Future Basic Science Directions Into Mechanisms of Neuropathic Pain

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The aim of this article is to outline mechanisms underlying generation and maintenance of pain arising from trauma to peripheral nerve fibers and to present an overview of our recent studies of animal models of peripheral neuropathic pain and pain of temporomandibular disorders (TMD). The former model was induced by placing a polyethylene cuff around the sciatic nerve of the Sprague-Dawley rat and the TMD model was induced by injection of complete Freund's adjuvant into the rat's temporomandibular joint. In cuff-implanted rats, ongoing activity of dorsal horn neurons was greater than in controls, the cutaneous receptive field size of the neurons was greater, and both noxious and innocuous mechanical stimuli to the receptive field elicited an excitatory response during stimulation but also a marked afterdischarge that lasted up to 30 minutes; this afterdischarge was never observed in control rats in response to innocuous stimulation. The model of TMD was characterized by joint space narrowing, bone remodeling, infiltration of immune cells, loss in the range of jaw opening, and signs of nociception. Alterations in the neural substrate of nociception in animal models, and therefore also possibly in humans, appear to include changes in peripheral as well as central neurons. In the periphery, changes include alterations in the phenotype and central projections of large-diameter sensory nerve fibers. At the level of the trigeminal brainstem and spinal cord, there appear to be several types of change. One type is an increased efficacy of synaptic transmission onto second-order neurons. Another type of change is a reduction in inhibitory mechanisms, including a shift of gamma-amino butyric acid ($GABA_A$) receptor activation to excitation. There is a need for further studies to focus on mechanisms for either the generation or the maintenance, or both, of neuropathic pain. J OROFAC PAIN 2004;18:306–310

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Neuropathic pain is characterized by a debilitating tactile allodynia, or an excruciatingly painful and persistent response to a normally innocuous mechanical stimulus. This type of pain is distinct because of its relative refractoriness to medical interventions. Despite extensive clinical and basic studies, people with neuropathic pain remain undertreated. Much progress has been made over the past 10 to 15 years in our understanding of mechanisms underlying neuropathic pain in particular,[1–4] because animal models have been developed that share these properties with the human condition.

Basic science investigations on animal models are designed to enable experimental manipulations that are not possible or ethical in humans to allow longitudinal studies and that can include the period before model induction to provide a means to analyze

efficacy of new drugs. An appropriate animal model should exhibit symptoms similar to the human condition and should respond in a predictable way to medications that are effective in humans. The overall objective, then, is to gain insights that may lead to important novel interventions. While there are many models of neuropathic pain, there are 4 primary models. The first was described by Bennett and Xie in 1988 and is induced by tying loose ligatures around a peripheral nerve.[5] Subsequent models were induced by partially cutting nerves,[6] by ligating spinal nerves,[7] and by placing fine polyethylene cuffs around a peripheral nerve.[8] The reader is referred for further reading on models of neuropathic pain to other articles in this issue of the *Journal of Orofacial Pain,* as well as to a recent book edited by Luo.[9]

This paper will describe some features observed by the author and his colleagues in their own laboratory, where they explored the mechanisms of neuropathic pain through studies on the Mosconi and Kruger animal model[8] and how these studies have led to view future basic science directions into mechanisms of neuropathic pain. In addition, some results from a recently introduced animal model of temporomandibular disorders (TMD) will be surveyed, as the trigeminal system may behave in ways different from those seen at the spinal level. The author hypothesizes that in at least some types of chronic pain, sensory nerve fibers and the peripheral tissues that they innervate exist in a dynamic state whereby each promotes the health of the other. Yet, under conditions that are still being explored, this state may be altered so that chemicals from the peripheral tissues activate nociceptive afferent fibers that lead to the perception of pain. In turn, the sensory nerves release transmitters by antidromic activation or by local release mechanisms that promote tissue pathology and thereby contribute to tissue damage.

Model of Neuropathic Pain in the Hind Leg

To understand the mechanisms underlying generation and maintenance of pain arising from trauma to peripheral nerve fibers, an animal model of peripheral neuropathic pain, induced by a modification of the Mosconi and Kruger method, was adopted.[10] Von Frey hair testing confirmed marked tactile allodynia in all animals.

Once the model had been established, more than 10 days after cuff implantation, animals were anesthetized and the spinal cords were transected for single-unit extracellular recording from dorsal horn neurons at the mid-lumbar spinal level. Ongoing activity was greater in cuff-implanted rats than in controls. The cutaneous receptive field size was also greater in cuff-implanted rats. In cuff-implanted rats, both noxious and innocuous mechanical stimuli to the receptive field elicited not only an excitatory response during stimulation but also a marked afterdischarge that lasted up to 30 minutes; this afterdischarge was never observed in control rats in response to innocuous stimulation.[11,12] Electrical stimulation of the sciatic nerve at 4 Hz and 20 Hz elicited the typical initial discharge but also an afterdischarge. The afterdischarge with 20 Hz stimulation was greater than that with 4 Hz, and this was interpreted to indicate a predominant role of myelinated afferents in eliciting this afterdischarge.[12] Importantly, in some experiments, when a distinct afterdischarge was elicited in a repeatable manner, in trials separated by 1 hour or more, the local anesthetic lidocaine was applied to the sensory nerve between the cuff and the spinal cord.[13] This led to an immediate decrease in the afterdischarge in all cases, which indicates that the afterdischarge was due to a dramatic drive from the primary sensory fibers.

Furthermore, behavioral experiments with this model have demonstrated that intrathecal administration of the NK-1 receptor (ie, substance P receptor[14]) antagonist CP-96,345 decreased the mechanical allodynia that characterizes this model. This suggests that activation of NK-1 receptors, presumably by release of substance P in the spinal cord, plays a role in the sensitivity of the neural substrate of nociception at the spinal level. Importantly, in pilot experiments to complement those described, intrathecal administration of the antiepileptic drug gabapentin, which is effective in some cases of neuropathic pain in humans,[15,16] was ineffective in this animal model (unpublished data), and intrathecal administration of morphine had a significant but minor effect (unpublished data, 2001–2002). This supports other findings of the relative ineffectiveness of opiates for treatment of neuropathic pain in humans, as well as the insensitivity of some people with neuropathic pain to treatment with gabapentin.[17]

These data are complementary to an extensive literature implicating central nervous system changes in nociceptive mechanisms.[1–3] These include increases in excitatory mechanisms,[18–20] decreases in inhibitory mechanisms,[21–25] and even a phenotypic switch in the signal of a receptor for the inhibitory amino acid gamma-amino butyric acid (GABA), whereby a change in the function of the chloride transporter shifts the chloride equilibrium potential positively, so that GABA acting on

the GABA receptor depolarizes and thus excites the membrane.[26]

In experiments designed to determine whether the model could be reversed, the cuff was removed 1 or 4 days after implantation. The allodynia reversed spontaneously in some animals in the 1-day group but not in the 4-day group; experiments are currently being done to investigate the mechanisms underlying this difference. The data indicate, however, that there is a reversible process that secures in time; therefore, novel interventions may be developed to block the development of this presumably central neuroplastic change.

When considering future directions for research, it is important to take the view then that alterations in the neural substrate of nociception in animal models, and therefore also possibly in humans, appear to include changes in both peripheral and central neurons. Changes include alterations in the phenotype and central projections of large-diameter sensory nerve fibers. A unifying hypothesis is therefore needed that synthesizes the data implicating both peripheral and central changes.

Model of TMD

In the trigeminal region, chronic pain syndromes include neuropathic disorders, musculoskeletal pain, vascular pain, and other chronic headaches. Therapy for neuropathic pain usually consists of polypharmacy, developed through a hit-or-miss strategy.[17] Therapy of chronic facial pain syndromes often relies on long-term psychotropic medications that cause severe side effects yet often offer only minimal pain relief. Thus, the approach taken by the author and his colleagues was that to understand TMD, studies were needed to address the peripheral changes in joint tissues, the neural substrate of nociception at the trigeminal level, and the neurological disturbances accompanying nerve damage.

Developing an animal model of TMD is important because the temporomandibular joint (TMJ) differs from other joints in a number of significant ways. For example, the articular surface of the TMJ is covered with dense fibrous tissue, whereas most other synovial joints are covered with hyaline cartilage.[27] In addition, the TMJ is capable of not only opening and closing, but extending and rotating in the 3 dimensions.[27] The joint is recruited not only for eating and drinking, but also for speaking and yawning.

Chronic models of TMD may be created by disc displacement or trauma to the joint. These pathologies may be accomplished by surgical manipulation to fracture the condyle or to create defects in the articular disc.[28,29] In addition, such models may be established by mechanical damage to the joint, physical impact,[30] or repeated hyperextension of the jaw.[31] Alternatively, the model may be characterized by the injection of an adjuvant containing bacterium, which can create joint pathology.[32,33] The authors adopted an animal model of TMD induced by injection of complete Freund's adjuvant (CFA) into the TMJ. The few animal models of chronic TMD that have been reported (eg, Dubner and Ren[2]), have demonstrated changes in meal patterns, external swelling, chromodacryorrhea, and heightened levels of proinflammatory markers such as interleukin-1β, tumor necrosis factor-α, and cyclooxygenase-2.[33,34] Changes in C-fos expression in the trigeminal brainstem sensory nuclei have also been observed at different times after model induction.[35] Shinoda et al[36] mapped changes in the distribution of nerve fibers containing calcitonin gene–related peptide and found an increase in total fiber density in the TMJs of rats with experimentally induced arthritis. Despite these advances, there is still a need for a systematic analysis of temporomandibular dysfunction through a well characterized and validated animal model of chronic dysfunction that can be used for longitudinal studies.

Such a model was established by the percutaneous injection of CFA into the intra-articular joint space of the jaw in rats anesthetized with 4% halothane in oxygen. CFA was prepared by adding 60 mg of Mycobacterium butyricum (Difco Laboratories) to an 11 mL mixture of mineral oil (6 mL), 0.9% sodium chloride (4 mL), and Tween 80 (1 mL; Uniqema). The solution was mixed thoroughly and autoclaved for 20 minutes at 120°C to rupture the cell walls of the mycobacterium. The site of injection was identified by palpating the zygomatic arch to the posterior aspect, where the condyle inserts into the temporal bone. A 27-gauge needle was then inserted from the posterior aspect of the TMJ into the area immediately inferior to its posterior border of the zygomatic arch at a 30-degree angle above the skin, and 25 μL of CFA was injected. Control rats were injected with 25 μL of vehicle. Tests of efficacy of the model were typically run from 7 days after model induction up to 60 days.

Clinical signs included loss of joint space in microcomputed tomographic analysis. Joint space was defined as the distance between the top of the condyle and the glenoid fossa. There was also evidence of bone resorption. Histologic analysis demonstrated infiltration of immune cells into the joint tissue, including lymphocytes, macrophages, and activated myofibroblasts. There was also a proliferation of synovial cells of the joint to the extent that a pannus was typically seen encroaching into the joint

space. In addition, CFA-injected rats showed marked villous hyperplasia extending into the joint space.

Since TMD are often characterized by mechanical dysfunction, the range of opening of the jaw with a force of 50 g was also determined. It was observed that this range was reduced from a mean of approximately 32 degrees to approximately 18 degrees as measured on a protractor in a mold that accommodated the fixed head of the rat.

Two tests were run to determine whether jaw opening might be painful in this model. First, the trigeminal subnucleus caudalis of the brainstem was examined for internalization of the NK-1 receptor. The rationale was that the internalization of this receptor would be indicative of the endogenous release of its natural ligand, substance P, presumably from primary afferent nociceptive fibers. Indeed, it was observed in confocal microscopic analysis that jaw opening in CFA-treated rats but not in control rats led to internalization of NK-1 receptors, identified with selective antibodies according to a protocol described elsewhere.[37]

The second test to determine whether jaw opening was a painful stimulus in this model examined the effects of jaw opening on the tail withdrawal reflex. In this case, the rationale was that if opening of the jaw activated nociceptive mechanisms this would inhibit the tail withdrawal reflex via extrasegmental descending inhibitory mechanisms that perhaps resemble diffuse noxious inhibitory controls (DNIC), as it has been demonstrated that this reflex is inhibited by extrasegmental nociceptive inputs.[38,39] It was observed that a 3-minute period of alternate opening and closing of the jaw with an opening stimulus of 100 g led to a transient inhibition of the tail withdrawal reflex lasting approximately 15 minutes. This effect was not seen in control animals injected with the vehicle for CFA.

To investigate further changes in the physiology of the joint tissue, extravasation of plasma proteins into the tissues was measured with a method based upon measurement of Evans blue dye, which binds to albumen. This test was run to determine any changes in the integrity of the vascular endothelial barrier because this is involved in regulation of tissue health. Animals injected with CFA showed a significant increase in plasma extravasation in the joint tissues compared to vehicle-treated animals. This increase was seen as early as 3 days after model induction and remained until at least day 14. On gross examination, the leakage of plasma proteins remained highly localized to the area immediately surrounding the joint.

Overall, the data indicate that this model may prove to be useful in gaining insights into mechanisms that contribute to or exacerbate TMD and TMJ disease. It is thus anticipated that future studies on this model will provide insights into novel interventions to treat people with TMD. In fact, the model may prove to be useful in preliminary testing in the development of such novel interventions. Pilot trials have been run to challenge the model to determine its potential applicability to such testing in the development of novel interventions. The loss of the range of jaw opening and the loss of integrity of the endothelial barrier were challenged with daily administration of meloxicam, a cyclooxygenase-2 inhibitor. It was found that both parameters showed significantly less severity than those in CFA-injected rats that did not receive meloxicam.

Future studies focusing on this model will address the changes in the neural substrate that serve nociception in order to identify specific changes that occur and the time course of these changes. In particular, the author's research will focus on intracellular signal transduction mechanisms that are involved in regulation of gene expression, as this may contribute to long-term sensitivity and pain. A further focus will be on the hypothesis outlined above, that the sensory nerves and the peripheral tissues that they innervate exist in a dynamic state. In this state, each promotes the health of the other. However, under conditions that are still being explored, this state may be altered so that additional chemicals are released from the peripheral tissues. These additional chemicals then activate nociceptive afferent fibers that lead to the perception of pain. Furthermore, the sensory nerves release transmitters by antidromic activation or by local release mechanisms that promote tissue pathology and thereby contribute to tissue damage.

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