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Immune-to-brain communication dynamically modulates pain: Physiological and pathological consequences

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Abstract

This review examines recently recognized roles of immunological processes in pain modulation and explores the potential implications of these immunologically derived phenomena for human chronic pain control. The focus is an examination of how activation of immune-like glial cells within the spinal cord can amplify pain by modulating the excitability of spinal neurons. Such glially driven enhancement of pain can be physiological, as occurs in response to peripheral infection or inflammation. Here, immune-to-brain-to-spinal cord communication leads to pain enhancement (hyperalgesia) as one component of the well-characterized sickness response. This sickness-induced hyperalgesia, like many sickness responses, is mediated by the activation of glia and the consequent release of proinflammatory cytokines. However, glially driven pain can also occur under pathological conditions, such as occurs following peripheral nerve inflammation or trauma. Here, immune- and trauma-induced alterations in peripheral nerve function lead to the release of substances within the spinal cord that trigger the activation of glia. Evidence is reviewed that such pathologically driven glial activation is associated with enhanced pain states of diverse etiologies and that such pain facilitation is driven by glial release of proinflammatory cytokines and other neuroexcitatory substances. This recently recognized role of spinal cord glia and glially derived proinflammatory cytokines as powerful modulators of pain is exciting as it may provide novel approaches for controlling human chronic pain states that are poorly controlled by currently available therapies.

Keywords: Glia; Proinflammatory cytokines; Interleukin 1; Interleukin 6; Tumor necrosis factor; Sickness responses; Pain facilitation; Sciatic inflammatory neuropathy

1. Introduction

While acute pain is a normal, physiological, and adaptive response to noxious stimuli, chronic pain is a maladaptive, pathological condition. Chronic pain in people fails, by-and-large, to be controlled by currently available drugs. An effective drug for human chronic pain is one that provides only partial relief for one out of five patients, while the remaining four patients experience no relief from pain (McQuay et al., 1995, 1996). Why these drugs fail is an enigma, but may be due to the fact that all of the currently available drugs target neurons. The present review examines why targeting neurons may not be an optimal strategy for treating this debilitating condition. Furthermore, it proposes that basic principles of neuroimmunology may provide novel approaches for resolving the immense human suffering caused by chronic pain.

Classically, the pain pathway has been conceived of as a chain of neurons from periphery to cerebral cortex, in which one neuron relays pain information to the next neuron in line, and so on. Decades of research have clearly shown that pain transmission along this neuronal path can be modulated by pain suppressive and pain enhancing circuitry, again comprising neurons. In the past few years, it has become clear that *non*-neuronal cells of the central

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nervous system (CNS) also play pivotal roles in pain facilitation and can result in hot, cold, and hard pressure pains being grossly amplified and even warm, cool, and light touch being perceived as pain. These key nonneuronal players in pain are glia; specifically, activated microglia and astrocytes. These are immunocompetent cells of the CNS. As such, they are activated by classical immune stimuli such as viruses, bacteria, and trauma. However, they are also now known to be activated by substances released by neurons within the spinal cord. These spinal neuronal signals are released in response to inflammation and damage in the body, conditions which lead to both glial activation and glially driven pain facilitation (see Fig. 1).

The thesis of this review is that glia act as a "volume control" for pain. As will be discussed below, glia are not involved in normal, everyday pain. But they are critically involved in pain enhancement. When glia become activated, they amplify pain by amplifying the messages relayed by sensory nerves to the spinal cord, and by amplifying the spinal pain message relayed to the brain.

This review will be organized in a series of sections, beginning with a brief overview of normal and abnormal pain processing. This will be followed by a discussion of how the study of immune-to-brain communication helped lead to the realization that spinal cord glia can powerfully modulate pain, both physiologically (e.g., as part of the sickness response) and pathologically (e.g., in response to peripheral inflammation and trauma).

2. Pain is normally adaptive and dynamic

The sensation of pain normally serves to protect the organism from harm, triggering immediate behavioral responses to prevent, or at least minimize, tissue damage. In addition, pain is a powerful motivator for learning about dangers and how to avoid them. Painful stimuli, such as acids, heat, cold, and hard pressure, activate specific receptors expressed on nerve endings. These receptors are expressed only by a select sub-population of peripheral nerves, called A-delta and C fibers, which are responsible for conveying information about painful stimuli from peripheral tissues to the spinal cord.

Upon reaching the spinal cord, these pain-responsive A-delta and C fibers synapse on neurons within a specialized spinal cord region called the dorsal horns. Within the dorsal horns are neurons that transmit the pain information to the brain. This description seems to imply that pain is passively relayed from the periphery to the brain, with each neuron in line maintaining an accurate representation of the painful stimulus encountered in the outside world. This is not the case. Pain signals can be dynamically modulated in the spinal cord dorsal horns, with pain signals being suppressed, amplified, or

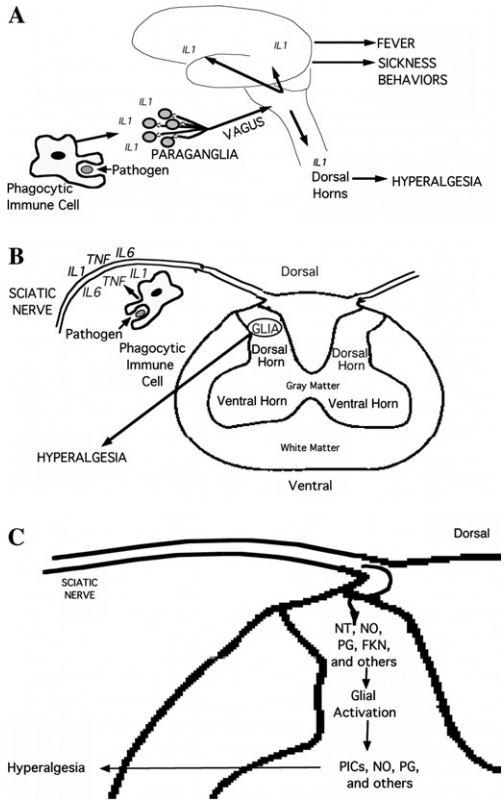

Fig. 1. Glial activation in the spinal cord dorsal horn leads to enhancement of pain, called hyperalgesia. (A) Peripheral immune activation results in sickness responses, including sickness-induced hyperalgesia, via immune-to-brain communication. This panel illustrates one well-characterized circuit. In this example, phagocytic immune cells (e.g., macrophages) release interleukin-1 (IL1) and other proinflammatory substances upon activation by pathogens (e.g., bacteria). The released IL1 binds to IL1 receptors expressed by sensory paraganglia associated with sensory fibers of the vagus. This, in turn, activates the vagus, leading to immune-to-brain communication. Once these signals reach the brain, fever and other sickness behaviors are created via the release of glial IL1 and other proinflammatory substances. Sickness-induced hyperalgesia is created by an immune-to-brain-to-spinal cord dorsal horn pathway, again being mediated by glial release of proinflammatory substances such as IL1. (B and C) show an alternative route leading to spinal cord dorsal horn glial activation and hyperalgesia. As illustrated in (B), activation of phagocytic immune cells (e.g., macrophages) around an otherwise healthy peripheral nerve (e.g., sciatic nerve) leads to the release of proinflammatory cytokines (TNF, IL1, and IL6), as well as other proinflammatory substances. These mediators act directly on the sciatic nerve. The sensory nerves of the sciatic terminate in the spinal cord dorsal horn and, as such, inflamed nerves lead to activation of glia in this region. Glial activation, in turn, results in hyperalgesia. (C) Provides a more detailed accounting of how such hyperalgesia occurs. Pain-responsive peripheral nerve fibers synapse within the spinal cord dorsal horn, releasing glial-excitatory substances such as neurotransmitters (NT; e.g., ATP, glutamate, and substance P), nitric oxide (NO), prostaglandins (PG), fractalkine (FKN), and others. Glia that become activated in response to these neurally derived signals, in turn, release neuroexcitatory substances, including proinflammatory cytokines (PICs), NO, PG, and others. The result is amplified sensitivity of pain neurons, resulting in hyperalgesia (Panel A is Reprinted, with permission, from the *Annual Review of Psychology*, Volume 51 © 2000 by Annual Reviews www.annualreviews.org).

relayed unaltered. Under normal, physiological situations, such pain modulation is adaptive.

It is adaptive to suppress pain, for example, when an organism encounters life-threatening events. In such a situation, there is a classic "fight or flight" stress response. One component of the constellation of changes that ensues is the release of a variety of neurotransmitters/neuromodulators within the spinal cord dorsal horns, including endogenous opiates (endorphins and enkephalins) (Kelly, 1986). Spinal release of these morphine-like peptides creates "stress induced analgesia," where "analgesia" means "without pain." Thus, during emergency situations, endorphins and enkephalins can act within the dorsal horns to prevent pain messages from ever reaching the brain. This is highly adaptive when pain from tissue damage may hinder success in escaping life-threatening situations.

Similarly, it is adaptive to amplify pain, such as in response to tissue injury. The exquisite sensitivity of sunburned skin to heat and light touch is one simple example of such enhancement. In this case, a normally warm sensation would be experienced as a burning sensation on sunburned skin, and light touch that is normally innocuous is now painful. The sensory information is being amplified in the spinal cord dorsal horn resulting in a much lower pain threshold. The heightened sensitivity reminds the organism to care for and protect the injury, and to avoid the cause of the pain in the future (Willis, 1992). This type of pain is referred to as "hyperalgesia," meaning "exaggerated pain." Hyperalgesia can result from increased excitability of peripheral pain fibers, which increases the release of neurotransmitters (such as glutamate and substance P) that signal pain to the spinal cord. Hyperalgesia can also result from the production and release of excitatory neuromodulators within the dorsal horns, such as nitric oxide and prostaglandins. These enhance pain both by increasing the amount of neurotransmitter released by pain fibers synapsing in the dorsal horns and by increasing the excitability of dorsal horn neurons that relay pain information to the brain.

3. Pain processing can become pathological

Normally, pain is experienced as an acute event. When the pain continues well after an injury has healed, such pain is no longer adaptive. It is at this point that pain is called pathological. Under such conditions, pain becomes grossly amplified to a point at which even warm, cool, and light touch are now perceived as painful. Thus, for example, chronic pain patients find clothing or bedsheets unbearable when these come in contact with the affected area. Body regions beyond the site of damage may also exhibit exaggerated pain responses, despite the fact that the area is not innervated by the damaged nerves. These sensations are referred to as "extra-territorial pain" because the pain arises outside the skin territory normally innervated by the damaged nerve. Additionally, chronic pain patients may report pain as arising from the corresponding body part on the opposite, healthy side of the body. This is called "mirror-image pain" (Tal and Bennett, 1994).

As described above, pain signaling has traditionally been described as being formed by a serial chain of neurons, with sensory neurons relaying pain information to spinal cord neurons, which then relay pain messages to neurons in the brain. In the light of this conception, it was completely logical to assume that pathological pain must be caused by malfunctioning neurons. Given this assumption, all the early studies (and the vast majority of recent studies as well) have sought to understand the etiology of pathological pain by identifying what goes wrong with neurons in the pain pathway. And, indeed, many neuronal changes have been identified in animal models of such pain states. For example, pain neurons can become spontaneously active, causing the spinal release of their neurotransmitters in the absence of a painful stimulus. Neuronal calcium channels can also be altered, increasing the excitability of A-delta and C fibers. These are just two of many plastic changes that are now well documented (Woolf, 2004). However, the troubling fact remains that drug therapies that target such neuronal changes do not, for the most part, control human pathological pain. We will present evidence for why such failure may occur. We will argue that glia are dynamic modulators of spinal pain transmission, and that glia, via their release of proinflammatory cytokines, may be critically involved in the creation and maintenance of pathological pain. The drug therapies that target neurons do not block or reduce these glial processes.

4. Historical overview: sickness, proinflammatory cytokines, and pain

While a number of presumed neuronally released neuromodulators, like nitric oxide and prostaglandins, have long been implicated in spinally mediated pain facilitation, substances released from activated glia, such as proinflammatory cytokines, have not. This is because glia have not been classically thought to regulate neuronal function. Only recently has it become clear that glia are able to significantly influence neuronal activity (reviewed in Araque et al., 1999). Indeed, neuronal synapses are dependent on astrocytes, as astrocytes are now recognized to be integrally involved in synaptic plasticity. As this new understanding of glia–neuronal interactions is emerging, coupled with the expression of proinflammatory cytokine receptors on the cell sur-

face of neurons, the ability of glia to modulate neuronal activity via the release of proinflammatory substances is necessary to consider.

The discovery that glia and glially derived proinflammatory cytokines may be involved in pain modulation arose from studies of the "sickness response" (reviewed in Maier and Watkins, 1998). The "sickness response" includes a wide-ranging constellation of changes triggered by peripheral immune challenge (Hart, 1987). These adaptive changes are evolutionarily old and occur across a wide range of species. Sickness responses include physiological (fever and increased white blood cell count), endocrinological (activation of the hypothalamo-pituitary axis and sympathetic nervous system), and behavioral changes (e.g., decreased social interaction and decreased food and water intake).

It is important to note that, similar to normal pain, these sickness responses are adaptive. The induction of these behaviors supports the survival of the organism during times of infection or injury. For example, fever enhances survival of the organism by increasing the organism's core body temperature to the point that some pathogens cannot replicate, while simultaneously stimulating the replication of the host's white blood cells (Kluger et al., 1998). We have reasoned that pain facilitation may actually be part of this sickness pattern and also function to promote recuperation during times of infection and injury. Indeed, a variety of agents that induce sickness (e.g., lipopolysaccharide) proved to produce hyperalgesia.

A substantial body of research has indicated that sickness responses to peripheral infection are induced by a cascade of events that begin with the release of proinflammatory cytokines in the periphery. These cytokines in turn signal the brain via both neural and blood-borne routes, initiating the brain processes that are involved in the mediation of host defense and that create the behavioral, and many other aspects of sickness. Interestingly, these brain processes include the production of proinflammatory cytokines, largely by glial cells, and these brain cytokines are critical in the mediation of sickness responses.

Because hyperalgesia seemed part of the sickness response, it was natural to wonder whether pain facilitation might also be mediated by glially derived proinflammatory cytokines, but this time in the spinal cord. Indeed, this possibility has been supported. During sickness, proinflammatory cytokines and glial activation are induced in the spinal cord, and these spinal cytokines are critical to the mediation of sickness-induced enhanced pain.

It is important to point out that, as with other components of the sickness response, sickness-induced hyperalgesia is physiological, and not pathological. It is a normal, naturally initiated, and naturally resolved alteration in organismic functioning. However, the question then becomes: how does a normal physiological response become pathological? One answer may be that there is simply more than one way to activate glia. The discussion above suggests that a normal, physiological pathway to activate glia is via the immune-to-brain-to-spinal-cord pathway initiated by the sickness response. The endproduct here is spinal cord glial activation, glial release of proinflammatory cytokines, and consequent pain enhancement. This suggests that anything that induces spinal cord glia to take on a proinflammatory profile could potentially create pain enhancement. Since spinal glial cells are activated in response to spinal inflammation, central or peripheral trauma, and other non-physiologic events, these glia could potentially induce pain enhancement under such circumstances. And, indeed they do. Evidence for this conclusion will be discussed in the following sections.

Interestingly, at the same time that the existence of sickness-induced hyperalgesia was becoming appreciated and the role of spinal cord glia and proinflammatory cytokines in this phenomenon were clarified, a completely independent line of research was also leading to the conclusion that glia play a modulatory role in pain. It had been known for many years that trauma or injury to peripheral nerves leads to glial activation in the CNS, both in the brain and spinal cord. Trauma to peripheral motor nerves leads to glial activation around the axotomized motor neurons, while trauma to peripheral sensory nerves leads to glial activation in the region of degenerating central terminals. However, this glial activation was not known to be related to the pain produced by nerve injury until the early-to-mid 1990s. Garrison et al. (1991) were the first to make the connections between nerve damage-induced glial activation and nerve damage-induced pain facilitation. They examined the expression of glial fibrillary acidic protein (GFAP), the major astrocyte structural protein that is upregulated during glial activation. GFAP expression was analyzed in rats that had undergone sciatic nerve chronic constriction injury (CCI), a classic rat model of neuropathic pain (that is, pathological pain arising as the result of inflammation or trauma to peripheral nerves). First, they found, not surprisingly, that CCI activates astrocytes (Garrison et al., 1991). However, Garrison et al. also reported that a pharmacological manipulation that blocked CCI-induced hyperalgesia blocked astrocyte activation (Garrison et al., 1994). This finding stimulated other laboratories to explore whether glial activation is strongly correlated with pain facilitation. Indeed, every animal model of pain facilitation that has been studied has shown a positive relationship between hyperalgesia and glial activation in the spinal cord.

5. Glia: immunocompetent cells of the CNS

As used here, the term "glia" refers to both microglia and astrocytes. These two cell types are very similar in terms of the stimuli that activate them, and both are activated by conditions that induce pain facilitation. When activated, each can further activate the other, leading to the release of a variety of neuroexcitatory products. Many of these products of activated microglia and astrocytes can enhance pain transmission in the spinal cord dorsal horns.

Before proceeding to a discussion of glial modulation of pain, it is important to first understand basic glial physiology. Glia are referred to as "resting" when they are in a normal, basal state. However, the level of basal activity differs between astrocytes and microglia. Resting astrocytes actively regulate extracellular ion and neurotransmitter concentrations, availability of neurotransmitter/neuromodulator precursors to nearby neurons (Schousboe and Westergaard, 1995), and extracellular pH (Ransom, 1992). Thus, normal, basal activity for astrocytes is active maintenance of homeostasis in their microenvironment. In contrast, microglia exhibit minimal activity in their basal state. To date, no function has been identified for resting microglia. Resting astrocytes and microglia are activated in response to CNS trauma, ischemia, tumors, neurodegeneration, viruses, and bacteria, as well as by a variety of neurotransmitters and neuromodulators.

The transition of astrocytes and microglia from their basal to activated states is associated with changes in morphology, receptor expression, and released substances. While resting astrocytes have many thin processes, activation is associated with hypertrophy and increased production of intermediate filaments (e.g., GFAP) (Ridet et al., 1997). Microglia undergo remarkable anatomical changes as well. At rest, microglia have a ramified morphology with many thin processes, and low receptor expression. Microglial activation is a graded event that is reflected in associated morphological changes (Kreutzberg, 1996). As microglia become increasingly stimulated, their processes gradually shorten and become plumper. With increasing stimulation, microglial processes can become completely retracted so that the cell assumes an amoeboid morphology. Microglial activation is also identified by upregulation of cell surface markers and receptors (e.g., complement 3 receptors), and/or functional changes (e.g., migration to area of injury and phagocytosis). Upon activation, both microglia and astrocytes can synthesize and release proinflammatory products.

Of particular relevance to our discussion here is the immunocompetent nature of these cells as they become activated. Glial cells are considered to be immunocompetent cells because they are activated by viral and bacterial components. The behavior of microglia following immunogen activation is very similar to that seen in stimulated peripheral macrophages in that they upregulate complement 3 receptors (activation of the complement system is an initial event in innate immune stimulation), increase phagocytosis, increase release of reactive oxygen and nitrogen species, increase release of chemokines, and induce the proinflammatory cytokine cascade (Kreutzberg, 1996). Also consistent with peripheral macrophage responses is the expression of major histocompatibility complex class II (MHCII) molecules and costimulatory molecules, B7 and CD40 by activated microglia, indicating that microglia are able to present antigen to T-cells in the CNS (Kreutzberg, 1996). The role of astrocytes as immunocompetent cells is similar to peripheral macrophages in that, when stimulated with an immunogenic substance upon activation, they respond by upregulating reactive nitrogen species, release of chemokines, and the induction of the proinflammatory cytokine cascade (Dong and Benveniste, 2001). Astrocytes have also been shown to be capable of phagocytosis, and expression of MHCII with costimulatory molecules. These abilities have been demonstrated under extreme conditions (i.e., direct stimulation with interferon-gamma), or in established pathological conditions, and as such it is still unclear if astrocytes act as antigen-presenting cells in less severe conditions.

A last point that bears noting is that glia show regional heterogeneity in their receptor expression and response profile. Within the spinal cord, glia can express receptors not found on glia in many other CNS regions. For example, the spinal cord is one of the few CNS sites where glia express receptors for substance P, a neurotransmitter released by pain fibers that synapse in the spinal cord dorsal horn (Marriott et al., 1991). Indeed, regional heterogeneity is also observed across sub-regions of the spinal cord. While few studies have focused on this issue, it is intriguing that glia in pain modulatory regions of the dorsal horn are selectively responsive to conditions leading to pain facilitation. Only these spinal cord glia alter their expression of chemokine receptors, glutamate transporters, and prostaglandin synthesis enzymes under pathological pain conditions.

6. Sciatic inflammatory neuropathy: hyperalgesia and glial activation

As already noted, hyperalgesia can be viewed as a component of the sickness response that results from peripheral immune activation. The immune-to-brain communication that initiates sickness employs both blood-borne and neural routes, with the vagus nerve having been shown to carry the peripheral nerve part of the "message"(Watkins and Maier, 1999). These immune-to-brain communication pathways induce the production of proinflammatory cytokines in the brain and

spinal cord, whereby spinal proinflammatory cytokine release mediates sickness-induced hyperalgesia, similar to other more classical sickness responses. This raises the question of whether there is something sacred about vagal communication with the CNS or whether other peripheral nerves may function in a manner similar to the vagus nerve, so as to activate spinal cord glia and release proinflammatory cytokines at this site, as well.

Prior to describing immune-to-CNS communication via nerves other than the vagus, one issue should be addressed. As noted in the "Introduction" section of this review, normal pain serves adaptive functions. In contrast, pathological pain is defined as amplification of pain well beyond the point of healing. That is, pathological pain no longer serves to signal active inflammatory or traumatic events. Where the line between adaptive and pathological pain lies in animal models is clouded, as animal models were developed to mimic human pathological pain states but on a greatly compressed timescale. Thus, inflammation and trauma are, by and large, still present in such animal models, despite the data being described as relevant to human pathological pain conditions. Because the animal models of neuropathic pain are studied as models of human pathological pain states, the term "pathological pain" will be ascribed to them, below. However, it should be recognized that these responses in laboratory animals are arguably still adaptive sickness responses to ongoing inflammation and trauma. This fact does not minimize the importance of the discovery that non-vagal neural pathways can tap into and drive pain facilitatory circuitries.

To examine whether peripheral nerves other than the vagus can induce sickness-like changes in pain, we have developed the sciatic inflammatory neuropathy (SIN) model. To induce SIN, an immunogenic stimulus is delivered directly around the sciatic nerve at mid-thigh level. This procedure creates an inflammation along a large nerve bundle, not at sensory nerve terminals in the skin. The design of the model is such that the stimulus is restricted to the sciatic nerve, resulting in an inflammatory response solely around this nerve bundle (Milligan et al., 2003). Gelfoam is placed around a section of the nerve, without damaging the nerve, and a catheter is placed into the gelfoam and brought to the exterior of the rat. Later, after the rat has recovered from the surgery, an immunogenic stimulus such as zymosan (yeast cell walls) is injected into the gelfoam. This brings immune cells such as macrophages and neutrophils to this site. When the immune cells contact the zymosan they release their normal inflammatory products near the nerve. A low dose of zymosan produces unilateral hyperalgesia ipsilateral to the injection site, while a higher dose of zymosan induces bilateral hyperalgesia. In other words, greater immune activation around one sciatic nerve caused the unilateral hyperalgesia to become "mirrored" on the contralateral side. This mirror-image pain is a result of spinal cord sensitization, and not due to systemic spread of the zymosan challenge. As noted previously, this mirror-image pain pattern is also reported by some chronic pain patients.

Similar to vagally mediated sickness-induced hyperalgesia, glial activation is causal to SIN-induced hyperalgesia. This conclusion derives from findings indicating that the peri-spinal administration of glial-specific inhibitors blocks and/or reverses SIN-induced hyperalgesia. For example, fluorocitrate blocks the activity of both astrocytes and microglia, but has no effect on neurons at appropriates dosages. When administered over spinal cord prior to peri-sciatic inflammation, fluorocitrate completely blocks the onset of hyperalgesia (Milligan et al., 2003). Minocycline allows a more selective method for analyzing glial involvement in pain. Minocycline is a semisynthetic, second generation tetracycline derivative that selectively inhibits microglial activity independent of its antibiotic effects. Similar to the effects seen with fluorocitrate, minocycline administered prior to peri-sciatic immunogen administration blocks the onset of hyperalgesia (Ledeboer et al., 2004). When administered shortly after the establishment of hyperalgesia, minocycline attenuates the existing hyperalgesia. However, after a week of ongoing SIN-induced hyperalgesia, minocycline no longer had any effect on behavior. These data suggest that microglial activation is essential for the initiation of SIN-induced hyperalgesia, but that a cascade occurs whereby microglial involvement in the ongoing pain state fades with time.

The relative contribution of microglia and astrocytes to pain facilitation is an active area of ongoing investigation. At this point, it appears that microglia initiate hyperalgesia, whereas astrocytes are responsible for maintaining the pain state. Consistent with these conclusions, immunohistochemical staining for the complement 3 receptor (microglial activation marker) and GFAP (astrocyte activation marker) indicates that microglia are most activated during the development of hyperalgesia, while astrocytes are most activated during the maintenance of hyperalgesia, with initial astrocyte activation delayed relative to that of microglia (Colburn et al., 1997).

It should also be noted that these glial inhibitors do not alter normal pain processing (Milligan et al., 2003; Ledeboer et al., 2004). Glia are not activated in response to acute normal pain, and thus do not release pain enhancing substances under this circumstance. It is only when glia become activated that they release substances leading to hyperalgesia.

7. Linkage between spinal proinflammatory cytokines and hyperalgesia

In response to specific neuronal signals activated glia can release the proinflammatory cytokines, tumor

necrosis factor (TNF), interleukin-1 (IL1), and interleukin-6 (IL6). Proinflammatory cytokines do not appear to be constitutively released in the spinal cord, at least to any degree that alters pain processing. Similar to results with the glial inhibitors, pharmacological blockade of the activity of proinflammatory cytokines does not interfere with normal acute pain (Milligan et al., 2003).

The release of proinflammatory cytokines is one key means by which glia signal neurons as well as other glia. In the spinal cord, proinflammatory cytokines have been implicated in the production of hyperalgesia. For example, spinally released TNF, IL1, and IL6 have each been demonstrated to be important for the expression of SIN-induced pain changes. Peri-spinal administration of agents that block the actions of proinflammatory cytokines blocks the induction and maintenance of SIN-induced hyperalgesia (Milligan et al., 2003). Consistent with these findings, studies employing spinally delivered cytokine antagonists indicate that proinflammatory cytokines are critical for the induction of exaggerated pain in diverse models of pain facilitation, including those arising from tissue inflammation, peripheral nerve inflammation, peripheral and spinal nerve trauma, spinal cord inflammation, and spinal cord trauma (Meller et al., 1994; Sloane et al., 2004; Sweitzer et al., 2001; Watkins et al., 1997). Thus, proinflammatory cytokines are broadly involved in spinally mediated pain facilitation. Furthermore, upregulation of spinal proinflammatory cytokines has been reported to be associated with pain facilitation of diverse etiologies. Lastly, peri-spinal administration of either IL1, TNF, or IL6 protein induces enhanced pain responses and/or enhanced electrophysiological indices of enhanced pain transmission (DeLeo et al., 1996; Falchi et al., 2001; Reeve et al., 2000). Taken together, the data reviewed above indicate that spinal proinflammatory cytokines may be necessary and sufficient for the induction and maintenance of hyperalgesia.

8. Glia and mirror image pain

It may be recalled that mirror image pain is a form of hyperalgesia that is perceived to arise from the healthy, opposite side of the body relative to the original injury site. As noted above, mirror image pain is observed in the SIN model of neuropathic pain following intense peri-sciatic inflammation. In addition, mirror image pain is observed following frank nerve trauma induced by CCI (Paulson et al., 2000). In CCI, the sciatic nerve is loosely ligated with immunogenic sutures at mid-thigh level, a manipulation that causes nerve trauma to, and an intense inflammatory response of, the sciatic nerve. The spread of pain to the mirror-image side of the body suggests that signals from one side of the spinal cord are somehow being conveyed to the other side. While it is tempting to assume that proinflammatory cytokines serve this role, proinflammatory cytokines have very short half-lives. Thus, some other mechanism is likely to be involved in the creation of mirror-image pain.

Interestingly, astrocytes are able to communicate with each other via inter-cellular gap junctions, independent of the release of excitatory substances. Such gap junctions are a possible mechanism by which glia can create mirror-image pain. Gap junctions are channels between cells that allow for the direct movement of ions and second messengers from one cell to the next (Dermietzel, 1998). Within the spinal cord, astrocytes share gap junctions with other astrocytes, allowing groups of astrocytes to act in syncytiums (group of cells sharing small cytoplasmic substances allowing for synchronous activity). Therefore, when one astrocyte is activated, the activation can spread along the syncytium. This spread of excitation can lead to the activation of distant glia and consequent release of neuroexcitatory substances from these newly activated cells. Thus, gap junctions provide a mechanism for the intra-spinal spread of excitation that could create mirror-image pain.

To date, only one study has explored the potential role of gap junctions in mirror image pain. Peri-spinal administration of the gap junction decoupler, carbenoxolone, blocked the mirror image pain associated with both SIN and CCI, while leaving hyperalgesia unaltered in the SIN- or CCI-affected limb (Spataro et al., 2004). As carbenoxolone is known to have non-specific effects beyond decoupling gap junctions, the effects of glycrrhetinic acid were examined as well. Glycrrhetinic acid shares all known activities of carbenoxolone except that it fails to decouple gap junctions. The failure of glycrrhetinic acid to mimic the effects of carbenoxolone on mirror image pain lends support to the conclusion that gap junctions mediate this pain phenomenon. Since neurons within the spinal cord appear to share very few gap junctions, the effects of peri-spinal carbenoxolone are most likely due to the decoupling of junctions among spinal cord astrocytes. These findings point to a new explanation of pain that arises in otherwise healthy body parts.

9. Summary and conclusions

Sickness responses include a broad constellation of adaptive changes that occur in response to immune challenge. Pain enhancement (hyperalgesia) is just one component of this survival-oriented response. It, like many more classical sickness responses, was discovered to be mediated by glial activation and release of proinflammatory cytokines. This was a novel and surprising finding from the perspective of the pain field, which had assumed that glia had no role in pain. This new view of glial cells as powerful enhancers of pain raised the ques-

tion of whether these immune-like cells may be importantly involved in pathological pain conditions. Indeed, they are. Glial activation and their release of proinflammatory cytokines are now recognized to be importantly involved in the creation and maintenance of enhanced pain states of diverse etiologies. Indeed, it is striking that there has yet to be a single report documenting that spinal cord glia are not involved in the mediation of enhanced pain responses. This pervasive involvement of glia in pain facilitation argues for the development of novel approaches to clinical pain control by targeting glia and their neuroexcitatory products.

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References

Araque, A., Parpura, V., Sanzgiri, R.P., Haydon, P.G., 1999. Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci. 22, 208–215.

Colburn, R.W., DeLeo, J.A., Rickman, A.J., Yeager, M.P., Kwon, P., Hickey, W.F., 1997. Dissociation of microglial activation and neuropathic pain behaviors following peripheral nerve injury in the rat. J. Neuroimmunol. 79, 163–175.

DeLeo, J.A., Colburn, R.W., Nichols, M., Malhotra, A., 1996. Interleukin-6-mediated hyperalgesia/allodynia and increased spinal IL-6 expression in a rat mononeuropathy model. J. Interferon Cytokine Res. 16, 695–700.

Dermietzel, R., 1998. Diversification of gap junction proteins (connexins) in the central nervous system and the concept of functional compartments. Cell Biol. Int. 22, 719–730.

Dong, Y., Benveniste, E.N., 2001. Immune function of astrocytes. Glia 36, 180–190.

Falchi, M., Ferrara, F., Gharib, C., Dib, B., 2001. Hyperalgesic effect of intrathecally administered interleukin-1 in rats. Drugs Exp. Clin. Res. 27, 97–101.

Garrison, C.J., Dougherty, P.M., Carlton, S.M., 1994. GFAP expression in lumbar spinal cord of naive and neuropathic rats treated with MK-801. Exp. Neurol. 129, 237–243.

Garrison, C.J., Dougherty, P.M., Kajander, K.C., Carlton, S.M., 1991. Staining of glial fibrillary acidic protein (GFAP) in lumbar spinal cord increases following a sciatic nerve constriction injury. Brain Res. 565, 1–7.

Hart, B.L., 1987. Behavior of sick animals. Vet. Clin. North. Am. Food Anim. Pract. 3, 383–391.

Kelly, D.D., 1986. Stress-induced Analgesia. New York Academy of Sciences, New York, NY.

Kluger, M.J., Kozak, W., Conn, C.A., Leon, L.R., Soszynski, D., 1998. Role of fever in disease. Ann. NY Acad. Sci. 856, 224–233.

Kreutzberg, G.W., 1996. Microglia: a sensor for pathological events in the CNS. Trends Neurosci. 19, 312–318.

Ledeboer, A., Sloane, E.M., Milligan, E.D., Frank, M.G., Maier, S.F., Watkins, L.R., 2004. Minocycline attenuates mechanical allodynia and proinflammatory cytokine expression in rat models of pain facilitation. Pain (submitted for publication).

Maier, S.F., Watkins, L.R., 1998. Cytokines for psychologists: implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. Psychol. Rev. 105, 83–107.

Marriott, D.R., Wilkin, G.P., Wood, J.N., 1991. Substance P-induced release of prostaglandins from astrocytes: regional specialisation and correlation with phosphoinositol metabolism. J. Neurochem. 56, 259–265.

McQuay, H., Carroll, D., Jadad, A.R., Wiffen, P., Moore, A., 1995. Anticonvulsant drugs for management of pain: a systematic review. Br. Med. J. 311, 1047–1052.

McQuay, H.J., Tramer, M., Nye, B.A., Carroll, D., Wiffen, P.J., Moore, R.A., 1996. A systematic review of antidepressants in neuropathic pain. Pain 68, 217–227.

Meller, S.T., Dykstra, C., Grzybycki, D., Murphy, S., Gebhart, G.F., 1994. The possible role of glia in nociceptive processing and hyperalgesia in the spinal cord of the rat. Neuropharmacology 33, 1471–1478.

Milligan, E.D., Twining, C., Chacur, M., Biedenkapp, J., O'Connor, K., Poole, S., Tracey, K., Martin, D., Maier, S.F., Watkins, L.R., 2003. Spinal glia and proinflammatory cytokines mediate mirror-image neuropathic pain in rats. J. Neurosci. 23, 1026–1040.

Paulson, P.E., Morrow, T.J., Casey, K.L., 2000. Bilateral behavioral and regional cerebral blood flow changes during painful peripheral mononeuropathy in the rat. Pain 84, 233–245.

Ransom, B.R., 1992. Glial modulation of neural excitability mediated by extracellular pH: a hypothesis. Prog. Brain. Res. 94, 37–46.

Reeve, A.J., Patel, S., Fox, A., Walker, K., Urban, L., 2000. Intrathecally administered endotoxin or cytokines produce allodynia, hyperalgesia and changes in spinal cord neuronal responses to nociceptive stimuli in the rat. Eur. J. Pain. 4, 247–257.

Ridet, J.L., Malhotra, S.K., Privat, A., Gage, F.H., 1997. Reactive astrocytes: cellular and molecular cues to biological function. Trends Neurosci. 20, 570–577.

Schousboe, A., Westergaard, N., 1995. Transport of neuroactive amino acids in astrocytes. In: Kettenmann, H., Ransom, B.R. (Eds.), Neuroglia. Oxford University Press, New York, pp. 246–258.

Sloane, E.M., Langer, S.E., Milligan, E.D., Wieseler-Frank, J., Mahoney, J., Levkoff, L., Cruz, P., Flotte, T.R., Leinwant, L., Maier, S.F., Watkins, L.R., 2004. Chronic constriction injury induced pathological pain states are controlled long term via intrathecal administration of a non-viral vector (NVV) encoding the anti-inflammatory cytokine interleukin-10 (IL-10). Proc. Am. Pain Soc..

Spataro, L.E., Sloane, E.M., Milligan, E.D., Wieseler-Frank, J., Schoeniger, D., Jekich, B.M., Barrientos, R.M., Maier, S.F., Watkins, L.R., 2004. Spinal gap junctions: potential involvement in pain facilitation. J. Pain (in press).

Sweitzer, S., Martin, D., DeLeo, J.A., 2001. Intrathecal interleukin-1 receptor antagonist in combination with soluble tumor necrosis factor receptor exhibits an anti-allodynic action in a rat model of neuropathic pain. Neuroscience 103, 529–539.

Tal, M., Bennett, G.J., 1994. Extra-territorial pain in rats with a peripheral mononeuropathy: mechano-hyperalgesia and mechano-allodynia in the territory of an uninjured nerve. Pain 57, 375–382.

Watkins, L.R., Maier, S.F., 1999. Implications of immune-to-brain communication for sickness and pain. Proc. Natl. Acad. Sci. USA 96, 7710–7713.

Watkins, L.R., Martin, D., Ulrich, P., Tracey, K.J., Maier, S.F., 1997. Evidence for the involvement of spinal cord glia in subcutaneous formalin induced hyperalgesia in the rat. Pain 71, 225–235.

Willis, W.D.J., 1992. Hyperalgesia and Allodynia. Raven Press, New York.

Woolf, C.J., 2004. Pain: moving from symptom control toward mechanism-specific pharmacologic management. Ann. Intern. Med. 140, 441–451.