This paper was presented at the National Academy of Sciences colloquium "The Neurobiology of Pain," held December 11–13, 1998, at the Arnold and Mabel Beckman Center in Irvine, CA.

Implications of immune-to-brain communication for sickness and pain

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ABSTRACT This review presents a view of hyperalgesia and allodynia not typical of the field as a whole. That is, exaggerated pain is presented as one of many natural consequences of peripheral infection and injury. The constellation of changes that results from such immune challenges is called the sickness response. This sickness response results from immune-to-brain communication initiated by proinflammatory cytokines released by activated immune cells. In response to signals it receives from the immune system, the brain orchestrates the broad array of physiological, behavioral, and hormonal changes that comprise the sickness response. The neurocircuitry and neurochemistry of sickness-induced hyperalgesia are described. One focus of this discussion is on the evidence that spinal cord microglia and astrocytes are key mediators of sickness-induced hyperalgesia. Last, evidence is presented that hyperalgesia and allodynia also result from direct immune activation, rather than neural activation, of these same spinal cord glia. Such glial activation is induced by viruses such as HIV-1 that are known to invade the central nervous system. Implications of exaggerated pain states created by peripheral and central immune activation are discussed.

Hyperalgesia and allodynia generally are viewed as purely neural phenomena that reflect changes in spinal cord dorsal horn neuronal excitability brought about by changes in afferent inputs. The pharmacology of exaggerated pain states also typically is viewed in purely neural terms, involving substances either released from sensory and/or centrifugal afferents of dorsal horn neurons or, like nitric oxide, from the dorsal horn neurons themselves. This paper will present a different view. The work to be reviewed illustrates that non-neuronal cells also can drive hyperalgesic and allodynic states. These non-neuronal cells are immune cells in the periphery and glia within the brain and spinal cord. Substances released by these immune and immune-like cells can dramatically alter pain processing.

Until recently, the central nervous system and immune system were thought to operate independently of each other. However, they do not. The first ideas about the dynamic inter-relationships of these two system arose from studies examining the cascade of events initiated by exposure to stressors (1, 2). Stress activates neural circuits in the brain. These stress-induced alterations in brain activity lead to activation of brain-controlled outflow pathways to the periphery, such as the hypothalamo-pituitary-adrenal axis and sympathetic nervous system. The hormones and transmitters released by these outflow pathways turned out to bind receptors expressed by immune cells and immune organs, thereby dramatically altering immune function (1, 2). Thus, the central nervous system proved to regulate immune function.

Within just the past few years, it has been recognized that the inter-relationship between the central nervous system and immune system is, in fact, bidirectional (1). That is, products of activated immune cells feed back to the brain to alter neural activity. The sections that follow focus first on the broad view of how and why the immune system communicates to the brain. The manner in which immune-to-brain communication impacts the pain response then will be explored. Finally, the role of immune-like glia in the spinal cord in exaggerated pain responses will be described and implications discussed.

Immune-to-Brain Communication in Sickness

The immune system responds to infection in two related, but differing, ways. One is slow and selective; the other is rapid and generalized (3). The slow response involves recognition of foreign invaders such as bacteria and viruses through binding to specific receptors expressed on specialized types of immune cells, resulting in the slow and prolonged production of antibodies directed specifically against that particular foreign entity. The other, very rapid and generalized, response is referred to as the sickness response or, alternatively, as the acute phase response (4). This sickness response is triggered by the recognition of anything foreign to the host. It serves as a rapid early defense mechanism until the much slower antibody response can be developed. The sickness response is an organized constellation of responses initiated by the immune system but orchestrated and partially created by the brain (1, 4, 5). The sickness response includes physiological responses (fever, alterations in plasma ions to suppress minerals required by bacteria/viruses to replicate, increases in white blood cell replication, increased sleep, etc.), behavioral responses (decreased social interaction and exploration, decreased sexual activity, decreased food and water intake, etc.), and hormonal responses (increased release of classic hypothalamo-pituitary-adrenal and sympathetic hormones). It has been argued that much of this constellation of changes is in the service of fever. Fever is a phylogenetically very old response that raises the core body temperature to the point where bacteria/viruses do not multiple rapidly, bacteria cannot form protective outer coats, where white blood cells do multiple very rapidly, destructive enzymes key for survival function most effectively, and so on. Every degree of fever requires a 10–15% increase in energy, and most of the components of the sickness response can be viewed as supporting this energetic requirement by either creating energy (hormones released by sickness free energy from bodily stores) or saving energy (increased sleep, decreased exploration and sex, decreased foraging/stalking, etc.) (1, 4, 5). This sickness response requires that immune-

to-brain communication must occur because only the brain can orchestrate such a pervasive array of changes.

The triggers for initiation of the sickness response are substances released by immune cells activated by the foreign entity. As a group, these proteins are referred to as proinflammatory cytokines (3). This name reflects the fact that these proteins orchestrate and augment inflammatory responses. Proinflammatory cytokines include IL-1, IL-6, and tumor necrosis factor. These proteins may be necessary and sufficient for sickness, because preventing their actions (using receptor antagonists, etc.) block sickness responses, whereas exogenous administration of these proteins can create sickness responses (1, 5).

Pain as a Natural Outcome of Immune-to-Brain Communication

The constellation of responses reviewed above constitute the classic view of sickness. Although changes in pain responsivity have not been considered in this classic view, it would be reasonable to suspect that hyperalgesia might be a natural part of this response profile because recuperative behaviors supportive of healing would be produced by enhanced pain (6). Furthermore, recuperative behaviors would be expected to decrease activity and so decrease energy expenditure, again in keeping with the view that sickness responses serve to save energy for use in the production of fever (5).

If such an argument has merit, then one would expect that hyperalgesia should occur after administration of agents known to induce sickness. In fact, hyperalgesia is produced both by i.p. administration of the cell walls of Gram-negative bacteria (endotoxin; also called lipopolysaccharide) (7) and by i.p. live bacteria (8), both of which are known to elicit the release of proinflammatory cytokines from a variety of immune cells. Hyperalgesia also can be elicited simply by administering either IL-1 or tumor necrosis factor alone (9, 10). The key importance of proinflammatory cytokines in sickness hyperalgesia is clear from the fact that it can be blocked by either an IL-1 receptor antagonist or tumor necrosis factor binding protein (9, 11). Thus, these cytokines can be both necessary and sufficient for sickness-induced hyperalgesia to occur.

The mechanism(s) by which proinflammatory cytokines activate the central nervous system is a matter of lively controversy. Both blood-borne and peripheral nerve-generated signals have been proposed (12). For localized tissue infection/inflammation and localized proinflammatory cytokine administration, at least, activation of peripheral nerves appears to be the most likely route (12–14). By using i.p. administration of sickness agents as the example, signals to the brain appear to be carried via the subdiaphragmatic vagus because: (*a*) sickness agents activate sensory vagal neurons, as supported by increases in cFos expression in these cells (15), and (*b*) subdiaphragmatic vagotomy blocks hyperalgesia induced by endotoxin, IL-1, and tumor necrosis factor (9, 10). Activation of the sensory vagus may be secondary to activation of specialized sensory structures called paraganglia (16). These sensory cells are thought to be chemoreceptors, based on their anatomy. They express IL-1 binding sites and are known to form afferent synapses onto sensory vagal fibers. Intriguingly, these IL-1 binding paraganglia are in close physical proximity to dense populations of immune cells that can create and release IL-1 (17). These accumulations of macrophages, mast cells, and dendritic cells embedded in connective tissue near paraganglia have been referred to as nerve-associated lymphoid cells (NALC) (17). The location of these immune cells and their ability to express IL-1 suggest that this NALC may rapidly recognize infection and signal the brain via IL-1 release onto neighboring paraganglia. In support of this notion, we have found that levels of IL-1 in the NALC associated with the

paraganglia rapidly and dramatically increase after i.p. illness agents (17). Thus, this arrangement of peripheral cells may underlie immune-to-brain communication arising from the abdomen.

The pathway by which abdominal sickness signals elicit hyperalgesia has been at least partially mapped in the central nervous system, by using a combination of discrete lesions and expression of cFos, an immediate-early gene product used as a neuronal activation marker (18). From this work, the neural circuitry underlying sickness hyperalgesia was found to involve a nucleus tractus solitarius—nucleus raphe magnus—spinal cord dorsolateral funiculus circuit. The involvement of the nucleus tractus solitarius is notable, in that this appears to be a common "hub" within the brain for creating sickness responses (19).

Whether hyperalgesia induced by peripheral inflammation is mediated by the same general pathway is not yet clear. Although s.c. inflammation (formalin) hyperalgesia is not affected by subdiaphragmatic vagotomy (10), it does require a brain-to-spinal cord circuit that, like sickness hyperalgesia, involves the nucleus raphe magnus (20). Whether the nucleus tractus solitarius is involved is uncertain. Although unilateral lesions of the nucleus tractus solitarius failed to affect s.c. formalin hyperalgesia (21), this may well be because dorsal horn laminae responsive to s.c. formalin send bilateral projections to this medullary structure (22). Bilateral lesions could not be tested given the survival problems such animals face. In addition to at least partial overlap in the neurocircuitry of inflammation- and sickness-induced hyperalgesias, there are similarities in their neurochemistry as well. Both depend on nitric oxide, excitatory amino acids, and substance P at the level of the spinal cord (20).

Role of Spinal Cord Glia in Exaggerated Pain

From the discussion above, it is clear that peripheral infection/inflammation leads to activation of a brain-to-spinal cord pathway, culminating in the creation of hyperalgesia. However, an intriguing aspect of this spinal circuitry is that it critically depends on activation of spinal cord microglia and astrocytes. Indeed, hyperalgesia produced either by s.c. inflammation (23) or i.p. bacterial infection (8) can be blocked by spinal administration of drugs that disrupt glial function. Further, anatomical examination of astrocytes and microglia show them to be clearly activated by peripheral infection/inflammation, as evidenced immunohistochemically by increased expression of glia-specific activation markers (8). Lastly, i.p. endotoxin at the same dose that elicits hyperalgesia rapidly increases dorsal spinal cord levels of IL-1, a product of glial activation (24).

So how are these glia activated and what role do they play in exaggerated pain states? Regarding activation, the glia may be activated by neurotransmitter(s) released by spinal projections of the nucleus raphe magnus. Candidate neurotransmitters to serve this role are substance P and glutamate, because: (*a*) as noted above, both sickness-induced hyperalgesia and s.c. formalin hyperalgesia are mediated, in part, by spinal cord substance P and excitatory amino acids, (*b*) the nucleus raphe magnus-to-spinal cord pathway mediating both sickness-induced hyperalgesia and s.c. formalin hyperalgesia contains substance P and glutamate as neurotransmitters (25), (*c*) microglia and astrocytes express substance P and glutamate receptors (26), and (*d*) glia are activated by substance P and glutamate *in vitro* (27). Once activated, astrocytes and microglia form a positive feedback circuit whereby substances released from microglia activate astrocytes to release substances that further stimulate microglia, and so forth (28). Many of the substances that can be released from microglia and astrocytes are known to be key mediators of hyperalgesia, including nitric oxide, excitatory amino acids [both *N*-methyl-D-aspartate (NMDA) and non-NMDA agonists], IL-1, pros-

taglandins, and nerve growth factor (28). Thus, once spinal cord microglia and astrocytes are activated in a perseverative positive feedback manner, the neuroexcitatory substances they release could drive exaggerated pain states.

However, astrocytes and microglia have not generally been viewed as cells whose major function is activation in response to centrifugal hyperalgesia circuitry. Rather, astrocytes and microglia are immunocompetent cells and thus can respond like immune cells within the central nervous system. Astrocytes and microglia express specific receptors for various bacteria and viruses and are activated on binding to these infectious agents. An example of a neurotropic virus (that is, a virus that can "home" to the brain and spinal cord) is HIV-1, which causes AIDS. HIV-1 invades the brain and spinal cord early in, and continuing throughout, disease progression (29) and this invasion leads to the activation of microglia and astrocytes (30). One reason for the prolonged microglial and astrocyte activation by HIV-1 is that currently available drugs used to treat AIDS do not readily penetrate the blood-brain barrier, so HIV-1 within the brain and spinal cord are not disrupted by such treatments.[†] Once within nervous tissue, HIV-1 binds to receptors on microglia and astrocytes that recognize one specific portion of the virus, namely a glycoprotein (called gp120) expressed on the outer surface of the viral coat (31).

The arguments developed above predict that intrathecal delivery of gp120 should be sufficient to produce hyperalgesia, if immune activation of glia initiates the same sort of positive feedback cascade as occurs for sickness-induced hyperalgesia. In our initial series of studies of this issue, we found that gp120 delivered over lumbosacral cord caused dose-dependent hyperalgesia as measured by the tail-flick test. Because the receptors identified on microglia and astrocytes require the complex three-dimensional conformational structure of native gp120 for receptor activation to occur (32), it follows that irreversible heat denaturation of gp120's natural conformation should disrupt the ability of this protein to create hyperalgesia, if microglia and astrocytes function as we hypothesize. In fact, such disruption of the normal three-dimensional structure of gp120 dose abolish the effects of this viral protein on pain.

Results using the tail-flick test can, at times, be confounded by drug-induced alterations in tail skin temperature (33). We have examined this issue by using two independent approaches. First, we monitored superficial tail temperature throughout tail-flick testing and found that there was no correlation between superficial tail temperature and tail-flick latency (24). Second, we examined the effect of intrathecal gp120 on withdrawal latency of the plantar surface of the hind paws in response to a radiant heat stimulus. This experiment was important because the paws, unlike the tail, are not used to regulate the organism's temperature. Therefore, paw withdrawal latencies are not subject to the skin temperature confounds inherent in the tail-flick test. Here again, robust hyperalgesia was observed, supporting the conclusion that intrathecal gp120 produces thermal hyperalgesia (34).

Intrathecal gp120 produces mechanical allodynia as well as thermal hyperalgesia. The first indication of this effect came from pilot studies in which we observed marked increases in vocalization of gp120-injected rats to light touch, compared with vehicle controls. These initial observations were followed by experiments that used two standardized tests. First, we used calibrated VonFrey monofilaments to examine withdrawal responses elicited from the plantar surface of the hind paws in response to low-threshold mechanical stimuli. This procedure

clearly demonstrated that gp120 induces allodynia (34). Second, we used light touch stimuli to the fur of the hindquarters and found that gp120 induced touch-evoked agitation as well (34).

Importantly, activation of spinal cord microglia and astrocytes appear to be critical for the hyperalgesic and allodynic effects of gp120. Pilot studies using immunohistochemistry suggest activation of glia in spinal cord after gp120. Furthermore, pretreatment of the rats with a drug that disrupts glial function prevented both gp120-induced thermal hyperalgesia and mechanical allodynia (24, 34).

The mechanisms underlying these effects are at present unknown. We are actively investigating this issue and predict that the effects will be similar to those previously defined for sickness-induced hyperalgesia. Recall that IL-1 was previously noted to rapidly increase in dorsal spinal cord after i.p. endotoxin, and that spinal cord IL-1 is a key mediator of hyperalgesia induced by peripheral inflammation. Thus, our initial pilot studies have focused on potential gp120-induced changes in spinal cord IL-1. To date, these data indicate that intrathecal gp120 produces a rapid and dramatic increase in dorsal spinal cord IL-1 mRNA and IL-1 protein (34). This gp120-induced increase in IL-1 protein does not simply reflect intracellular content, but rather is indicative of increased IL-1 release into extracellular space, because levels of IL-1 in lumbosacral cerebrospinal fluid dramatically increase as well (34). Although we have not yet directly tested the effect of IL-1 receptor antagonist on gp120-induced effects, these preliminary data are certainly consistent with the view that gp120-induced alterations in glial function are likely to be important for hyperalgesia and/or allodynia.

Conclusions and Implications

The evidence reviewed above places exaggerated pain in a new framework. This view conceptualizes hyperalgesia not as an entity in and of itself, but rather as a single element in a much larger constellation of physiological, behavioral, and hormonal changes, orchestrated by the central nervous system in response to bodily infection and inflammation. This sickness response enhances survival of the organism in the face of immune challenges. Hyperalgesia, like all of the other components of the sickness response, is triggered by proinflammatory cytokines released by activated macrophages and other immune cells. For localized immune challenges, at least, this proinflammatory cytokine release leads to activation of peripheral nerves that signal the brain. Activation of a centrifugal pathway then occurs, resulting in the activation of microglia and astrocytes within the spinal cord dorsal horn. Neurotransmitters released by the centrifugal pathway combined with neuroexcitatory substances released by astrocytes and microglia create exaggerated pain responses. Thus, for this form of hyperalgesia at least, glia assume a new, pivotal role in the generation of exaggerated pain.

The importance of microglia and astrocytes in spinal cord hyperalgesia mechanisms has potentially important implications. Most important of these is that glia may exaggerate pain when these immune-like cells respond to infection/inflammation within the central nervous system. Clearly, this is the case after intrathecal administration of the HIV-1 envelope protein, gp120. Given that a number of viruses and bacteria can invade the central nervous system, such observations suggest that glia may have a far more important role in pain modulation than previously recognized.

[†]Sham, H., Kempf, D., Molla, A., Marsh, K., Betebenner, D., Chen, X., Rosenbrook, W., Wideburg, N., Chen, C., Kati, W., *et al.*, Fourth Conference on Retroviruses and Opportunistic Infections, Jan. 22–26, 1997, Washington, DC, abstr. 396.

1. Maier, S. F. & Watkins, L. R. (1998) *Psychol. Rev.* **105,** 83–107.
2. Maier, S. F., Watkins, L. R. & Fleshner, M. (1994) *Am. Psychol.* **49,** 1004–1018.
3. Kuby, J. (1992) *Immunology* (Freeman, New York).
4. Hart, B. L. (1988) *Neurosci. Biobehav. Rev.* **12,** 123–137.
5. Kent, S., Bluthe, R.-M., Kelley, K. W. & Dantzer, R. (1992) *Trends Pharmacol. Sci.* **13,** 24–28.
6. Watkins, L. R., Maier, S. F. & Goehler, L. E. (1995) *Pain* **63,** 289–302.
7. Mason, P. (1993) *Neurosci. Lett.* **154,** 134–136.
8. Watkins, L. R., Deak, T., Silbert, L., Martinez, J., Goehler, L., Relton, J., Martin, D. & Maier, S. F. (1995) *Proc. Soc. Neurosci.* **21,** 357.1 (Abstr.).
9. Watkins, L. R., Wiertelak, E. P., Goehler, L. E., Smith, K. P., Martin, D. & Maier, S. F. (1994) *Brain Res.* **654,** 15–26.
10. Watkins, L. R., Goehler, L. E., Relton, J., Brewer, M. T. & Maier, S. F. (1995) *Brain Res.* **692,** 244–250.
11. Maier, S. F., Wiertelak, E. P., Martin, D. & Watkins, L. R. (1993) *Brain Res.* **623,** 321–324.
12. Watkins, L. R., Maier, S. F. & Goehler, L. E. (1995) *Life Sci.* **57,** 1011–1026.
13. Sommer, C., Lindenlaub, L., Petrausch, S. & George, A. (1998) *Proc. Soc. Neurosci.* **24,** 495.4 (Abstr.).
14. Sorkin, L. S., Xiao, W.-H., Wagner, R. & Myers, R. R. (1997) *Neuroscience* **81,** 255–262.
15. Goehler, L. E., Gaykema, R. P. A., Hammack, S. E., Maier, S. F. & Watkins, L. R. (1998) *Brain Res.* **804,** 306–310.
16. Goehler, L. E., Relton, J. K., Dripps, D., Kiechle, R., Tartaglia, N., Maier, S. F. & Watkins, L. R. (1997) *Brain Res. Bull.* **43,** 357–364.
17. Goehler, L. E., Gaykema, R. P. A., Nguyen, K. T., Lee, J. E., Tilders, F. J. H., Maier, S. F. & Watkins, L. R. (1999) *J. Neurosci.*, **19,** 2799–2806.
18. Watkins, L. R., Wiertelak, E. P., Goehler, L., Mooney-Heiberger, K., Martinez, J., Furness, L., Smith, K. P. & Maier, S. F. (1994) *Brain Res.* **639,** 283–299.
19. Ritter, S., Ritter, R. C. & Barnes, C. D. (1992) *Neuroanatomy and Physiology of Abdominal Vagal Afferents* (CRC, Ann Arbor, MI).
20. Wiertelak, E. P., Furness, L. E., Horan, R., Martinez, J., Maier, S. F. & Watkins, L. R. (1994) *Brain Res.* **649,** 19–26.
21. Wiertelak, E. P., Roemer, B., Maier, S. F. & Watkins, L. R. (1996) *Brain Res.* **748,** 143–150.
22. Menetrey, D. & Basbaum, A. I. (1987) *J. Comp. Neurol.* **255,** 439–450.
23. Watkins, L. R., Martin, D., Ulrich, P., Tracey, K. J. & Maier, S. F. (1997) *Pain* **71,** 225–235.
24. Watkins, L. R. & Maier, S. F., eds. (1999) *Cytokines and Pain* (Birkhauser, Amsterdam), pp. 39–58.
25. Menetrey, D. & Basbaum, A. I. (1987) *Neuroscience* **23,** 173–187.
26. Porter, J. T. & McCarthy, K. D. (1997) *Prog. Neurobiol.* **51,** 439–455.
27. Martin, F. C., Anton, P. A., Gornbein, J. A., Shanahan, F. & Merrill, J. E. (1993) *J. Neuroimmunol.* **42,** 53–60.
28. Zielasek, J. & Hartung, H.-P. (1996) *Adv. Neuroimmunol.* **6,** 191–222.
29. Diederich, N., Acermann, R., Jurgens, R., Ortseifen, M., Thun, F., Schneider, M. & Vukadinovic, I. (1988) *Eur. Neurol.* **28,** 93–103.
30. Tyor, W. R., Glas, J. D., Griffin, J., Becker, P. S., McArtheru, J. C., Bezman, L. & Griffin, D. E. (1992) *Ann. Neurol.* **31,** 349–360.
31. Ma, M., Geiger, J. D. & Nath, A. (1994) *J. Virol.* **68,** 6824–6828.
32. Sundar, S. K., Cierpial, M. A., Kamaraju, L. S., Long, S., Hsieh, S., Lorenz, C., Aaron, M., Ritchie, J. C. & Weiss, J. M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 11246–11250.
33. Tjolsen, A. & Hole, K. (1993) *Am. Pain Soc. J.* **2,** 107–111.
34. Milligan, E. D., Nguyen, K. T., Hansen, M., Xu, D., Lee, J., Martin, D., Hinde, J. L., Mehmert, K., Bohl, M., Maier, S. F. & Watkins, L. R. (1998) *Proc. Soc. Neurosci.* **24,** 155.1 (Abstr.).