BACK TO BASICS (PAIN RESEARCH)

Note: The following is a summary of the Australian Pain Society Basic Pain Research Special Interest Group (SIG) Pre-Conference Workshop. The Basic Pain Research SIG aims to improve, share, and promote scientific knowledge and understanding of nociception and pain at the cellular and molecular level. The Pre-Conference Workshop was held as part of the Australian Pain Society 39th Annual Scientific Meeting, which took place from earlier this year on the Gold Coast, Queensland, Australia.

The Basic Pain Research Special Interest Group (SIG) of the Australian Pain Society (APS) was approved by the APS Board in 2016 and launched in 2017. The Basic Pain Research SIG aims to bridge the gap between basic and clinical research pain research to benefit patients by improving our understanding of the components of nociception and pain at the molecular and cellular level. 2019 marked the third year the Basic Pain Research SIG had held a pre-conference workshop, following successful outings in Adelaide and Sydney. Outgoing SIG Chair, Rainer Haberberger, welcomed attendees to the workshop before seven fantastic speakers presented the latest happenings of basic pain research. Read on for a summary of a selection of the pre-conference workshop presentations. A complete list of the pre-conference workshop speakers can be found here.

Tachykinins, Inflammation, and Pain

Professor Allan Nimmo, James Cook University, kicked off the workshop by providing a historical perspective of substance P and its role in both inflammation and pain. Inflammation and pain have been linked to one another since the Greek encyclopaedist Celsus (not to be confused with Celsius, the Swedish astronomer after whom the temperature scale is named) described the cardinal signs of inflammation—warm, *painful*, swollen, and red. This then poses the question of whether pain is a unique symptom, or whether it is just part of the inflammatory process.

Substance P was discovered after being isolated from the equine gut in the 1930's. Substance P is a tachykinin that has long been implicated in inflammation and has had an up-and-down history with respect to its associations with nociception and pain. The entire notion of an association between substance P and pain was based around circumstantial evidence—no one had shown that substance P was actually involved in nociceptive processing or transmission!

Substance P is closely related to neurokinin A, and therefore it is no surprise that substance P binds to the neurokinin 1 (NK1) receptor. Pharmaceutical companies spent many years and millions of dollars developing NK1 receptor antagonists to block the actions of substance P and its subsequent involvement in inflammatory processes. However, clinical trials testing the effects of NK1 receptor antagonists in nociception and chronic inflammatory conditions all yielded poor and inconsistent results.

Much of research relating to substance P was driven by the desire to use it as a clinical treatment, namely for chronic inflammatory conditions, rather than what the evidence showed that substance P did. It wasn't until the late 1990's that Nimmo and his colleague Professor Robert Vink (University of South Australia) began to investigate the role of substance P in a more acute setting, such as traumatic brain injury and stroke.

After displaying that substance P was released by neurons following a traumatic brain injury, Nimmo and Vink set out to find an NK1 receptor antagonist with assistance from Roche. The compound—tagged as EU-C-001—can be administered intravenously and was shown to reduce intracranial pressure and increase brain oxygen levels in a sheep model of traumatic brain injury. The NK1 receptor antagonist was also found to reduce levels of inflammatory mediators IL-1β, IL-6, and TNF-α, suggesting a broader inhibitory effect on inflammatory processes. Nimmo and colleagues are now in Phase II clinical trials in both Australia and the United States for EU-C-001.

Therefore, substance P appears to be an appropriate target for the acute inflammatory response. "This is where we need to focus more when considering the role of substance P in pain transmission", Nimmo said.

Can Mice Teach us about Vulvodynia?

Dr Christine Barry, Flinders University, Adelaide, discussed her research relating to the development of a mouse model of vulvodynia—a common condition typically characterised by pain at the entrance to the vagina. While vulvodynia can affect women of all ages, it is most common in women under the age of 25. Vulvodynia exerts a significant impact on sexual function, relationships, and fertility.

The pathophysiology of vulvodynia is quite well known; commonly described as hyperinnervation of the symptomatic tissue—meaning that there are more nerves that are more sensitive to external stimuli. There are a variety of treatments recommended for vulvodynia, including psychological interventions, pelvic floor physiotherapy, and surgery. While each of these treatments can provide some degree of benefit, none of these treatments specifically targets the underlying pathophysiology.

Barry explained how parallels can be drawn between vulvodynia and atopic dermatitis—the most common form of eczema—as they are both disorders of hyperinnervation. The development of highly reproducible animal models of atopic dermatitis not only aided the understanding of its pathophysiology, but also helped identify potential treatment targets. Unfortunately, this is where the commonalities end—there is yet to be a reproducible animal model of vulvodynia.

In the vaginal hyperinnervation model that Barry has been developing, the injection of complete Freund's adjuvant (CFA)—a pro-inflammatory agent—into the vagina of mice leads to swelling, vaginal hyperinnervation of multiple types of nerve fibres, and abundant macrophage inflammation. These CFA-induced changes replicate the pathophysiologic changes seen in human cases of vulvodynia. The infiltration of macrophages was of interest to Barry and her team—they wondered what role the macrophages were playing with respect to the hyperinnervation and nociceptor sensitisation.

Macrophages regulate pain sensitivity through a variety of different mechanisms. The M1 phenotype is the classic pro-inflammatory macrophage that promotes hyperalgesia, while the M2 phenotype is anti-inflammatory and promotes analgesia. This led Barry and her team to

question whether macrophages influenced nociceptor sensitivity and axonal sprouting in their hyperinnervation model of vulvodynia. So, they set out to identify the phenotype of the macrophages in their vaginal hyperinnervation model at seven- and 28-days post-injury, before examining whether nociceptor sensitivity was altered in their model.

Barry and her team observed an abundance of the M1—or pro-inflammatory—macrophage phenotype at the seven-day post-injection mark. Barry and her team also found some evidence of an increase in the M2—or anti-inflammatory—macrophage phenotype at the 28-day mark. However, further work is required to examine the 28-day post-injury tissue so be sure to keep an eye out for this! Hyperinnervation of nerve fibres was seen at both seven- and 28-days post-injection. Therefore, it is possible that macrophages contribute to hyperinnervation in this animal model. Further work is also needed to examine the role of macrophages in altered nociceptor sensitivity.

Chronic Constriction Injury: More than just a Pain Model?

Preet Makker, a PhD candidate from the University of New South Wales and MD student at the University of Sydney, gave a fascinating presentation on nerve excitability in peripheral neuropathy. Makker's doctoral research focuses on mapping chronic pain and motor axonal damage in chronic constriction injury (CCI).

While CCI is a commonly used model of both acute and chronic pain due to its effects on sensory neurons, there is a small amount of evidence to suggest that spatial motor axonal degeneration also occurs following CCI. However, much less is known about the effects of CCI on motor neurons. Makker believes that improving our understanding of the pattern of degeneration and reinnervation in CCI will provide new insight into how the injury progresses from acute to chronic pain. "I want to present the CCI model in a new way—not just presenting it as a model of pain—but more to see it as a progressive disease with distinct sensory and motor characteristics, many of which mimic clinical neurological syndromes."

Makker uses a technique called threshold tracking to measure the excitability of myelinated axonal neurons. Threshold tracking was originally developed for use in the clinic but has since become a gold standard in vivo technique in clinical and pre-clinical research. As the name implies, threshold tracking involves tracking the axonal response to subthreshold polarising currents to measure a range of outputs that can be used to make inferences about biophysical properties of the axon. There are three key outputs Makker captures—the first tracks the axonal response to subthreshold depolarising and hyperpolarising currents, the second tracks the recovery of the axonal internode (the bit of the axon where there are gaps in the myelin sheath) after the axon potential has fired, while the third tracks the response of the axon to strong, hyperpolarising currents.

Acute and transient ischemia occurs following a CCI. The constriction of the peripheral nerve leads to a reduction in blood flow of the epineurium—the outermost layer of connective tissue wrapped around a peripheral nerve. The decrease in blood flow reduces the amount of available ATP, which is needed to drive the sodium-potassium pumps. The end result is membrane depolarisation, which leads to a pronounced change in the threshold tracking outputs. Interestingly, at seven-days post-injury approximately 60% of Makker's animals had recovered their neuronal excitability, *despite signs of injury still being present in the axons*. The remaining 40% of the animals developed a

complete conduction block. “This is most likely due to the ischemia becoming worse and worse over time”, Makker summarised.

A conduction block occurs when a nerve impulse fails to propagate through a structurally-intact axon. The idea of a conduction block is important to consider, as the CCI model does not sever the nerve—there is only constriction. Makker identified two patterns of conduction blocks in his CCI-inflicted mice. Most of the mice displayed a complete block initially but slowly recovered, whereas the remainder of the mice displayed an incomplete block at all follow up timepoints. The onset and the recovery of conduction block were sporadic, with no clear pattern. Interestingly, all mice recovered from their complete conduction block by 20-weeks post-injury. Makker is confident that the ischemia from the CCI contributes to the conduction block but acknowledges there are other mechanisms that play a role, such as demyelination and hyperpolarisation. There are multiple clinical correlates of this line of research—peripheral neuropathies, Guillain-Barre Syndrome, and amyotrophic lateral sclerosis—meaning that there are aspects of CCI that can be considered to examine other clinical conditions and can be used as a model of a range of diseases, not just acute and chronic pain.

The Basic Pain Research SIG is organising another pre-conference workshop for the 2020 Australian Pain Society ASM. Keep an eye out for it to hear more exciting reports of basic pain research being conducted in Australia.

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