Instituto Juan March
de Estudios e Investigaciones

1

CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

# What do Nociceptors Tell the Brain?

Organized by

C. Belmonte and F. Cerveró

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G. J. Bennet
J. N. Campbell
F. Cerveró
A. W. Duggan
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H. O. Handwerker
M. Koltzenburg
R. H. LaMotte
R. A. Meyer
J. Ochoa
E. R. Perl
H. P. Rang
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# I N D E X

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## WHAT DO NOCICEPTORS TELL THE BRAIN?

Monday, February 24th, 1992

Welcome & Introduction: C. BELMONTE & F. CERVERO

1st Session - Chairman: C. BELMONTE
**Setting the stage.**

E.R. Perl - Nociceptors as separate classes of primary afferent units.

J.N. Campbell - Hyperalgesia and Sensitization: an overview of neural mechanisms.

J. Szolcsányi - Efferent functions of nociceptors: reevaluation of axon reflex theory.

W.D. Willis Jr. - Central processing.

2nd Session - Chairman: E.R. PERL
**Nociceptors and the signalling of injury.**

H.O. Handwerker - Pain and itch mediated by cutaneous nociceptors.

R.F. Schmidt - The signalling of injury by muscle and joint afferents.

C. Belmonte - Corneal nociceptors: a model to study polymodality.

M. Koltzenburg - When do "silent" nociceptors start to talk to the brain?

Tuesday, February 25th, 1992

3rd Session - Chairman: J.N. CAMPBELL
**Sensitization and hyperalgesia: Part I.**

R.A. Meyer - Sensitization of mechanically insensitive afferents (MIAs) from skin.

E.R. Perl - Induction of novel adrenergic excitation of cutaneous nociceptors after nerve injury.

P.W. Reeh - Nociceptors - hot and sour.

H.P. Rang - Effects of bradykinin on sensory neurones.

4th Session - Chairman: J. SZOLCSANYI
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Poster Oral Presentations: P. Marchettini, S.J.W. Lisney, F.J. Alvarez, J.A. López-García, M.A. Pozo, J.F. Herrero.

J. Gallar - Polymodal nociceptors and neurogenic inflammation in the cornea.

R.H. LaMotte - Allodynia and Alloknesis: a model.

E. Torebjörk - Pain and hyperalgesia from nociceptive and non-nociceptive inputs.

H.-G. Schaible - Nociceptive processing in the spinal cord during acute and chronic monoarthritis.

Wednesday, February 26th, 1992

5th Session - Chairman: W.D. WILLIS Jr.
**Central processing.**

F. Cerveró - Functional properties of nociceptor-driven cells in the spinal cord.

J. Ochoa - What does the brain hear from nociceptors in disease?

A.W. Duggan - The central release of neuropeptides following peripheral stimuli in normal and inflammatory states.

G.J. Bennett - Does nociceptor activity cause transsynaptic damage to spinal cord dorsal horn neurons?

# Session 1:
# SETTING THE STAGE

# NOCICEPTORS AS SEPARATE CLASSES OF PRIMARY AFFERENT UNITS

Edward R. Perl
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As a step in the logic attempting to define a generic stimulus quality for pain, equivalent to those existing for other classes of sensory modalities, Sherrinton (1900) pointed to the widely accepted relationship between tissue damage and pain. He suggested the common feature of pain-producing stimuli to be events threatening or producing physical damage of tissue and labeled such stimulation "nocuous" (noxious). This reasoning led to his coinage of the terms noci-ceptor/noci-ceptive to designate sense organs and reactions evoked by noxious stimuli. These terms and concepts did not achieve wide currency probably because primary afferent units with the correct characteristics had not been uncovered. The terminology was revived to describe a class of relatively slowly-conducting myelinated fibers innervating skin that are effectively and promptly excited only by strong cutaneous mechanical stimulation (Burgess and Perl, 1967). High threshold cutaneous mechanoreceptors (MyHIMs) are found throughout mammalia including human beings and exhibit a constellation of features indicating that they are a unique and coherent set of primary afferent units: receptive fields consisting of multiple small spot-like excitable areas, distinctive peripheral receptive terminals, a singular central termination pattern including distinctive synaptic articulations, and an unusual combination of glyco-protein membrane epitopes.

Skin and several other tissues have more than one type of nociceptor. The most common nociceptor in many mammals (C-fiber polymodal nociceptor or CPM) is readily excited by noxious heat, irritant chemicals or strong mechanical stimuli applied to the skin surface. Differences between CPM and MyHIM nociceptors are more than receptive properties, e.g. conduction velocity of primary afferent fiber, central projection pattern, molecular constituents.

The functional importance of nociceptors becomes clear when the ability of other types of sense organs to signal or code differences between innocuous and noxious stimuli is systematically tested. Low-threshold mechanoreceptors and specific" cooling or warming thermoreceptors produce maximal signals to strong, but not injurious stimuli. Tissue-damaging stimulus intensities either inactivate such low threshold afferent units or do not evoke a unique frequency or pattern of discharge making it impossible for them to provide information to the CNS distinguishing noxious from innocuous events. In contrast, nociceptors such as MyHIMs or CPMs are only activated by strong stimuli, those near the saturation level of low-threshold afferent units and provide graded signals for graded noxious stimulation unequivocally indicating noxious events.

The responses of neurons of the spinal cord demonstrate that part of the central projection of nociceptors is selective. In the superficial dorsal horn of the spinal cord, some neurons have an excitatory input dominated by one kind of nociceptor, others by nociceptors of more than one type. This selective connectivity is in addition to multiceptive excitatory convergence on still other sets of dorsal horn neurons that receive supraliminal low-threshold and nociceptive primary afferent input. Spinal neurons with such dissimilar combinations of nociceptive and other primary afferent input exhibit differing patterns of inhibitory effects from peripheral stimuli as well. The variety of nociceptors and their connections are one indication of complexity in the roles these primary afferent units have in normal and pathological pain.

**Hyperalgesia and Sensitization:**
**An overview of neural mechanisms**

James N. Campbell, Johns Hopkins University, Baltimore MD USA

Hyperalgesia is defined here as a leftward shift of the stimulus-response function that relates magnitude of pain to stimulus intensity. Recent evidence suggest several different peripheral and central mechanisms that might account for hyperalgesia. Two peripheral mechanisms merit consideration: (1) Injury could lead to enhanced transmission of stimulus energy from the skin surface to the nociceptor. (2) The nociceptor could itself sensitize. Thus, a given stimulus affects a greater neural response via sensitization of the transduction process. Central mechanisms also play a role in hyperalgesia. The various means by which this happens fall into three categories; the first two of these constitute a form of central sensitization, whereas the latter constitutes a alteration of tonic inhibitory influences: (1) there may be an enhanced response of central neurons concerned with pain to peripheral nociceptive inputs; (2) inputs from new channels may acquire the capacity to activate central neurons concerned with pain (e.g., low threshold mechanoreceptors may acquire the capacity to evoke pain); (3) peripheral injury might decrease inputs from channels that normally inhibit pain.

Two factors seriously complicate analysis of the neural mechanisms of hyperalgesia: firstly, injury induces hyperalgesia not only at the site of injury (primary hyperalgesia), but also at an area removed from the injury (secondary hyperalgesia). Secondly, hyperalgesia may be present with regard to one stimulus modality, but not another. For example, in the zone of secondary hyperalgesia mechanical hyperalgesia can be demonstrated to coexist with heat hypalgesia. Another example, is that intradermal bradykinin in man produces heat, but not mechanical hyperalgesia. The neural basis for these dissociated hyperalgesic states probably resides primarily in central mechanisms. Recent evidence with cutaneous nociceptors in monkey, however, suggest that after exposure to inflammatory mediators some nociceptors sensitize to mechanical but not heat stimuli, and still others sensitize to heat but not mechanical stimuli.

The final issue to be considered concerns a framework by which one may decipher between mechanisms that account for primary hyperalgesia versus mechanisms that account for secondary hyperalgesia. Two variables are critical: where is the injury with regard to the receptive field of the neuron from which recordings are obtained, and where is the testing performed. The injury and the test site may coincide within or outside the receptive field of the neuron being recorded. Alternatively, the test site may be outside of the injury site with four possibilities: both are outside the receptive field, both are inside the receptive field, the test site is inside but the injury is outside the receptive field, and fourthly, the test site is outside but the injury is inside the receptive field. When the test site and injury site coincide, one is studying mechanisms for primary hyperalgesia. When they do not coincide, one is addressing mechanisms for secondary hyperalgesia.

**Efferent functions of nociceptors: reevalutation of axon reflex theory.**

J. Szolcsányi
Department of Pharmacology, University Medical School of Pécs, Hungary

Capsaicin elicits nociceptive reactions from a variety of organs. The activated receptors are the polymodal nociceptors, other chemoceptive receptors with unmyelinated (C) or slowly conducting myelinated (A-delta) axons. They contain multiple, often coexisting neuropeptides (tachykinins, CGRP, somatostatin, opioid peptides). Characteristic feature of these capsaicin-sensitive nerve terminals is that by antidromic or orthodromic stimulation mediators are released from them, which elicit various tissue responses. They include arteriolar vasodilation with enhanced blood flux, neurogenic inflammation in various organs, trophic effects in the skin and stomach in vivo as well as various visceromotar changes (gastrointestinal, respiratory, urogenital tracts), positive chrono- and inotropic effects on the heart, contraction of the iris sphincter both in vivo and in vitro conditions.

It has been suggested that a separate class of primary afferent neurons classified by pharmacological means (capsaicin-sensitive sensory neurons) subserve a dual sensory-efferent function with local neuroregulatory significance. In most types of preparations the above neural responses to chemical stimuli are not inhibited by blocking the axonal conduction with tetrodotoxin or local anesthetics. These responses are not mediated by axon reflexes.

It is concluded that varicosities of peptidergic capsaicin-sensitive fibers at the periphery are sites for both signal initiation and transmitter secretion. Under certain areas and conditions axon reflexes also take places and operate through three types of arrangements: axonal arborisations, coupling between two fibers and conduction between varicosities of the sames fiber. The efferent, non-nociceptive functions of C-polymodal nociceptors and other capsaicin-sensitive nociceptors is emphasized.

CENTRAL PROCESSING. Wm. D. Willis, Jr. University of Texas Medical Branch, Galveston, TX, 77550, U.S.A.

Information from peripheral nociceptors normally reaches the spinal cord through the dorsal roots. However, after chronic nerve injury, some gains access through ventral root afferents (Chung, et al.). Primary afferent terminals, possibly from nociceptors, are found on dorsal horn interneurons and on projection neurons, including spinothalamic tract (STT) cells (Carlton, et al.). Interneurons may be excitatory or inhibitory, and inhibition can be pre- or postsynaptic; inhibitory mediators include GABA and glycine (Carlton). Numerous synaptic terminals on STT cells contain excitatory (Westlund, et al.) or inhibitory amino acids, and a few contain peptides, such as substance P and CGRP (Carlton, et al.).

The responses of STT cells to cutaneous stimulation, like those of many interneurons, can be described in terms of their dominant input. Some show the greatest responses to tactile afferents, whereas others respond best to nociceptive afferents. The nociceptive STT cells can be further subdivided into 2 groups by their nociceptive thresholds. However, the vast majority of STT cells receive at least some convergent input from both tactile and nociceptive afferents (Owens, et al.).

Selective pharmacological blocking agents can be used to investigate the neurotransmitters released during nociceptive transmission to STT cells. CNQX, a non-NMDA antagonist, almost completely blocks transmission from cutaneous afferents to STT cells, whereas AP7, an NMDA antagonist, reduces nociceptive transmission, including the prolonged discharges evoked by intradermal (i.d.) capsaicin administration (Dougherty, et al.). These observations indicate a dominant role of excitatory amino acids (EAAs) in excitatory pathways to STT cells. AP7 also prevents sensitization of STT cells following i.d. capsaicin. An NK1 receptor antagonist, CP966345, also reduces the discharges and prevents the sensitization produced by i.d. capsaicin (Dougherty, et al.).

Changes can be produced in the responsiveness of STT cells in a number of models of human disease, including experimental arthritis (see poster by Westlund, et al.). In addition to the sciatic nerve loose ligation model of Bennett and Xie, signs of peripheral neuropathy can be produced by tight ligation of 1 or more spinal nerves (Chung, et al.). In monkeys, this results in behavioral hyperresponsiveness to von Frey hairs (see poster by Carlton, et al.) and in increased responses of STT cells to mechanical and thermal cutaneous stimuli (Palecek, et al.). In rats, at least, the behavioral hyperresponsiveness is sympathetically maintained (Chung, et al.).

# Session 2:
# NOCICEPTORS AND THE SIGNALLING OF INJURY

## Pain and itch mediated by cutaneous nociceptors

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It is known from experiments employing differential nerve blocks by pressure or ischemia that different classes of nerve fibres mediate different sorts of cutaneous sensations. Both, pain induced by pin prick or by heating stimuli, and itching sensations elicited by histamine are apparently mediated by slowly conducting, mainly unmyelinated afferent nerve fibres. However, it is unclear whether a uniform class of "polymodal" nociceptors or separate classes of units are accountable for burning and itching sensations. Microneurographic studies have shown that most chemosensitive unmyelinated afferent units are responsive to both, mustard oil application (inducing burning pain sensations) and histamine iontophoresis (inducing itching sensations).

In contrast to normal skin in which pain is provoked by punctate pressure or heating via excitation of nociceptors, in sensitized skin painful sensations may be elicited not only by lower levels of usually painful stimuli than normally required, but also by slightly stroking the skin e.g. with a brush. This phenomenon is called allodynia and can be elicited not only in an inflamed skin area itself ("primary allodynia") but also in the surroundings ("secondary allodynia"). Recent experiments have shown that allodynia is mediated by fast conducting sensitive mechanoreceptor units rather than by nociceptors (see E.Torebjörk,this meeting). A similar phenomenon has been observed in the realm of itching sensations: itching may become kindled e.g. in and around a skin area treated with histamine by slight mechanical stimulation (alloknesis).

A hypothesis will be presented on the cooperation of peripheral and central mechanisms that may possibly contribute to primary and secondary hyperalgesia, itch and alloknesis.

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# The Signalling of Injury by Muscle and Joint Afferents

Robert F. Schmidt

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Present day hypotheses about the origin of pain in deep tissues, implicit in the title of this symposium, are based on the idea that pain is an independent sensation with its own specialized apparatus of sensors, conduction pathways and centers. The neurobiology of joint pain is used to exemplify the processes of excitation (transduction) in nocisensors (nociceptors), the subsequent step of transformation, and the peripheral conduction of noxious signals. Results from work on fine afferents from skeletal muscle will be compared to that done on the knee joint to highlight the similarities and dissimilarities in the transmission of noxious signals from these two types of deep tissue.

The cat`s knee joint capsule is densely innervated by fine afferent nerve fibers. Their noncapsular endings ("free nerve endings") consist only of the sensory axon and associated Schwann cells (no myelin sheath, no perineurium). The sensory axon divides into several branches and forms a terminal tree. Each axons forms a series fo spindle-shaped thick segments ("beads") connected by waist-like thin segments (string-of-beads appearance). Evidence will be presented that these beads and the end bulb at the tip of the axon all carry receptive sites (transduction sites), and preliminary data will be presented relating the ultrastructural appearance of these sites to their threshold of activation (which according to our physiological findings ranges from very low to very high).

Some remarks will be devoted to the recent finding that healthy tissues contain nocisensors with threshold so high that they cannot be excited by acute noxious stimuli (silent or "sleeping" nocisensors). However, sensitization of these nocisensors as a consequence of pathophysiological tissue alterations (e.g. by inflammation) will "awaken" them. Sensitization is probably brougth about by algesic substances (mediators of inflammation, e.g. prostaglandins, bradykinin). Some examples of more detailed studies from our laboratory will be presented which deal with the mode of action of several inflammatory mediators, e.g. PGE2, PGE1, bradykinin, serotonin, when applied alone and in various combinations to normal and inflamed tissue.

Finally, reference will be made to the possible involvment of several neuropeptides, such as calcitonin-gene-related peptide, somatostatin, substance P and neurokinin A, in the peripheral encoding of nociceptive events and in the efferent functions which these fibers may have. At last, it will be shown that in a population of slowly conducting joint afferents protein kinase C is likely to be involved in the process of transduction.

CORNEAL NOCICEPTORS: A MODEL TO STUDY POLYMODALITY.

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The cornea of the eye receives a rich sensory innervation from trigeminal ganglion neurons. Pain is the sole sensation that can be elicited by stimulation of the human cornea. Based on their responses to mechanical, thermal and chemical stimuli, corneal A-delta and C sensory units were classified as polymodal nociceptors, mechano-nociceptors and 'cold' nociceptors. Membrane mechanisms underlying polymodality of nociceptive fibers are unknown. We explored whether $Ca^{2+}$ channels are involved in chemical and thermal excitation of polymodal nociceptors, using corneal sensory afferents of the cat as a model. Impulse responses of polymodal nociceptors to application on the cornea of 10 mM acetic acid (pH=4.5) were reduced to about 20% of control by topical application of the $Ca^{2+}$ channel blockers diltiazem (1 mM) and cadmium (2.5 mM) and also by high $Ca^{2+}$ (40 mM), while mechanical sensitivity remained unaffected. Sensitization induced by repeated heating cycles applied on the cornea (35°C to 45°C in 2°C steps) and ongoing activity following noxious heat were also blocked by 1 mM diltiazem. Finally, expression of c-fos protein in trigeminal complex neurons of the rat following chemical irritation of the cornea with 10 mM acetic acid was diminished by ocular pretreatment with diltiazem. These results suggest that a modified $Ca^{2+}$ channel (Konnerth et al. J. Physiol. 386: 603, 1987) is involved in the excitation of nociceptors by chemical stimuli. The suppression by $Ca^{2+}$ channel blockers of chemical sensitivity of nociceptive terminals without affecting mechanical responsiveness, opens a possibility for the use of these agents in the treatment of ocular pain and neurogenic inflammation.

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## When do "silent" nociceptors start to talk to the brain?

Martin Koltzenburg

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The conceptual approach to the neural basis of pain has long been dominated by Sherrington´s definition of the adequate stimuli causing pain. He emphazised the sensory discriminative and protective nature of pain summarized in the statement that "pain seems the psychical adjunct to protective reflexes" (*Cutaneous sensation,* in E.A.Schäfer (ed.), Textbook of Physiology, Vol.2, Pentland, London, 1900, pp.920-1001). It is now taken for granted that specific receptors exist in the skin which could act as the body´s alarm system sensing immediate threat and tissue damage. Although this conventional view can successfully explain many important aspects of pain such as magnitude, spatial and temporal profiles, the generality of this clear concept is now in need for revision with the recent emergence of new data which cannot easily be fitted into the Sherringtonian concept.

In the viscera some forms of severe tissue injury, like perforation of hollow organs, are not necessarily painful and the protective function of visceral pain is not always clear as the body can often not implement useful strategies to counteract internal threats. Furthermore, direct electrophysiological recordings have failed to provide uneqivocal evidence for specific classes of nociceptors in some, but probably not all, viscera. For the large bowel and lower urinary tract, distension stimuli which typically give rise to pain appear to be encoded by an increased discharge intensity of afferent fibres which also respond to non-painful stimuli which are important for the storage and reflex function of these organs.

Evidence also indicates that the structural and functional properties of nociceptive terminals are highly malleable suggesting that the peripheral nociceptive nervous system can adapt dynamically in response to tissue perturbations. One important mechanisms is the recruitment of previously unresponsive afferents. The recruitment of these novel receptors is not achieved by transient noxious stimuli, but requires excessive or sustained tissue damage typically exceeding the working range of conventional nociceptors. It is possible that the receptors are added as a further protective mechanism once the immediate alarm system of the classical nociceptors has failed and permanent tissue damage has been inflicted. The excitability change of the receptor which do not respond initially to noxious mechanical or thermal stimuli have now been directly observed in many tissues, notably, joint, urinary bladder and skin and in many species including rat, cat and primates. The receptors are often activated in inflammatory conditions suggesting that they are preferentially chemosensitive. Importantly, after having been activated some previously unresponsive afferents begin to express a sensitivity for previously ineffective stimuli. So far little is known about the factors which activate the silent afferents, but they appear to be similar, if not identical, to those which are known to sensitize conventional nociceptors. This could mean that the silent afferents do not differ in their basic repertoire of transduction mechanisms from conventional nociceptors. Nevertheless the receptors could entail different functional consequences. Whilst sensitization of classical nociceptors would entail a temporal summation centrally, the recruitment of previously unresponsive afferents would add a component of spatial summation. Furthermore, much of the effectiveness of transmission will obviously depend on the potency of central connections and the type and amount of transmitter released, yet little is known about difference of such properties between conventional and silent nociceptors.

# Session 3:
# SENSITIZATION AND HYPERALGESIA:
# Part I

## Sensitization of Mechanically Insensitive Afferents (MIAs) from Skin

Richard A. Meyer, Johns Hopkins University, Baltimore, MD USA

The properties of conventional nociceptors do not appear to account for several aspects of pain and hyperalgesia after cutaneous injury. This has prompted the speculation that a class of cutaneous nociceptors exists that have not yet been fully characterized. Since these "missing" nociceptors likely do not respond to the mechanically stimuli normally used to search for nociceptors, an electrical search technique was developed to locate the receptive field of cutaneous nociceptors.

In the hairy skin of anesthetized monkey, we used this technique to locate the receptive field of 63 Aδ fibers and 22 C fibers that had extremely high thresholds (greater than 6 bars) or were unresponsive to mechanical stimuli. We refer to these afferents as mechanically insensitive afferents (MIAs). Ten Aδ-fiber MIAs had a short latency response to stepped heat stimuli and could be responsible for first pain sensation. Five Aδ-fiber MIAs and one C-fiber MIA did not respond to mechanical or heat stimuli but did respond to intradermal injection into the electrical receptive field of an artificial inflammatory mixture containing histamine, bradykinin, prostaglandin $E_1$, and serotonin. These chemo-only fibers could be responsible for chemogenic pain. Two MIAs had large, complex receptive fields that might account for the large flare that surrounds an injury.

Since the MIAs in this initial study represented a large proportion of the Aδ-fiber (48%) and C-fiber (30%) population, we sought to determine if the properties of MIAs differed from those of mechanically sensitive nociceptive afferents (MSAs). In a recent series of experiments, we characterized the response of 25 MSAs and 26 MIAs to an intradermal injection of the artificial inflammatory mixture. Differences between MIAs and MSAs were most apparent when the Aδ-fiber and C-fiber nociceptors were considered separately. Aδ-fiber MIAs responded vigorously to the chemical injection and became sensitized to mechanical stimuli after the chemical injection in a manner similar to that observed with Aδ-fiber MSAs. However, Aδ-fiber MIAs had slower conduction velocities and were less likely to become sensitized to heat stimuli. Many C-fiber MIAs responded vigorously to the chemical injection, whereas all of the C-fiber MSAs responded weakly. However, C-fiber MIAs and MSAs were equally likely to become sensitized to mechanical and heat stimuli after the chemical injection.

In conclusion, although MIAs and MSAs exhibit some differences other than mechanical threshold, many properties of MIAs and MSAs are similar. Correlational psychophysical studies are needed to determine whether MIAs and MSAs have unique roles in pain sensation.

INDUCTION OF NOVEL ADRENERGIC EXCITATION OF CUTANEOUS NOCICEPTORS AFTER NERVE INJURY, Edward R. Perl, Dept. of Physiology, University of North Carolina at Chapel Hill, CB 7545, Chapel Hill, NC 27599.

Myelinated fiber mechanical nociceptors (MyHTM) and C-fiber polymodal nociceptors (CPM) from normal nerves innervating normal skin rarely, if ever, are excited by sympathetic stimulation or adrenergic substances. Such observations have led to proposals suggesting activity from low threshold mechanoreceptors in combination with complex central processing for the basis of sympathetically-aggravated (causalgic) pain. Recently we showed several types of peripheral nerve damage to induce a remarkable excitatory reaction of CPM units to both sympathetic stimulation and close arterial injection of norepinephrine. The induced adrenergic excitation of CPM units appears too soon after nerve injury to have involved units that had incurred sufficient damage to have degenerated and regenerated. The adrenergic excitation takes place at the peripheral receptor terminals, not at the point of nerve injury, and enhances sensitization produced by repeated noxious stimuli. In the original rabbit model, these excitatory changes were shown by pharmacological characteristics to be mediated by an $\alpha_2$ type of adrenergic receptor. To examine the generality of the phenomena, the effects of partial-cut nerve injuries was tested on the adrenergic responsiveness of MyHTM units. Under conditions that clearly were associated with development of adrenergic responsiveness by CPM units, MyHTM units showed only a very rare appearance of adrenergic excitation. As part of an effort to determine the processes underlying development of the adrenergic excitation, the presence of mRNAs encoding $\alpha$ adrenergic membrane receptors were examined by <u>in situ</u> hybridization histochemistry on dorsal root ganglia. A DNA probe for the mRNA of $\alpha_2$ receptors was found to label very few DRG neurons in normal animals; however, in preliminary studies an increase of a specific hybridization of this probe was observed in some DRG neurons of injured sciatic nerve segments in some rats. The data suggest an increase in an $\alpha_2$ mRNA in a subset of DRG neurons related to the nerve damage but not in simply damaged neurons with partial sciatic nerve lesions. These observations favor an upregulation of an $\alpha_2$-like receptor in some cases of peripheral nerve damage as a possible explanation of sympathetically-enhanced activity in certain classes of nociceptors. The apparent adrenergic receptor upregulation takes place in uninjured or minimally injured primary afferent neurons and only some nociceptor categories are heavily involved. Thus, in this situation, what nociceptors tell the brain changes and represents a pathophysiological alteration. Such adrenergic excitation in a subpopulation of cutaneous nociceptors could represent the initiating factors in the development of human causalgic-like states.

## Nociceptors - Hot and Sour

Peter W. Reeh, Michael St.Pierre, Kay H. Steen and Astrid Hanisch

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Nociceptors in skin are specifically excited and sensitized by hydrogen ion concentrations that normally appear in inflamed or ischaemic tissues. These effects are of long duration and do not show obvious tachyphylaxis. Previous psychophysical work fails to support this crucial observation, since it did not account methodically for the buffering capacities and counterregulations of the tissue stimulated with low pH-solutions. Thus, the pain sensation produced by experimental tissue acidosis falsly appeared as transient. Using continuous infusion by a syringe pump of buffered saline (pH 5.2), however, allows to produce dose - dependant, burning cutaneous pain for as long as the flow is maintained. This localized sensation is accompanied by impressive hyperalgesia and allodynia to mechanical stimulations.

In inflammation, low pH meets with a number of inflammatory mediators. Such agents combined in an "inflammatory soup" ($10^{-6}$ BK, HIS, 5-HT and $PGE_2$ ) excite only about one third of the polymodal nociceptors in rat skin, *in vitro*, and they only act transiently. Lowering the pH of this compound solution to 6.1 increases the proportion of units driven to 75%, enhances the mean discharge activity by a factor of 3.6 and prevents the tachyphylaxis completely.

Recent evidence from cultured DRG cells (patch-clamp) and from a urinary bladder preparation (neuropeptide release) suggests a close similarity between the excitatory action of capsaicin and of low pH. Results from the rat skin-nerve preparation are conflicting in that distinct numbers of nociceptive afferents are responsive to acid pH but not to capsaicin and vice versa. Ruthenium Red, reported to antagonize both stimulants, readily blocks the capaicin induced nociceptor excitation (and desensitisation) but has no influence at all on the pH and on the heat sensitivity. In addition, Ruthenium Red ($> = 10^{-5}$ M) has excitatory (and sensitizing) effects of its own due to its known interference with multiple ion channels. A more specific antagonist would be needed to elaborate a conclusive picture.

(Supported by the Deutsche Forschungsgemeinschaft)

# EFFECTS OF BRADYKININ ON SENSORY NEURONES

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At the physiological level, bradykinin, a peptide inflammatory mediator, both excites and sensitizes nociceptive afferent nerve terminals, causing pain and hyperalgesia. When administered intrathecally, it has complex effects. Initially, the animals respond with an aversive reaction, which is followed by an antinociceptive effect (Laneuville et al, 1989). Isolated sensory neurones are sensitive to bradykinin, but the relationship between its actions at a cellular level and its physiological role is still unclear.

At the cellular level, the main effects of bradykinin on sensory neurones include: membrane depolarisation, associated with an increase in $Na^+$ conductance (Burgess et al, 1989); activation of phospholipase C, leading to IP3 formation, intracellular $Ca^{2+}$-release (Thayer et al, 1988, Burgess et al, 1989) and activation of protein kinase C (Boland et al, 1991; Francel & Dawson, 1988); activation of phospholipase $A_2$, leading to prostanoid formation (Gammon et al, 1989), and inhibition of slow $Ca^{2+}$-mediated after-hyperpolarisations (Weinreich, 1986). All of these effects may contribute to the excitation and sensitization observed.

Nociceptive afferent neurones are known to express bradykinin receptors of the B2-type at their central, as well as their peripheral terminals (Steranka et al, 1988), and electrophysiological studies show that the central terminals are depolarised by bradykinin acting on B2-receptors(Dunn & Rang, 1990). We have now measured the effect of bradykinin on the release of the sensory neuropeptide, CGRP, from slices of rat spinal cord in response to stimulation of the attached dorsal roots. Bradykinin does not evoke any detectable release of CGRP, but strongly increases the stimulation-evoked release. This action of bradykinin is also mediated through B2-receptors, since it is blocked by the B2-antagonist, HOE140, but not by the B1-antagonist, Des-Arg$^9$-Leu$^8$-bradykinin. It is also prevented by the cyclo-oxygenase inhibitor, indomethacin, suggesting that the bradykinin may be acting through prostanoid release. Consistent with this hypothesis is the finding that various prostanoids, including $PGE_1$, $PGE_2$, and $PGF_{2\alpha}$, mimic the effect of bradykinin. Agents that increase intracellular cAMP, such as forskolin, and the phosphodiesterase inhibitor, IBMX, have a similar effect on stimulation-evoked CGRP release, suggesting that prostanoid-induced activation of adenylate cyclase may account for the effects of bradykinin and prostanoids.

The efficacy of intrathecal aspirin-like drugs in causing analgesia (Devoghel, 1983, Taiwo & Levine, 1988), suggests that spinal prostanoid synthesis may exert a modulatory effect on the nociceptive pathway. However, further experiments will be needed to test the possibility that enhanced transmitter release from the central terminals of nociceptive neurones, mediated through local production of bradykinin and/or prostaglandins, plays any part in the mechanism of hyperalgesia.

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# Session 4:
# SENSITIZATION AND HYPERALGESIA:
# Part II

POLYMODAL NOCICEPTORS AND NEUROGENIC INFLAMMATION IN THE CORNEA.

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Stimulation of sensory fibers by injury leads to local release of neuropeptides that mediate neurogenic inflammation. Excitation and sensitization of nociceptors following lesive stimuli are due in part to the presence of inflammatory mediators. We explored whether topical application of inflammatory substances (BK, 'inflammatory soup') excites polymodal nociceptors in the cornea. Also, the possibility that selective blockade of chemical sensitivity of nociceptors to these mediators leads to a reduction of neurogenic inflammation was studied. Electrophysiological experiments were done in anesthetized cats. Single unit activity was recorded from corneal and corneoscleral sensory fibers. Polymodal nociceptors were identified by their response to 10 mM acetic acid. Topical application in the cornea of the 'inflammatory soup' (BK, $PGE_2$, SP, 5-HT & histamine at $10^{-5}$ M; 7 mM $K^+$) produced, after a variable latency, a vigorous firing of nerve impulses. Pretreatment with $Ca^{2+}$ channel blocker diltiazem (1 mM) significantly reduced the response of polymodal fibers to the 'inflammatory soup'. These results suggest that $Ca^{2+}$ antagonists block the sensitizing action exerted by inflammatory mediators released after tissue injury. Decreased sensitivity of nociceptors to endogenous mediators could reduce neuropeptide release and, thus, neurogenic inflammation. To test this possibility, the effects of $Ca^{2+}$ blockers on two models of corneal neurogenic inflammation were tested. In albino rabbits, diltiazem reduced pain reaction to anterior segment irritation (assessed by motor responses: wiping movements, blepharospasm) and also attenuated pupillary and conjunctival inflammatory reaction to the chemical irritant capsaicin (3.3 mM). Diltiazem also decreased corneal and conjunctival inflammatory signs produced by exposure to ultraviolet light; UV-induced inflammation was similar in both eyes when they were pretreated with TTX, and diltiazem was applied to one side, thus suggesting that the $Ca^{2+}$ antagonist attenuates ocular inflammation through a neurogenic mechanism. (Supported by DGCYT No. PB90-0113 and PTR90-0088).

# Allodynia and Alloknesis: A model

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Experimental evidence for peripheral and central mechanisms contributing to two qualitatively different cutaneous dysesthesias to lightly stroking the skin is reviewed. In one series of experiments, an intracutaneous injection of capsaicin into the human forearm produced allodynia, or tenderness, to stroking within a large area of skin surrounding the injection site (i.s.). In another series, an intracutaneous injection of histamine produced alloknesis (de novo itch or exacerbation of an ongoing itch) to the same mechanical stimulus applied to the skin surrounding the i.s. In both experiments, the spread of the dysesthesia up the arm was blocked by locally anesthetizing a narrow mediolateral strip of skin 1 cm proximal to the i.s. In electrophysiological recordings in human and/or monkey, 1° afferent nociceptive nerve fibers were found that responded to one, both or neither chemical but none of the fibers developed an enhanced sensitivity to stroking the skin. Additional evidence suggested that the sensitization occurred within the central nervous system: (1) When a short acting proximal nerve block was given prior to capsaicin injection into the anesthetized skin, the allodynia was absent or reduced in area, compared with controls, after the block wore off, (2) intraneural electrical microstimulation, evoking touch referred to a restricted region of skin, evoked pain in addition to touch after capsaicin was injected outside, and allodynia developed within, the region, and (3) identified nociceptive spinothalamic tract cells in monkey developed enhanced responses to stroking the skin after capsaicin injection inside their receptive fields.

In further, but preliminary, studies of central mechanisms of alloknesis: (1) pain and/or hyperalgesia blocked, for hours, both itch and alloknesis previously produced by exposure to poison ivy or by subsequent intracutaneous injections of histamine, and (2) electrophysiological recordings from single neurons in the dorsal horn of the cat revealed wide dynamic range cells some of which responded to intracutaneous injections of capsaicin, but not histamine, others to either chemical and still others to neither -suggesting one possible neural code for itch, i.e. the absence of activity in capsaicin (pain mediating) cells coupled with the discharges in histamine (itch mediating) cells.

A model is proposed to explain the above findings. Chemically evoked itch and pain are mediated by two different subpopulations of nociceptive 1° afferent nerve fibers that project to different populations of spinothalamic tract cells in the dorsal horn. Alloknesis and allodynia are produced by activity in two types of noceffective 1° afferents that, rather than mediating sensation, have an effector role in producing a prolonged sensitization of two types of interneurons in the dorsal horn each receiving convergent input from low threshold mechanoreceptive 1° afferents. These interneurons project to correspondingly different types of spinothalamic tract neurons. Peripherally, these types of noceffective afferents project widely in the skin (or are functionally coupled over a wide area of skin). It is proposed that the term "nocifensor neurons" be applied generically to all classes of dorsal root fibers that serve to protect the body including neurons which have roles that are sensory (nociceptors) and/or effector (noceffectors) -the latter modulating physiological functions in the peripheral tissue or within the CNS.

# Pain and hyperalgesia from nociceptive and non-nociceptive inputs

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This presentation reviews present knowledge on the physiological properties of human nociceptors and their capacity to signal pain. It is shown that nociceptors in the skin become sensitized following tissue injury and that such sensitization largely accounts for hyperalgesia to heat and to sustained pressure at the site of the lesion. It is also shown that hyperalgesia to moving tactile stimuli both at the site of a lesion and in a wide surround area is due to an altered central processing of signals in non-nociceptive, probably low-threshold mechanoreceptive afferents that, in the presence of an ongoing input from nociceptive fibres, evoke unpleasant sore sensations described as pain. The central changes are critically dependent on the amount of ongoing afferent input from nociceptive fibres, being increased by warming and reduced by cooling, and the central abnormalities are quickly normalized when the nociceptive input is abolished.

These experimental findings in normal human subjects have interesting clinical implications. Allodynia to gentle touch is a common symptom in neuralgia, regardless of whether the pain condition is relieved by sympathetic blockade or not, suggesting that the tactile allodynia is a consequence of the ongoing pain but not linked with its pathophysiological cause. The reversible character of the experimentally induced pain and tactile hyperalgesia has its clinical counterpart in the rapid relief of background pain and tactile allodynia after sympathetic blockade in patients with sympathetically maintained pain. It is rewarding from the therapeutic aspect that the central changes can be reset very quickly in these patients, even if the pain syndrome has lasted for decades.

While most patients with neuralgia have allodynia to gentle touch, others are bothered by firm pressure, and both forms of mechanical hyperalgesia may coexist. The experimental findings described here suggest that the underlying mechanisms are distinctly different; the tactile allodynia being due to central changes whereas the hyperalgesia to static pressure probably depends on peripheral sensitization of nociceptors to mechanical indentation.

# NOCICEPTIVE PROCESSING IN THE SPINAL CORD DURING ACUTE AND CHRONIC MONOARTHRITIS

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In cats and rats noxious information arising in joints is processed in a subset of spinal neurons which receive either convergent input from skin, deep tissue such as muscle and joint (these cells are usually wide dynamic range neurons) or convergent input from deep tissue and joint (a proportion of these cells are nociceptive specific neurons).

Experiments in acutely spinalized cats have shown how the development of an acute inflammation in the knee leads to considerable hyperexcitability in spinal neurons with joint input. Most neurons with knee input showed increased responses to stimuli applied to the injected knee, to areas adjacent to the knee and to regions remote from the knee such as the ipsilateral paw and the contralateral hindlimb. Further studies revealed similar phenomena in intact cats but the changes were less dramatic. The repeated cooling of the spinal cord demonstrated that the effectiveness of descending inhibition progressively increased during developing inflammation counteracting the development of hyperexcitability on the spinal level. Thus there are at least 3 components which determine the modifications of spinal discharges, i.e. the afferent, the spinal and the supraspinal component.

The experiments with acute arthritis give unambiguous information about the reaction patterns of particular neurons but they do not necessarily allow statements about the neuronal activity in a chronic situation where afferent, intraspinal and descending influences may all be active. We have therefore performed a series of experiments in rats in which we produced a unilateral inflammation in the ankle by injections of Freund's complete adjuvans in the ankle region. These injections evoked a localized inflammation with an acute phase (1-2 days after inoculation) and a chronic phase (several weeks). Populations of neurons have been recorded from in control animals and in animals with unilateral inflammation lasting up to 20 days. Within 20 days there was a progressive reduction in the proportion of neurons which appeared as nociceptive specific. Receptive fields of neurons in rats with inflammation were markedly larger than in control rats with spreading into the abdomen and the tail. In addition there was an increase in the proportion of neurons with contralateral excitatory receptive fields. The mechanical thresholds at the ankle joint were reduced in rats with inflammation. The proportion of spontaneously active neurons was also increased but there was no significant enhancement in the discharge frequency. These experiments show that there are changes in the receptive field and response properties in rats with acute inflammation similar to those described previously in spinalized cats with acute inflammation. It is presumed that similar afferent and spinal mechanisms are at work under acute and chronic inflammation which produce hyperexcitability in spinal neurons with joint input in spite of a presumed increase in the effectiveness of descending inhibition.

# Session 5:
# CENTRAL PROCESSING

## Functional properties of nociceptor-driven cells in the spinal cord

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We have recently argued that the perception of pain is not mediated by a single neurophysiological mechanism and proposed that the different pain states represent diverse expressions of a nociceptive system that can bring into play several different mechanisms (Cervero & Laird, *NIPS*, **6**, 268-273; 1991). In particular, we have considered three different pain states or "phases" and suggested that different neurophysiological mechanisms underlie these pain states. In this talk the functional properties of nociceptive systems in the spinal cord will be discussed in the light of our recent proposal.

*Phase 1 pain: The processing of a brief noxious stimulus.*

Two types of dorsal horn neurones receive inputs from peripheral nociceptors: i) Nociceptor-specific neurones, and ii) Multireceptive or wide-dynamic range neurones. Multireceptive neurones in the dorsal horn receive very convergent inputs from a variety of sensory receptors innervating a large area of skin. These cells are not obvious candidates for a role in distinguishing noxious from innocuous stimuli. However, Multireceptive neurones are very responsive to changes in descending control, good at encoding small changes in the intensity of noxious stimuli and show long-lasting increases in excitability in response to minor noxious inputs. Nociceptor-specific neurones have small receptive fields from which they can only be excited by noxious stimuli. They are well suited to convey precise information about the peripheral location of a noxious stimulus, and to discriminate noxious from innocuous stimuli. The responsiveness of Nociceptor-specific neurones in the superficial dorsal horn are largely unaffected by descending modulation or by non-damaging noxious stimuli, although those located in the deeper layers of the dorsal horn are more like Multireceptive neurones in these respects. The neurophysiological mechanism subserving Phase 1 pain can therefore be viewed as a fairly simple route of transmission from peripheral nociceptors to the brain, with possibilities for modulation occurring at synaptic relays along the way. The simplicity of this mechanism reflects the observation that in humans undergoing Phase 1 pain, there is a close correlation between discharges in nociceptors and the subjective appreciation of pain.

*Phase 2 pain: Nociceptive systems and prolonged stimuli: tissue damage and inflammation.*

Injury and tissue damage evoke an inflammatory reaction as part of the healing process. The pain state produced by tissue damage and inflammation is qualitatively different from Phase 1 pain since in this injured state the responses of the peripheral nociceptors change. As the input to the CNS changes, the responses of the central components of the nociceptive system would also be expected to change. However, it is now clear that nociceptive neurones in the dorsal horn modify their responsiveness in ways that are not merely a reflection of the changes in their inputs. Multireceptive neurones show changes in their excitability that are very easily induced by relatively minor stimuli. Furthermore, the more resistant Nociceptor-specific neurones can also alter their properties when the periphery is damaged or inflamed. These changes in excitability may last for hours, even in the absence of further ongoing stimuli. Some descending control systems acting on the dorsal horn are also altered during the development of a peripheral inflammation. Under these conditions, a close correlation between discharges in peripheral nociceptors and the perception of pain is lost. Phase 2 pain is characterized by its central drive; initially triggered by peripheral inputs but not necessarily maintained by them.

*Phase 3: Abnormal pain states. peripheral neuropathies and central pain.*

Chronic pain syndromes are often the consequence of damage to peripheral nerves or to the CNS itself. These abnormal pains include spontaneous pain, reduced pain thresholds and painful sensations evoked by light touch. These new pain states are characterised by a complete lack of correlation between injury and pain. These pains are expressions of alterations in the normal nociceptive system induced by peripheral or central damage. Under these circumstances the pains are maintained by anomalous CNS activity, driven by either abnormal nociceptors or by low threshold peripheral inputs. The mechanism responsible for each one of the various Phase 3 pain states is probably unique to the individual disease.

## WHAT DOES THE BRAIN HEAR FROM NOCICEPTORS IN DISEASE?

Metaphor in the title of this International Workshop invites to answer with metaphor. We all know what nociceptors tell the brain: a fairly monotonous repetitive code, as illustrated by Zotterman half a century ago.

The question that matters here is: *What does the brain hear out of that message?*

The normal response is consistent: a steady percept, devoid of the intermittency contained in the frequency code that is the message, and of a certain magnitude. But its *quality* is dependent on a number of variables:

Compromise associations with co-activated inputs, established along the itinerary of the afferent volleys.

Excitatory and inhibitory central processing.

Nature of the modality-specific brain station that decodes the input.

Mood of the sensing brain.

While these variables impose a private bouquet on nociceptor-evoked sensations, their consistent common denominator is that they are painful.

Experimental studies on human volunteers have revealed:

Excitation of receptors of cutaneous C-nociceptors by heat, certain chemicals or electrical stimulation of their nerve fibers evokes *burning pain.*

Excitation of receptors of cutaneous nociceptors by low temperature evokes *cold pain.*

Excitation of receptors of cutaneous C-nociceptors by low temperature, in absence of cold specific input, evokes a *burning pain.* Excitation of muscle nociceptors by electrical stimulation of their nerve fibers evokes a muscle *cramp-like pain.*

Experimental studies on patients have revealed:

Excitation of sensitized cutaneous C-nociceptors evokes spontaneous *burning pain,* mechanical hyperalgesia and heat hyperalgesia; both these hyperalgesias are "burning" and are relieved by passive cooling.

Pathological removal of cold specific input releases low temperature-induced pain, which now expresses the *pure burning quality* that C-nociceptors evoke when excited in absence of co-activation with other inputs.

Studies of receptor-response characteristics of muscle nociceptors in patients with chronic muscle pains are not available.

The complaint of chronic spontaneous burning pain, mechanical hyperalgesia (particularly the dynamic subtype) and thermal hyperalgesia (particularly cold subtype) are relatively common in patients with psychogenic painful syndromes.

Examples of human nociceptor syndromes will be presented.

José Ochoa, M.D., Ph.D., D.Sc.

## The Central Release of Neuropeptides following Peripheral Stimuli in Normal and Inflammatory States

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A decade ago a predominant view was that a neuron contained and released one neurotransmitter and hence an important task was to identify the transmitter released from the central terminals of nociceptors. With the recognition of extensive coexistence of neuroactive compounds within primary afferent fibres a more modern phrasing of this problem is to define (a) which compounds are released centrally when noxious stimuli are applied to differing tissues in the normal state, (b) how this alters with the development of pathological states such as inflammation or nerve injury, (c) the half lives of the released compounds, (d) spread following release and (e) the functional consequences of these differing release patterns. This is clearly a more complex question than was originally envisaged.

The tachykinins substance P and neurokinin A have been most extensively studied. Peripheral cutaneous noxious stimuli produce central release of immunoreactive neurokinin A (NKA) which persists for a remarkable period beyond the stimulus and diffuses widely from the site of release. The functional significance of this persistance is obscure but it could act to produce wide spread alterations in neuronal excitability. Central release of ir substance P (SP) is better seen with the development of peripheral inflammation. Thus in experiments studying release in a model of acute arthritis, release of irNKA occurred immediately the joint was injected with kaolin and carageenan, whereas irSP release did not occur for some hours after injection and required joint flexion to elicit release. The release of irSP was then relatively massive.

Recent experiments have shown that microinjection of peptidase inhibitors into the superficial dorsal horn results in a persistence and wide diffusion of released irSP, a situation normally seen with irNKA. Thus an important difference between NKA and SP may be resistance to peptidases and this may determine the sites accessed following release.

Calcitonin gene-related peptide (CGRP) is also released centrally by peripheral noxious stimuli but the relationship to inflammation has not been examined. CGRP inhibits enzymes degrading SP and it is possible that the receptors accessed by SP following release are in part determined by the amount of co-released CGRP.

Spinal release of somatostatin has been observed following peripheral thermal but not mechanical noxious stimuli but the significance of this distinction is unknown. Galanin was not released by peripheral nerve stimulation in the anaesthetized cat but this has not been examined following peripheral nerve section, a condition which results in greatly elevated levels of galanin in dorsal root ganglia. Visceral stimuli have been little investigated for possible central release of neuropeptides.

The relationship of release of these neuropeptides to release of L-glutamate has yet to be defined. Peripheral noxious stimuli do produce release of L-glutamate in the dorsal horn but differentiation between release from primary afferents and that from spinal neurons is more difficult than with neuropeptides.

Collectively these data suggest that a severe peripheral noxious stimulus releases a number of neuroactive compounds in the dorsal horn of the spinal cord and that this is altered by peripheral pathology.

**Does nociceptor activity cause transsynaptic damage to spinal cord dorsal horn neurons?** (G.J. Bennett, A.K. Nachemson and J.M.A. Laird. Neurobiol. and Anesthesiol. Branch, NIDR, NIH, Bethesda, MD 20892, U.S.A.)

Previous work has shown that a painful peripheral mononeuropathy in the rat is accompanied by signs of transsynaptic degeneration or atrophy (pyknosis and hyperchromatosis) in spinal cord neurons (Sugimoto et al., Pain 42:205-213, '90). These "dark neurons" (DNs) were detected 8 days after the injury; they were of small or medium size, and concentrated in the injured nerve's territory within laminae I-III. They were also present, in reduced numbers, in a comparable position in the opposite dorsal horn. There was no increase in the incidence of DNs in rats sacrificed 8 days after a bilateral control surgery (exposure without nerve injury). In an attempt to determine the earliest appearance of DNs, we began by examining a control group composed of rats sacrificed 2 days after bilateral control surgery. To our surprise, there were many DNs in the lumbar dorsal horn of these rats. The experiments reported here confirmed this finding and show that surgery by itself is sufficient to produce DNs.

Four groups (each n=5) of rats were prepared using sodium pentobarbital anesthesia (50 mg/kg, i.p.) and sacrificed two days later. Group 1 received bilateral surgery to the thighs (a blunt dissection, about 3 cm long, through biceps femoris) and a mobilization of the sciatic nerve. Group 2 was similar except that the nerve was not manipulated. Group 3 received a unilateral surgery with nerve manipulation. Group 4 was anesthetized but not operated. Blocks from the lumbar (L4-L5 junction) and cervical (C6) cord were processed as described by Sugimoto et al. DNs were identified by a survey of the entirety of each dorsal horn using a 100X objective and plotted onto camera lucida drawings of each section. The incidence of DNs for each region of each rat was estimated by the mean of counts from 3-5 sections. Group data are given as the mean $\pm$ S.D. of the number of DNs per hemisection).

All four groups had a uniformly small number of DNs in the cervical sections (Groups 1-4, respectively: $2.8 \pm 1.8$; $3.6 \pm 1.5$; $2.5 \pm 1.0$; $3.0 \pm 1.4$). There were no statistically significant differences in the cervical counts in between-group or within-group (side-to-side) comparisons. The counts of lumbar DNs in Group 4 (anesthesia without surgery) were not different from the cervical counts ($2.8 \pm 1.0$). Statisticlly significant increases (with respect to a within-group comparison to the cervical counts) in the incidences of lumbar DNs were found in all rats receiving surgery. The lumbar DNs found after surgery were identical in appearance and laminar distribution to those found in the neuropathy cases. Groups 1 and 2 (bilateral surgery) had the highest counts ($8.1 \pm 2.3$ and $6.6 \pm 2.5$, respectively; no between-group or side-to-side differences). Group 3 (unilateral surgery) had significant increases on both sides (ipsilateral: $5.3 \pm 2.0$; contralateral: $4.3 \pm 1.2$; significant side-to-side difference). The increases on both sides found in the animals with unilateral surgery were significantly smaller than the increases found with bilateral surgery.

Unilateral surgery produced DNs on both sides of the cord. The incresed counts in the animals with bilateral surgery are thus almost certainly due to summation of the direct and contralateral effects of each surgery. The uniformly low cervical counts, and the absence of any lumbar increase in the unoperated group, indicate that the lumbar increases following surgery are not due to postmortem artefact or a generalized stress response. We prepose that the activation of nociceptive primary afferents by surgery causes a transient excitotoxic insult to spinal cord neurons. This effect may be involved in the production of postoperative pain and iatrogenic neuropathies.

Titulo: EFFECTS OF MORPHINE ON GAIT PATTERN AND NOCICEPTION IN AN EXPERIMENTAL MODEL OF NEUROGENIC PAIN

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After preoperative measurements of the Sciatic Functional Index (SFI) and withdrawal threshold to noxious radiant heat in both hind paws, a mononeuropathy was produced in 6 rats by loosely constrictive ligatures around the common sciatic nerve in the right hind paw. At the same time a sham operation was performed in the left hind paw. Comparing with preoperative values, a severe disruption of the pattern of walking, indicated by low scores of SFI, was observed in the postoperative (PO) days 1 and 2. Simultaneously, reduced thresholds for withdrawal response to noxious radiant heat in the right hind paw were observed at the same time points, but normal responses in the contralateral paw. Saline i.p. injection in the third PO day did not modify the previous sensorial and locomotoric findings. A single dose of Morphine (2 mg/kg, i.p.) in the seventh PO day improved the walking performance when recorded 60 min after the injection ($P < 0.05$), and induced a normalization in the threshold to noxious heat in the right hind paw (no significant difference vs basal values) togheter with hypalgesic responses in the contralateral paw ($P < 0.05$). The present study reveals the efficacy of morphine in controlling the hyperalgesia due to nerve constriction. The partial, but statistically significant, improvement in the pattern of gait under the effect of morphine support the role of a functional component for the disturbances of walking in this experimental model, perhaps related to allodynia elicited by weight bearing on the affected paw.

# ELECTRON MICROSCOPIC IMMUNOCYTOCHEMICAL DEMONSTRATION OF THE NEUROPEPTIDE CONTENT AND THE PRESYNAPTIC AND/OR POSTSYNAPTIC MODULATION BY GABA-CONTAINING TERMINALS OF UNMYELINATED AND MYELINATED SINGLE IDENTIFIED MONKEY AND CAT NOCICEPTIVE PRIMARY AFFERENTS.

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Lamina I and lamina II (LI and LII) of the spinal cord are known to contain the terminal arborizations of small diameter primary afferents including those responsible for the transmision of nociceptive information from the periphery to the central nervous system. Small calibre primary afferents projecting to LI and LII have conduction velocities in the A-delta (small myelinated) or C (unmyelinated) ranges. Some of these small calibre primary afferents are known to contain a number of neuropeptides including calcitonin gene-related peptide (CGRP) and substance P (SP), however usually it is not possible to correlate peptidergic primary afferent terminals found in ultrastructural immunocytochemical studies with the type of sensory input that they transmit. In addition a third of LI and LII cells are GABAergic and their axons probably arborize in the vicinity of the primary afferent synaptic boutons, however the types of synaptic interactions that GABA-containing terminals establish with the synaptic boutons of different physiological or neurochemical types of small primary afferents are not known.

In this study we examined the morphology, neuropeptide content and synaptic interactions with GABAergic terminals of single physiologically identified primary afferents filled with HRP and analyzed at the ultrastructural level using postembedding immunocytochemical techniques to reveal the presence of CGRP, SP and GABA. Three A-delta High Threshold Mechanoreceptors (HTM) from the monkey and one from the cat and two monkey C-fibres were included in this study. One of the C-fibres was characterized as a polymodal nociceptor. All HRP-filled terminals were studied in LI and LII through serial sections. A number of major differences were noted between A-delta HTM fibres and the two C-fibres: 1) A-delta HTM terminals made more synapses than C-fibre terminals. 2) A-delta HTM terminals contained clear synaptic vesicles of variable sizes but very rarely showed large dense-cored vesicles (LDCVs). In contrast C-fibres contained large numbers of both clear synaptic vesicles and LDCVs. 3) LDCVs inside the C-fibre terminals showed immunoreactivity for CGRP but not for SP. Neither CGRP nor SP immunoreactivity were unequivocally present in our sample of A-delta HTM primary afferent terminals. 4) Two to three GABA-immunoreactive (GABA-IR) terminals usually surrounded the terminals of A-delta HTM fibres and were presynaptic to both the primary afferent terminal and to dendrites postsynaptic also to the primary afferent. Between 20-60% of the terminals in a single A-delta HTM fibre were found to receive GABAergic presynaptic input. Non GABAergic terminals were not found presynaptic to A-delta HTM primary afferent terminals. In contrast C-fibre terminals did not receive any presynaptic input although GABA-IR terminals frequently converge synaptically onto the same dendrites that were postsynaptic to C-fibre terminals.

These differences indicate that the first synapse between nociceptive primary afferents with A-delta or C-conduction velocities probably involve a different assembly of neurotransmitters and different types of modulation by GABAergic neurons.

*(In case of lack of space the first introductory paragraph can be suppressed)*

# EFFECTS OF NERVE INJURY AND SYMPATHECTOMY ON ADRENERGIC RESPONSIVENESS OF DIFFERENT TYPES OF CUTANEOUS NOCICEPTORS

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Several studies have established that neither sympathetic stimulation (SS) nor norepinephrine (NE) excite cutaneous nociceptors of normal animals or produce pain in normal subjects. However, a proportion of partial injuries of human peripheral nerves are followed by a syndrome in which burning pain referred to the partially denervated region is a prominant symptom. The mechanisms underlying such post-traumatic causalgic pain have been controversial. In a recent study, Sato and Perl (Science 251:1608, 1991) showed that over 20% of intact C-fiber polymodal nociceptors (CPMs) in a partially injured nerve are excited by SS and/or NE. This adrenergic excitation is blocked by alpha$_2$ catecholamine receptor antagonists and takes place at the receptive terminal region of the CPM units. In order to further explore the extent and the basis of the change in nociceptor adrenergic responsiveness, two sets of experiments were carried out.

The response to SS or NE of single A$\delta$ high threshold mechanoreceptors (A$\delta$-HTM) was assessed in intact and partially injured nerves. A partial cut was made in the great auricular nerve of anesthetized rabbits. One to 4 weeks later, under deep anesthesia, the distal part of the cervical sympathetic trunk was arranged for electrical stimulation (SS) and a branch of the great auricular artery was cannulated for close arterial injection of norepinephrine in the pinna. The great auricular nerve was transected proximal to the lesion and fibers were teased until discharges could be identified from single A$\delta$-HTM units (mechanical threshold range from 3.25gr/mm$^2$ to 153.75gr/mm$^2$). Each unit was tested for its response to graded mechanical stimulation, cooling, SS, NE, and noxious heat (>45$^{\circ}$C) in that order. None of 19 A$\delta$-HTM tested in intact nerve responded to either SS or NE. In a sample of 48 A$\delta$-HTM (unresponsive to cooling or noxious heat) recorded from 29 rabbits 4-28 days after partial nerve lesion, two were excited by SS and one by NE. One of four A$\delta$ units that responded to innocuous cooling was excited by SS and NE, and another was excited only by SS. The adrenergic excitation of these two A$\delta$ cooling units consisted of 12 to 50 impulses at latencies of 3 to 20 sec.

A second set of experiments is examining the effects of interuption of the sympathetic innervation. Under deep anesthesia, the superior cervical ganglion was removed in adult rabbits 1-4 weeks before recording the response of CPM and A$\delta$-HTM units to von Frey stimulators, cooling, NE, and noxious heat. In preliminary observations, about one third of 23 CPM units respond weakly (1-4 spikes) to NE. Some of these CPM units began marked spontaneous activity 10 to 20 min following NE injection. (Conduction velocity ranged from 0.67 to 1.21 m/s and mechanical thresholds between 3.25gr/mm$^2$ and 9.9gr/mm$^2$.) All of another six C-fiber units responding vigorously to cooling were excited by NE. None of 5 A$\delta$-HTM identified in the sympathectomized series were excited by NE.

In conclusion, A$\delta$-HTMs only rarely undergo the changes in adrenergic responsiveness after nerve injury seen for CPM units. Preliminarily, it appears that disruption of the sympathetic innervation alone produces some form of adrenergic excitatory influence on CPM units, but this seems to be less striking than that observed after mixed nerve injury.

## ANALYSIS OF BEHAVIORAL, ELECTROPHYSIOLOGICAL AND ANATOMICAL CHANGES IN A PRIMATE MODEL OF EXPERIMENTAL PERIPHERAL NEUROPATHY

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In humans, chronic pain following nerve injury represents a difficult therapeutic problem since the underlying pathophysiological mechanisms are unknown. In the present study, we adapted a recently developed rat model of experimental peripheral neuropathy (Chung et al.,'91) to the primate. Elucidating the neuroplasticity occurring with this injury may clarify underlying neuromechanisms which might be clinically exploited. In two monkeys (M. fascicularis), the $L_7$ spinal nerve was tightly ligated. Compared to presurgery levels, both animals exhibited increased behavioral responsiveness bilaterally to application of innocuous stimuli (Von Frey hairs or brush) as well as noxious stimulation on the ventral surface of the feet. This increased responsiveness occurred throughout the 14 day survival period and was more dramatic on the operated side. Electrophysiological studies demonstrated that spinothalamic tract cells ipsilateral to the injury showed increased responsiveness to brush, cold and heat. Presumably such changes would underlie mechanical and cold allodynia and hyperalgesia. Immunohistochemical studies in the dorsal horn demonstrated a decrease in SP and CGRP and an increase in glial and galanin immunostaining ipsilateral to the injury. These preliminary findings suggest that we have a successful adaptation of a neuropathy model in this species. More importantly, the behavioral and electrophysiological data support the presence of allodynia and hyperalgesia associated with this model.

# A SCIATIC LOOSE LIGATURE PRODUCES IPSI- AND ADJACENT NEUROPATHIC HYPERALGESIA IN RATS

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The loose ligature mononeuropathy is a novel animal pain model that has recently become a focus of research interest. When ligatures are loosely tied around the common sciatic nerve of a rat, a sciatic mediated hyperalgesic response to heat rapidly develops over the plantar surface of the hindpaw (PSH) (Bennett and Xie 1988). A pressure hyperalgesia over the dorsum of the hindpaw has also been reported, but the peripheral nerve mediating this hyperalgesia has not been identified (Attal et al 1990).

This study addresses the question of whether the loose ligature mononeuropathy can induce a saphenous nerve mediated heat and/or pressure adjacent neuropathic hyperalgesia (ANH) in the rat. We also examined the effect of sciatic loose ligature on sciatic nerve mediated heat and pressure withdrawal thresholds. We produced a mononeuropathy in rats by unilateral sciatic loose ligation. A prolonged reduction in the mean withdrawal threshold to heat and pressure over the medial dorsum of the hindpaw (MDH), and to heat over the plantar surface of the hindpaw (PSH), was observed on the loose ligature side. There was no significant side to side pressure threshold difference over the lateral dorsum of the hindpaw (LDH). MDH hyperalgesias induced by sciatic loose ligature were mediated by the saphenous nerve. This ANH resembles the saphenous mediated ANH observed over the MDH following sciatic transection. The heat hyperalgesia over the PSH and the LDH pressure threshold difference, following loose sciatic ligation, are mediated by the sciatic nerve.

## BEHAVIORAL CHANGES INDUCED BY MORPHINE IN RATS PLACED ON THE HOT PLATE

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The objective was to analyse, from an ethological point of view, the evolution of behavioral changes induced by morphine-treatment in rats placed on the hot plate. Forty male Wistar rats (200-300 g) were employed. They were divided into saline (n=10), 3 mg/kg (n=10), 6 mg/kg (n=10) and 9 mg/kg morphine-treated (n=10) groups. Morphine ($SO_4H$) was injected subcutaneously. Five hot plate tests were carried out for each rat: the first one inmediately before injection and thereafter four tests at 60, 120, 180 and 240 min. The platform (25x25 cm) of the hot plate (Socrel DS27) was maintained at 55±.5°C and the cut-off time was 25 s. The compartment of the apparatus (15x15x45 cm) was covered to prevent the escape of the rat. Behavior was videotaped and analysed by a 13-pattern ethogram, a computer and a software package made up to this end. Several descriptive parameters were quantified and a cluster analysis based on similarity values between patterns was employed. Statistic treatment was based on ANOVA and Newman-Keuls tests. Significant differences were found in patterns such as leaning posture, immobile exploration, forepaw-licking, hindpaw-licking, stamping, jumping off, limb withdrawal and resting. They were affected during different tests: second and third ones (immobile exploration, stamping, resting), second and fifth tests (hindpaw-licking), last three ones (forepaw-licking), fourth test (limb withdrawal) or throughout all the experiment (leaning posture, jumping off). A clear hypoalgesic effect was found during the second test in all patterns evoked by the noxious stimulus. Moreover the resting element was displayed after morphine treatment. An unexpected hyperalgesic effect was observed beyond the second test in three patterns: forepaw-licking (third, fourth and fifth tests), limb withdrawal (fourth test), and hindpaw-licking (fifth test). This phenomenon was not noticed in other elements which were also evoked by the heat noxious stimulus such as stamping or jumping off. Exploratory (walk-sniff, rearing), self-grooming (face-washing, body-cleaning) and alert (freezing) elements were not modified by opioid treatment. Cluster analysis revealed that the structure of behavior was dramatically changed after morphine injection. Thus clear-cut normal categories were lost and patterns such as limb withdrawal and resting became highly associated with immobile exploration. Results suggest that: i) neural systems related to above mentioned elements are modulated in a different way by opiates, and ii) there is an hyperalgesic rebound effect after morphine analgesia that is revealed by changes of paw-licking elements and limb withdrawal. In conclusion, ethological methods are very useful to assess behavioral rat's changes after morphine treatment during hot plate tests (supported by a Spanish-French Integrated Action).

"ANTINOCICEPTIVE EFFECTS OF KAPPA OPIOIDS: FUNCTIONAL EVIDENCE FOR DIFFERENT KAPPA RECEPTOR SUBTYPES".

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Intravenous kappa opioids are as effective analgesics as morphine after peripheral noxious stimuli (Parsons & Headley, 1989; Herrero & Headley, 1990). However, the potency of kappa agonists is greater in sham spinalized rats than in those with the cord transected (Herrero & Headley, 1991). There have been suggestions that kappa receptor can be subdivided, although the evidence is equivocal (Traynor, 1989). We have now examined this issue comparing the relative potencies of five kappa compounds on reducing spinal reflexes elicited by noxious stimulation. U50,488, U69,593, PD117,302, CI977 (Hunter et al, 1990) and GR103,545 (Hayes et al, 1990) were all given i.v. in a log2 cumulative regime, in rats anaesthetized with alpha-chloralose (Herrero & Headley, 1991). Reflexes were recorded as single motor unit (s.m.u.) responses to 15 sec noxious pinch, repeated every 3 min, in rats submitted to either spinalization (n=43) or sham spinalization surgery (n=48).

ED50 values in spinalized animals were 6mg/kg for U50,488, 5mg/kg for U69,593, 3mg/kg for PD117,302, 0.3mg/kg for GR103,545 and 0.2mg/kg for CI977. These values were lower when the cord was kept intact: 1.5 fold for PD117,302, 4-8 fold for U-50,488, U69,593 and CI977 and 60 fold for GR103,545. The parallelism showed by the dose-response curves in sham versus spinalized rats was different, with a ratio of <=1 for U50,488, U69,593 and PD117,302 and >40 for CI977 and GR103,545. All drug effects were reversed by naloxone at different doses. 0.1mg/kg of naloxone caused more than 50% reversal of all agents except GR103,545 (25%). Lastly, U50,488 and U69,593 showed a clear hypertensive effect whereas PD117,302, CI977 and GR103,545 did not produce a clear variation in the blood pressure

These differences suggest that two kinds of behaviour were elicited with these five kappa opioids. One was observed with U50,488 and U69,593 whereas the other was with GR103,545. Although PD117,302 and CI977 shared common characteristics. PD117,302 had some similarities with U50,488 whereas CI977 did with GR103,545.

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CALCIUM SPIKES AND CALCIUM PLATEAUX EVOKED FROM DISTAL DENDRITES OF TURTLE SPINAL MOTONEURONES BY APPLIED ELECTRIC FIELDS. J. Hounsgaard* and O. Kiehn*, Inst. of Neurophysiology, Univ. of Copenhagen, Blegdamsvej 3C, DK-2200 Copenhagen N., Denmark.

In motoneurones in transverse slices of the turtle spinal cord nefidipine insensitive Ca spikes are promoted by TEA while nefidipine sensitive Ca plateaux are promoted by 5-HT and apamin (Hounsgaard et al. J. Physiol. 398: 575-589, 398: 591-603, 414: 265-282). We have used differential polarization by applied electric fields (Chan et al., J. Physiol. 402: 751-771 and 409: 145-156) to determine the compartmental origin of the two Ca mediated regenerative responses. During experiments the transmembrane potential was measured at the motoneuronal soma while electric fields were established by passing current between plate electrodes on either side of the preparation. Synaptic responses were minimized by the presence of TTX, 2APV, CNQX bicuculline, strychnine and picrotoxin.

In the presence of TEA electric fields in the ventrodorsal or the mediolateral direction could evoke Ca spikes independent of the polarity of the field and of the membrane polarization at the soma. In the presence of apamin Ca plateaux were generated by the same regime of differential polarization that was used to generate Ca spikes.

The results show that distal dendritesin motoneurones can support Ca spikes and Ca plateaux. This suggests that voltage dependent current generators are involved in local processing of synaptic responses in the dendrites of motoneurones.

**EVIDENCE FOR A PROJECTION FROM THE POSTERIOR NUCLEUS OF THE THALAMUS TO THE LATERAL ENTORHINAL CORTEX IN RAT. A BASIS FOR PAIN MEMORY?.** R. Insausti[1] and J.L. Gil[2]. Department of Anatomy, University of Navarre, Pamplona [1] and Department of Neurosurgery, Hospital of Navarre, Pamplona[2].

The memory processing of painful sensations, although highly relevant for the survival of animal species and man, remains largely unknown.

Anatomical evidence exists that some midline and intralaminar thalamic nuclei innervate the entorhinal cortex and the hippocampus . However, a more direct connection between nociceptive-related structures of the brain and the hippocampal formation is still lacking.

We have explored the possibility of such link by systematically placing iontophoretic deposits of the retrograde tracer horseradish peroxidase conjugated to wheat germ agglutinin (WGA-HRP) into various portions of the rat entorhinal cortex, as it is known that different cortical and subcortical structures project to the hippocampus through the perforant path, which originates in the entorhinal cortex.

The entorhinal cortex was directly exposed through a craniectomy in fourteen sodium pentobarbital anesthetized animals, and a glass micropipette (O.D.>10$\mu$m) was lowered into the brain under visual guidance. A 3% solution of WGA-HRP in saline was injected (4-12 $\mu$A for 10-20 min) at various rostrocaudal and mediolateral levels of the entorhinal cortex. After survival times under 24 h, the animals were perfused transcardially with 1% paraformaldehyde and 1.25% glutaraldehyde in phosphate buffer. The brains were sectioned at 50 $\mu$m in a freezing microtome and reacted according to Mesulam's protocol.

We found retrogradely labeled neurons and indication of anterograde transport of the tracer in the dorsolateral portion of the posterior nucleus of the thalamus (Po) in the group that had the deposits in the proximity of the rhinal fissure. In contrast, more medially placed deposits did not show labeling in Po, although they labeled, as in the previous group, midline thalamic nuclei such as centralis medialis, paraventricular and reuniens. In addition, a few retrogradely labeled neurons were present in mid and rostral portions of the suprageniculate nucleus of the medial geniculate nucleus of the thalamus (SG). Deposits located in the immediately adjacent perirhinal cortex also resulted in labeled neurons in Po and SG, the latter being heavier than after entorhinal cortex injections.

Our findings suggest that thalamic nuclei that receive nociceptive input are able to directly relay this input to the entorhinal cortex, thus making feasible the processing of this information through memory related circuits. This assumption is reinforced by the fact that a similar projection was found to perirhinal cortex, a region known for projecting heavily to the entorhinal cortex. The specificity of this projection to the strip of entorhinal cortex closest to the rhinal fissure favors the involvement of the septal pole of the hippocampus, as this region projects preferentially to this part of the hippocampus.

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INCREASED NEURONAL LABELING FOR FOS PROTEIN IN THE SPINAL CORD AFTER NERVE INJURY. Kajander, K.C. and A.M. Madsen. Departments of Oral Science, and Cell Biology and Neuroanatomy, Schools of Dentistry and Medicine, University of Minnesota, Minneapolis, MN 55455, USA.

The product of the *c-fos* proto-oncogene, Fos protein, which binds to DNA, has been shown to increase in neurons in the spinal cord in response to painful peripheral stimuli. We were interested in the effects of peripheral nerve injury on expression of Fos protein in spinal neurons. Two different models of nerve injury were used. In one model, the chronic constriction injury (CCI), the left common sciatic nerve was loosely tied with four chromic gut ligatures. In the other model, the sciatic nerve was transected.

Immunohistochemical techniques were used to evaluate changes in Fos labeling in the spinal cord at times between 1 and 20 days after nerve injury. At selected times, rats were deeply anesthetized and perfused transcardially with 4% paraformaldehyde. After post-fixing, L-4 and L-5 spinal segments were sectioned (30 μm thick) and Fos expression was localized with an antibody directed against the M-peptide sequence of Fos protein and visualized using the peroxidase-antiperoxidase method. Locations of labeled cells were mapped using a drawing tube at 20x.

There was an increase in labeling for Fos protein in both models in the ipsilateral spinal cord one day after injury. This increase primarily occurred in the dorsal spinal gray matter and was significant for the CCI up to and including day five ($p<0.05$, ANOVA). A significant increase was not maintained after transection.

These data suggest that Fos expression increases and is at its greatest soon after a peripheral nerve injury, and that the increase is greater after CCI than after transection. In addition, the increase occurs in areas of the spinal cord thought to be important in sensory processing. This research was partially supported by NIH Training Grant #DEO7098.

## EVANS' BLUE PLASMA EXTRAVASATION AFTER ANTIDROMIC STIMULATION OF FINE NERVE STRANDS

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Antidromic stimulation of unmyelinated afferents leads to vasodilatation associated with plasma extravasation. This results in the leakage of Evans' blue dye from vessels in the vicinity of the stimulated peripheral nerve ending. The possible occurence of blue spots after simulation of unresponsive units was expected to provide hints to the localization of cutaneous terminals of these presumptive "sleeping" nociceptors.

Ten male Wistar rats were anaesthetized using 100 mg/kg thiopental (7 animals) or 120 mg/kg thiobutabarbital (3 animals). The left saphenous nerve was exposed in a paraffin pool and small filaments containing up to 5 C-fibres in one case 8 unmyelinated afferents were prepared. After intravenous Evans' blue injection (50 mg/kg) single filaments were electrically stimulated (2Hz, 0.5ms, 3V, 2min) using the nerve strand as an anode. Spots of dye leakage were documented on a schematic drawing of the hindlimb. Receptive fields were determined following antidromic stimulation (with special respect to the blue spots). Peripheral nerve terminals of unresponsive units were localised using transcutaneous electrical stimulation with maximal strength of 100V.

In 10 filaments 23 unmyelinated fibres were studied in detail. Antidromic stimulation did in no case result in ongoing activity. Out of 10 mechano-heat sensitive C units 7 produced dye leakage at their receptive fields. Another 5 mechanoreceptive fields not responsive to heat or cold were detected inside stained spots. Six units (3 mechano-heat- 1 mechano-cold- and 2 mechanosensitive) failed to produce plasma extravasation. Mechanical thresholds were within a range of 1 to more than 362 mN. Heat thresholds ranged from 44 to 49 °C. No mechano- or thermoreceptive field was found for 5 fibres associated with blue spots. For these fibres action potentials could be elicited using transcutaneous electrical stimulation applied to the blue spots.

In conclusion, we find that the majority of mechano-heat sensitive C-fibres can produce plasma extravasation after antidromic stimulation. However, dye leakage was also observed to result from units belonging to other classes of sensory C-fibers, so from purely mechanosensitive and from unresponsive units.

## Effects of an experimental peripheral neuropathy on neurogenic plasma extravasation in the rat

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Peripheral nerve injury may produce painful syndromes known as causalgia, reflex sympathetic dystrophy etc., particularly if the injury is to a nerve supplying a limb. The affected area often shows abnormal temperature regulation, oedema and trophic changes. These characteristic signs are usually ascribed to dysfunction of the sympathetic nervous system, but it is also possible that they could be related to the efferent actions of fine afferent fibres and the peptides they contain. A sub-group of patients with hot skin, oedema and pain relieved by cooling have been described; in these patients spontaneous activity in the damaged nerve appears to produce sensitized nociceptor endings and also vasodilatation and plasma extravasation via an axon reflex mechanism (Cline et al 1989).

The induction of a constriction injury of one sciatic nerve in the rat (by loose ligation of the nerve) results in evidence of abnormalities in pain sensation in the animals, and also in temperature asymmetries and trophic changes similar to those seen in patients (Attal et al 1990, Bennett & Xie 1988, Wakisaka et al 1991).

In the present study, plasma extravasation in the territory of the sciatic nerve has been examined (by extracting Evan's Blue dye from the skin) in normal rats and in rats 4, 10 and 28 days after the induction of a constriction injury of one sciatic nerve. The amount of extravasation was measured after a period of no intentional stimulation, after electrical stimulation of the sciatic nerve at C-fibre intensity, and after the application of a 5% mustard oil solution to the skin.

We find that : 1) There is unlikely to be a large contribution from an axon reflex mechanism to the temperature abnormalities seen in this model since the time course of changes is different, though there was a small increase in the amount of dye extracted from the injured side after a period of no intentional stimulation in the 10 and 28 day groups as compared to the control group. 2) A marked decrease in the amount of plasma extravasation evoked by electrical stimulation of the nerve in the 10 and 28 day groups as compared to the control group was seen, and is likely to be due in part to the known decrease in the number of unmyelinated fibres in the injured nerve, but also perhaps to a decrease in the level of peptides in the nerves. 3) There was no change in the amount of extravasation evoked by the application of 5% mustard oil in the nerve-injured animals compared to control, despite the known loss of unmyelinated fibres, which indicates the possibility of some change in the properties of the receptors or of the skin in the affected area.

# Studies on the pre-synaptic control of primary afferent nociceptive neurotransmission by endogenous inhibitory mechanisms.

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With their high spatial resolution (100 $\mu$m), antibody microprobes provide an excellent tool for the study of neuropeptide release in the spinal cord following peripheral noxious stimulation. As the tachykinins substance P (SP) and neurokinin A (NKA) are released locally in the region of the substantia gelatinosa in response to tibial nerve stimulation at C-fibre strength, it is probable that this release is from primary afferent terminals of nociceptive origin.

Presynaptic control of transmitter release from the central terminals of nociceptors has been proposed as being important in several mechanisms of analgesia, and the present studies have thus examined a number ofcompounds for effects on the spinal release of either immunoreactive (ir) SP or ir NKA following noxious peripheral stimulation in the spinalized cat. We have shown that systemic analgesic doses of morphine (5 mg $kg^{-1}$ i.v.) were ineffective in reducing noxious stimulus evoked release of ir NKA. Intraspinal injections of the $\alpha$-2 adrenoceptor agonists noradrenaline ($10^{-3}$M) or medetomidine ($10^{-3}$M), were both ineffective in inhibiting noxious stimulus evoked release of ir SP, whereas neuropeptide Y (NPY) ($10^{-5}$), administered in the same manner produced a statistically significant reduction in the release of ir SP in response to the same stimulus parameters.

We conclude that there is no evidence from these studies to support the involvement of a pre-synaptic mechanism at the first synapse in the spinal analgesia produced by morphine or the $\alpha$-2 adrenoceptor agonists. Such pre-synaptic mechanisms may however, explain the analgesic action of NPY at the level of the spinal cord.

## Transneuronal signals affecting the properties of polymodal nociceptor afferents after peripheral nerve injury in rats

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In previous experiments we have shown that transection and subsequent regeneration of the saphenous nerve on one side of a rat reduces the ability of the contralateral saphenous nerve to evoke plasma extravasation in response to antidromic nerve stimulation (Allnatt et al., 1990). This effect is not due to a reduction in axon numbers on the contralateral, unoperated side and so the implication is that the properties of the nerve fibres themselves - in this case the polymodal nociceptor afferents - have been changed. This effect is evident at 2 weeks after injury and it persists until at least 20 weeks. It occurs after nerve transection and regeneration but not after transection and ligation, where regeneration is deliberately prevented. We have also found that the effect is restricted to homologous pairs of nerves: for example, injury of the saphenous nerve on one side does not affect the plasma extravasation evoked by sural nerve stimulation on the contralateral side and *vice-versa*. Our proposal is that a transneuronal signal is involved in initiating this effect, and at the moment we are trying to find out how specific is this signal.

Careful topical application of capsaicin to the saphenous nerve of a rat results in a long-term reduction in the number of unmyelinated polymodal nociceptor afferents in the nerve (Pini et al., 1990). By carrying out experiments on rats treated in this way we hope to see if transneuronal effects occur after transection of peripheral nerves depleted of polymodal nociceptor afferents. If there is no effect in these treated animals we will have evidence that the transneuronal signal is restricted to polymodal nociceptor afferents in paired nerves on the two sides of the body.

These experiments are in progress at the moment and the poster presented at the meeting would report the up-to-date results.

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INTRACELLULAR CHARACTERIZATION OF NOCICEPTIVE INPUTS TO THE DORSAL HORN USING THE RAT SPINAL CORD-HINDLIMB PREPARATION.

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The isolated spinal cord-hindlimb preparation has enabled the analysis of membrane potential changes in ventral horn neurons during natural stimulation of cutaneous mechanoreceptors in rats (King et al, 1990). Briefly, this technique involves the surgical isolation of the lumbosacral portion of the spinal cord along with the spinal nerves, the dorsal root ganglia and the sciatic nerve attached to the hind limb. The preparation is perfused by warm, oxygenated Krebs in a dual chamber bath. We have used this model in order to characterize intracellularly the responses of dorsal horn neurons to natural noxious and innocuous cutaneous afferent input.

Intracellular recordings from 33 deep (III-VI) dorsal horn neurons were made. The mean resting membrane potential was -68$\pm$6 mV (range -60 to -84 mV) and the mean input resistance was 79$\pm$32 MOhms (range 30 to 140 MOhms). Twenty three neurons responded to a wide range of mechanical stimuli from light touch to strong pinch and were classified as multireceptive. Ten neurons responded exclusively to pinch and were classified as nociceptive specific. The characteristic response of a multireceptive neuron to pinch consists of an initial depolarization of 10-16 mV followed by a sequence of lower amplitude and longer latency EPSPs. In ten of these neurons the depolarization was accompanied by high frequency spiking, in the remaining neurons the response profile was entirely subthreshold. Nociceptive specific neurons responded to noxious stimuli with an initial depolarizations of 8-12 mV, this was accompanied by spiking in 4 cells. The duration of the entire response was extremely long-lasting, up to 20 seconds in some cases and far outlasted the period of mechanical stimulation. The typical response of a multireceptive cell to light touch consists of a depolarization of 5-10 mV with cell firing in 6/23 neurons.

A striking feature of this study is that 50% of the all the neurons recorded from never fired action potentials following cutaneous stimulation, the postsynaptic response consisted entirely of subthreshold EPSPs. This finding raises the question of the physiological significance of such afferent inputs and their possible contribution , if any, to sensory modulation eg. hyperalgesia.

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# CO-INDUCTION OF JUN-B AND C-FOS IN A SUBSET OF NEURONS IN THE SPINAL CORD

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Noxious stimulation in vivo provokes the transciptional activation of several genes which are thought to play an important role in the phenomena of stress and pain. In the rat the expression of the *c-fos* proto-oncogene is rapidly induced upon noxious stimulation in some well defined neurons in the dorsal horn of the spinal cord. Fos proteins are known to associate in transcriptional complexes with the products of the *jun* family constituting nuclear factor AP-1 through a direct interaction which involves the leucine-zipper domain. These considerations prompted us to analyse the expresion of the jun gene family members *c-jun, jun B* and *jun D* in rats subjected to noxious stimulation. We present data indicating that in unstimulated animals the transcipts of the three genes are differentially expressed and abundant within the various laminas of the lumbar spinal cord. Surprisingly, upon induction only the *jun B* transcript is augmented, being co-localized with Fos in a subset of neurons of the medial dorsal horn.

# *MUSCLE NOCICEPTORS IDENTIFIED IN HUMANS: INTRANEURAL RECORDINGS, MICROSTIMULATION AND PAIN*

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*In spite of extensive information available on high-threshold mechanoreceptors and chemoreceptors in animal muscles, receptor response characteristics of the sensory apparatus encoding muscle pain perception in humans remain unknown to date. Using intraneural microstimulation and recording in nerve fascicles supplying human muscle, we have identified sensory units with characteristics of nociceptors.*

*During intraneural stimulation of common peroneal nerve fascicles, seven subjects localized and mapped the areas of deep cramp-like pain projected to muscle. By applying mechanical pressure on the projected fields, receptive fields of mechanoreceptive units with moderate to high threshold could be identified within or adjacent to the areas of projected pain. Through electrical stimulation of the receptive fields and intraneural recording, conduction velocities were measured that ranged 3.1 - 13.5 m/sec for Group III (n = 7) and 0.6 - 1.9 m/sec for Group IV (n = 6) fibers. None of the Group III or Group IV units were spontaneously active. Mean receptive field areas, mapped with a 3 $mm^2$ blunt probe , were 3.2 (± 0.84) and 4.7 (± 1.03) $cm^2$ for Group III and Group IV afferents, respectively. The initial area of projected pain experienced immediately upon intraneural stimulation at threshold for pain sensation ranged from 0.78 - 20.4 $cm^2$. Continuous intraneural stimulation at 10 Hz and steady intensity resulted in gradual enlargement of the painful area. Continuous intraneural stimulation at increasing intensity reaching the limit for bearable pain, resulted in progressive enlargement of the original area of pain, plus referral of painful sensation to areas remote from the receptive field of the stimulated nerve.*

*The present study constitutes the first electrophysiological characterization of both Group III and IV human muscle nociceptors, together with direct endorsement that their activity evokes cramp-like pain. The psychophysical evidence indicates that spatial localization function of muscle pain at threshold intensity can be remarkably accurate initially but it becomes diffuse following further temporal or spatial recruitment of muscle-nociceptors.*

THE N NEURON OF THE LEECH: AN "IN VITRO" MODEL FOR THE STUDY OF POLYMODAL NOCICEPTION.

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The N neuron of the leech segmental ganglion has been described as a high threshold mechanoreceptor that responds to body wall stretching (lateral N cell) or to injury of the connective tissue that surrounds the gut (medial N cell).

A modified preparation of segmental ganglion of the leech attached to the skin (Nicholls, 1968) was developed. Tissues were placed in a perfusion chamber for intracellular recording of nociceptive N-neuron; the ganglion was located in the upper level of the chamber, while the skin, connected to the ganglion by nerve filaments, was placed in a lower inclined platform, to allow perfusion and test solutions to flow downstream, away from the ganglion. Noxius mechanical, chemical and thermal stimuli were applied to the skin. N neurons exhibited a mechanical threshold of 120 mN; they responded with a train of impulses to topical 50 mM acetic acid and were excited by heat (thermal threshold 38°C). Repeated heating sensitized the respond to subsequent thermal and chemical stimuli. These results indicate that N-neuron of the leech behaves as polymodal nociceptive neurons in mammals, making them a suitable model for the study of membrane mechanisms involved in polymodality of nociceptors.

## DISCHARGE BEHAVIOR OF RAT TRIGEMINAL BRAINSTEM NEURONS FOLLOWING CONTROLLED NOXIOUS CHEMICAL STIMULATION OF THE NASAL MUCOSA: EFFECTS OF STIMULUS INTENSITY AND DURATION, INTERSTIMULUS INTERVAL AND HETEROTOPIC CONDITIONING

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The application of defined $CO_2$ pulses to the nasal mucosa provides a method for controlled and repeatable noxious chemical stimulation [Anton et al. 1991, Neurosci. Lett. 123: 208-211]. We recorded from medullary dorsal horn neurons of halothane anesthetized rats to test their response behavior.

In a first series of experiments we used this method to examine the effects of stimulus intensity, duration and interstimulus interval:

1. The neurons could be characterized and classified as WDR or NS cells.
2. The neurons encoded the intensities of the $CO_2$ pulses (2s; 25-100%). In general WDR neurons showed more pronounced discharges than NS neurons, although the average discriminatory capacities of both categories of neurons were not significantly different. Stimulus response functions (SRFs) obtained with short interstimulus intervals (30s) were flatter than those obtained with longer interstimulus intervals (120s). This probably reflects the fatigue of the respective nociceptive primary afferents caused by rapid stimulus repetition rates.
3. When stimulated with longer lasting $CO_2$ pulses (8s; 100%) the neurons showed 3 different types of response behavior: a) strictly phasic, b) phasic-tonic or c) complex discharges (rapidly decaying discharges followed by a second increase in activity outlasting the stimulus duration). NS neurons tended to display phasic-tonic behavior, whereas WDR neurons in most cases responded in a phasic or complex mode.

Our electrophysiological examinations show, that the noxious chemical $CO_2$ stimulation itsself does not evoke hypersensitivity or any signs of inflammation. Previous psychophysical and immunhistochemical data using the same stimulation technique confirm this finding [Anton et al. 1991, Pain, in press; Anton et al. 1991, Neuroscience, 41, 629-641].

In a further series of experiments we examined whether the discharge behavior is altered following a heterotopic irritation. For this conditioning we either applied a mixture of different inflammatory agents (the so-called "inflammatory soup") onto the surface of the ipsilateral cornea or we applied strong radiant heat (55$^{o}$C; 100s) onto the ipsilateral upper lip. Comparing the SRFs obtained before and after the heterotopic conditioning, we observed two different effects:

1. The SRFs of some neurons became steeper following the peripheral irritation, possibly due to central sensitization.
2. In other cases the SRFs became flatter, possibly reflecting the domination of descending inhibitory mechanisms caused by the irritation.

We are performing further experiments to elucidate these phenomena in a more detailed fashion.

# RESINIFERATOXIN AND RUTHENIUM RED AS PHARMACOLOGICAL TOOLS IN STUDIES ON NOCICEPTIVE MECHANISMS

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Resiniferatoxin (RTX) is a naturally occuring substance structurally related to capsaicin. Both RTX and capsaicin act on the same specific pharmacological receptive site situated on primary sensory neurones but RTX is 100-10.000-fold more potent than capsaicin. A new type of nonselective cation channel is operated by this capsaicin receptor. In vitro studies have revealed that the inorganic dye Ruthenium red (RR) inhibits the opening of this cation channel in response to capsaicin or hydrogen ions. The aim of the present paper is to get new information about nociceptive mechanisms and receptorial processes in vivo with the aid of RTX and RR.

Thermonociception of rats was investigated by the leg withdrawal test by measuring the noxious heat threshold (Szolcsányi, 1975). The technique is more sensitive to analgesics than the classical hot plate and tail flick tests where latency for supramaximal stimulation is determined. Subcutaneously applied RTX (100-300 $\mu$g/kg) or capsaicin (150-400 mg/kg) evoked an increase in noxious heat threshold lasting for several days. Similar thermoanalgesia was observed after intrathecal administration of RTX (0.15-0.3 $\mu$g) or capsaicin (15 $\mu$g) and the effect of RTX lasted over two weeks. These data show that primary sensory neurones affected by capsaicin and RTX are activated by noxious heat stimulation and these substances are capable of inhibiting not only the peripheral but also the central endings of these nociceptive afferents. Interestingly, a reversible antinociception was observed after systemically administered RR (4 mg/kg sc.). It is concluded that the cation channel operated by the capsaicin receptor is activated by noxious thermal stimuli at the sensory nerve endings.

Intravenous injection of capsaicin induces substernal pain in man (Winning et al. 1986) and evokes vagal pulmonary chemoreflex consisting of bradycardia, fall in blood pressure and apnea in anaesthetized rats. This latter reflex response was used as a model for determining in vivo pharmacological interactions at the level of sensory nerve endings. In rats RR (0.5-2 mg/kg iv.) inhibited the triple response evoked by capsaicin in a dose dependent manner. Stimulation of the regenerative region of vagal capsaicin-sensitive afferents by intravenously applied veratridine also elicited the pulmonary chemoreflex and this response was not antagonized by RR (0.5-2 mg/kg). It is suggested that in contrast with the hypothesis of Paintal (1988) the primary site of action of capsaicin is at the generator region of sensory receptors. Intravenous injection of RTX failed to evoke the pulmonary chemoreflex up to 5 $\mu$g/kg but dose-dependently (0.01-1 $\mu$g/kg) inhibited the capsaicin-induced reflex response. It is concluded that the function of capsaicin-sensitive sensory receptors can be inhibited by RTX without initial spike generation. This might form the conceptional basis for developing new type peripherally acting analgesics.

**Corneal inputs to neurones in the spinal trigeminal nucleus of anaesthetized rats**

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The mammalian cornea is richly innervated by nociceptive primary afferents (Belmonte *et al*, 1991) whose cell bodies are located in the trigeminal ganglion. In the present study we have recorded the electrical activity of neurones in the subnucleus caudalis of the spinal trigeminal nucleus responding to noxious stimulation of the rat's cornea. Changes in the receptive field properties of these neurones induced by corneal nociceptive stimulation were also examined.

Adult rats were anaesthetized initially with halothane (2.5% in 100% O2) and maintained with sodium pentobarbitone (15 mg/Kg/h i.v.). The animals were paralysed with pancuronium bromide (2mg/kg i.v.) and artificially ventilated. Electrical activity was recorded from neurones in the lateral region of the subnucleus caudalis of the spinal trigeminal nucleus.

We recorded from 49 neurones all of which were located in the superficial layers of the spinal nucleus and were driven by mechanical stimulation of the ipsilateral cornea (threshold responses in the range 0.1 to 2 mN). Thirty-seven of these neurones showed, in addition, a cutaneous receptive field around the eye from which they could be activated by either noxious stimulation of the skin (n=30) or by both, noxious and innocuous stimulation (n=7).

The responses of 5 neurones to graded thermal stimulation of the cornea (from 39 to 51 °C) were also examined. Four of these cells were driven by corneal stimulation only and one by corneal stimulation and by noxious stimulation of the adjacent skin. All cells responded to thermal stimulation of the cornea with thresholds in the range 41 to 43 °C. At the end of the series of thermal stimuli all five cells showed enlarged receptive fields (this effect lasting for at least 30 minutes) that now included the eyelids and adjacent skin. Only noxious stimulation of the skin could drive the cells from their enlarged receptive fields.

These results show that most neurones in the spinal trigeminal nucleus with a corneal input are nociceptive-specific and that their receptive fields can increase in size following corneal noxious stimulation. However, these plastic changes do not seem to alter the basic nociceptive-specific properties of the cells.

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The C-fibre input to secondary sensory neurons in the spinal dorsal horn: amino acidergic and non-amino acidergic components.

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Separate activation of large and small calibre primary afferents (Yoshimura and Jessell, 1990; Urban and Dray, 1991) has made it possible to study the synaptic input of fibres with different modalities to single dorsal horn neurons in *in vitro* experiments.

The hemisected spinal cord/horizontal spinal slice - DRG complex was prepared from young rats and mice. The cord and the DRGs were separated in different compartments of a recording chamber. Primary afferents were excited by chemical and electrical activation of the DRG and the dorsal root, and simultaneous intracellular recordings were made from DRG cells and dorsal horn neurons.

For the selective activation of small calibre fibres with slow conduction velocity two methods were used: (1.) Capsaicin (0.2-1.0*u*M). (2) Electric pulses of high intensity in the presence of 1.0-10 *u*M TTX perfused to the DRG.

To activate large fibres selectively, dorsal roots were stimulated by low intensity electrical pulses. In some experiments DRGs were pretreated with capsaicin (100 *u*M) to inactivate small calibre fibres.

Capsaicin (0.4-1.0 *u*M, 30 sec) excited C-cells in the DRG and evoked a prolonged postsynaptic excitation in dorsal horn neurons. Superfusion of 1.0 *u*M TTX to the DRG did not alter the response. The postsynaptic response in the superficial dorsal horn was attenuated but not blocked by kynurenic acid (100 *u*M) or 2APV (20-50 *u*M) applied to the spinal cord. Brief applications of substance P (0.4-1.0 *u*M, 30 sec) mimicked the effect of capsaicin obtained in the presence of EAA receptor blockers ( 2APV and kynurenic acid).

Repetitive electrical stimulation of the dorsal roots at high intensity and low frequency (0.1-0.5Hz) produced "wind up" in the dorsal horn cells. During the repetitive stimulation (tetanic phase) the membrane gradually depolarised and the number of evoked action potentials increased. After the stimulation was stopped (post-tetanic phase) the membrane potential slowly repolarised to the resting level. In the presence of 2APV the posttetanic phase was partially blocked, but the depolarisation during electrical stimulation was not affected. Pretreatment of the DRGs with capsaicin (100 *u*M, 10 sec) blocked both phases, while TTX did not.

These data suggest that synaptic activation of dorsal horn cells by C-fibers has a EAA- and a non-EAA component. Furthermore it is proposed that the post-tetanic phase of "wind up" is sensitive to 2APV, while 2APV does not prevent the developement of the tetanic phase. Capsaicin pretreatment blocks both phases emphasising the role of C-fibres and the involvement of non-EAA transmitters in the development of "wind up".

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# THE EFFECTS OF OSMOTICALLY ANISOTONIC SOLUTIONS ON SENSORY NERVE ENDINGS IN RAT SKIN, IN VITRO

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The presence of an anisotonic extracellular milieu in inflamed tissues has long been strengthened (cf. Schade 1923). To evaluate possible effects of osmotic stimuli on the endings of primary afferent nerve fibres, we exposed the receptive fields in rat skin in vitro (Reeh 1986) to hypertonic (0.6-1.2osm/l) solutions of sucrose in distilled water or in synthetic interstitial fluid and to hypertonic saline in equal concentrations; half isotonic (0.15osm/l) solutions and Aqua destillata also belonged to the panel of stimuli we applied.
Hypertonic sucrose solutions excited only 9% of the Adelta- and C-fibres tested irrespective of their receptive properties, whereas 55% were activated, i.e. exhibited low frequency ongoing discharge. Thirty-six percent were not affected at all. Hypertonic saline was much more effective as exciting 82% of the Adelta- and C-fibres irrespective of their receptive properties in reproducible and temporally distinct responses. Only 8% of the fine afferents were not affected. However, also half of the 8 Aß-SAI-units, but only 1/4 SAII- and 0/4 RA-fibres tested with hypertonic saline were excited, whereas hypertonic sucrose was ineffective except for a weak activation of 2/4 Aß-RA-units. Hypotonic stimuli and even Aqua destillata had only little effect on the population of thinly and of unmyelinated afferent fibres in that they evoked a stimulus response in only 8% of the units tested; 42% were activated, 50% were unaffected. Ongoing activity appeared in 14% of the fibres after hypertonic saccharose and in 17% after hypertonic saline. The mechanical (von Frey-)sensitivity often underwent a dramatic impairment following the application of hypertonic solutions as well as of distilled water. This was also true for most of the Aß-fibres tested.
Our data deny osmotic hypertonicity by itself to strongly excite nociceptors or other fibres in inflamed tissue. The stimulating effects of hypertonic saline are consistent with the literature (cf. Keele and Armstrong 1964); yet, elevated concentrations of sodium chloride have never been observed in inflamed tissue. Our findings with Aqua dest. leave a certain discrepancy to the results of psychophysical experiments (Keele and Armstrong 1964). Probably, the weak activity elicited thereby and by hypertonic sucrose accumulates to a relevant nociceptive input via spatial summation on convergent spinal neurones.

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## ANATOMICAL, PHYSIOLOGICAL, AND RELEASED AMINO ACID CHANGES PARALLELING THE GENERATION OF ACUTE ARTHRITIS IN MONKEYS

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Glutamate and other excitatory amino acids have been shown to play a key role in nociception and the hyperalgesia associated with the acute inflammatory response. In an effort to more fully understand the role of glutamate in this process, we determined the percentage of glutamate axons in the medial articular nerve (MAN) of monkey, a source of preterminal afferent fibers innervating the knee joint. Glutamate-positive axons were unmyelinated (12%) or were included in the small, thinly myelinated (29%) group in control nerves. After induction of the experimental knee joint inflammation with a kaolin/carrageenan mixture, a doubling in the percentage of small, thinly myelinated glutamate-positive axons corresponding to A delta fibers, was observed on the side of the experimental arthritis as compared to the MAN of the other side or uninjected controls. These increases were greatest after 4hrs of inflammation and were observed only when injection of kaolin/carrageenan was combined with joint flexion and mechanical stimulation in the anesthetized preparation. The effects of the experimentally induced arthritis on immunoreactivity of putative primary afferent neurotransmitter/neuromodulators in the dorsal horn of the spinal cord were examined in the same monkeys. While a significant decrease was found for substance P after 4hrs and CGRP after 8hrs, an increase in immunoreactive staining for glutamate terminals was noted. Extracellular levels of amino acids were measured via a microdialysis probe placed in the lumbar dorsal horn during the development of experimental arthritis in the anesthetized monkeys. Glutamate, aspartate, glycine and serine increased transiently following intra-articular injection of kaolin/carrageenan, while glutamine levels decreased. A second prolonged and often greater phase of release of the same amino acids occurred at about 2hrs after the joint injection. Simultaneous recordings from identified spinothalamic tract neurons in the monkeys during the development of inflammation showed a potentiation of their responses to mechanical stimuli and to iontophoretically applied excitatory amino acids, particularly those acting at non-N-methyl-D-aspartate receptors. The enhancement of both responses, the release of excitatory as well as inhibitory amino acids, and the increased stainability of glutamate in the spinal cord and MAN suggest that glutamate is a major contributor to the hyperalgesia associated with the inflammatory state.

*Workshop on*

*WHAT DO NOCICEPTORS TELL THE BRAIN?*

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247 **Workshop on Pathogenesis-related Proteins in Plants.**
Organized by V. Conejero and L. C. Van Loon. Lectures by L. C. Van Loon, R. Fraser, J. F. Antoniw, M. Legrand, Y. Ohashi, F. Meins, T. Boller, V. Conejero, C. A. Ryan, D. F. Klessig, J. F. Bol, A. Leyva and F. García-Olmedo.

248 Beato, M.:
**Course on DNA - Protein Interaction.**

249 **Workshop on Molecular Diagnosis of Cancer.**
Organized by M. Perucho and P. García Barreno. Lectures by F. McCormick, A. Pellicer, J. L. Bos, M. Perucho, R. A. Weinberg, E. Harlow, E. R. Fearon, M. Schwab, F. W. Alt, R. Dalla Favera, P. E. Reddy, E. M. de Villiers, D. Slamon, I. B. Roninson, J. Groffen and M. Barbacid.

251 **Lecture Course on Approaches to Plant Development.**
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252 **Curso Experimental de Electroforesis Bidimensional de Alta Resolución.**
Organizado por Juan F. Santarén. Seminarios por Julio E. Celis, James I. Garrels, Joël Vandekerckhove, Juan F. Santarén y Rosa Assiego.

253 **Workshop on Genome Expression and Pathogenesis of Plant RNA Viruses.**
Organized by F. García-Arenal and P. Palukaitis. Lectures by D. Baulcome, R. N. Beachy, G. Boccardo, J. Bol, G. Bruening, J. Burgyan, J. R. Díaz Ruiz, W. G. Dougherty, F. García-Arenal, W. L. Gerlach, A. L. Haenni, E. M. J. Jaspars, D. L. Nuss, P. Palukaitis, Y. Watanabe and M. Zaitlin.

254 **Advanced Course on Biochemistry and Genetics of Yeast.**
Organized by C. Gancedo, J. M. Gancedo, M. A. Delgado and I. L Calderón.

255 **Workshop on The Reference Points in Evolution.**
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256 **Workshop on Chromatin Structure and Gene Expression.**
Organized by F. Azorín, M. Beato and A. A. Travers. Lectures by F. Azorín, M. Beato, H. Cedar, R. Chalkley, M. E. A. Churchill, D. Clark, C. Crane-Robinson, J. A. Dabán, S. C. R. Elgin, M. Grunstein, G. L. Hager, W. Hörz, T. Koller, U. K. Laemmli, E. Di Mauro, D. Rhodes, T. J. Richmond, A. Ruiz-Carrillo, R. T. Simpson, A. E. Sippel, J. M. Sogo, F. Thoma, A. A. Travers, J. Workman, O. Wrange and C. Wu.

257 **Lecture Course on Polyamines as modulators of Plant Development.**
Organized by A. W. Galston and A. F. Tiburcio. Lectures by N. Bagni, J. A. Creus, E. B. Dumbroff, H. E. Flores, A. W. Galston, J. Martin-Tanguy, D. Serafini-Fracassini, R. D. Slocum, T. A. Smith and A. F. Tiburcio.

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259 **Workshop on Transcription and Replication of Negative Strand RNA Viruses.**
Organized by D. Kolakofsky and J. Ortín. Lectures by A. K. Banerjee, M. A. Billeter, P. Collins, M. T. Franze-Fernández, A. J. Hay, A. Ishihama, D. Kolakofsky, R. M. Krug, J. A. Melero, S. A. Moyer, J. Ortín, P. Palese, R. G. Paterson, A. Portela, M. Schubert, D. F. Summers, N. Tordo and G. W. Wertz.

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261 **Workshop The Regulation of Translation in Animal Virus-Infected Cells.**
Organized by N. Sonenberg and L. Carrasco. Lectures by V. Agol, R. Bablanian, L. Carrasco , M. J. Clemens, E. Ehrenfeld, D. Etchison, R. F. Garry, J. W. B. Hershey, A. G. Hovanessian, R. J. Jackson, M. G. Katze, M. B. Mathews, W. C. Merrick, D. J. Rowlands, P. Sarnow, R. J. Schneider, A. J. Shatkin, N. Sonenberg, H. O. Voorma and E. Wimmer.

263 **Lecture Course on the Polymerase Chain Reaction.**
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264 **Workshop on Yeast Transport and Energetics.**
Organized by A. Rodríguez-Navarro and R. Lagunas. Lectures by M. R. Chevallier, A. A. Eddy, Y. Eilam, G. F. Fuhrmann, A. Goffeau, M. Höfer, A. Kotyk, D. Kuschmitz, R. Lagunas, C. Leão, L. A. Okorokov, A. Peña, J. Ramos, A. Rodríguez-Navarro, W. A. Scheffers and J. M. Thevelein

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266 **Workshop on Innovations on Proteases and their Inhibitors: Fundamental and Applied Aspects.**
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268 **Workshop on Salt Tolerance in Microorganisms and Plants: Physiological and Molecular Aspects.**
Organized by R. Serrano and J. A. Pintor-

Toro. Lectures by L. Adler, E. Blumwald, V. Conejero, W. Epstein, R. F. Gaber, P. M. Hasegawa, C. F. Higgins, C. J. Lamb, A. Läuchli, U. Lüttge, E. Padan, M. Pagès, U. Pick, J. A. Pintor-Toro, R. S. Quatrano, L. Reinhold, A. Rodríguez-Navarro, R. Serrano and R. G. Wyn Jones.

269 **Workshop on Neural Control of Movement in Vertebrates.**
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