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NEUROPATHIC PAIN – A FEMALE PERSPECTIVE ON PAIN

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ABSTRACT

Neuropathic pain is a specific type of pain that results from injury to a nerve fibre or part of the nervous system. Due to unique qualities of nerves, neuropathic pain often becomes chronic and a long-term economic and societal challenge. The prevalence of neuropathic pain in the clinical population is 5% in men and 8% in women, but pre-clinical animal research has focused mainly on male models. Microglia, immune cells in the nervous system, are the main mediators of the neuropathic pain pathway in males and increase pain signaling to the brain. Recent initiatives to include female animals in pre-clinical research revealed that microglia are not necessary to cause female neuropathic pain.

The aim of this literature search was to examine where the pain pathway differs in males and females and where it converges back together to cause, what both sexes know as neuropathic pain. The results confirm that blocking microglia was not sufficient to alleviate pain in females with nerve injury, but pain was alleviated in animals in which microglia and T-cells were deleted. The present state of knowledge suggests that T-cells are the cells contributing to central sensitisation in females, but the site at which they interfere is still to be determined. More complex investigation of the role of T-cells in pain and analgesia will be beneficial to clarify how they can interfere with pain treatments, and help us design new, more effective treatments for females and males suffering with chronic pain.

INTRODUCTION

What is neuropathic pain and how can it be studied?

Pain is commonly experienced as a result of damage to some part of our body. The most frequently encountered pain lasts a couple of minutes, perhaps a few hours or days in the case of more severe injury such as surgical procedures or broken bones. The good news is that this pain eventually subsides. This type of pain is called acute (i.e. in the moment) and is protective pain (it protects you from repeatedly touching the harmful object or overusing the damaged body part). We are also mostly able to pinpoint the source of the pain – where the pain is coming from, and which body part we should take care of.

In contrast to this, there is chronic pain. Chronic pain persists past the healing of the initial injury or results from years of gradual, mild damage. Over time, you may lose sensitivity to where the pain is coming from, making correct targeting of the source of the pain more difficult. On average, 20% of the population in the United States was reported to struggle with some sort of chronic pain, making it a serious societal problem in terms of healthcare, well-being and lost productivity costs (Dahlhamer et al., 2018).

Neuropathic pain is often confused with chronic pain. Neuropathic pain is a specific subset of pain that you feel once a nerve fibre – the electrical impulse carrying 'wires' in our body that allow us to feel and move – is injured (Figure 1). Examples of when nerves are injured include conditions such as multiple sclerosis, specific viral infections or following stroke. While at the surface neuropathic pain may feel like chronic pain, what is happening underneath the skin in the body and spinal cord is very different. This is why neuropathic pain, pain caused by a nervous system injury, is a different type of pain compared to when a different body part is injured.

My research question was to study how neuropathic pain is caused, and to examine the emerging evidence suggesting that female neuropathic pain is mediated differently to male neuropathic pain due to differences in the immune system.

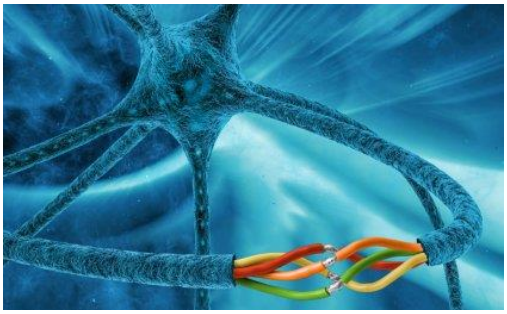

Figure 1: Injured nerve fibres (seen here as the frayed parts of the wires) are more difficult to heal than other body parts. Image source: University of Queensland 2015.

The distinction between neuropathic pain and pain caused by injury of other tissue such as bone, muscle or skin, is because nerve fibres are very different to other kinds of body tissue (Costigan, Scholz and Woolf, 2009). Their unique structure enables them to carry signals between distant parts of the body through a special electrical code. This electrical signal is carried down the axon, which is the long wire section of the nerve (pictured in Figure 1). Pain is also a message that is communicated from the location where the injury is (the wound, broken bone or

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bruise), through the spinal cord and into the brain along axons of neurons. Since the structure of the nerves is so complex (Figure 1), they take a long time to heal and, unlike skin and bones, rarely heal to their pre-injured states. This makes it very likely for neuropathic pain to become chronic, and therefore a long-term issue for many people.

One of the main challenges encountered when studying pain is how subjective each person's experience of pain is. This means that the amount of pain experienced and how bad it is will depend on your previous experience and present feelings. For example, have you ever experienced an injury while being drunk? You probably only realised the extent and pain the following morning. This experience-dependent feeling of pain makes it difficult to study pain scientifically. Just like in other areas of science, a lot of the pre-clinical work (studying what is going on in the mammalian body before we can apply this to treating humans) is done on animals (see Mogil, 2012 for a complex review). Yet, since we are unable to monitor the emotions and experiences of the experimental animals, there are some limitations to the knowledge we can apply from these models to humans, which is the method of quantifying how much pain the animal is experiencing.

Pain investigators have developed two main measures of identifying pain in animals. The first of these is hypersensitivity. This is increased sensitivity to slightly painful stimuli. This would be the change between saying 'Ouch' before the nerve injury and 'OUCH' after the nerve injury. In animals, this can be measured by how much they withdraw after a stimulus of fixed intensity (Mogil, 2020).

The second behaviour that we can measure is allodynia. Allodynia means that non-painful stimuli are experienced as painful. This is most commonly achieved through gently brushing the limb of an animal, they withdraw their limb, as this was painful. This is significantly easier to measure than hypersensitivity, because you know that the brushing, under normal circumstances, is not painful. The outcome measure of allodynia and hypersensitivity is the latency with which the animal withdraws. This helps us measure and compare how much pain the animal is in. Withdrawing faster indicates a higher degree of pain (Mogil, 2020).

Sex differences

The key aspect of biological research that has long been overlooked is the sex difference between male and female organisms. Clinical trials, which are carefully designed and regulated studies of how humans respond to promising drugs, have been obliged to include both males and females in their studies since the last century (Mogil, 2020). However, before these drugs or ideas can be verified using humans, there must be extensive pre-clinical trials on animals and/or cells that indicate they are safe. In these pre-clinical trials, researchers have predominantly used males. Up to 80% of the research carried out before 2005 was carried out on male only animals (Figure 2; Mogil, 2012).

This created a knowledge gap with regards to what was happening in female bodies. As previously mentioned, chronic and neuropathic pain may appear similar on the surface, but they are very different on the inside. Due to the neuropathic pain being different to chronic pain in regards to what goes on in the spinal cord, we are unable to study this on humans. Therefore, for many years we did not know whether the drugs we were testing on males and females, developed only on males, were doing the same thing in both sexes. Researchers assumed they did.

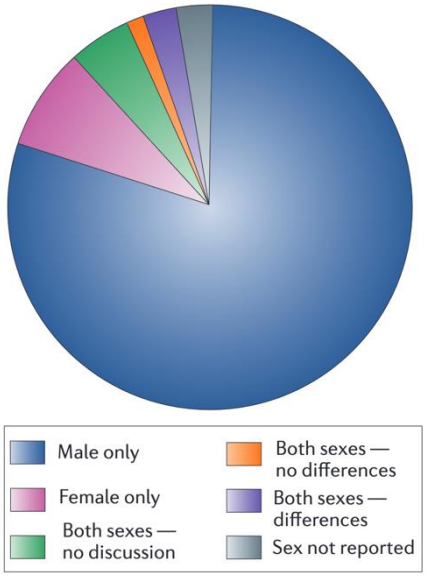

Figure 2: The majority of pre-clinical studies in pain were carried out in males only. Pie chart showing the proportions of studies carried out on males only, females only, both sexes or unreported sex in the journal Pain up to 2005. Mogil (2012).

Why were females excluded? The predominant reason was that females undergo what (in animals) is called an oestrous cycle (the menstrual cycle in women). The researchers thought it would be difficult to align all their research so that they recorded data at fixed time points in the cycle. They correctly argued that females are differently sensitive to pain, depending on which phase of their oestrous cycle they are in. Nonetheless, the variability between hormones, and consequently pain perception, in males is just as high. This is due to dominance status of the male in the 'pack' of animals which changes testosterone levels (Mogil, 2012). So researchers' assumptions were misogynistically biased.

Since 2016, there has been an international effort to start including female animals in investigating the causes and cures to disease. Owing to this policy, the interest and engagement in female pre-clinical research has gradually increased, with only 50% of articles using male-only models by 2019 (Mogil, 2020). However, the knowledge gap that has been created by decades of research towards male-biased ideas will take a long time to close.

Fortunately, the pain field had recognized the need for sex-dependent investigation of pain just before it became a global effort. In the general population, 8% of women, but only 5% of men struggle with chronic neuropathic pain. In

Table 1: Key words used in the literature search. One of the terms from Column 1 was combined with one of the terms from Column 2 and one from Column 3.

Column 1	Column 2		Column 3
Neuropathic pain	P2X4 receptor	BDNF	Female
OR	P38	T-lymphocyte/T-cells	OR
	NMDA receptor	KCC2	
Peripheral Nerve Injury	TLR4	Microglia	Sex

self-reports about 20% of women reported experiencing some sort of chronic pain (Steingrímsdóttir, et al., 2017). One likely explanation are the social influences exerted on males to supress their pain, whereas females are less stigmatized when they express pain. However, the leading scientific groups suggested that the observed differences in pain cannot be explained solely by cultural factors (Chesler et al., 2002).

Qualitative vs. Quantitative Variables

To understand the importance of the discovery of the difference in neuropathic pain between males and females, it is important to first understand the type of data that a scientific investigation can measure. There is quantitative data, or differences that can be counted or measured numerically. An example of this is the measurement of hormone levels in the body: Both males and females have oestrogen and progesterone, but at different relative amounts.

Things that cannot be compared by numbers or values are qualitative variables. These measures are *absolutely* different between males and females, so data can be placed into a category, rather than on a measurable scale. An obvious example are the genitalia – biological males and females can be categorised as having either a penis or a vagina. What was proposed by Mogil (2020), is that pain and how it is caused is not only a difference of pain *magnitude*, but rather that pain is caused by *distinct mechanisms* in males and females (with specific overlap) to result in the common experience of pain.

Research Question

The aim of this research was to examine where the pain pathway differs in males and females, and where it converges back together to cause, what both sexes know as neuropathic pain.

METHODS

To investigate the present state of knowledge about the mechanisms of male and female neuropathic pain, a literature search was conducted of the terms outlined in Table 1 on Web of Science and PubMed databases. These key words were identified by analysing the themes in immunity and neuropathic pain in complex reviews by Costigan, Scholz and Woolf (2009) and Mogil (2020).

Consequently, articles that focused on more complex forms of nerve injury, such as those caused by chemotherapy, diabetes, or multiple sclerosis were excluded. These are diseases that affect the whole body, and so it could interfere with the function of the immune system, distorting the effects of nerve injury.

What is Peripheral Nerve Injury?

Our nerves are essential components of sensing pain. Pain on the skin is detected by the 'nociceptor', derived from the words *nociception*, meaning pain, and *receptor*, meaning sensor. These nociceptors are packaged together with other nerves that carry different types of information from the outside of the body towards the spinal cord. When any of these nerves is injured, the injury will start to 'call for help' using chemical signals.

One type of helper that is attracted by these signals is the macrophage, the debris 'eater' in the body, and the T-cell, an important immune cell that protects our body against specific intruders or specific damage. Together, these cells cause the typical redness and swelling that one may experience after a moderately severe injury. This is called inflammation and is part of the healing process. A similar reaction happens around the injured nerve fibre.

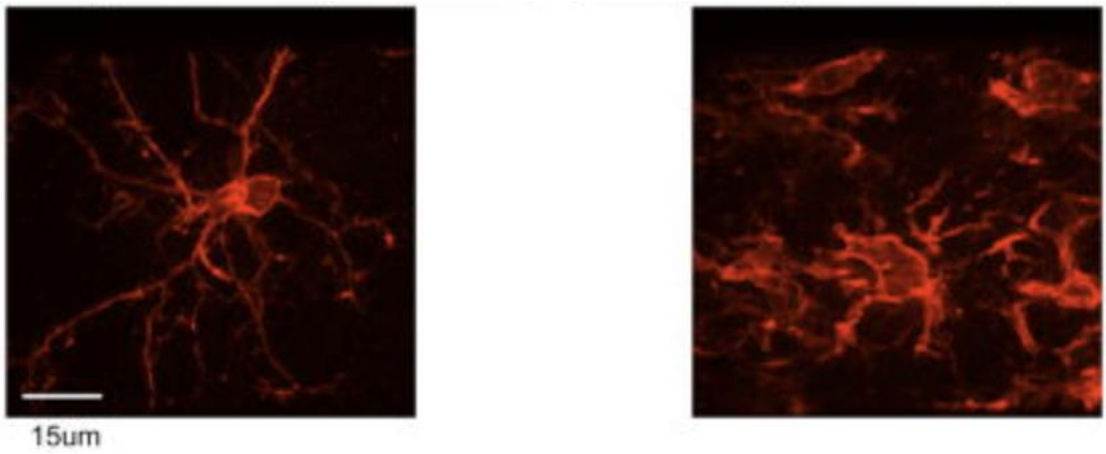

Figure 3: Changes to microglial shape and number following nerve injury. Left panel show a microglia prior to nerve injury in the spinal cord. The right panel shows several activated microglia in the same area of the spinal cord, following nerve injury

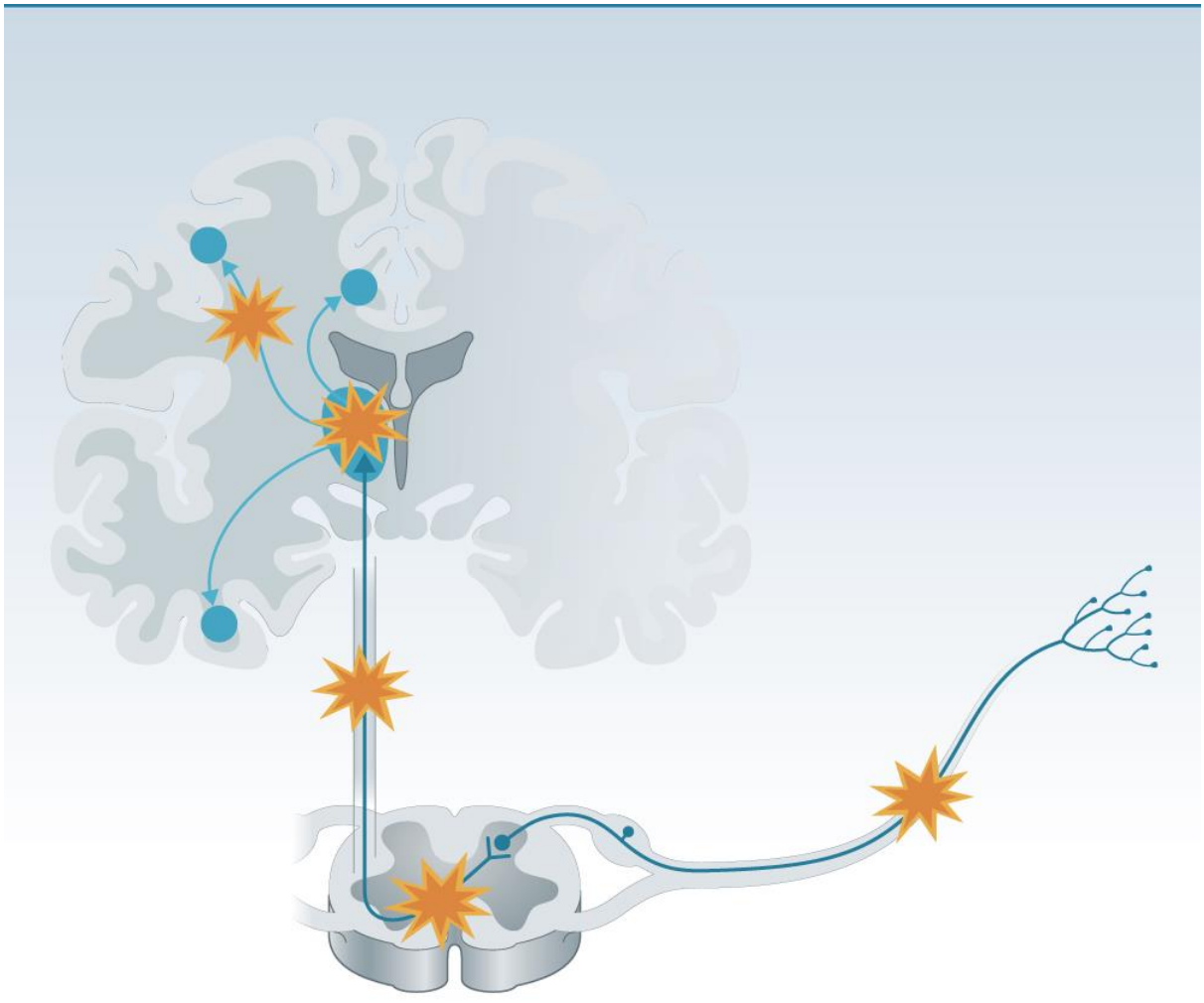

Figure 4: The neural pathway of pain messages. Upon nerve injury, the messages that are sent by the nerves are muddled up, resulting in more signals to the brain being interpreted as pain. Adapted from (Finnerup, Kuner and Jensen, 2021)

While the 'help signals' are sent to the macrophages and T-cells, the nerves that have been injured and their close neighbours are still active. Since they themselves are message carriers (carrying messages of texture, heat etc.), the damage means that their message gets muddled. They start sending 'spam' signals into the spinal cord, which is the relay station for messages going into the brain (Figure 4), and this spam is mis-interpreted as pain, even when it is just touch. This is called allodynia. These 'spam' messages are what makes neuropathic pain different to pain resulting from injury of the skin or a muscle (Costigan, Scholz and Woolf, 2009). Most commonly this is linked to changes to the nerves themselves. Yet new discoveries suggest that immune cells called microglia could be contributing to and causing these spam messages in the spinal cord (Coull et al., 2005; Chen et al, 2017).

Immune cells, microglia and female pain

The microglia are close relatives of the macrophages that are the first line 'worriers' at the injury site. Just like macrophages, they eat debris from the brain and spinal cord, and are crucial for pre-natal brain development and during early childhood (Pont-Lezica et al., 2011). They engulf brain circuits we do not need to make space for brain circuits we do, or will, need. Since microglia are the immune cells of the central nervous system, they are important actors in causing neuropathic pain. They are located in the spinal cord and when there is not injury or danger, they float around within the spinal cord (Figure 3).

The theory of microglia being involved in neuropathic pain dates to the onset of this century, with several research groups looking at how microglia cause allodynia or hypersensitivity (Coull et al., 2005; Beggs and Salter, 2010). Research found that when the microglia are 'switched on' using ATP (a chemical that has multiple functions in our body, including providing energy and carrying important messages) the animals behaved as if they were in pain. Consistently, in animals that had experienced nerve injury, the amount of microglia in their spinal cords was higher and their shape resembled the 'on' or 'activated' shape that also caused the pain symptoms (Figure 4; Beggs and Salter, 2007).

Once activated, microglia can dramatically affect what is happening in their surroundings. In the spinal cord after nerve injury, microglia release chemicals that increase the frequency of the pain messages being sent up the spinal cord, into the brain. There are complex changes that occur at the intersection between the nociceptor and the spinal cord neuron, which result in the body *getting better* at sending pain messages through the spinal cord. This process is called central sensitisation, from the word *central* referring to spinal cord, and *sensitisation*, becoming more sensitive to painful stimuli (Mapplebeck et al., 2019).

The discovery that microglia might cause the problematic features of neuropathic pain was great news for developing treatments (Inoue and Tsuda, 2018). Research groups could block microglia in animals in three ways. The first is pharmacologically, by injecting animals with chemicals that block the microglia; genetically by deleting the genes responsible for making microglia through modern viral methods; or thirdly by blocking the chemicals that are released by the microglia and were linked to central sensitisation (Sorge et al., 2015; Berta et al., 2016; Chen et al., 2017). All of these interventions reduced the pain sensitivity of male animals that had experienced nerve injury. Indeed, clinical trials have begun that test some of these approaches to reduce neuropathic pain, with varying levels of success (Inoue and Tsuda, 2018).

Microglia in Females

Even though there are countless contributors to neuropathic pain at the level of the cell or whole body, microglia have received the most attention. The feature that stands out about microglia is that they seem to be necessary only for male neuropathic pain. A series of recent studies reported that microglia might not be necessary for the production of female pain following nerve injury (Sorge et al., 2015). Looking back to the initial research into microglia, all of the interventions were carried out on male only animals, which was common at the time. These studies were therefore missing an important piece of information about what is happening in the bodies of around half of the human population – in females (Coul et al., 2005; Beggs and Salter, 2007).

The first study to suggest that female pain was different from male pain was in a study by Sorge *et al*. in 2011. Their group found that the TLR4 receptor – a specific receptor used by the immune system to detect damage – on a spinal cord immune cell was only responsible for causing pain in males, but not females. Building on this extraordinary observation, in 2015 Sorge *et al*. conducted a series of experiments trying to block microglia in females using the three methods of blocking microglia described above.

They found that only blocking the factors released by the microglia, a chemical called brain-derived neurotrophic factor (BDNF), eased nerve-injury induced pain in female mice. BDNF was later measured in male and female spinal cords following nerve injury, and the amounts were similar between males and females (Mapplebeck et al., 2019). However, specifically blocking or deleting microglia did not relieve allodynia caused by nerve injury in female mice. What they concluded was that females do not use microglia as their 'default' pain pathway (Sorge et al., 2015).

Interestingly, the same was true in castrated males, which indicates that the cause of this difference could be linked to sex hormones. Similarly castrated females, with the addition of male hormone testosterone, did show reduced allodynia after blocking microglia. These observations indicate that testosterone might be the switch that determines whether microglia will cause pain or not.

These observations raised a more important question, which started the search into what is the main contributor to pain in females, if it is not the microglia.

T-cells

T-cells have already been indicated as important cells that gather at the site of the injured nerve to remove and heal damage. Due to their assumed importance in nerve, and other forms of, injury, Sorge et al. (2015) removed the T-cells from male and female mice. These mice were called

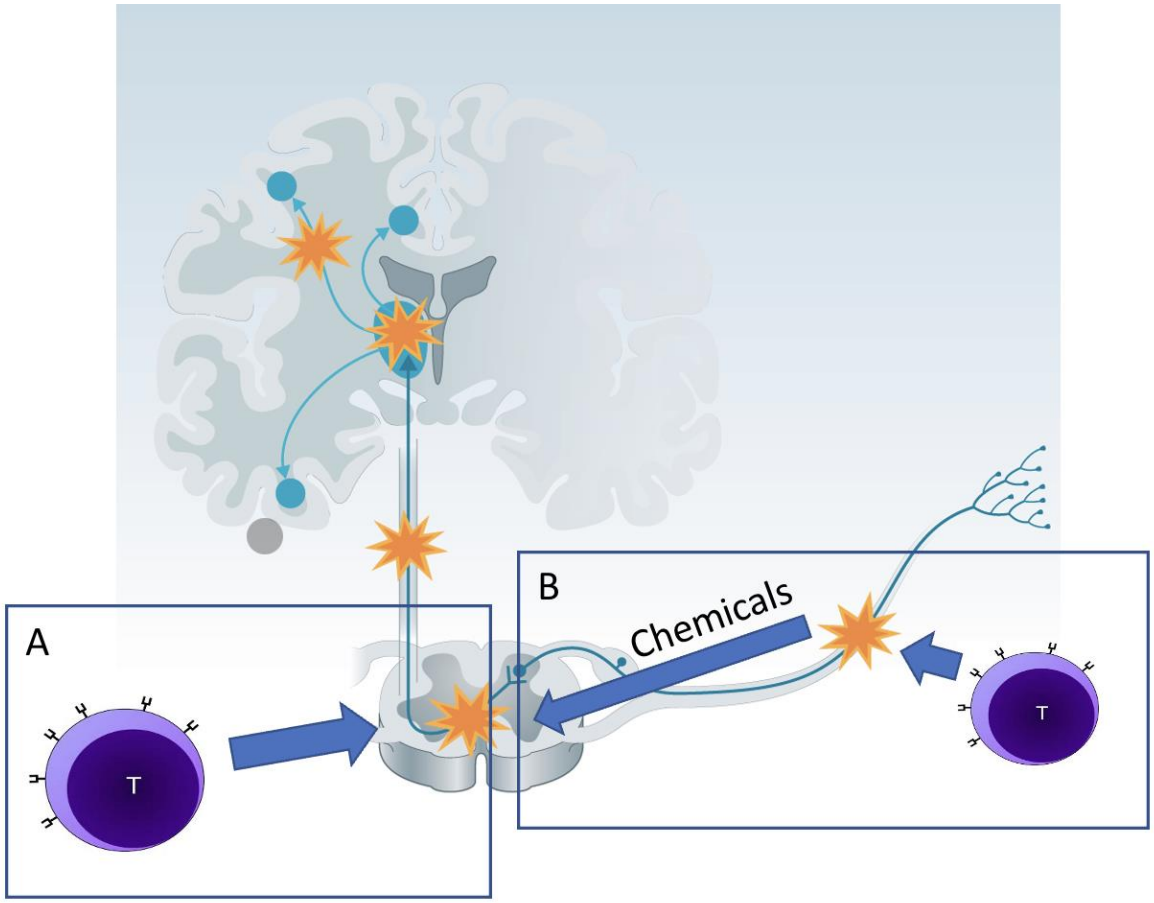

Figure 5: The possible mechanisms of how T-cells contribute to female neuropathic pain. A) T-cells (purple) infiltrate the spinal cord. B) T-cells gather at the site of nerve injury and release chemicals that then travel to the spinal cord. Adapted from (Finnerup, Kuner and Jensen, 2021)

immunodeficient because they lacked these specific immune cells. These mice were found to feel equal pain than unaltered mice. The difference between immunodeficient and unaltered mice was most prominent when they removed the microglia through a chemical called minocycline. Females that lacked T-cells *and* microglia had reduced hypersensitivity and allodynia following nerve injury, which could mean that they felt less pain. The group therefore suggested that females have two pathways through which neuropathic pain can be experienced. One is the microglial route, which is shared with males, but the other is the biologically default route that includes T-cells.

Evidence for the importance of T-cells in females has been increasing for some time. Even prior to this discovery, we knew that women are three times more likely to suffer from autoimmune disease – diseases when the immune system (especially T-cells) becomes too active and starts to attack the healthy body (Fairweather and Rose, 2004). Examples of these diseases include arthritis, when immune cells attack the joints, or multiple sclerosis, when immune cells attack healthy nerves. In both cases these locations become inflamed and painful.

Now that there was a plausible contender for the source of pain in females, the question is as to where the T-cells act to produce female neuropathic pain. There are two ways that the T-cells could selectively cause neuropathic pain in females only. One possibility is that the T-cells themselves enter into the spinal cord to act alongside the microglia (Figure 5A). The second is that the cells that are called to the nerve injury site become more reactive in females and release more chemicals that irritate the nerve and spinal cord, increasing the amount of pain felt (Figure 5B).

Inflammation is the process that surrounds the injury to help it heal. After nerve injury, the immune cells of the nervous system (the microglia) also multiply, which means that also the spinal cord becomes inflamed. This would make it easier for other immune cells, such as T-cells to enter the site of injury or infection, since the microglia would be sending out attracting signals to macrophages and T-cells. When the spinal cord itself is damaged (spinal cord injury), T-cells can enter to help the microglia repair the damage (Gattlen et al., 2016).

In the past, there have been multiple suggestions that T-cells are present in the male spinal cord after nerve injury (Sweitzer et al., 2002; Costigan et al., 2009). However, more recent studies have argued against the possibility of T-cells in the spinal cord (Gattlen et al., 2016; Lopes et al., 2017). The main argument against this, is the difficulty with which T-cells would enter. The spinal cord has a tight and very selective layer surrounding it, which would not allow the T-cells to enter. To this day, there is still a controversy whether T-cells can be present in the male spinal cord after nerve injury. More importantly, to the

extent of the current study, there were no fully justified reports of T-cells in the female spinal cord after peripheral nerve injury.

What is more likely is that the T-cells mediate neuropathic pain by acting throughout the body, outside the spinal cord. Females have nearly twice as many T-cells at the nerve injury site than males, as well as higher T-cell levels in the blood after injury (Lopes et al., 2017). Similarly to microglia, T-cells can produce chemicals that could be interpreted as signals of injury and cause increased pain perception. It is possible that these chemicals could be transported to the spinal cord and contribute to central sensitisation, similarly how chemicals from microglia cause central sensitisation in males.

No doubt, the role of T-cells in pain is very complex, far beyond the scope of this review. One additional finding that must be mentioned to introduce the holistic picture of T-cell interaction in pain is their role in analgesia, which is the reduction of pain. Analgesia can be induced from the brain, depending on our mental state, such as the drunk injury example in the introduction, or it can be mediated by processes in our body. One form of analgesia is that experienced during pregnancy. In late stages of pregnancy, the female's body becomes ready for birth and therefore numbs feelings of pain and injury called pregnancy analgesia (Rosen et al., 2017). This sort of analgesia was reported to be caused by T-cells, as it was absent in immunodeficient mice (mice without T-cells). Pregnancy analgesia is considered to be a result of opioids, pain suppressing chemicals that can also be artificially injected to counteract moderate to severe pain. The T-cells tend to become more sensitive to our body's own opioids during pregnancy, and so can actually counteract pain. Clearly T-cells are important for multiple aspects of female pain, but we still do not know exactly *where* they act and the exact molecular pathway through which they cause pain. Our understanding of the exact interaction of T-cells and opioids will be extremely helpful to inducing analgesia in the clinic.

Conclusion

It is important to understand the limitations that constrain what we know about nerve injury responses in animals and how this can be applied to treat neuropathic pain clinically. At present, we are unable to be able to accurately study directly what is happening in the human spinal cord. We can therefore only use informed assumptions to extrapolate what might be happening in humans. Even then, there is a large time gap between the discovery of what is happening, to being able to test possible methods of correcting this in humans. This is one of the reasons that, at present, there are few pain treatments targeting microglia. One of these, a drug called minocycline, is also used to treat depression and acts against microglia in general. In animal trials this drug only works against pain in males or immunodeficient females. The data is yet to be collected on whether this drug is effective in females and males, but this information would tell us more about how translatable the animal studies are to human pain.

Nonetheless, the conclusion based on the pre-clinical findings examined in this investigation is, that there are two pathways responsible for neuropathic pain. There is the male-biased microglial pathway and the female T-cell mediated pathway. Females have the capability to use the microglial pathway, but only in the absence of T-cells or in the presence of testosterone. These immune cells can then release the chemicals that have been equally measured in both males and females, that increase the capability of nociceptors to send pain signals through the spinal cord (Mapplebeck et al., 2019).

As has been demonstrated, the field of female pain, and scientific research in general, has been neglected for a long time. In recent years, breakthrough discoveries made by groups in the field, have helped shed light on some of the sex differences in pain that have been overlooked in the past. Thanks to initial discoveries about differences in immune cells after nerve injury, there are now increasing efforts to understand the female neuropathic pain mechanism. To date, there is an understanding that these two different sex-specific pathways. There is the male-microglia mediated route that is generally well defined and understood. And there is also the female neuropathic pain pathway, that most likely involves T-cells. The location and precisely what they do to cause pain is much less understood. Equally, understanding what it is that makes T-cells so important for analgesia will be vitally important to being able to enhance the present pain treatments to better address female pain.

In future years there should be more work done to find out how neuropathic pain is caused in females, and this can help us develop more targeted treatments to treat pain in female patients. The field of pain is an excellent example of why personalised medicine is important to improve treatment outcomes, and why research must overcome historical and socially ingrained prejudice to and consider both sexes to avoid detrimental bias in pain treatment.

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