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Colloquium Paper

This paper is the introduction to the following papers, which were 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.

The neurobiology of pain

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This is a very exciting time in the field of pain research. Major advances are occurring at every level of analysis, from development to neural plasticity in the adult and from the transduction of a noxious stimulus in a primary afferent neuron to the impact of this stimulus on cortical circuitry. The molecular identity of nociceptors, their stimulus transduction processes, and the ion channels involved in the generation, modulation, and propagation of action potentials along the axons in which these nociceptors are present are being vigorously pursued. Similarly, tremendous progress has occurred in the identification of the receptors, transmitters, second messenger systems, transcription factors, and signaling molecules underlying the neural plasticity observed in the spinal cord and brain stem after tissue or nerve injury. With recent insight into the pharmacology of different neural circuits, the importance of descending modulatory systems in the response of the nervous system to persistent pain after injury is being reevaluated. Finally, imaging studies have revealed that information about tissue damage is distributed at multiple forebrain sites involved in attentional, motivational, and cognitive aspects of the pain experience.

These major advances in pain research were the subject of a National Academy of Sciences colloquium entitled "The Neurobiology of Pain," held at the Beckman Center of the Academy in Irvine, California on December 11–13, 1998. The meeting was organized by John Liebeskind (deceased), Ronald Dubner, and Michael Gold. Its purpose was to bring together pain research scientists and those in related fields who have made recent major advances in the development, cellular, and molecular biology and integrative neurosciences related to the neurobiology of pain. The colloquium was organized into six sessions, each with a separate theme: channels, receptors, imaging and systems neuroscience, growth factors and cytokines, development and plasticity, and molecular genetics. There was ample opportunity for the discussion of the most fruitful and exciting lines of research and the identification of important future directions. One hundred and sixty scientists attended the colloquium. We are indebted to GlaxoWellcome, Inc. for its generous support that helped defray the expenses of graduate students and the social events, as well as to Fran Addison for her invaluable assistance in the organization of the colloquium.

This colloquium was held because of John Liebeskind's commitment to the study of pain. Elected to the Academy after more than 20 years of pioneering research in the field, John always maintained that a critical component to progress in this or any field was a forum in which leaders in the field could assemble to discuss recent advances and future directions. Given the tremendous advances that have occurred in the field of pain research over the last decade, John felt that a colloquium held under the auspices of the Academy would be both timely and appropriate. Over two years ago, he approached us and asked that we help him organize this colloquium. Soon after the program was approved and the date was set, John learned that he had terminal cancer, and he died in September, 1997. He would have been very pleased by the depth and breadth of research covered as well as the lively interactions of all the participants. While John was remembered by many of the speakers, Greg Terman, one of his former students, delivered a moving and informative tribute (1).

The colloquium got underway with a spirited discussion of the role of ion channels in peripheral nerve, particularly their expression in nociceptors. Researchers have long since appreciated that, in the presence of injury, nociceptors may become hyperexcitable. A change in the expression of ion channels is one mechanism that may contribute to this hyperexcitability. Steve Waxman (2) summarized data from an elegant series of experiments indicating that sodium channel expression in dorsal root ganglion neurons is dynamic, changing markedly after tissue or nerve injury. Importantly, different forms of injury induce different changes in the expression of sodium channels. For example, nerve injury in the form of axotomy results in a decrease in the expression of tetrodotoxin (TTX)-resistant currents and an increase in a rapidly repriming TTX-sensitive sodium current. In contrast, inflammation results in an increase in the expression of TTX-resistant sodium currents and a decrease in the expression of a TTX-sensitive current. Utilizing a different nerve injury model than that employed by Waxman and colleagues, in combination with antisense oligodeoxynucleotides, Frank Porreca (3) presented evidence indicating that a TTX-resistant sodium channel called SNS/PN3 is critical for the initiation and maintenance of nerve injury-induced hyperalgesia and allodynia. In contrast, NaN, another TTX-resistant sodium channel recently identified by Waxman and colleagues (4), does not appear to contribute to the maintenance of nerve injury-induced changes in nociceptive thresholds. Michael Gold (5) reported on the role of the TTX-resistant sodium currents in inflammation and showed that the current is modulated by inflammatory mediators such as prostaglandin E_2, 5-HT, and adenosine, consistent with its role in peripheral sensitization. Gold provided additional data indicating that TTX-resistant channels are not only present and functional in the peripheral terminals of nociceptors, but that modulation of these channels contributes to prostaglandin-induced mechanical hyperalgesia. Daniel Weinreich (6) switched the focus of the discussion to other channels by addressing the role of a calcium-dependent potassium current in controlling the excitability of vagal afferents. Through a beautiful series of experiments, Weinreich was able to assess the relative contribution of various sources of calcium responsible for the gating of the potassium currents.

Abbreviations: TTX, tetrodotoxin; NGF, nerve growth factor.

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Peter McNaughton (7) reviewed the data collected in his laboratory demonstrating for the first time the existence of an ion channel specifically activated by heat. Pursuing observations indicating that bradykinin modulates the heat activated channel, McNaughton presented evidence implicating activation of the epsilon isoform of protein kinase C in this process. McNaughton's findings are of even greater interest because of the similarity of this ion channel to the properties of the recently cloned capsaicin/heat receptor by Julius and colleagues (8).

Research on receptors involving the transduction, transmission, and modulation of nociceptive information is clearly one of the most exciting and rapidly advancing areas in the field of pain research today. With the molecular characterization of many of the receptors involved in the transmission of nociceptive stimuli as well as the cellular elements necessary for synaptic transmission, researchers have begun to piece together the essential elements necessary for the first steps ultimately leading to the perception of pain. Amy MacDermott started the session by describing results from recent experiments performed in her laboratory designed to investigate the role of presynaptic non-*N*-methyl-D-aspartate receptors at the first synapse in the nociceptive pathway. Utilizing a dorsal root ganglion neuron/dorsal horn neuron co-culture, MacDermott and her colleagues obtained evidence indicating dorsal root ganglion neurons express functional α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and kainate receptors. Importantly, activation of these receptors appears to influence glutamate release from the sensory neuron and therefore the activation of the dorsal horn neurons. Edwin McCleskey described an exciting series of experiments performed in his laboratory utilizing a combination of electrophysiology and single-cell PCR. Through beautifully controlled reverse transcription–PCR reactions, McCleskey and his colleagues were able to determine the number of mRNA copies encoding the μ-opioid receptor in a single cell in which the presence of functional μ-opioid receptors had previously been investigated. Their results provide a mechanistic explanation for some perplexing aspects of opioid analgesia. Michael Salter (9) provided evidence supporting a revolutionary hypothesis concerning the cellular events underlying the development of long-term potentiation in the hippocampus and, by analogy, central sensitization of spinal cord dorsal horn neurons after tissue injury. Salter's compelling evidence indicates that the initial steps underlying these two phenomena may involve an increase in the intracellular concentration of Na^+, activation of the nonreceptor protein tyrosine kinase, Src, and the subsequent phosphorylation of *N*-methyl-D-aspartate receptors. Edward Perl (10) pointed out that changes in the expression of receptors involved in the transmission of nociceptive stimuli may contribute to the pathophysiology of pain. Perl cited evidence obtained in his laboratory supporting the hypothesis that an increase and/or change in the expression of α-adrenergic receptors present in sensory neurons is an underlying mechanism of adrenergic excitation of sensory neurons often observed after nerve injury.

The session on imaging and systems neuroscience examined some of the most recent exciting findings on pain pathways and their modulation. William Willis reported on a new visceral pain pathway that ascends in the dorsal column of the spinal cord (11). Postsynaptic dorsal column neurons in the rat sacral spinal cord transmit visceral signals to the gracile nucleus, and this information is then relayed to the ventral posterior lateral thalamic nucleus. Functional MRI studies have revealed that dorsal column lesions eliminate blood volume changes in the thalamus produced by noxious pelvic visceral stimulation, suggesting the importance of this pathway. More studies are needed to determine the functional significance of the spinothalamic and spinoparabrachial visceral pathways in comparison with this newly discovered dorsal column pathway. Tony Yaksh (12) described studies with different animal models that support the importance of spinal cord processing in pain states; he emphasized the functional and pharmacological comparability of symptoms across species and pointed out that these models are an important source of information for the development of novel clinically relevant analgesics. Howard Fields described his elegant findings on specific brain stem networks involved in potent pain modulation. μ-opioid receptor agonists activate neurons in the periaqueductal gray and the rostral ventral medulla by inhibiting GABAergic inhibition. The behavioral antinociception and inhibition of dorsal horn neurons is mediated by the release in rostral ventral medulla of an endogenous opioid peptide acting at the μ-opioid receptor. Fields also presented exciting data indicating that κ-opioid receptor selective ligands have actions in rostral ventral medulla that oppose those of the μ-opioid receptor-selective ligands and block the antinociceptive effect of periaqueductal gray-administered morphine. Interestingly, this effect was only observed in male rats. Descending modulation systems were summarized by Gerry Gebhart (13), who provided evidence that supraspinal structures make a significant contribution to the development and maintenance of hyperalgesia associated with tissue injury. He suggested that persistent input engages spinobulbospinal facilitatory mechanisms that contribute to secondary hyperalgesia that occurs outside the site of injury. The findings by others of descending inhibitory systems contributing to hyperalgesia emphasizes the bimodal nature of these descending systems in the modulation of persistent pain. Ken Casey (14) described the role of forebrain mechanisms of pain in imaging studies in humans and reviewed convincing evidence that the perceived intensity of unilateral pain evoked by different inputs correlates with increases in regional cerebral blood flow in primarily five structures: bilaterally in the thalamus, the contralateral insula, the bilateral premotor cortex, the contralateral anterior cingulate, and the cerebellar vermis. In contrast, results on the role of primary somatosensory cortex are somewhat inconsistent. Cathy Bushnell (15) reviewed the factors contributing to this inconsistency including cognitive modulation, average-related degradation of signal due to anatomical variability in sulcal anatomy and differences in methodology. She provided behavioral evidence indicating that manipulations that altered pain discrimination altered activity in primary somatosensory processing regions of the cerebral cortex. In contrast, manipulation that preferentially altered the affective or motivational dimension of pain produced changes in the anterior cingulate cortex. The combined use of psychophysical testing and brain imaging in humans should help reveal the functional role of these different forebrain structures that have direct corticofugal projections to the thalamus, brain stem, and spinal cord and thereby modulate the pain experience at those levels.

The role of trophic factors and cytokines in the development and maintenance of pain in response to various forms of tissue injury is an area of research that has virtually exploded in the last several years. William Snider opened the session by describing recent experiments performed in his laboratory designed to distinguish trophic influences of nerve growth factor (NGF) from its role in cell survival. Through the use of knockout mice, Snider and his colleagues obtained striking results suggesting that, although activation of the high affinity NGF receptor was necessary to establish proper innervation of peripheral targets, activation of this receptor was not necessary for the growth and guidance of central terminals. In addition to its role in development, NGF and other growth factors and cytokines have been shown to mediate pain and hyperalgesia associated with tissue injury. Lorne Mendell (16), the first to describe the

link between NGF and pain, presented results obtained from experiments performed in his laboratory identifying the mechanisms underlying the initial hyperalgesic response to NGF. The initial hyperalgesia in response to systemic or peripherally administered NGF depends on indirect mechanisms, specifically mast cell degranulation. Mendell presented resent evidence indicating that NGF also is capable of potentiating capsaicin-evoked currents in isolated sensory neurons. Utilizing this intriguing observation, Mendell presented a model that would account for the initial NGF-induced thermal hyperalgesia. Focusing on the interaction between the immune system and the nervous system, Linda Watkins (17) described additional pathways through which activation of the immune system results in changes in multiple sites throughout the nervous system. Watkins described the molecules involved in the signaling pathways as well as how activation of this system results in changes in behavior. Steve McMahon (18) brought trophic factors back to center stage with his summary of a growing body of data implicating a critical role for brain derived neurotrophic factor in the altered nociceptive processing observed in the presence of inflammation. Brain derived neurotrophic factor appears to function as a neurotransmitter/neuromodulator in the dorsal horn of the spinal cord, where it is released from the central terminals of small-caliber afferents and increases the excitability of dorsal horn neurons.

The session on development and plasticity explored plasticity that occurs in the central nervous system after tissue and nerve injury. Maria Fitzgerald (19) reported on changes in the neonatal spinal cord that are not simply immature or incomplete versions of what occurs in the adult. Central sensitization occurs in the normal immature spinal cord in response to electrical stimulation of A β fibers whereas activity-induced plasticity in the adult spinal cord takes place only in response to C fiber strength stimulation, unless the dorsal horn is primed by previous peripheral injury. *N*-methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors are distributed in higher density in the neonatal cord, and the receptor subunit composition in the neonatal spinal cord maximizes non-*N*-methyl-D-aspartate calcium influx. Clifford Woolf (20) provided an outstanding, concise, and up-to-date review of activity-induced and signal-induced plasticity in sensory neurons after tissue and nerve injury. He showed the interaction of these mechanisms in the role of brain derived neurotrophic factor in the generation of central sensitization. Jianren Mao (21) suggested that hyperalgesia and morphine tolerance may be interrelated by common neural mechanisms involving excitatory amino acid receptor activation and subsequent intracellular events, such as protein kinase C translocation and nitric oxide production. This hypothesis is supported by experiments showing that hyperalgesia develops when animals are made tolerant to morphine and that both the hyperalgesia and morphine tolerance develop as a consequence of peripheral nerve injury. Gary Bennett (22) reported on a new model of inflammation in which a focal neuritis is produced in the rat sciatic nerve. The results suggest the presence of a neuroimmune interaction that occurs at the onset of nerve injury and contributes to the development of neuropathic pain.

While the tools of molecular genetics were employed by many of the researchers who spoke throughout the colloquium, the issue was addressed formally in the final session. Alan Basbaum (23) discussed the use of knockout mice to investigate the role of specific receptors and second messengers in nociceptive processing. Basbaum eloquently illustrated how this powerful approach has shed new light on our understanding of the mechanisms of action of molecules such as substance P and the γ isoform of protein kinase C. For example, the contribution of substance P and neurokinin A to central sensitization may be considerably less than previously suspected while, in contrast, activation of the γ isoform of protein kinase C appears to be vital to the development of nerve injury-induced hyperexcitability of dorsal horn neurons. Michael Moskowitz focused the discussion somewhat by describing several approaches that have been employed in the study of migraine. Moskowitz reviewed the data implicating the involvement of a specific class of serotonin receptors in migraine headache. Based on an exciting series of functional MRI studies, Moskowitz demonstrated that brain metabolism and blood flow may be uncoupled before the onset of headache. In contrast to the approach utilized by many researchers attempting to identify a role for a specific protein in nociception (a bottom-up approach), Jeffrey Mogil (24) described a top-down approach in which genetic mapping may be employed to identify genes responsible for specific behavioral phenotypes. Such an approach is readily applied to pain research, where it provides a mechanism for the identification of unique molecules critically involved in nociceptive processing. To illustrate a case in point, Mogil described the identification of a specific serotonin receptor subtype involved in the expression of morphine analgesia. George Uhl (25) discussed the integration of the top-down and bottom-up approaches through experiments performed with the μ-opioid receptor knockout mouse as well as populations of humans. His results suggest that polymorphisms in the gene encoding the μ-opioid receptor may explain much of the variability observed among people with respect to their responsiveness to opiate analgesia. Identification of the underlying mechanisms controlling opioid responsiveness may enable the development of individualized treatment programs for ongoing pain.

The exciting new advances in pain research emphasize the importance of this field of neuroscience. The neural apparatus responsible for the perception of pain includes mechanisms that clearly are prototypic components of all mammalian sensory systems. These mechanisms include specialized receptors, stimulus transduction mechanisms, ion channel modulation, rapid and slow activity involving excitatory and inhibitory transmitters and their receptors, amplification of relevant signals at peripheral and central nervous system sites utilizing activity-dependent and signal-dependent mechanisms of neuronal plasticity, and, finally, distributed processing of environmental signals and their interaction with learned memories at higher centers. The field of pain research has made giant steps in putting together important segments of this puzzle. It is, of course, the hope of all of us that these advances will lead to an improvement in the quality of life of acute and chronic pain sufferers.

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