A PERIAQUEDUCTAL GRAY MODEL OF PAIN INHIBITION

FOR PHANTOM LIMB PAIN

By

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A Periaqueductal Gray Model of Pain Inhibition for Phantom Limb Pain

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The study of nociception has been a very electrifying field in neuroscience, pharmacology, and medicine over the past several decades. Pain in living organisms is a homeostatic, sensory-based process that alerts beings to possibly damaging or noxious stimuli from the environment. However, this sensory mode is extremely dynamic and can be influenced by cognitive states, reflex responses, inhibitory or faciliatory neurons, etc. This literature review that addresses the currently understood system of pain transmission, the anatomy/nociceptive functionality of the periaqueductal gray, and phantom limb pain to reinvest research towards top-down treatment of chronic pain syndromes.

Introduction

The sensory system is a set of neurons that collects information from the environment and sends it to the central nervous system (CNS) for processing. It includes the visual, auditory (listening), olfactory (smell), gustatory (taste), and somatosensory (touch). Each respond to different stimuli and project to different cortical regions of the brain to calculate an appropriate response. In this paper, I focus on the somatosensory system and specifically pain with an emphasis of the role of the periaqueductal gray in pain processing and its implicative role in phantom limb pain.

The sensation of pain is termed nociception. Nociception can be underappreciated by humans because it causes discomfort, however, this system is extremely important in transmitting information of potentially threatening or damaging stimuli. Without a pain sensory system an individual could sustain an infected cut that could go untreated if it were not brought to attention. Therefore, nociception is an invaluable tool for defense responses and survival for an organism.

Nociceptors are the neurons that recognize noxious or painful stimuli and communicate that with the CNS. There are several different classes of nociceptors that are optimally activated by diverse types of noxious stimuli. Higher order sensory neurons can be activated or suppressed by different transmitters, peptides, molecules, and chemicals which allow for different responses based on the circuitry.

An ineffective pain system can lead to several pain related conditions or disorders. These include chronic pain, allodynia, and others that will be addressed in this paper. Understanding the mechanisms behind these conditions is of high interest for clinical research in finding effective methods for treatment. However, this requires continued research of how nociception works and where inadequacies can lead to disorders.

This paper will focus specifically on the role of the periaqueductal gray (PAG) and its function in pain inhibition, termed antinociception. After understanding the anatomy and functionality of the PAG, I take a deeper look at the characterization of phantom limb pain (PLP) and the possible

clinical relevance of the PAG in studying PLP.

Characterizing Pain

There are several ways to characterize pain in an individual. The first classification is between acute, persistent, and chronic pain. Acute pain is a result of disease or injury and is caused by a stimulus activating the nervous system to alert an individual to a potentially threatening factor. Acute pain is normal, biological perception of pain in organisms.[1] Persistent pain and chronic pain are relatively interchangeable and often chronic pain is used to represent both. Persistent pain is considered the activation of pain modalities that span a longer time because of some sort of tissue injury or disease. Chronic pain is an extreme case of persistent pain and is defined as the perception of pain with no biological function.[2]

The distinction between persistent pain and chronic pain is subtle important. Persistent pain is considered more biological while chronic pain is ill-understood and appears to only cause unwarranted discomfort. In this paper, we will refer to both types of pain as chronic pain. The reason for this is that some research acknowledges the distinction between the two, while others do not. For the sake of consistency between literature, chronic pain will suffice to represent both types of pain.

Chronic pain can further be divided into two categories: nociceptive pain and neuropathic pain. Nociceptive pain is the perception of pain due to activity of nociceptors found in soft tissue, joints, and skin. The domain for this class of pain ranges from ligament tears to arthritis.[2] Neuropathic pain, on the other hand, is due to direct damage to neurons and nerves in the nervous system.[3] Neuropathic pain can include things such as PLP due to amputation and postherpetic neuralgia. Postherpetic neuralgia is caused by inflammation and damage to peripheral nerves after a bout of herpes zoster.[4,5]

Neuropathic pain that results from damage to neurons of the spinothalamic tract or its targets is called central pain. Central pain can be very dramatic in intensity and its perception can be scattered throughout the body.[2,6] In comparison to central pain, neuropathic pain that involves damage to the nociceptors of the somatosensory nervous system is called peripheral pain. This can arise from conditions such as polyneuropathy and peripheral nerve injury.[7]

Some popular examples of painful conditions that occur across several neuropathic and nociceptive pain paradigms are hyperalgesia, allodynia, and spontaneous pain. Hyperalgesia, as opposed to hypoalgesia, is an increased sensitivity to painful or noxious stimuli. This includes a persistent sensation of pain outside of the presence of a stimuli. Allodynia is a phenomenon where previously non-painful (nonalgogenic) stimuli, such as a light touch, will cause pain. Spontaneous pain arises in the complete absence of any stimuli at all.[8]

The characterizations of pain are plentiful in the scientific literature. Understanding the classes of pain and the mechanisms behind each can be fundamentally important to drawing conclusions about clinical treatment.

Classes of Nociceptive Neurons and Relevant Ion Channels

The neurons that collect and transmit pain signals are called nociceptors. These neurons respond to noxious activity that can be perceived as threatening and are often specialized for specific signals. This occurs by receptor activation from its appropriate stimulus. There are four important classes of nociceptors: thermal, mechanical, polymodal, and silent. Each of these nociceptors can have different myelination thicknesses and receptors. The silent nociceptor is considered

more puzzling and less understood as compared to the other three, so it is often considered separately but it is important to discuss in the context of hyperalgesia.[2]

Thermal nociceptors are neurons that are activated by extremely warm and cool temperatures. The domain for the activation of these are often either above 115ºF or less than 41ºF.[2] Most neurons that are activated by noxious heat are C-fibers while those activated by noxious cold are A-delta.[9,10] Figure one reveals how the difference in myelination between these neurons can modulate conduction velocities. First pain is a quicker, sharper pain, while second pain is long, dull pain.

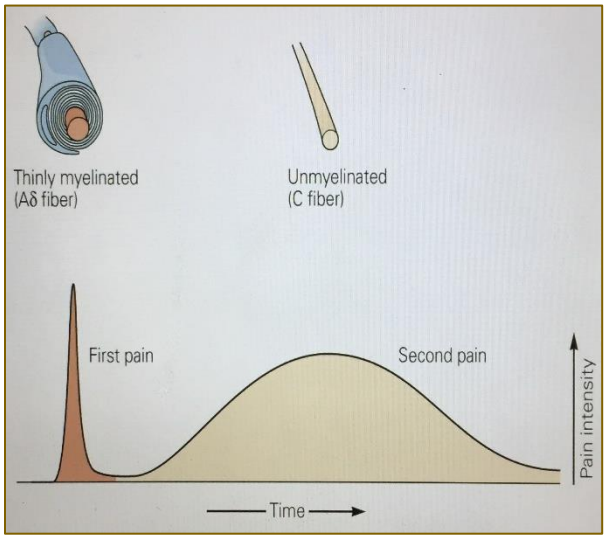

Figure 1 This visual shows that conduction speed between noxious cold and noxious hot nociceptors. Most nociceptors are either Aδ- or C-fiber neurons (Kandel et al, 2013).

Mechanical nociceptors are activated by noxious pressure like getting punched or your finger getting stuck in a door. These nociceptors are thinly myelinated, Aδ-fiber neurons.[11] Other more damaging stimuli such as cuts can also transiently activate these nociceptors.[12]

Polymodal neurons are the most dynamic form of nociceptor as they are activated by noxious mechanical, chemical, and heat/cold stimuli such as mechanonociceptors and thermonociceptors. These neurons are the most abundant C-fiber nociceptors and are affiliated with the mechanism behind second pain as seen in figure 1.[2]

Silent nociceptors are the least understood form of pain neurons. These neurons do not usually respond to simple noxious stimulation and their domain for maximal activation are extreme and narrow. However, when these nociceptors are "awoken" they are often correlated with spontaneous pain, hyperalgesia, and allodynia making it possibly important nociceptor for the study of chronic pain.[13]

The different receptors and ion channels that are found on each nociceptor are responsible for the depolarization of the neuron and transmission of an action potential based on the appropriate stimuli. These receptors and channels can be voltage, mechanical, thermal, and/or chemically gated and the gating type defines the function of the nociceptors.

Three receptors/channels associated with noxious heat stimuli are the capsaicin receptor, vanilloid receptor (VR), and transient receptor potential (TRP) channels. These all have similar morphology and are activated both by extremely hot temperature and the chemical in spicy peppers, capsaicin.[14] These receptors/channels are found on nociceptive neurons that respond to heat such as the thermonociceptors and polymodal nociceptors.[15]

TRP channels are an interesting family of temperature sensitive channels. There are about eight or so channels that are well understood. Most of these channels respond to both chemicals and temperature.[2] (Figure 2) The two most discussed and studied TRP channels happen to be TRPM8 and TRPV1, which are cold and hot receptors respectively. As can be seen in figure 2, TRPM8 is also

responsive to methanol along with cold temperatures and TRPV1 is activated by capsaicin and hot temperatures.[16,17]

Mechano-receptors often have mechanically gated ion channels.[18] When noxious pressure causes tissue damage, however, nearby nociceptors expressing an ionotropic purinergic receptor called PTX3 can depolarize. The PTX3 receptor is activated by ATP released from the damaged tissue.[2] The other ion channel that detects damage are the acid-sensing ion channels (ASIC), which activated when hydrogen ions increase in the tissue.[19] The PTX3 and ASIC channels can explain the persistent feeling of pain after a stimulus has caused damage to tissue by mechanical means.

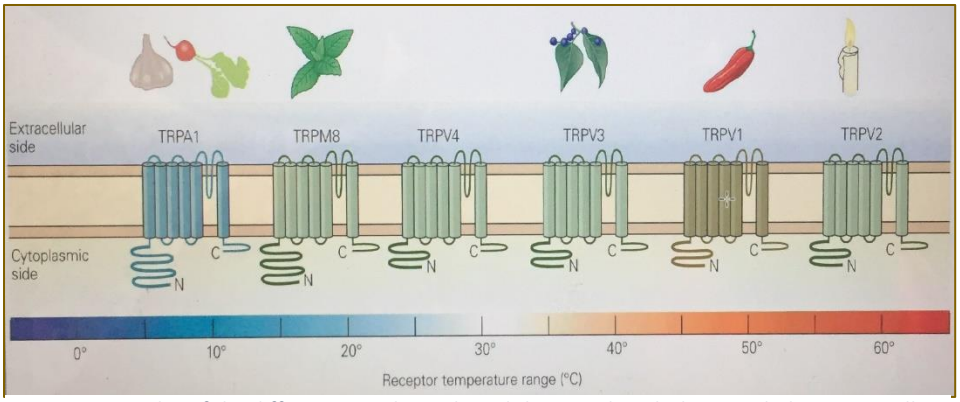

Figure 2 Examples of the different TRP channels and the stimuli with the stimuli that maximally activate each (Kandel et al, 2013).

While there are many different receptors and channels that can be activated by noxious stimuli the ones discussed above have been hallmarks in the study of pain. There several studies investigating different protein and RNA markers of pain, whether it is on the nociceptors or ascending sensory neurons.[20,21]

Relevant structures for pain processing and modulation

The number of nervous system structures involved in the processing and modulation of pain is numerous. Being one of the most important sensory systems for survival, the information from nociceptors is important for many CNS regions. It becomes important for cardiovascular, respiratory, skeletal, and endocrine response among others.

The first region of interest in the processing of nociceptive information is the dorsal root ganglia (DRG) of the spinal nerve. This structure is found alongside the spinal cord and is the cell-body hub for nociceptors. These cell bodies contain the DNA that codes for the necessary proteins expressed by nociceptors. They project to the dorsal horn of the spinal cord, which then projects to several other structures through several pathways (discussed in the next section).

Another hub for sensory neuron cell bodies is the trigeminal ganglia (TG). The trigeminal ganglia provide sensory information for cranial nerve five, the trigeminal nerve. This structure provides sensory input from regions of the head and face.[22]

The most relevant target of DRGs and TGs is the thalamus. The thalamus serves as an important relay station in the brain. It receives information from the second order sensory neurons and proceeds to transmit that information to other cortical areas for processing. These high order structures can than coordinate a response and send that information back down for modulation.

The thalamus is also included in a set of brain structures characterized to be activated consistently with exposure to pain. These structures comprise the pain matrix, which has been defined through advancements in function magnetic resonance imaging (fMRI).[23] The nervous system regions that are often included in the pain matrix along with the thalamus are the rostral anterior cingulate

cortex, pregenual cingulate cortex, somatosensory cortices (S1 & S2), PAG, insula, and amygdala.[24,25]

The somatosensory cortices S1 and S2 collect information about the type and location of sensory input. These regions project to other areas to integrate function related with sensation. Although this cortical area is unsurprisingly included in the pain matrix the other cortical structures provide interesting insight into how pain can used to affect other cognitive, emotional, and physical processes.

The cingulate cortex is important for integrating both the physiological brain (sensation and movement) and the psychological brain (cognition and emotion) and influences other areas of the brain to coordinate a response.[26] The anterior cingulate cortex (ACC) is known to be involved in three types of attention: motivated attention, attention allocation, and error detection.[27] Being able to direct attention and respond quickly to a painful stimuli is important for the quick response and survival of an organism, making the ACC's function crucial. The pregenual cingulate cortex (pCC) is activated during negative decision and negative emotion, which often can be a result of an organism being in a stressful environment.[28,29] Considering that pain is a warning signal, the activity of this region is important to allow an organism to be emotionally prepared to react in a dangerous situation.

The insula and amygdala are highly connected structures both anatomically and functionally.[30] The insula is known to represent painful and nonpainful sensation as found by electrical stimulation.[31] The amygdala is popularly known for emotion and affective states but also has a nucleus devoted to pain-related emotion – the central nucleus of the amygdala (aka the nociceptive amygdala).[32] These two structures are important for processing information about the sensation of pain and expressing an appropriate emotion. This function is very similar to the pCC and indicates the importance of assessing the gravity of danger for a painful stimulus.

The PAG receives ascending information about pain and sends a descending response. The functionality of the PAG will be discussed in a later section but it's important to note the raphe nuclei of the reticular formation, locus ceruleus, and the rostral ventromedial medulla for serving as important relay structures for the PAG even though they are not part of the pain matrix.

Pain is influenced by emotion more than the other sensory modalities. This fact justifies the structures that comprise the pain matrix and often activate with the presence of a stimulus.[2]

Spinal Pathways that Process Pain

Communication from the sensory neurons to higher-order cortical and subcortical areas is accomplished through ascending spinal tracts. These tracts originate in the dorsal horn of the spinal cord where second-order neurons and interneurons are innervated by the primary afferent neurons. Although there are several ascending spinal tracts, these three serve to communicate nociceptive information – the spinothalamic, spinoreticular, and spinomesencephalic tracts.

The dorsal horn is anatomically compartmentalized into six laminae. Lamina I and II receive the greatest input from nociceptive afferents. Lamina I attains strong input from myelinated Aδ fibers that to project to lamina II and V weakly. Lamina II receives greater input from C fiber interneurons which can directly and indirectly project to lamina I as well.[2,33] These laminae communicate with the other lamina through interneurons which all have projection neurons that can cross the midline of the spinal cord.[34] The neurons arising from the

dorsal horn are called secondary neurons with primary neurons being the nociceptors.

The spinothalamic tract is highly implicated in sending nociceptive, thermal, and mechanical sensations from the dorsal horn to the thalamus.[35] This tract is divided into two pathways. The anterior pathway conveys mechanical information and the lateral pathway conveys information about pain and temperature. These secondary neurons travel contralaterally up the spinal cord through the medulla and pons before terminating on the thalamus. From the thalamus third-order neurons project to S1.[2,36,37]

The spinoreticular tract is also involved in sending pain signals to the primary somatosensory cortex. This tract ascends contralaterally in the spinal cord and the secondary neurons project to the reticular formation at the levels of the medulla and pons.[2,38] It is important to know that the nucleus raphe is found in these areas. The neurons of this area will then project to the thalamus and eventually to S1.[37]

The most direct and relevant tract for the PAG is the spinomesencephalic tract. This pathway projects from the secondary neurons in the dorsal horn of the spinal cord to the midbrain and mesencephalon structures. Several studies by Dr. Robert Yezierski and others showed that this tract sends information from the skin, joints, organs, and muscles and is functionally important for analgesia, cardiovascular, reproductive, respiratory, and vocalization.[2,37,39] Each of these functions are all integrated and modulated at the PAG, which relays a response through descending output.

Relevant Neurotransmitters and Neuropeptides

Like most of the nervous system, there are countless amounts of neurotransmitters and neuropeptides that can be released from presynaptic neurons. In the nociceptive system, neurotransmitters have several clearance mechanisms from the synapse while neuropeptides clear through diffusion. This difference is important because it can lead to poor localization of pain when a neuropeptide is the effector on a postsynaptic neuron. The most relevant neurotransmitters and neuropeptides for the discussion of the PAG are glutamate, aspartate, glycine, GABA, substance P, CGRP, and endorphins.

Glutamate and aspartate are very important amino acids in a cell but also play a neurotransmitter role in the nervous system. These molecules are often excitatory but in the PAG, glutamate appears to be more effective.[40] Double-labeling immunostaining experiments of PAG neurons showed that 95.2% of neurons have both amino acids and other experiments revealed that both molecules are releasable as neurotransmitters in the PAG.[41,42] Other work has indicated the excitatory input to the PAG is largely from the cerebral cortex and that the PAG itself may release both neurotransmitters to the nucleus raphe magnus (NRM) as an excitatory signal.[41,43,44]

Glycine and GABA serve inhibitory functional roles in the PAG and entire nervous system. Although evidence shows that glycine is found across the entire extent of the PAG, it appears that its most significant role is a modulatory one for vocalization in the PAG. However, localization of the glycine receptor is extremely scarce in the PAG.[45,46]

Gamma-aminobutyric acid (GABA) is the neurotransmitter released by GABAergic inhibitory neurons. They serve as interneurons within the PAG and descending projection neurons to the medulla and reticular formation.[47,48] The interneurons are tonically active and inhibition of those GABAergic neurons drives the activation of the PAG.[49] GABA release in the PAG and the its downstream targets is known to influence nearly all the functions of the PAG including defense reaction, vocalization, and

analgesia.[46,50,51] This sheds light on the importance of this neurotransmitter and the inhibitory circuitry of the PAG.

The neuropeptides substance P has a relatively simple role in the PAG. Substance P release in the PAG is associated with lordosis and analgesic activity.[52,53] Substance P and CGRP release at sites of neurogenic inflammation leads to dilation of blood vessels as seen by heat and redness.[2] Although CGRP does not have a direct role in PAG functionality, it is released by peptidergic nociceptors and can be found *de novo* in Aβ mechanoreceptors, which can provide a possible mechanism for allodynia after neuropathic damage.[54,55]

Endorphins and opiates are important to mention because they are involved in pain processing and antinociception. Several studies use morphine as an exogenous opiate to understand analgesic functions of the PAG and these molecules can have a global function when they are found in the bloodstream.[56,57,58]

Anatomy of the Midbrain and Location of the PAG

The midbrain (also known as the mesencephalon) is the most rostral part of the brainstem and sits between the spinal cord and cerebellum. In the brainstem, the midbrain is accompanied by both the pons and medulla. The brainstem is important for several autonomic functions such as respiration and cardiac activity.[59] In cases where only the brain stem develops, such as hydraencephaly, an individual can live but for a very short lifespan.[60]

The midbrain itself is divided into three main structures: the tectum, tegmentum, and cerebral peduncles (Figure 3). The tectum includes the colliculi and sits dorsally to the tegmentum which includes the red nucleus, substantia nigra, and PAG. The cerebral

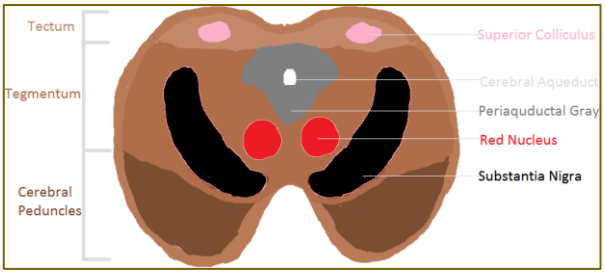

Figure 3 Simple representation of the midbrain

peduncles are the most ventral section and are known to relay information between the cerebellum and cerebrum and spinal cord and cerebrum.[61]

There are lots of other nuclei, tracts, and cranial nerves that can be located anatomically along the entire brainstem and midbrain. These mostly contribute to autonomic activity throughout the body, relay stations for ascending or descending information, or just passing axons.[60]

Anatomical Organization of the PAG

The PAG itself can be further divided into anatomical and functional parts. Although there has been cytoarchitectonic studies using Nissl, Golgi, and Weil stainings that suggest that the PAG is a homogenous unit[62], Dr. Andrew Gundlach showed that there are four columnar subdivisions by using *in vitro* receptor autoradiography: dorsomedial, dorsolateral, lateral, and ventrolateral.[45]

Each of these four subdivisions are defined based on the classes and densities of several receptor types in the PAG. These columns extend along the rostrocaudal extent of the PAG and the lengths/areas at any section are variable.[50] This rostrocaudal orientation has been shown to have loosely defined topography based off cardiovascular functions between the dorsal and lateral/ventral columns.[63]

The projections to and from each column are different and can indicate the role these columns have for information processing by the PAG. The dorsolateral column does not project very much in the brain stem but does

receive some input from structures in the brainstem. The dorsolateral column is not well understood but its high number of GABA interneurons makes it an attractive region for PAG self-inhibition.[45] The dorsomedial column project strongly to areas of the medulla. [63,64,65] Both the dorsolateral and dorsomedial columns receive input from cortical and subcortical regions of the brain but not spinal.[66,67,68]

The lateral column receives input from several regions based off where in the rostrocaudal axis neurons are found. The more rostral-intermediate and caudal-intermediate receive input from the trigeminal nucleus and cervical enlargement while the intermediate PAG also get input from cortical and subcortical regions.[66,67,68] In the caudal PAG, the circuitry is like the intermediate third, but it also receives some input from the lumbar enlargement.[50,63].

Finally, for the ventrolateral column, it appears that the afferent and efferent connections from this column is nearly the same at the lateral column. The biggest difference, however, is that while the lateral column is known to cause more somatomotor and autonomic activity of the PAG, the ventrolateral column causes decreased activity.[50] The other major difference is that the effect of analgesia in the ventrolateral column can be influenced by opioids and in the lateral column they cannot.[69,70,71]

Relevant PAG Pathways and Connections

The synaptic connections to the PAG, from the PAG, and within the PAG contribute to the functionality of this midbrain structure. Earlier in this review, we discussed the spinal tracts that provide sensory information and the cortical/subcortical regions that provide higher-order cognitive details to the PAG. These different inputs are all integrated at the PAG and descending projections activate appropriate responses. Three descending pathways discussed largely in the literature of the PAG are the noradrenergic (NA) pathway, serotonergic (5-HT) pathway (Figure 4), and the PAG-RVM-Dorsal Horn pathway.

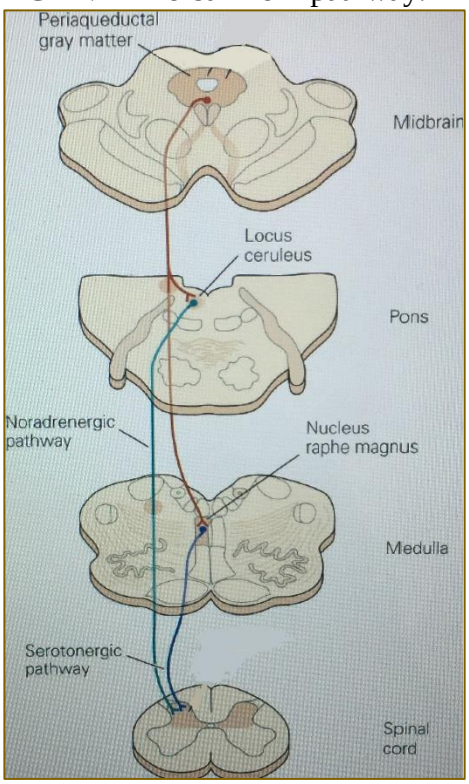

Figure 4 Representation of the PAG connections to the locus ceruleus and nucleus raphe magnus though their respective pathways (Kandel et al, 2013).

The effect of norepinephrine on the human body can be global and potent. Its release mobilizes several biological systems and drives the sympathetic nervous system.[72] Although the PAG does not express NA neurons, there is work that shows that PAG neurons innervate the locus ceruleus which does.[73] Norepinephrine from the locus ceruleus has been shown to influence arousal, behavior execution, task-switching, stress, and antinociception.[2,74]

The 5-HT pathway is similar to the NA pathway in that the PAG doesn't not express serotonergic neurons although its activation leads to 5-HT release in the spinal cord.[75] Scientific findings revealed that these serotonergic projections arose from the nucleus raphe magnus (NRM), which happens to be a target of the PAG.[70,76] The

ventrolateral PAG is actually implicated in activating the NRM, which when lesioned blocks analgesia and its activation evokes it.[77,78]

The final PAG-rostral ventromedial medulla (RVM)-dorsal horn pathway is critical for antinociception. Since the PAG does not have direct connections to the dorsal horn, it projects to the RVM which then projects to the dorsal horn. Interestingly, activation of the RVM can facilitate or inhibit nociception based on the cell activated.[71] The role of these on- and off-cells is discussed in a later section, but this pathway is important in the PAG's descending modulatory role.

Functional Roles of the PAG

Although I focus on the antinociceptive role of the PAG in this paper, the PAG is located at the cross bridge between the spinal cord and brain which makes it a critical integrator of several functions. The different behaviors and responses that the PAG influences, besides analgesia, is lordosis, vocalization, respiration, vascular control, and defense response.

Lordosis is a behavior elicited by female organisms that includes raising their head and rump. This is a very well understood behavioral feature and its influence by the PAG is strongly supported. This behavior is shown to be excited by substance P which is released by male stimulation. Lordosis behavior requires several other hormones and systems to be elicited. The other higher order structure involved in lordosis behavior is the ventromedial hypothalamic nucleus. Within the PAG, GABAergic neurons of the dorsal columns transmit lordosis related activity.[79,80,81]

Vocalization in the PAG is believed to be activated in the dorsolateral PAG, where vocalization neurons share the column with analgesic neurons. The PAG does project this information to higher brain regions but it does not directly project to lower spinal regions involved in activating the appropriate muscles for speaking. The dorsolateral PAG interneurons project to lateral PAG, which can allow this information to be passed down to the medullary regions. [82,83,84] The muscles used to vocalize are unsurprisingly related to respiration, which alludes to the importance of the PAG for integrating this information.[85] The sort of vocalization and levels of inspiration/muscles involved are variably influenced by stimulation of the PAG.[86]

The PAG's role in cardiovascular function is one of the more well understood and characterized functions. Activation of the PAG can either cause increase blood pressure (hypertension) or decrease blood pressure (hypotension) through changing the tone of either the iliac artery or renal artery. These stimulation sites were found in columns along the rostrocaudal extent of the PAG; the dorsomedial and dorsolateral are hypertensive and the lateral and ventrolateral are hypotensive. Another fascinating characterization of the PAG was that in these columns there also appeared to have viscerotopy, like the somatotopy of the S1. This organization showed that the more hindlimb vasculature is activated in the rostral region of these columns while the renal vasculature is affected at the caudal areas (Figure 5, shows this representation).[87,88,89]

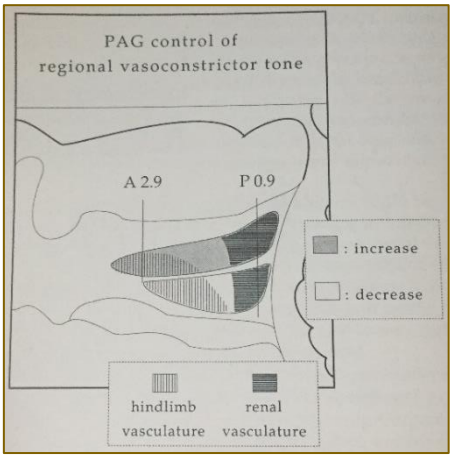

Figure 5 Viscerotopic organization along the rostrocaudal extent of the PAG (Carrive, P., 1991)

Activity of the dorsolateral column triggers defensive behavior in the form of aggression or flight.[63,87,90] This column, as described above, receives input from higher order brain structures including the ACC and amygdala, each of which contribute to the cognition and emotion of an organism. Another interesting response is that analgesia evoked by the PAG often allows organisms to escape a dangerous situation without the distraction of wounds. Vocalization can allow an individual to send warning signals or freeze a predator.[46,71] It is also important to note that these seemingly related functions are all activated in the dorsolateral PAG, which likely integrate these functions.

PAG Antinociceptive Role with RVM

The PAG-RVM-dorsal horn pathway is the central pathway thought to send an antinociceptive signal. Studies have shown that in the PAG itself, there are very few purely analgesic sites.[91] Most of the PAG that causes analgesia is fraught with side effects by its other functions.[92,93] This, along with the fact that the PAG doesn't have a significant amount of direct projections to dorsal horn neurons, make its parallel investigation of the PAG-RVM-dorsal horn pathway necessary.

The PAG relies on the RVM to make connections with the spinal cord. Much like the PAG, the descending input from the RVM is also GABAergic, inhibiting the dorsal horn secondary neurons.[94] Therefore, inhibition of the PAG projecting neurons (by cortical/subcortical or PAG interneurons) would lead to disinhibition of RVM inhibitory neurons on the spinal cord. An interesting phenomenon, however, is the hyperalgesia caused by PAG stimulation. There is not much literature on this occurrence, but one likely reason is the presence of faciliatory cells in the RVM. [91]

The RVM has three classes of cells: neutral, on-cells, and off cells.[81] Neutral cells are unresponsive to any nociceptive testing models and are not discusses greatly in the literature. The on- and off-cells project to the first, second, and fifth laminae of the dorsal horn and modulate the secondary nociceptor targets. On- and off-cells functions are complementary.

The on-cells are activated during increased noxious responsiveness and predicted to facilitate nociception. These cells are inhibited by morphine and the latency for the tail flick responsiveness assay of nociception is very short with its stimulation. Off-cells, on the other hand, are active when nociceptive reflexes are inhibited and known to cause inhibition at the dorsal horn. These cells are activated by morphine and have a long latency.[57,95,96]

Although hyperalgesia and analgesia are both possible effects from the RVM, analgesia dominates. The reason for this is because of the possible circuitry of these neurons. The on-cells demonstrated a very planar dendritic and axonal projections. These cells are parallel to one another and perpendicular to the rostrocaudal axis. Off-cell axons extended and terminated throughout the rostrocaudal extent (Figure 6).[96,97,98] Therefore activation of off-cells influenced on- and off-cells more globally.

Figure 6 Representation of the RVM on- and off-cells morphology (Mason et al, 1990).

Although RVM on-cell activity is influenced heavily by their off-cell cousins, they still have an important function. Immediately after a noxious stimulus activates a nociceptor, these on-cells turn on while the off-cells are still silent.[99] This mechanism may be established to allow organisms to direct their attention quickly to an injury, so they may be able to respond

quicker. Even a short hyperalgesic response would allow a multitude of these pain matrix structures to respond more effectively cases of life or death.

Characterization of Phantom Limb Pain

Phantom limb pain (PLP) is a form of chronic pain in an individual's missing limb after an amputation. A study in 2015 indicated that about 1.7 million Americans live with a missing limb and by 2050 this number will rise to about 3.5 million. The reasons that people undergo an amputation surgery include injuries, cancer, vascular issues, trauma and congenital deformities.[100] Although not every person who has an amputation suffers from phantom pain, it is relevant enough that a search for a clinical remedy is in need.[101]

Some related conditions to PLP are phantom limb sensation, residual-limb pain, and non-painful residual-limb phenomena. Residual-limb pain is a sensation of pain in the stump end of a limb where the amputation occurred.[102] The non-painful sensations or phenomena have been described as feeling touch, itches, tingles, brushes, and involuntary movements.[101]

There are several possible mechanisms of PLP that will be described in the next section. It is interesting to note that PLP is more likely in individuals that suffered from chronic pain prior to an amputation.[103,104] Although there may be peripheral and central mechanism that may perpetuate PLP, the peripheral mechanism of this neuropathic pain is not elaborate enough to cause this disorder alone. Also, the psychological factors cannot be ignored, as both placebos and noceboes have proven significantly effective in modulating pain, but these aspects will not be discussed for the purposes of this paper. [25,101]

Possible Mechanisms of Phantom Limb Chronic Pain

PLP is a very diverse condition and its mechanism can be different for each person. This is a major reason why people who suffer from PLP have different ranges of subjective experiences. The different ways that PLP may arise after an amputation include regenerative plasticity, collateral sprouting, and central sensitization.

Regenerative plasticity is the repair mechanism of axon to reinnervate the region it originally projected. When this occurs, the peripheral nerve can maladaptively form a neuroma, which is a common cause of PLP (Figure 7). These neuromas are very sensitive and can cause both spontaneous and ectopic pain.[105,106] Neuroma's do not always occur in amputees but when they do, surgical removal is one way try to deal with the corresponding PLP.[107]

Collateral sprouting is a second maladaptive repair mechanism of an injury. This occurs when nearby healthy neurons

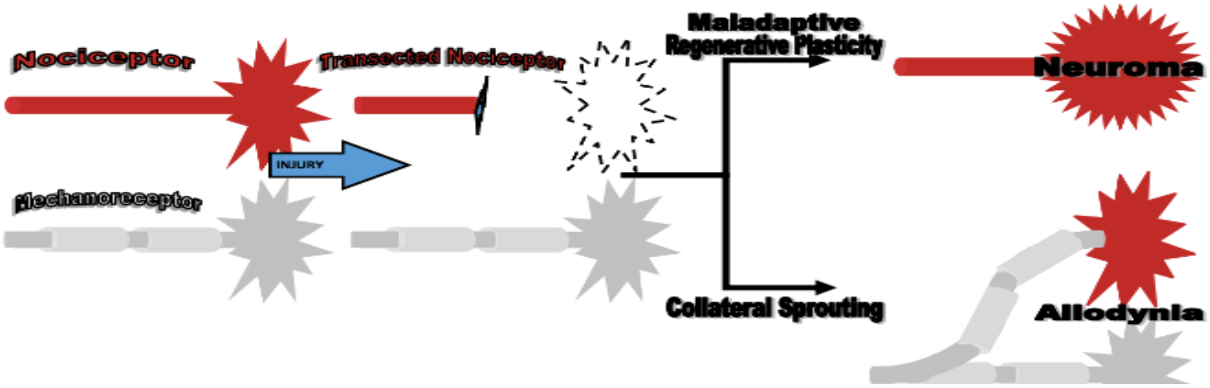

Figure 7 Visual representation of the repair mechanism of a damaged nociceptor. The maladaptive regenerative plasticity can cause a neuroma to form and collateral sprouting can lead to allodynia.

sprout axons to innervate a formerly innervated area (Figure 7). This is a very likely mechanism for allodynia because non-nociceptive somatosensory neurons can sprout in this formally nociceptive region. Stimulation of this neuron can transmit information of both pain and touch and since these neurons are Aβ myelinated neurons, this pain is transmitted quickly.[105, 106, 108] Aβ neurons also contain CGRP (a pain related protein) and release of protein has a possible role in allodynia.[54,55]

Central sensitization is an increased response from the central nervous system due to nociceptive activity. Although this is not a mechanism of repair, it is important because it is a major mechanism in some chronic pain conditions. Central sensitization is an increased response to noxious stimuli at the dorsal horn of the spinal cord between the primary afferent and secondary ascending neuron. Central sensitization can be caused by a phenomenon called wind-up. Wind-up occurs when there is a constant, low frequency stimulation of nociceptors that causes increased pain for individuals suffering from chronic pain. This mechanism depends on both NMDA channels and L-type calcium channels.[109]

For individuals that already suffer from chronic pain in a limb before getting that limb amputated, they are more likely to suffer from PLP. PLP arises more readily in these patients because of this central sensitization mechanism. This is not the only way that a person can develop PLP by central sensitization. The other way is that during surgery, the neurons around the amputation can discharge causing wind-up to occur. Both mechanism increase the likelihood for an individual to suffer from PLP.

Although like LTP, wind-up is not the same. LTP occurs from brief, high frequency, tetanic stimulation and the electrical response lasts between hours and days, while windup shows enhanced activation for only a few minutes.[110] A study by Svendsen et al, showed that LTP can be induced in dorsal horn neurons and this work revealed that both phenomena are differentially implemented based on their criteria.[111]

All these plasticity-based mechanisms have instrumental roles in the level and type of pain an amputee is suffering from. Understanding each of these are important for preventing PLP and treating the symptoms in the instances that they arise.

Treating Phantom Limb

Current clinical care of PLP is split into two measures: prevention and treatment. Preventative measures are accomplished by taking preoperative steps to reduce the chance of an individual developing PLP from an amputation. Treatment of PLP is meant to resolve the cause (if this is known) or to deal with the side effects.

The most effective preventative measure for PLP is to provide both general and peripheral anesthesia before and throughout surgery. Although this does not have perfect efficacy, the idea in addressing a possible mechanism of PLP is sound.[101,112] By providing anesthesia to the peripheral and central nervous system, this would effectively restrict the possibility of wind-up in the dorsal horn neurons. Although this wouldn't be effective for individuals who were already suffering from chronic pain prior to the amputation, this measure should still be taken to reduce the likelihood of PLP worsening.[101]

The treatments for PLP are plentiful but often, rather ineffective.[113] These treatments include pharmacological, surgical, anesthetic, psychological, and others.[101] Many pharmacological therapies include opioids, calcitonin (serotonergic neuron agonist), and ketamine (NMDA antagonist), which were effective but with some aversive side-effects.[114,115,116,117]

Myoelectric prosthesis is a method that used the electric signals of an individual's

body to navigate a prosthetic limb. This treatment is shown to both reduce cortical reorganization and PLP.[118] A famous psychological study using mirror training allowed patients to navigate their intact limb and look at a mirror which made it appear as if they had their missing limb. This technique helped to reduce some pain for patients.[119]

Examples of surgical methods include neurectomy/sympathectomy (removing nerve tissue), rhizotomy (spinal nerve root removal), cordotomy (spinal cord transection), and stump revision (tissue removal and plastic remodeling of stump). These methods do not have significant clinical research devoted to their effectiveness, but they are utilized by physicians when the neuropathy is predicted.[101]

Some other unique treatments include acupuncture, ultrasound, prosthesis training, and hypnosis.[101] Still though none of these methods have resolved the sensation of PLP completely and those that do tend to cause undesirable side-effects.

Conclusion and Future Directions

This review has looked at the structure of the nociceptive sensory module, role of the periaqueductal gray matter in analgesia, and the details of chronic phantom limb pain. Although, the somatosensory nervous system is a very important and well-investigated neurological tool, there is a great deal of research that still needs to go into it. This includes characterizing the neuropeptides, receptors, channels, nociceptors, and the pathways.

The function of different cortical, subcortical, and brainstem structures are abstract and underappreciated. By devoting more energy in revealing how these systems function properly, clinical research can dial down on where malfunctions are occurring.

The periaqueductal gray matter is a very dynamic and imperative structure. Its collection of sensory information and projection to the rostral ventromedial medulla is critical for allowing top-down modulation of pain. Although this pathway has been investigated in former research labs, using the novel contemporary methods can prove to reveal more fruitful information about nociceptive modulation. With improvements to technology, there can be greater localization of the exact neurons that produce analgesic-only projections to the RVM and likewise from the RVM to the spinal cord.

Phantom limb pain is a prominent form of chronic pain and its need for effective clinical treatment is very clear. The mechanisms behind this condition are elusive and medical investment into understanding these mechanisms are imperative. By further characterizing this form of chronic pain, researchers can create more direct and effective preventative protocols and treatment methods for this issue.

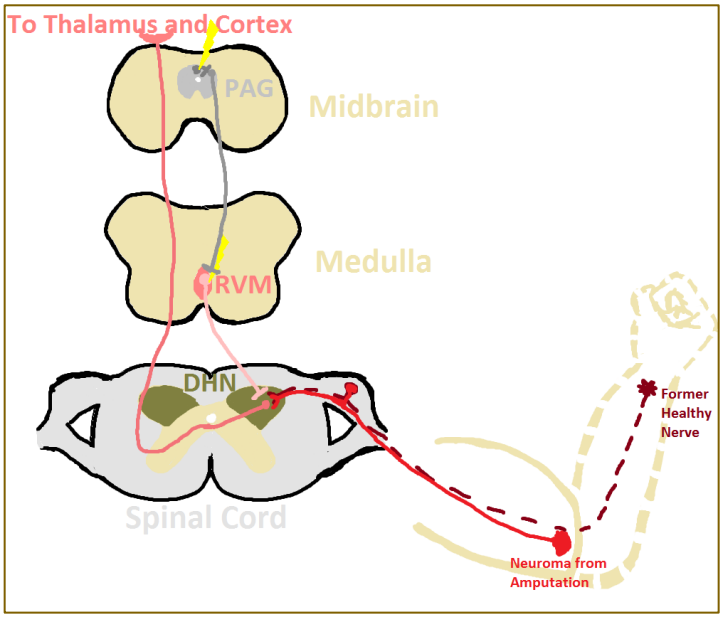

Figure 8 Diagram showing the circuitry of interest. The lightning bolts represent neurons that would be of interest to identify and stimulate to inhibit the phantom limb pain arising from a neuroma.

The possibility of utilizing a technique to stimulate the PAG-RVM-dorsal horn system to inhibit chronic pain is not impossible. Although this is not a feasible method currently, as technology allows physicians to find the exact afferent neurons transmitting chronic pain and the exact inhibitory neurons that project to it, stimulation-based therapies can be a frontier in resolving phantom limb pain and chronic pain alike (Figure 8).

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