial for pain sensing and the mammalian development of the DRG. They also noted that the gene expression profile of the DRG is distinct from that of the CNS (Ray et al., 2018). The observed outcomes in the nervous system are not only model-dependent but can also differ depending on the region, time, and dose used (Jaggi et al., 2011) and the respective sex, as Li & Ward (2024) demonstrated in their research. They examined the effects of the neuropathy-inducing anti-cancer drug (Aghili et al., 2024) paclitaxel on mechanical sensitivity and neuroinflammation in the DRG and PAG (Li & Ward, 2024). Early on, both sexes exhibited similar sensitivity in the DRG and disappeared earlier in males, while in the PAG, females had more neuroinflammation at later time points (Li & Ward, 2024). It is worth noting that translational success is unpredictable, as it's difficult to say in advance if the results of preclinical animal studies will apply to human metrics (Leenars et al., 2019). However, in reverse studies with animals, most drugs have shown good effectiveness after being approved and tested on humans (Abbout et al., 2021).

10.1. Pain models and their translational validity

Preclinical rodent models accurately mirror human response, as most transcriptomes are conserved between humans and mice. This explains the use of preclinical and translational animal and human tissue models for preclinical findings and clinical achievements in a translational fashion (Ray et al., 2018). Preclinical pain models can be categorized into peripheral nerve injury models, such as the chronic constriction injury (CCI) model, the sciatic- or spinal nerve ligation (SNL) model (Guha & Shamji, 2016) and central nerve injury models, such as the spinal cord injury (SCI) model (Bouali-Benazzouz et al., 2021). For example, "central neuropathic pain develops in" more "than 75% of individuals suffering a SCI" (Gensel et al., 2019), which explains the importance of this validated and easily reproducible central nerve injury animal model of NP (Jaggi et al., 2011). Another critical aspect of the sex-dependent characteristics and changes is the use of different mice strains (Al-Hadlaq & Masocha, 2023). For example, the laboratory mouse strain C57BL/6 as a mouse model indicates a strong translational capacity. It accurately predicted DRG-specific genes by human dorsal root ganglion (hDRG)- specificity (Ray et al., 2018). This further highlights the linkage between sex differences, the used model, and the type of NP (Al-

HadlaQ & Masocha, 2023), indicating that it's not dependent on microglial molecules only (Inoue & Tsuda, 2018).

While an underrepresentation of females in preclinical studies (Boullon et al., 2021) has been known in the literature, different reasons can be implicated. For example, in Canada, only 40% of post-mortem organ donors are female, which is reasonable for previously using male donor samples only (Dedek et al., 2022). Because of the evident and significant similarities between mouse and human tissues, important transcriptomic changes can be identified and explained using a cultured DRG system as a model, from which a comparison between native and cultured mouse and human DRG can be drawn. Nevertheless, important differences also have to be considered (Wangzhou et al., 2020). These differences relate to a discrepancy between preclinical and clinical trials in the pain-relieving effects of molecules. This may be due to genetic and molecular differences between humans and animals, making predictions in humans difficult (Abbout et al., 2021).

Preclinical, translational evidence

A comparison across tissues, such as human and mouse tissue, mouse DRG (mDRG), and tibial nerve (TN), allowed the researchers to disclose the species- and tissue-based similarities of the corresponding transcriptomes (Ray et al., 2018).

For example, Ray et al. (2019) analyzed human tibial nerve (hTN) transcriptomes from donors aged 21-70 found in the Genotype Tissue Expression (GTEx) dataset. They found sex differences in genes linked to chronic pain. Males showed a higher ratio of pro- and anti-inflammatory genes in comparison to females. They also displayed an abundance of expressed genes associated with macrophages, like C-C motif chemokine ligand 2 (CCL2), while females had higher gene- levels associated with T-cells, like CD8+ (Ray et al., 2019). Then, to constitute shared characteristics of radicular/neuropathic pain between animal models and humans, North and his colleagues (2019) used hDRG- neurons from patients of both sexes. Out of the three known maladaptations regarding spontaneous neuronal activity, such as more positive membrane potential, more hyperpolarized action potential (AP) threshold, and spontaneous depolarizing fluctuations, the last two were also identified in hDRG. This could be explained by the increased levels of genes in the pain samples associated with pain- pathways, transcription factors (TF), cytokine-ligands, neuroimmune, and Schwann cell genes (North et al., 2019).

Further evidence of conserved sexually dimorphic pain mechanisms between rats and humans was presented by Dedek et al. (2022). They combined BDNF (Dedek et al., 2022), which is usually responsible for neuronal differentiation, maturation, survival, modulation of neurotransmission, and neuronal plasticity (Ismail et al., 2024), and an in vivo inflammatory rodent model with a human spinal cord tissue model. This demonstrated molecular effects on "N-methyl-D-aspartate receptor (NMDAR) potentiation" and spinal dorsal horn (SDH) hyperexcitability. In this study, an intraplantar CFA injection revealed a male-specific

pathway in the lumbar spinal cord. This involved decreased levels of KCC2, striatal-enriched protein tyrosine phosphatase-61 (STEP61), and increased levels of Fyn and GluN2B. Additionally, ex vivo BDNF treatment of human SDH tissue showed that males had reduced KCC2 membrane intensity and increased KCC2 intracellular intensity, indicating male-specific NMDAR potentiation. Similar results were observed in females who had undergone ovariectomy, highlighting the role of hormones in these mechanisms (Dedek et al., 2022).

In a CIPN model using paclitaxel, neuropathy was induced in both sexes. Administering inducible co-stimulatory molecule agonist antibody (ICOSaa) led to a quick resolution of mechanical hypersensitivity in both sexes, but only females exhibited a significant reduction in pain. This was due to ICOSaa causing higher IL-10 production and a greater percentage of T-cells in the DRG. Blocking IL-10 with an IL-10R antibody fully blocked the ICOSaa effect in females. The translational potential of ICOSaa treatment is supported by the presence of CD4⁺ and CD8⁺ T-cells in human DRG, suggesting applicability in NP- patients (Sankaranarayanan et al., 2023).

Again, it's important to be cautious about interpreting animal data and translating it into human metrics, as Shiers et al. (2023) mentioned in their comparative study of human and mouse DRG and TG. In their research, Shiers and team examined the mRNA expression of mitogen-activated protein kinase interacting kinase (MNK) 1 and 2, along with sodium voltage-gated channel alpha subunit 10 (NCBI, 2024, August 17) (SCN10A). They found that the mRNA of both isoforms, MNK1 and MNK2, were expressed differently in each species in different regions (Shiers et al., 2023).

Preclinical, translational, and/or animal-based (e.g., tissue, cells) findings

Target Molecule/ Protein	Mechanism	Model	Procedure	Species & Age	Finding (sex-dependent)	Reference
P2X4R	Inhibition P2X4R, allodynia	SNI	Minocycline administration	Mice, 7-12 weeks	M, not F	Sorge et al., 2015
BDNF	Inhibition BDNF, allodynia	SNI	Y1036 or TrkB-Fc	Mice, 7-12 weeks	M, not F	
CSF-1	Inhibition DRG-macrophages expansion	SNI	Deletion of CSF-1 in sensory neurons	Mice, 4-12 weeks	M, not F	Yu et al., 2020
Chemokine receptor	Response of: -CXCL10 -CCL2 -TNF α -IL-1 α -IL-1 β -IL-4 -IL-6 IFN α ↑ IFN γ ↑	SNL	polyinosinic:polycytidylic acid (poly I:C) injection; intracerebroventricular	Mice, 8-9 weeks	M>F	Posillico et al. 2021
					M, not F	
CGRP	Reduction of total duration of ultrasonic vocalization	SNL	CGRP 8-37 (CGRP receptor Antagonist)	Rats, 12 weeks	M<F	Presto & Neugebauer, 2022
	Abundance of TrkB+ & TrkC+ neurons				M<F	
	Abundance of TrkA+ neurons				M>F	
TLR9	Reduction of pain	CIPN	ODN2088 (TLR9 antagonist)	Mice, not stated	M, not F	Luo et al., 2019
IL-23R	Reduction of mechanical pain	CIPN	P2305 intraplantar (i.pl) (IL-23R antagonist)	ORX-mice, 8-16 weeks	M, not F	Luo et al. 2021
GPR171	Restoration of GPR171 protein levels & Reduction of chronic NP	CIPN & CFA	MS15203 (GPR171 agonist)	Mice, 6-8 weeks	M, not F	Ram et al., 2021
TRP	TRPM8 mRNA in ganglia	CIPN	Oxaliplatin injection; intraperitoneal	Rats, not stated	M<F	Miguel et al., 2022
IL-10	Significant reduction in pain due to -IL-10 production↑ -T-cell %↑	CIPN & SNI	ICOSaa (on last paclitaxel- day) injection; intrathecal	Mice, 8-12 weeks	F, not M	Sankaranarayanan et al., 2023
Tlr4	Prevention of neuroinflammation in PAG	CIPN	Cannabigerol administration (30min prior to paclitaxel)	Mice, 6-8 weeks	M<F	Li & Ward, 2024
p38	Inhibition p38/MAPK Mechanical allodynia	CCI	Skepinone administration; intrathecal	Rats, 8-10 weeks	M, not F	Taves et al., 2016
	Inhibition inflammatory pain	Formalin-induced pain				
Leptin, FasL	Upregulation of Leptin & FasL	CCI	17 β -estradiol administration	Mice, 12 weeks	M<F	Vacca et al., 2021
COX-2	Levels of macrophages & CD8 ⁺ cells	CCI	CXB-NE-treatment; intravenous	Rats, not stated	M<F	Deal et al., 2022
	Drug potency				M>F	
Microglial genes	microglial activation & inflammatory response	CCI	Injury	Mice, 8-12 weeks	M>F	Fiore et al., 2022

IL-10, TGFβ	Reduction of microglial reactivity & neurotoxic astrocytes	CCI	Treg injection; intrathecal	Mice, 8-12 weeks	M, not F	Fiore et al., 2023
	inhibition of hypersensitivity & Increased expression of neuroprotective astrocytes				F, not M	
IL-1β	Expression of IL-1β proteins	CCI	Injury	Rats, 10 weeks	M>F (14 dpi)	Green- Fulgham et al., 2023
	Expression of IL-1β mRNA				M<F (7&14 dpi)	
VGCC	Deletion of mechanical hypersensitivity	CIBP	Progesterone & pregabalin administration	Rats, not stated	M<F	Ungard et al., 2020
	Pain thresholds	NP			M<F	
HMGB1	Increase of anti-inflammatory & anti- nociceptive associated proteins:	HMGB1-induced mechanical hypersensitivity	TLR4 ablation or/and Minocycline	Mice, 10-12 weeks	M, not F	Agalave et al., 2021
Iba1, TMEM119	Activity of Iba1 & TMEM119	CRPS	Immunostaining	Mice, 11-12 weeks	M>F (1 dpi)	Huck et al., 2022
Iba1	Iba1 protein levels	antiretroviral-induced pain model	2'-3'- dideoxycytidine (=antiretroviral drug, NRTI)	Mice, 8-12 weeks	M>F	Al-HadlaQ & Masocha (2023)
Cd11b	Cd11b & Phosphorylated p38 MAPK				M<F	
P0, PMP22	Stimulation of P0 & PMP22	Cultured Schwann cells	Progesterone & DHP	Rats, not stated	M, not F	Magnaghi et al., 2006
			THP		F, not M	
Prlr	Reduction of hypersensitivity	Hyperalgesic priming	ablation of Prlr in sensory neurons & ΔPRL administration (PRL antagonist)	Mice, 8-12 weeks	F, not M	Paige et al., 2020
Trk ⁺	TrkA ⁺ expression	Tissue comparison	DRG isolation	Mice, not stated	M>F	Vradenburgh et al., 2023
	TrkB ⁺ expression				M<F	
	TrkC ⁺ expression				M<F	
Prlr	Expression of Prlr	DRG	Ablation or PRL antagonist administration	1.Mice, 8-12 weeks	M<F	Stratton et al., 2024
	Inhibition of hypersensitivity			2.Monkeys, 10&12 years	F, not M	
Hcrr	Expression of Hcrr			3.Humans	M, not F	
ORXB	OrexinB- induced sensitization				M, not F	

TNF α	Release of Cytochrome C & Caspase 3 cleavage	EAE	MOG injection; subcutaneous & inoculation with pertussis toxin	Mice, 8-10 weeks	M>F	Maguire et al., 2022
	ATF3, pCREB				M<F	
TNFR2	Elimination of motor symptoms	EAE	P53- sc- mTNFR2 (TNFR2 agonist); intraperitoneally	Mice, 10-12 weeks	F, not M	Nguyen et al., 2024
TLR4	Levels of IL-1 β		LPS injection (TLR4 agonist)	Rats, 60-90 days	M<F	Doyle et al., 2017
	Levels of IL-10				M>F	
TLR2	central pain sensitization		GT1b; intrathecal	Mice, 8-10 weeks	M, not F	Lee et al., 2023
CGRP	CGRP-induced migraine, hypersensitivity	Behavioural	CGRP injection	rats, 12-14 weeks mice, 6-8 weeks	F, not M	Avona et al., 2019
CB2	Reversion and prevention of morphine-induced tolerance	PINP	LY2828360 (CB2 agonist)	Mice, not stated	M, not F	Guenther et al., 2024

Clinical and/or human-based (e.g., tissue, cells) findings

Mechanism	Model	Procedure	Species & Age	Finding (sex-dependent)	Reference
Nerve impairment	Large & small nerve fiber test	<u>Large nerve fiber</u> : NCS, stimulation and recording of surface, VPT <u>Small nerve fiber</u> : heat- induced axon reflex-mediated neurogenic vasodilation, CDT	Type II diabetic NP- patients with and without polyneuropathy, ≥ 18	M>F	Abraham et al., 2018
NP-effects				M<F	
PAF $\uparrow$	MEG analysis	Analysing alpha brain activity/PAF	NP-humans,	M: ANP F: ANP, DPC, SN	Fauchon et al., 2021
PAF $\downarrow$				M<F (sgACC)	
Potentialtion of NMDAR	Human tissue	Ex vivo BDNF (human SDH tissue)	Humans, 20-75 years	M, not F	Dedek et al., 2022
Potentialtion of NMDAR		CFA; intraplantar	Rats, 3-4 months rats	M, not F	
Rac1 polymorphism	DNA (blood) samples	SNP-identification associated with DN	T2D-patients, ≤ 35	F, not M: allele, rs7784465-C	Azarova et al., 2023
Expression of CALCA (encodes CGRP)	hDRG		Humans	M<F	Tavares-Ferreira et al., 2022
	hDRG	RNA-profiling of DRG from thoracic vertebrectomy patients, comparison of pain & no-pain samples	Humans, 34-77 years	Findings can be seen below	Ray et al., 2023*

*Ray et al., 2023		
Category	Male	Female
Key pathways	JUN-FOS (including ↑ expression of FOS, FOSB, FOSL1, FOSL2, JUNB, ATF3, EGR1, EGR3)	JAK-STAT
	OSM	INF I & II
	TNFα	TLR
	IL-1	PIK3C WEG??
Upregulated genes	IL-1β, TNF, CXCL14, EGRB, TRPV4, LIF, CCL3/4, OSM	CCL1, CCL19, CCL21, PENK, TRPA1, ADORA2B, GLRA3
co-expression module	CSF1, IL32, TNFSF9 (co-expressed with OSM)	IFIT1, IFIT2, IFIT1B, IFIT3, IFIT5, CASP3, CASP8
Immune cells-sensory neuron interaction	OSM, LIF, CCL3, TNF, AREG, ICAM3	CCL19, GZMB, CXCL10, IGF1
Decreased markers	CD2, CD96	

Table 3: main findings in male and female pain research (sorted by pain model);

Abbreviations in alphabetical order: ATF3, activating transcription factor 3; BDNF, brain- derived neurotrophic factor; CALCA, calcitonin- related polypeptide alpha; CCI, chronic constriction injury; CFA, complete Freund's adjuvant; CGRP, calcitonin- gene related polypeptide; CIBP, cancer- induced bone pain; CIPN, chemotherapy induced peripheral neuropathy; CDT, cooling detection threshold; CRPS, complex regional pain syndrome; CSF-1, colony stimulating factor 1; CXB-NE, celecoxib theranostic nanoemulsion; CXCL, CXC motif chemokine ligand; DHP, dihydroprogesterone; EAE, experimental autoimmune encephalomyelitis; hDRG, human dorsal root ganglion; Iba1, Ionized calcium-binding adapter molecule 1; LPS, lipopolysaccharide; MEG, magnetoencephalography; MOG, myelin oligodendrocyte glycoprotein; NCS, Nerve conduction study; NMDAR, N- methyl- D- aspartate receptor; PRL, NP, neuropathic pain; prolactin; ORX, orchietomized; Prlr, prolactin receptor; SNI, spared nerve injury; SNL, spinal nerve ligation; TMEM119, Transmembrane Protein 119; THP, tetrahydroprogesterone; TLR, toll like receptor; Trk, tyrosine kinase receptor; VPT, vibration perception threshold; VGCC, voltage gated calcium channel

11. Conclusion

The overall unsatisfying results of pain treatment in neuropathic pain has a lot of different and mostly very complex reasons. First of all, researchers did not do themselves any favours by dismissing female rodents or humans in their research for decades. Even though, both sexes had been frequently incorporated later on, females are still underrepresented. There is a huge gap in knowledge until today and it seems rather difficult and even impossible to compensate years of research that had been lost by dismissing sex as a biological variable (SABV). Notably, there are still some studies that fail to incorporate both sexes in their research. This can be due to various factors, such as simple as the convenience of using males, who don't undergo cyclic changes. Nevertheless, at this point it's only fair to mention other crippling factors which influenced pain- research' progress. So is the limited availability of female post- mortem donors (e.g. in Canada) one of such obstacles, especially because an equal representation of both sexes is crucial for preclinical, translational studies. Also of high importance are the time consuming aspects of pain research, which in itself are extremely costly due to pains' complex and heterogeneous nature. One also has to keep in mind the journey of an analgesic, from target to molecule to an actual drug, which provides the necessary effectiveness and safeness and subsequently its actual success in relieving pain in both sexes and all ages. Secondly, even when both sexes are being included, the majority of studies don't compare the results in a sex- oriented manner. The reason for this approach is mostly unclear but it does have an impact on the quality of the respective study.

Similar problems occur when analysing age- related causes for NP. A classification into age groups varies between studies and isn't all too specific, nor uniform. On the other hand, when age- dominated studies are made, the age gaps aren't big enough. However, most of the studies fail to address age- related factors (e.g. hormonal status, metabolism and comorbidities), which only allows general conclusions in the prevalence for chronic NP. Such conclusions are very unsatisfying, knowing that these aspects generally occur in the elderly more often, compared to younger ones. At this point, a disclosure of molecular mechanism would be profitable for both sides, the researchers and patients. However, it's important to mention that the lack of age- related information in a study is more understandable than the ignorance of SABV. The grounds for that is that chronic neuropathic pain in (very) young children and (very) old patients is challenging, due to reasons that have already been addressed (e.g. non- verbal, mental decline). Especially problematic is the treatment of children, which is often based on adult guidelines due to a lack of controlled trials in this population group. Common treatments include off-label use of anticonvulsants and tricyclic antidepressants. Despite being confronted with various challenges, significant age differences such as microglial activity in regard to their markers (e.g. Iba1) could be found in pain research. Again, also these differences can't be

generalized and easily translated into categories, such as “younger” and “older”, as the attribution to different age groups varies between studies. Additionally, some studies don’t mention the specific age at all, but use instead the terms “young” and “old”, assuming it’s an universal definition. Regarding such studies. it’s only legitimate to say, that it can be quite confusing to make valid conclusions. Therefore, studies would profit from a standardized and unified classification of all ages and of course, the inclusion of the respective sex.

The importance of including SABV becomes even more evident when looking at the most prescribed pain drugs, such as opioids. Even though the data on morphine’s efficacy seems somewhat contradictory, it’s been established that males require generally higher opioid dose than females. However, it’s important to distinguish between the used opioids (e.g. morphine, tramadol, oxycodone) and their effects on the respective sex. For example, some studies report that morphine’s analgesic effect is less in females, compared to males. It is suspected to root in opioid receptor- independent actions, such as morphine’s ability to bind to microglial TLR4. In this regard, females could profit from a TLR4 block to enhance the drugs’ efficacy.

Meanwhile, other studies mention a greater analgesic effect in females, hence require lower dose. Also here it’s important to take into consideration that clinically, females might indeed require lower opioid dose. This doesn’t have to necessary point to greater analgesic effect but rather the fear of side effects. Additionally and in preclinical context, analgesic effect might depend on the area and molecules on which the study concentrates on, as well as the pain model itself. Yet, the data is clear on women using more opioids than males, especially when stressed, making them more susceptible to opioid abuse. Also the use of cannabinoids should be done in a sex- specific manner. The reason for this is that a CB2 agonist inhibits and reverses morphine tolerance in males only. This seems plausible when taking into account that microglia express CB2 receptors and can be modulated in their activity through this receptors, again underlining the male-dominated microglial pain pathway. At this point it’s only valid to require new studies to elicit the same effects in females when treating with morphine, for example using adjuvants or new strategies after all. Also, the treatment with anticonvulsants would profit from new methods regarding the CGRP- expressing neurons, as well as P2X3 neurons which inhabit $\alpha 6$ subunit of GABA_A receptors. As allosteric modulators they were able to increase antiallodynic effect in females, rather than in males. Women also more frequently use NSAIDS, even though a preclinical study showed them having a weaker and shorter pain- relief response than males, but also here pointing to contradictory evidence. Taking together, it makes sense that women, even though not experiencing as satisfying antiallodynic effect as men, take more of these medications, as they experience NP more frequently and intense.

It's more than possible that sexual differences in experiencing pain might be also due to brain-based functions. For example, an abnormal functional connectivity with sgACC, which regulates pain in an emotional-dependent manner was found. Hence women possibly having a stronger emotional connection to pain than males. Additionally, females have a faster PAF in ANP, while in males it's increased. This could also suggest the different processing of pain and why males have an allegedly stronger antinociceptive outcome. Another brain region, such as amygdala, which is also related to emotions, is connected to female pain. Amygdala is affected by CGRP, which was found to be higher in female pruritogen-enriched neurons, indicating higher pain levels. CGRP seems to be a female-specific pain modulator, as its antagonization decreases certain female pain behaviour, while CGRP administration elicits hypersensitivity in females only. A probably more consistent evidence for sexual dimorphism, with females suffering from more frequent and severe clinical pain, especially when older, arises from hormonal differences. Such can be attributed to estrogen fluctuations in females. Female-specific, estrogen-driven pathology involves decreased and increased pro-nociceptive estrogen levels, meaning that either too high or too low levels can facilitate neuropathic pain in females. Further, estrogen regulates Prrl, TRPV1, and P2X3-dependent hypersensitivity in females. Inhibition of those targets could decrease pain in females. In terms of molecular differences, females produce more IL-23 and IL-17A than males, making them more susceptible to mechanical allodynia. Although differences in gene and protein expression profiles between the sexes are common, they often result in mechanical allodynia to the same extent in both, males and females, indicating sexual dimorphism in pain pathways that ultimately leads to a similar outcome. After metabolic stress (e.g. fasting), LKB1 is crucial for regulating neuronal energy in females but not in males, as both sexes with missing LKB1 had lower energy capacity, and fasted males exhibited greater pain sensitivity regardless of LKB1.

Meanwhile, male-specific effects can be attributed to molecules which are present exclusively in males (e.g. Hctr2) or to hormones which can be found in both sexes (e.g. progesterone, testosterone). However, such hormones follow different pain patterns and responses in the respective sex, which in turn depend on their concentration.

Also receptors exhibiting male-specific effects could be found (e.g. P2X4, TrkB). Interestingly, females may shift to male-specific (microglial) pain pathways under certain conditions and males can switch to a female-specific morphine response following specific spinal lesions.

Another challenge in the pain field is that even though there is a variety of well-established pain models, such as rodent models, they do have limitations in replicating all human pain mechanisms. Therefore, they can't be fully translated into human metrics, as there are species-related differences in genetic and molecular responses. Naturally, these different expression patterns of pain-related genes in rodents and humans can affect the efficacy of

treatments derived from animal studies. For instance, pain models (e.g. CCI, SCI) mimic human neuropathic pain but do not perfectly replicate all human aspects. Additionally, sex-specific results also depend on the used mouse strain or animal species in general. Notably, results are also region- dependent (e.g. PAG, DRG) and time- dependent. Nevertheless, gene expression profiles in rodents and humans are largely conserved, which helps in translating findings from rodent models to human conditions. However, translational success is uncertain, and ongoing research is needed to bridge the gap between animal models and human clinical applications. In this regard, we can't ignore that the quality and representativeness of a clinical study in NP depends on both, the researchers and the patients, therefore their corporation with each other.

Nevertheless, it's important to urge practitioners and clinicians to stick to valid methods in diagnosing and subsequently treating NP. Of course, interindividual differences in patients are always going to influence the progression of pain but it's crucial to recognise the importance of established methods, as well as international guideline's. It's especially them that offer a strong foundation for all involved individuals. This becomes even more important when taking into account that according to a study, less than 50% of authorities used the IASP criteria. However, it should be noted that the practice for diagnosis and treatment may vary from region to region. Treatment practices may be influenced by the fact that women often report experiencing more pain and greater pain sensitivity than men. This could be a reason for women being more likely to receive guideline-recommended treatments for neuropathic pain.

On the contrary, men might receive different treatment practices, reflecting less frequent use of sedatives compared to women.

In conclusion, authorities have to be clear on sex differences in the treatment of chronic NP and subsequently keeping the guidelines updated. Both, guidelines and treatment recommendations need frequent updates to remain effective and accurate, as more evidence for sexual dimorphism in chronic neuropathic pain arise. Even though it's been mentioned that female- pain might be associated with gender- based expectations and when manipulating the gender, they reported less pain, it's still undeniable that sex differences exist. Social expectations on a specific sex or gender do certainly contribute to the outcome but it definitely isn't a determinative factor. Personalized and sex- based treatment has the potential to significantly bridge gaps in care, provided that it is made accessible to all individuals, regardless of their socioeconomic status

12. References

12.1. Literature

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12.2. Figures

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12.3. Tables

Table 1. Overview of (pain- related) fibers types
Table 2. Demonstration of hDRG subtypes according to their group.

Table 3. Main findings in male and female pain research (sorted by pain model)

12.4. Online links

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13. Abbreviations

(s)TNF	(soluble) tumour necrosis factor
10X CXB- NB	celecoxib theranostic nanoemulsion
AED	antiepileptic drug(s)
ACC	anterior cingulate cortex
AMPA	α -amino-3-hydroxy-5-methyl-4 isoxazolepropionic acid
ANP	antinociceptive pathway
AP	action potential
APC	antigen-presenting cells
ASCICs	acid sensing ion channels
ASO	antisense oligonucleotide
AT	adipose tissue
ATF3	activating transcription factor 3
BAFF	B- cell activating factor
BDNF	brain- derived neurotrophic factor
BL	base level
BLA	basolateral
BMI	body mass index
Ca	Calcium
CALCA	calcitonin- related polypeptide alpha
CAPOX	capecitabin
CBG	Cannabigerol
CBZ	carbamazepine
CCI	chronic constriction injury
CCI-ION	constriction of the infraorbital nerve
CCL2	C-C motif chemokine ligand 2
CD4	cluster of differentiation 4
CD8	cluster of differentiation
CeA	Central amygdala
CFA	complete Freund's adjuvant
CGRP	calcitonin- gene related polypeptide
CIPN	chemotherapy- induced peripheral neuropathy
CNCP	chronic non- cancer patients
CNS	central nervous system
cNTS	commissural nuclei of the solitary tract
COX (1,2)	Cyclooxygenase
CPM	conditioned pain modulation
CRPS	complex regional pain syndrome

CSF	colony- stimulating factor
CT	cortical thickness
CXCL	CXC motif chemokine ligand
DA	Dopamine
DC	dendritic cells
ddC	dideoxycytidine
DE	differentially expressed
DEG	Differentially expressed genes
DF-NE	drug-free nanoemulsion
DHEA	dehydroepiandrosterone
DHT	dihydrotestosterone
DIPN	diabetes-induced neuropathic pain
DN	diabetic neuropathy
DN4	Douleur neuropathique
DPC	dynamic pain connectome
dpi	day(s) post injury
DRG	dorsal root ganglion
DSP	diabetic sensorimotor neuropathy
EAE	experimental autoimmune encephalomyelitis
ED50	Effective dose 50
EE	energy expenditure
eIF	eukaryotic initiation factor
ER	estrogen receptor
FasL	Fas Ligand
FC	functional connectivity
FDA	Food and Drug Administration
FOLFOX	fluorouracil
GABA	γ - aminobutric acid
GAH	gender affirming hormone
GAHT	gender affirming hormone therapy
GAS	gender affirming surgery
GdX	gonadectomized
GEF	guanine exchange factor
GFAP	glial fibrillary acidic protein
GMV	grey matter volume
GTE _x	Gentype Tissue Expression
hDRG	human dorsal root ganglion
HMGB1	high mobility group box 1

HPCA	Hippocalcin
hTN	human tibial nerve
HIV	human immunodeficiency virus
i.p	intraperitoneal
IASP	International Association for the Study of Pain
Iba1	Ionized calcium-binding adapter molecule 1
ICOSaa	inducible co-stimulatory molecule agonist antibody
IEFND	intra- epidermal nerve fiber density
IENF	intraepidermal nerve fiber
Ig (G)	immunoglobulin (G)
IL (10)	interleukin (10)
IL-10R	interleukin 10 receptor
INF(γ)	interferon (gamma)
IRF	interferon regulatory factor
ITS	intercalated cells
K	potassium
KCC2	potassium chloride cotransporter 2
LANSS	Leeds assessment of neuropathic symptoms and signs
LD	length-dependent
LKB1	liver kinase B1
LMTR	low threshold mechanoreceptor
LPS	lipopolysaccharide
LTP	long-term potentiation
M3G	morphine-3-glucuronide
MAPK	mitogen-activated protein kinase
MBP	myelin basic protein
MD-2	myeloid differentiation factor-2
MDD	major depression disorder
mDRG	mouse dorsal root ganglion
MEG	magnetoencephalography
MMP	matrix metalloproteinase
MNK	Mitogen-activated protein kinase interacting kinase
MOG	myelin oligodendrocyte glycoprotein
mPFC	medial prefrontal cortex
MRT	mean residence time
MUSTN1	musculoskeletal, embryonic nuclear protein 1
MyD	myeloid differentiation primary response
Na	sodium

NAA	N-acetyl aspartic acid
NAC	N-acetyl-L-cysteine
NIH	National Institutes of Health
NCS	nerve conduction study
NeuP	Neuropathic pain
NFκB	nuclear factor kappa B
NLD	non-length dependent
NLRP	NOD-like receptor
NMDAR	N-methyl-D-aspartate receptor
NoCP	nociceptive pain
Non- NeuP	non- neuropathic pain
NP	neuropathic pain
NRPS	numeric rating pain scale
NRTI	nucleoside reverse transcriptase inhibitor
NSAIDs	non-steroidal anti-inflammatory drugs
NTRK3	neurotrophic receptor tyrosine kinase 3
OB	olfactory bulbs
OIH	opioid-induced hyperalgesia
OIPN	oxaliplatin-induced peripheral neuropathy
OR	Opioid receptor
ORXB	Orexin B
OVX	ovariectomized
P0	glycoprotein zero
P450scc	cytochrome P450 side chain cleavage
PAF	peak alpha frequency
PAG	periaqueductal gray
PAR2	protein-activated receptor 2
PENK	proenkephalin
PG(E ₂)	prostaglandin (E ₂)
PINP	paclitaxel-induced neuropathic pain
PMP22	peripheral myelin protein 22
PNI	peripheral nerve injury
PNS	peripheral nervous system
PPAR	Peroxisome proliferator-activated receptors
PPT	perception pain threshold
PPT	pressure pain threshold
PRDM12	PR domain zinc finger protein 12
PREG	pregnenolone

PRLR	prolactin receptor
PROs	patient-reported outcomes
PSN	primary sensory neurons
PVALB	parvalbumin
QoL	quality of life
QST	quantitative sensory testing
RA	rapidly adapting
REE	resting energy expenditure
RER	respiratory exchange ratio
RVM	rostral ventromedial medulla
S1PR1	sphingosine-1-phosphate receptor 1
SA	slowly adapting
SABV	sex as a biological variable
SC	spinal cord
SCN10A	sodium voltage-gated channel alpha subunit 10
SDH	spinal dorsal horn
SFN	small fiber neuropathy
sgACC	subgenual anterior cingulate cortex
SGC	satellite glia cells
SLR	straight leg rise
SN	salience network
SNI	spared nerve injury
SNL	spinal nerve ligation
SNP	single nucleotide polymorphism
SNRI	serotonin- and norepinephrine reuptake inhibitor
SS	Sjögren´s Syndrome
SSRI	selective serotonin reuptake inhibitor
StAR	steroidogenic acute regulatory
STEP ₆₁	stratal-enriched protein tyrosine phosphatase- 61
T1D	type 1 diabetes
T2D	type 2 diabetes
TCA	Tricyclic antidepressant
TEAE	treatment-emergent adverse event
TF	Transcription factor
TG	trigeminal ganglia
THA	total hip arthroplasty
Tiam1	T-cell lymphoma invasion and metastasis 1
TLR(2)	toll like receptor (2)

TMEM	Transmembrane Protein
TN	Tibial nerve
Trem	triggering receptor expressed on myeloid cells
Trk(B)	Tropomyosin receptor kinase (B)
TRP	Transient receptor potential
TSPO	translocator protein
Vglut	vesicular glutamate transporter
VGNac	voltage-gated sodium channel
VHA	Veterans Health Administration
vIPAG	ventrolateral midbrain periaqueductal gray
WT	Wild type
WUR	wind- up ratio