affect brain receptors for progesterone (*17*). Noradrenergic systems display diurnal variations in activity (*18*), and a similar interaction of neurotransmitter activity and steroid binding could occur with estrogens.

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Myelinated Nociceptive Afferents Account for the Hyperalgesia That Follows a Burn to the Hand

Abstract. *Monkeys and human subjects were exposed to a series of thermal stimuli before and after a 53°C, 30-second burn to the glabrous skin of the hand. The responses of C- and A-fiber nociceptive afferents in the monkeys and subjective responses by the humans were compared. The burn resulted in increased sensitivity of the A fibers, decreased sensitivity of the C fibers, and increased pain sensibility (hyperalgesia) in the human subjects.*

Tissue injury, inflammation, and certain nerve injuries may lead to hyperalgesia, which is characterized by spontaneous pain and a decrease in the pain threshold. The neural mechanism of hyperalgesia is probably based on sensitization of the receptors of primary nociceptive afferents. Much attention has been given to the sensitization that may occur in C-fiber nociceptive afferents (*1*) sensitive to mechanical and heat stimuli (CMH), but little attention has been given to the sensitization that may occur in A-fiber nociceptive afferents (*2, 3*) responsive to mechanical and heat stimuli (AMH). In this study we present evidence that AMH's, not CMH's, mediate the hyperalgesia that results from a 53°C, 30-second thermal injury to the hand.

The responses of single nociceptive afferents in anesthetized monkeys were compared with subjective judgments of pain in human subjects. The receptive field of the nociceptive afferents and the human subjects were exposed to an identical sequence of thermal stimuli before and after a 53°C, 30-second burn to the glabrous skin of the hand (*4*). A laser thermal stimulator under radiometer feedback control (*5*) provided stepped increases in skin temperature superimposed on a 38°C base temperature; the area stimulated was 7.5 mm in diameter. The test sequence consisted of ten 3-second stimuli. The stimuli were delivered every 30 seconds. The first stimulus was always 45°C, and the remaining nine stimuli were presented in random order and ranged from 41° to 49°C in 1°C increments. Stimuli in both psychophysical and neurophysiological studies were delivered on the following schedule: test, 10-minute rest, test, 5-minute rest, burn, 10-minute rest, test, 10-minute rest, test. Subjective judgments of pain were measured with the magnitude estimation technique (*6*). Subjects assigned an arbitrary number (the modulus) to the magnitude of the pain evoked by the first stimulus (45°C) of the first test sequence. This stimulus was considered painful by all the subjects. Subjects then judged the painfulness of subsequent stimuli in that run and subsequent runs relative to the modulus. Nonpainful stimuli were assigned a value of zero.

Standard techniques (*2, 7*) were used to record from single fibers of the median and ulnar nerves that innervate the hand in monkeys (*Macaca fasicularis* and *M. mulatta*), which were first anesthetized with pentobarbital. Firm squeezing of the skin was used to search for nociceptive afferents, which could be readily differentiated from low-threshold mechanoceptive units by the vigorous response of the latter to light touching. Threshold for mechanical sensitivity was determined with calibrated nylon monofilaments. Conduction velocity of particular fibers was determined at the end of the experiment by measuring the latency of responses to suprathreshold electrical stimuli applied to the receptive field with intradermal electrodes.

Only fibers whose receptive fields were restricted to the glabrous skin were considered. Fifteen CMH's and 14 AMH's were studied with the protocol described here. An additional 23 CMH's

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and 39 AMH's were studied with other stimulus protocols with comparable results (*8*). The mean conduction velocity, mechanical threshold, heat threshold, and receptive field area for the fibers were similar to those reported previously (*9*).

To compare the various responses, the data were normalized (*10*) by dividing the response to a given stimulus by the response to the first 45°C stimulus (*11*). Before the 53°C, 30-second injury, the normalized response of the CMH's matched that of the human subjects (Fig. 1, A and C) whereas the AMH's showed only a nominal response (Fig. 1B). After injury in the human subjects the skin became hyperalgesic within minutes. Pain was present in each subject without further stimulation. The pain evoked by the test stimuli increased substantially after the burn (Fig. 1A). All the test stimuli were painful, and the mean rating of the 41°C stimulus after the burn was greater than the mean rating of the 49°C stimulus before the burn. Hyperalgesia in response to test stimuli persisted for as long as 2 hours after the burn. This response change parallels that seen in the AMH's but not that seen in the CMH's. As shown in Fig. 1B, the threshold for AMH response to heat was greatly decreased after the burn and the response to suprathreshold stimuli was increased. In contrast, the CMH's showed an increased heat threshold and a decreased response to suprathreshold stimuli.

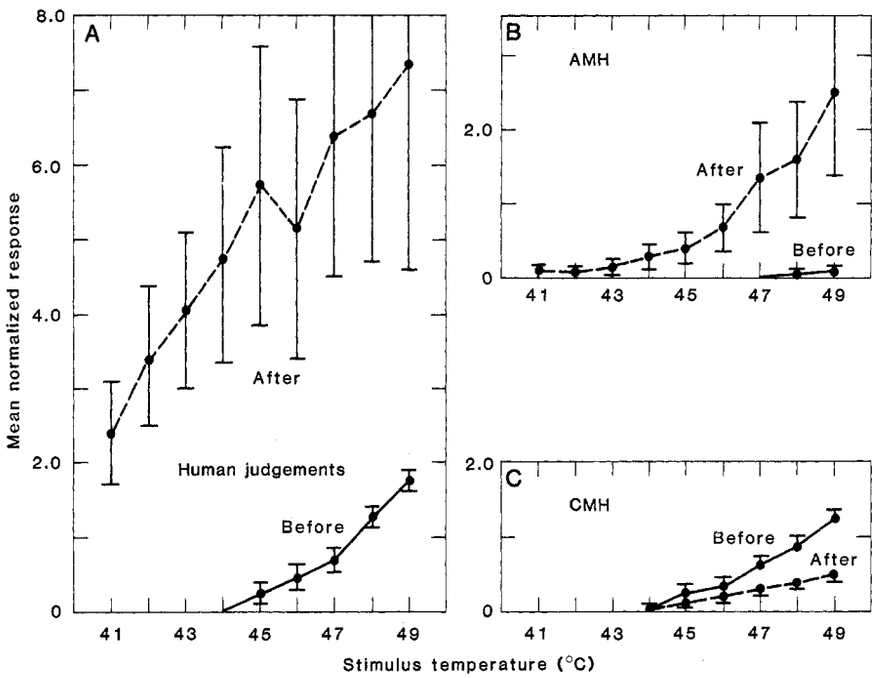

Fig. 1. Normalized responses (*10*) to thermal test stimuli presented in random order 5 minutes before and 10 minutes after the burn to the hand. (A) Human judgments of pain (N = 8). (B) Responses of AMH's (N = 14). The mean response to the initial 45°C stimulus after the burn was 22 ± 11 impulses. (C) Response of CMH's (N = 15). The mean response to the initial 45°C stimulus before the burn was 10.3 ± 1.5 impulses. The burn increased the magnitude of pain (hyperalgesia) perceived by the human subjects; this was matched by an enhanced response (sensitization) in the AMH's. The burn lowered the sensitivity of the CMH's.

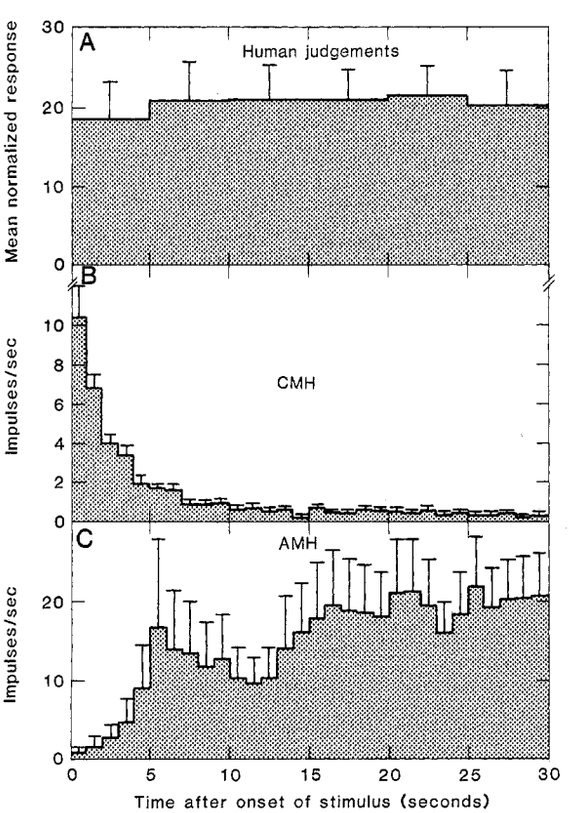

Fig. 2. Histograms showing mean responses during the burn to the hand. (A) Human judgments (N = 8). (B) Response of CMH's (N = 15). (C) Response of AMH's (N = 14). The pain was intense (53°C) throughout the stimulus. The brisk response of the CMH's at the beginning of the stimulus changed to a low rate after 5 seconds. The response of the AMH's increased during the first 5 seconds and remained high throughout the stimulus.

The mean responses during the 53°C, 30-second burn are shown in Fig. 2. The human subjects rated the pain every 5 seconds during the burn. Throughout the burn the pain remained at a level more than ten times higher than that evoked by the 49°C, 3-second stimulus before the burn. The response of the AMH's increased during the first 5 seconds and remained at a high level for the remainder of the stimulus. The CMH's showed a significant initial response that diminished to a nominal level within 5 seconds. Therefore, AMH's appear to code not only for hyperalgesia but also for the pain present during a sustained intense heat stimulus.

If A fibers rather than C fibers are chiefly responsible for the hyperalgesia that follows a thermal injury, then a block of A-fiber function should eliminate hyperalgesia. To test this, a sphygmomanometer cuff was placed on the upper arm in two human subjects and inflated to 250 mmHg 20 minutes after the 53°C, 30-second burn. Motor function and sensitivity to light touching and coolness were gone 40 minutes after initiation of the block, suggesting that action potential conduction in the A fibers was at least partially blocked. Hyperalgesia at the injury site was markedly decreased, although pain evoked by the test stimuli applied to uninjured skin 5 cm from the burn was not reduced. Because a nerve block performed in this

manner preferentially blocks activity in A fibers over that in C fibers (*12*), these data further support the view that hyperalgesia is signaled by activity in A fibers. Since pain sensation in uninjured skin was not attenuated by the block, C-fiber conduction evidently is not interrupted by this block and the pain evoked is probably signaled by activity in the C fibers.

These results indicate that C-fiber nociceptive afferents code for the intensity of thermal pain near pain threshold (43° to 48°C) on the glabrous skin of the uninjured hand. Above 48°C, myelinated nociceptive afferents also contribute to pain sensation. Their activity accounts for the pain during a prolonged, intense heat stimulus and for the hyperalgesia that results minutes after a burn (*13*).

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8. Other burn protocols that have been used include application of 53°C heat for 3 or 20 seconds, 49°C for 20 seconds, and 49°C for 1 minute. Following these stimuli the AMH's consistently showed an enhanced response whereas the CMH's consistently showed a suppressed response. Some of these results were reported previously (*2, 7*).
9. The AMH's had a conduction velocity of 34.7 ± 1.8 m/sec (mean ± S.E.; $N = 58$), a receptive field area of 37.3 ± 2.8 mm^2 ($N = 59$), and a mechanical threshold of 3.60 ± 0.26 bars ($N = 62$). The CMH's had a conduction velocity of 0.81 ± 0.05 m/sec ($N = 45$), a receptive field area of 20.8 ± 1.8 mm^2 ($N = 67$), and a mechanical threshold of 5.35 ± 0.44 bars ($N = 67$). These data are consistent with data we reported previously (*2, 7*).
10. The psychophysical data were normalized by dividing each subject's rating for a given stimulus by his rating for the initial 45°C stimulus and then averaged across subjects. The total impulse count during the stimulus interval was used as a measure of the response for the nerve fibers. The CMH data were normalized by dividing each fiber's response by the average response of the CMH's to the initial 45°C stimulus and then averaged across fibers. Since most AMH's did not respond to the initial 45°C stimulus, the AMH data were normalized by dividing each fiber's response by the average response of the AMH's to the first 45°C stimulus after the burn and then averaged across fibers.
11. Two 45°C stimuli were presented during the test sequence. Since the first 45°C stimulus was used to normalize the data, only the response to the second presentation of the 45°C stimulus is shown in Fig. 1. The decreased response to the second 45°C stimulus relative to the response to the first 45°C stimulus (as indicated by normalized values less than 1.0 for the response to 45°C shown in Fig. 1) was reported previously (*7*).
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13. Our preliminary experiments in other areas of the body indicate that these conclusions for glabrous skin may not have general applicability. Following a 53°C, 30-second burn to the hairy skin of seven human subjects, the threshold for pain decreased but the responses to stimuli above 46°C were not significantly changed. Although AMH's innervating the hairy skin became sensitized, the relative density of AMH's in hairy skin appears to be substantially lower than in glabrous skin. In addition, we found that CMH's innervating hairy skin show signs of sensitization not only after the burn stimulus but also after the test stimuli. It is not known whether the mechanism for hyperalgesia proposed here holds for other types of injury, such as mechanical insults, ultraviolet irradiation, and burns caused by less intense heat.
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Lithium Increases Serotonin Release and Decreases Serotonin Receptors in the Hippocampus

Abstract. *The effects of long-term lithium administration on pre- and postsynaptic processes involved in serotonergic neurotransmission were measured in rat hippocampus and cerebral cortex. Long-term lithium administration increased both basal and potassium chloride–stimulated release of endogenous serotonin from the hippocampus but not from the cortex. Serotonergic receptor binding was reduced in the hippocampus but not in the cortex. These results suggest a mechanism by which lithium may stabilize serotonin neurotransmission.*

Lithium is the most specific drug used for the treatment and prevention of recurrent manic-depressive disorders (*1*). The molecular mechanisms related to the therapeutic actions of lithium are not known, but determination of the neuronal effects of this ion may help to elucidate the regulation of neurotransmission and improve our understanding of the pathophysiological processes underlying affective disorders.

Alterations in neurotransmission at serotonin (5HT) synapses have been implicated in affective disorders, and lithium has been reported to affect several serotonergic processes, including synthesis (*2, 3*), release (*4*), and uptake of 5HT (*5*). Long-term administration of lithium exerts specific effects on the binding of [^{3}H]5HT to receptors in the rat brain (*6, 7*), resulting in a reduction of the density of [^{3}H]5HT binding sites in the hippocampus, but not in the cerebral cortex (*6*).

Two types of 5HT receptors can be distinguished in the rat brain: $5HT_1$ receptors to which [^{3}H]5HT binds and $5HT_2$ receptors in the cortex and possibly in the hippocampus to which [^{3}H]-spiperone binds (*8, 9*). We studied the effects of long-term administration of lithium on both [^{3}H]5HT and [^{3}H]spiperone receptor binding sites and on release and uptake of 5HT in rat cortex and hippocampus. We report that long-term administration of lithium exerts brain region specific effects on both types of 5HT receptor binding sites and on the release of endogenous 5HT.

Male Sprague-Dawley rats (220 to 240 g) were housed five to a cage in a light-controlled room (12 hours light, 12 hours dark; lights on at 7 a.m.) with temperature maintained at 22°C. The experimental groups were fed a diet containing 0.24 percent lithium carbonate mixed in powdered rat food. Control groups were fed the powdered food without added lithium. Both groups had water and hypertonic saline (0.46*M*) available to drink at all times. After being on the diet for 4 to 6 weeks, the rats were decapitated, and serum lithium and sodium concentrations were measured by flame photometry. The brain was dissected over ice and was either used immediately for studies of the uptake of [^{3}H]5HT and the release of endogenous 5HT or was frozen at −80°C for later study of the binding of [^{3}H]5HT and [^{3}H]spiperone to receptors.

Serum lithium concentrations in the experimental groups ranged from 0.9 to 1.1 meq/liter. There was no significant difference in serum sodium between the groups receiving lithium and the control groups. The lithium-treated rats gained weight more slowly than the controls, but appeared healthy; however, the lithium-treated rats showed the polyuria and polydipsia that are associated with therapeutic concentrations of lithium.

Serotonin receptor binding was measured in homogenate fractions after two centrifugations at 49,000*g* for 10 min-

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