Differential Sensitization of Amygdala Neurons to Afferent Inputs in a Model of Arthritic Pain

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Neugebauer, Volker and Weidong Li. Differential sensitization of amygdala neurons to afferent inputs in a model of arthritic pain. J Neurophysiol 89: 716–727, 2003; 10.1152/jn.00799.2002. Pain is associated with negative affect such as anxiety and depression. The amygdala plays a key role in emotionality and has been shown to undergo neuroplastic changes in models of affective disorders. Many neurons in the central nucleus of the amygdala (CeA) are driven by nociceptive inputs, but the role of the amygdala in persistent pain states is not known. This study is the first to address nociceptive processing by CeA neurons in a model of prolonged pain. Extracellular single-unit recordings were made from 41 CeA neurons in anesthetized rats. Each neuron’s responses to brief mechanical stimulation of joints, muscles, and skin and to cutaneous thermal stimuli were recorded. Background activity, receptive field size, and threshold were mapped, and stimulus-response functions were constructed. These parameters were measured repeatedly before and after induction of arthritis in one knee by intraarticular injections of kaolin and carrageenan. Multireceptive (MR) amygdala neurons (n = 20) with excitatory input from the knee joint responded more strongly to noxious than to innocuous mechanical stimuli of deep tissue (n = 20) and skin (n = 11). After induction of arthritis, 18 of 20 MR neurons developed enhanced responses to mechanical stimuli and expansion of receptive field size. These changes occurred with a biphasic time course (early peak: 1–1.5 h; persistent plateau phase: after 3–4 h). Responses to thermal stimuli did not change (7 of 7 neurons), but background activity (16 of 18 neurons) and electrically evoked orthodromic activity (11 of 12 neurons) increased in the arthritic state. Nociceptive-specific (NS) neurons (n = 13) showed no changes of their responses to mechanical, thermal, and electrical stimulation after induction of arthritis. A third group of neurons did not respond to somesthetic stimuli under control conditions (noSOM neurons; n = 8) but developed prolonged responses to mechanical, but not thermal, stimuli in arthritis (5 of 8 neurons). These data suggest that prolonged pain is accompanied by enhanced responsiveness of a subset of CeA neurons. Their sensitization to mechanical, but not thermal, stimuli argues against a nonspecific state of hyperexcitability. MR neurons could serve to integrate and evaluate information in the context of prolonged pain. Recruitment of noSOM neurons increases the gain of amygdala processing. NS neurons preserve the distinction between nociceptive and nonnociceptive inputs.

INTRODUCTION

Pain has a strong affective component, and arthritis-related pain results in depression and anxiety (Huys and Parker 1999). Conversely, affective states can modulate pain sensitivity and behavior in chronic pain patients (Haythornthwaite et al. 1991; Wilson et al. 2001). It has been suggested that the amygdala may be a neural substrate of the reciprocal relationship between pain and emotion (see Meagher et al. 2001).

The amygdala plays a key role in emotionality, the emotional evaluation of sensory stimuli, emotional learning, and memory, as well as affective disorders (Aggleton 2000; Blair et al. 2001; Cahill 1999; Davidson et al. 1999; Davis 1998; Gallagher and Schoenbaum 1999; LeDoux 2000; Maren 1999; Martin et al. 2000; Peper et al. 2001; Rasia-Filho et al. 2000; Rolls 2000). The amygdala, particularly the lateral and basolateral nuclei, has been shown to exhibit a high degree of plasticity in various models of long-term synaptic and behavioral modification (Bauer et al. 2001; Blair et al. 2001; Chapman et al. 1990; LeDoux 2000; LeDoux et al. 1990; Lin et al. 2000, 2001; Maren 1999; Martin et al. 2000; McKernan and Shinnick-Gallagher 1997; Neugebauer et al. 1997; Rainnie et al. 1992; Wang and Gean 1999). Recently, synaptic plasticity has also been shown in the central nucleus of the amygdala (CeA) in the kindling model of epilepsy and the chronic cocaine model of drug addiction (Neugebauer et al., 2000), and behavioral data implicate the CeA in fear conditioning (Nader et al. 2001).

It is not known if persistent pain states can lead to neuroplastic changes in the amygdala. Several lines of evidence implicate the amygdala in pain processing. Electrical stimulation of the amygdala elicits vocalizations that are accompanied by emotional reactions (Jurgens 1982; Jurgens et al. 1967). Lesions or inactivation of the amygdala decrease emotional pain reactions (Borszcz 1999; Calvino et al. 1982; Charpentier 1967; Werka 1997), without affecting normal behavior or baseline nociceptive responses (Calvino et al. 1982; Charpentier 1967; Fox and Sorenson 1994; Grimalva et al. 1990; Helmstetter 1992; Helmstetter and Belligowan 1993; Maier et al. 1993; Pavlovic et al. 1996; Tershner and Helmstetter 2000; Watkins et al. 1993, 1998). In humans, moderate levels of fear/anxiety increase pain, whereas intense fear/anxiety attenuates pain, and this is likely to be mediated through circuits involving the amygdala (see Meagher et al. 2001; Rhudy and Meagher 2000).

Nociceptive information reaches the amygdala through the....
spino-parabrachio-amygdaloid pathway, which originates from lamina I neurons in the spinal cord and trigeminal nucleus caudalis and provides purely nociceptive input to the CeA (Bernard and Bandler 1998; Bernard and Besson 1990; Bernard et al. 1993, 1996; Bourgeais et al. 2001b; Buritova et al. 1998; Jasmin et al. 1997). The CeA also receives polymodal, including nociceptive, information from thalamic and cortical areas through connections with the lateral and basolateral amygdaloid nuclei (Bourgeais et al. 2001b; Doron and LeDoux 1999; LeDoux 2000; Li et al. 1996; Linke et al. 1999; Pitkanen et al. 1995, 1997; Savander et al. 1995; Shi and Cassell 1998; Shi and Davis 1999; Smith et al. 2000). In addition, spinal neurons in the deep dorsal horn and/or the area around the central canal form monosynaptic connections with amygdala neurons and may provide sensory, including nociceptive, input to the amygdala (Burstein and Potrebic 1993; Newman et al. 1996; Wang et al. 1999). As the output nucleus for major amygdala functions, the CeA modulates various effector systems involved in the expression of emotional responses through widespread connections with the forebrain and brain stem (Aggleton 2000; Bourgeais et al. 2001a; Cassell et al. 1986; Gray 1993; Krettek and Price 1979; LeDoux 2000; Price and Amaral 1981).

The role of the amygdala in prolonged or chronic pain is largely unknown. CeA lesions produced a nonsignificant reduction of pain behavior in the formalin test (Manning 1998). Extracellular single-unit recordings in anesthetized rats show that the majority of neurons in the lateral and capsular divisions of the CeA respond exclusively or preferentially to brief noxious stimulation of the skin (Bernard et al. 1990, 1992) and deep tissue (Neugebauer and Li 2002). Interestingly, changes in background activity and evoked responses, but not somatic receptive field size, have been observed in parabrachial neurons in polyarthritic rats compared with controls (Matsumoto et al. 1996). The present study is the first to address nociceptive processing by individual amygdala neurons with input from the parabrachial area in prolonged pain. The knee joint arthritis pain model has been thoroughly characterized in our previous studies of peripheral and spinal cord neurons (Neugebauer and Schaible 1990; Neugebauer et al. 1993–1996). The advantages of this model are 1) the arthritis is confined to one joint so that the processing of inputs from arthritic and normal tissue can be compared and 2) the arthritis develops within a few hours, and thus, allows the analysis of changes in the same neuron recorded before and after induction of arthritis. In this study, we address changes in the processing of mechanos- versus thermo-nociceptive information in different types of CeA neurons in the arthritis pain model. Preliminary results have been reported in abstract form (Neugebauer 1999).

**METHODS**

**Animal preparation and anesthesia**

Adult male Sprague-Dawley rats (220–400 g) were anesthetized with pentobarbital sodium (50 mg/kg, ip). A cannula was inserted into the trachea for artificial respiration and to measure end-tidal CO₂ levels. A catheter was placed in the jugular vein for continuous administration of anesthesia (see following paragraph) and for fluid support (3–4 ml/kg/h lactated ringer solution). The carotid artery was catheterized for blood pressure monitoring. Depth of anesthesia was assessed by regularly testing the corneal blink, hindpaw withdrawal and tail-pinch reflexes; by continuously monitoring the end-tidal CO₂ levels (kept at 4.0 ± 0.2%), heart rate, arterial blood pressure (kept at 135 ± 5 mmHg) and ECG pattern; and by checking for abnormal breathing patterns. Core body temperature was measured with a rectal thermometer and maintained at 37°C by means of a homeothermic blanket system.

Animals were mounted in a stereotaxic frame, paralyzed with pancuronium (induction: 0.3–0.5 mg, iv; maintenance: 0.3 mg/h, iv) and artificially ventilated (3–3.5 ml: 55–65 strokes/min). Constant levels of anesthesia and paralysis of the musculature were maintained by intravenous infusion of a mixture of pentobarbital sodium (50 mg) and pancuronium (5 mg) in 30 ml NaCl (at approximately 40 μl/min). A unilateral craniotomy was performed at the sutura fronto-parietalis level for the recording of amygdala neurons and at the ipsilateral sutura occipito-parietalis level for electrical stimulation in the lateral pons, using the stereotaxic coordinates of the lateral pontine parabrachial area, where the monosynaptic connections of the spino-ponto-amygdaloid pain pathway to the central nucleus of the amygdala (CeA) originate (Bernard et al. 1993, 1996; Paxinos and Watson 1998). The dura mater was opened and reflected; the pia mater was removed over the recording site to allow smooth insertion of the recording electrodes.

**Electrophysiological recording and identification of amygdala neurons**

Extracellular recordings were made from single neurons in the CeA with glass insulated carbon filament electrodes (3–5 MΩ) using the following stereotaxic coordinates (cf. Paxinos and Watson 1998): 1.6–3.2 mm caudal to bregma; 3.8–4.4 mm lateral to midline; depth of 7,000–9,000 μm. The recorded signals were amplified and displayed on analog and digital storage oscilloscopes. Signals were also fed into a window discriminator, whose output was processed by an interface (CED 1401) connected to a Pentium III PC. Spike2 software (CED, version 3) was used to create peristimulus rate histograms on-line and to store and analyze digital records of single-unit activity off-line. Spike size and configuration were continuously monitored on the storage oscilloscopes and with the use of Spike2 software.

CeA neurons were orthodromically activated by electrical stimulation (square-wave current pulses, 50–500 μA, 150 μs; monopolar stimulation electrode) in the ipsilateral pons, using the stereotaxic coordinates that correspond to the lateral pontine parabrachial area, where the monosynaptic connections of the spino-ponto-amygdaloid pain pathway to the CeA originate (Bernard et al. 1993, 1996): 1–2 mm rostral to the lambda and 2.2 mm lateral to midline at the depth of 7.3 mm. We refer to the activity evoked by these orthodromic electrical stimuli as parabrachial input for simplicity. Once an individual CeA neuron was identified and its spike size optimized, we carefully searched for a receptive field in the knee joint(s) and determined size and threshold of its total receptive field in the deep tissue and skin.

Configuration, shape, and height of the recorded action potentials were monitored and recorded continuously using a window discriminator and Spike2 software for on-line and off-line analysis. Only those neurons were included in this study whose spike configuration remained constant and could be clearly discriminated from background activity throughout the experiment, indicating that the activity of one neuron only and from the same one neuron was measured.

**Experimental protocol**

Background activity was recorded for ≥10 min to calculate mean ± SE and 95% confidence intervals (CI) using Prism 3.0 software (GraphPad Software, San Diego, CA). Size and thresholds of the receptive fields in deep tissue and skin were mapped. Response thresholds for mechanical stimulation of the knee joint and other deep tissue (e.g., ankle joint and muscles) were determined as follows:
mechanical stimuli of gradually increasing intensity (steps of 50 g/30 mm²) were applied to the deep tissue (joints and muscles) by means of a forceps with a force transducer, whose calibrated output was amplified and displayed in grams on a LCD screen. The output signal was also fed into the CED interface and recorded on the Pentium III PC for on- and off-line analysis.

The mechanical threshold was defined as the minimum stimulus intensity that evoked an excitatory response (spike frequency higher than the upper 95% CI of background activity) or an inhibitory response (spike frequency less than the lower 95% CI of background activity). The threshold stimulus intensity was then tested again three times to verify the presence of a response in ≥50% of trials. A neuron was classified as receiving input from deep tissue if careful stimulation of overlying skin evoked no response or a response that was clearly distinct from that produced by stimulation of the deep tissue. Similarly, mechanical test stimuli were considered to activate deep tissue if the stimulation of overlying skin did not evoke any response. Only responses that were distinctly evoked by selective stimulation of deep tissue were included in the analysis of the processing of information from the deep tissue. Stimulus–response relationships were measured by applying graded mechanical test stimuli of 100 and 500–3,000 g/30 mm² intensity in increments of 500 g/30 mm² (15 s duration each; 15-s intervals).

Cutaneous receptive fields were mapped using the following stimuli: BRUSH (brushing the skin with a soft-hair artist’s brush in a stereotyped manner), PRESS (firm pressure using a large arterial clip to apply 1,005 g/8 mm², which is marginally painful when applied to the skin in humans), and PINCH (using a small arterial clip to apply 1,005 g/8 mm², which is clearly painful without causing overt damage to the skin). The most responsive site of the receptive field was then stimulated using a series of von Frey monofilaments with bending forces ranging from 60 mg to 178.5 g to measure stimulus-response relationships. Each filament was applied repeatedly for a period of 15 s followed by a 15-s pause. Cutaneous input was distinguished from deep tissue input by selective stimulation of skinfolds gently raised from the underlying deep tissue.

Thermal stimuli of innocuous and noxious intensity (37–53°C) were applied by a feedback-controlled contact Peltier thermode with an active area of 36 mm². Adapting temperature was set to 35°C; cycles of 5-s stimuli were delivered at intervals of 35 s. The temperature at the thermode was continuously measured, and with the use of the CED interface, recorded on the Pentium III PC for on- and off-line analysis of the stimulus-response relationships.

Heterosensory (visual and auditory) stimuli included shining a bright light into each pupil, snapping fingers, clapping hands, and whistling.

Classification of neurons and thresholds

According to the classification that we proposed previously for CeA neurons with deep tissue input (Neugebauer and Li 2002), CeA neurons in this study were nociceptive specific (NS), multisensitive (MR), or nonresponsive to somesthetic stimuli (noSOM) neurons. The classification was primarily based on the neurons’ responses to mechanical stimulation of the knee joint and other deep tissue, although the responses to cutaneous and other natural somesthetic stimuli (see Experimental protocol) were also characterized.

MR neurons consistently responded to low-intensity stimuli (deep tissue, <500 g/30 mm²; skin, <1.3 g von Frey filament and/or BRUSH) but were more strongly activated by noxious stimuli (deep tissue, >1,500 g/30 mm²; skin, >4.8 g von Frey filament, PRESS, PINCH). A stimulus intensity of 100–500 g/30 mm² applied to the knee and other deep tissue was considered innocuous; it did not evoke hindlimb withdrawal reflexes in awake rats (unpublished observations) and was not felt to be painful when tested on the experimenters. Pressure stimuli >1,500 g/30 mm² applied to the knee joint and other deep tissue were considered noxious; they evoked hindlimb withdrawal reflexes in awake rats (unpublished observations) and were distinctly painful when applied to the experimenters. NS neurons responded exclusively to noxious stimuli. Nonresponsive noSOM neurons were not activated by any mechanical and thermal stimuli.

Arthritis

In each experiment, background activity of one CeA neuron and its responses to graded mechanical stimuli and thermal stimuli were recorded before and for several hours (≥6 h, maximum 18 h) after the induction of arthritis in one knee joint. Background activity, evoked responses, and receptive field size had to be stable for several hours before the arthritis was induced. Throughout the experiment we carefully monitored a variety of parameters (body temperature, blood pressure, heart rate, ECG, CO₂ levels) to ensure a stable recording situation.

Arthritis was induced as described in detail previously (Neugebauer et al. 1993–1996). A kaolin suspension (4%, 80–100 μl) was slowly injected into the joint cavity through the patellar ligament with the use of a syringe and needle (1 ml, 25G/8). After repetitive flexions and extensions of the knee for 15 min, a carrageenan solution (2%, 80–100 μl) was injected into the joint knee cavity, and the leg was flexed and extended for another 5 min. This treatment paradigm reliably leads to inflammation and swelling of the knee within 1–3 h and persists for more than 24 h (see Neugebauer and Schäible 1990; Neugebauer et al. 1993–1996).

Histology

At the end of each experiment, the recording site in the CeA was marked by injecting DC (250 μA for 3 min) through the carbon filament recording electrode. The brain was removed and submerged in 10% formalin and potassium ferrocyanide. Tissues were stored in 20% sucrose before they were frozen and sectioned into 50-μm slices. Sections were stained with neutral red, mounted on gel-coated slides, and cover-slipped. The boundaries of the different amygdala nuclei were easily identified under the microscope. Lesion/recording sites were verified histologically and plotted on standard diagrams (from Paxinos and Watson 1998) of coronal brain sections (see Figs. 1, 7, and 8).

Data analysis

Recorded activity was analyzed off-line from peristimulus rate histograms using Spike2 software (CED, version 3). The neurons’ responses to mechanical and thermal stimuli were measured and expressed as spikes per second (Hz). Background activity, if present, was subtracted from the evoked responses. Stimulus–response relationships for mechanical and thermal inputs were measured for each neuron and then averaged across a sample of neurons. Stimulus–response functions were analyzed using models of nonlinear regression (Prism 3.0, GraphPad Software). Sigmoid curves were fitted to the stimulus-response data using the following “four parameter logistic equation” for nonlinear regression (Prism 3.0, GraphPad Software):

\[ y = A + \frac{(B - A)}{1 + (x/C)^D} \]

where \( A = \text{bottom plateau}, \) \( B = \text{top plateau}, \) \( C = \text{log(half-maximal intensity)}, \) and \( D = \text{slope coefficient}. \) Stimulus–response functions before and after induction of arthritis were compared statistically using a two-way ANOVA followed by Bonferroni posttests (Prism 3.0, GraphPad Software). All averaged values are given as the mean ± SE. Statistical significance was accepted at the level \( P < 0.05.\)

RESULTS

Sample of neurons

Extracellular single-unit recordings were made from 41 neurons in the central nucleus of the amygdala (CeA) in 39
anesthetized rats. Neurons were recorded in the posterior portion of the CeA (2.2–3.2 mm caudal to bregma), and particularly, in the lateral capsular subdivision (see Figs. 1, 7, and 8, insets; nomenclature according to Paxinos and Watson 1998).

Data from experiments in which histological analysis revealed recording sites outside the CeA were not included in this study. All CeA neurons in this study responded to parabrachial input evoked by orthodromic electrical stimulation in the lateral pons. Latencies ranged from 8 to 14 ms (mean = 11 ± 0.7 ms), corresponding to conduction velocities of approximately 1 m/s or less (see examples in Figs. 1F and 6). The mean threshold for monosynaptic orthodromic activation was 220 ± 28 μA (50–450 μA; 150 μs). The inputs activated by electrical stimulation were considered monosynaptic because the neurons’ responses followed 20-Hz stimulation with relatively constant latencies; they did not follow electrical stimulation at high frequencies of 333 and/or 500 Hz, a test for antidromic activation. No apparent differences were found in the distribution of latencies and electrical thresholds for the individual types of CeA neurons.

Primarily based on their responses to mechanical stimulation of the knee joint and other deep tissue (see METHODS), 20 CeA neurons were classified as multireceptive (MR) neurons, which responded significantly to innocuous but more strongly to noxious stimuli; 13 neurons were nociceptive-specific (NS), i.e., activated exclusively by noxious stimulation; 8 nonresponsive neurons were not activated by any somesthetic mechanical and thermal stimuli and were classified as noSOM neurons. All MR and NS neurons had a receptive field in the knee joint and responded to noxious mechanical stimulation. Noxious thermal stimuli activated 7 of 11 MR neurons, 9 of 11 NS neurons, but none of 8 noSOM neurons. The majority of CeA neurons (30) showed background activity, which ranged from 0.5 to 19.5 Hz (mean = 5.3 ± 1.8 Hz): 18 of 20 MR neurons, 4 of 13 NS neurons, and 8 of 8 noSOM neurons. None of the CeA neurons responded to visual or auditory stimuli (see METHODS).

**Sensitization of MR neurons in the arthritis pain model**

**ENHANCED PROCESSING OF MECHANOSENSORY INPUTS FROM DEEP TISSUE.** In 18 of 20 MR neurons, the responses to mechanical stimulation of the knee joint and other (noninflamed) parts of the body increased after induction of arthritis in the knee. A typical example is shown in Fig. 1. This MR neuron was recorded in the lateral capsular part of the CeA (Fig. 1E). The neuron responded initially to innocuous (100 g/30 mm²) and noxious (2,500 g/30 mm²) mechanical stimulation of the deep tissue (see METHODS) in the knee, ankle, and hindpaw, but not forearm (Fig. 1A). After induction of arthritis in the knee contralateral to the recording site, the neuron’s background activity and evoked responses increased within 1 h, and a receptive field appeared on the forearm (Fig. 1B). After returning almost to control levels at 2 h (Fig. 1C), the responses increased again (Fig. 1D) and remained elevated throughout the remainder of the experiment (to 10 h postinduction of arthritis).

The biphasic time course of enhanced responsiveness after induction of arthritis was a consistent finding in all 18 MR neurons that became sensitized and is illustrated in another MR neuron in Fig. 2. Evoked responses to mechanical stimuli of innocuous (Fig. 2A, 100 g/30 mm²) and noxious (Fig. 2B, 2,500 g/30 mm²) intensity increased at 1 h and again after 3–4 h. The second phase of enhanced responsiveness lasted for the remainder of the experiment. Interestingly, the increase in background activity (Fig. 2C) did not follow a biphasic time course but developed continuously to reach a plateau after 4 h. Similar changes in background activity were observed in 16 MR neurons.

The analysis of stimulus-response relationships for mechanical stimulation of the deep tissue showed a significant change in the arthritis pain model (Fig. 3). The responses of an individual MR neuron to graded mechanical stimulation of the
FIG. 2. Biphasic time course of arthritis-evoked changes in a MR CeA neuron. Evoked responses (A and B) and background activity (C and D) of 1 neuron in the lateral capsular part of the CeA in the right hemisphere. Brief (15 s) mechanical stimuli of innocuous (A: 100 g/30 mm²) and noxious (B: 2,500 g/30 mm²) intensity are applied to the deep tissue before and after induction of arthritis in the left knee joint. Spikes are counted per second; background activity preceding the evoked response (C) has been subtracted from the total activity during stimulation. After induction of arthritis, evoked responses increase with a biphasic time-course, showing an early peak at 1–1.5 h and a prolonged phase of hyperexcitability after 4 h (A and B). Background activity increases continuously until it stabilizes in the plateau phase (C). D: original traces (2 s duration each) showing the background activity of the same neuron in the control period (top) and the increased background activity 6 h postinduction of arthritis (bottom).

FIG. 3. Stimulus-response relations for mechanical stimulation of deep tissue are altered in the arthritis pain model. Stimulus-response functions (C and D) were constructed from the responses of each neuron to brief (15 s) graded mechanical stimuli in the innocuous and noxious range (see METHODS). A and B: individual example showing the increased responses of a MR neuron in the CeA (lateral capsular part) to graded mechanical stimulation of the knee joint 6 h after induction of arthritis (B) compared with control (A). Bin width of histograms: 1 s. Individual action potentials shown on the right next to corresponding histograms on the left illustrate that spike configuration, shape, and size remained constant throughout the experiment. Stimulus-response curves for mechanical stimulation were best described by a sigmoid nonlinear regression model using Prism 3 software (see METHODS). The stimulus-response relationships before and after induction of arthritis were significantly different (C; stimulation of the knee, n = 18 neurons, F(1,204) = 93.74, P < 0.0001, 2-way ANOVA followed by Bonferroni posttests; D: stimulation of the ankle, n = 13, F(1,144) = 117.68, P < 0.0001; 2-way ANOVA followed by Bonferroni posttests, see METHODS). Note the logarithmic scale. Each symbol represents the mean ± SE. * P < 0.05, ** P < 0.01, *** P < 0.001.
knee are shown in Fig. 3, A and B. After induction of arthritis (Fig. 3B), the responses of this neuron had increased compared with the control period before arthritis (Fig. 3A). Stimulus-response curves (Fig. 3, C and D) were constructed from recordings like those shown in the histograms of Fig. 3, A and B. In line with our previous study (Neugebauer and Li 2002), the stimulus-response curves for graded mechanical stimulation were best described by a sigmoid nonlinear regression model rather than a monotonically increasing linear function (using Prism 3 software; see METHODS). The stimulus-response relationships before and after induction of arthritis were significantly different [Fig. 3C, stimulation of the arthritic knee, n = 18 neurons, F(1,204) = 93.74, P < 0.0001, 2-way ANOVA followed by Bonferroni posttests; Fig. 3D, stimulation of the noninjured ankle, n = 13, F(1,144) = 117.68, P < 0.0001; 2-way ANOVA followed by Bonferroni posttests, see METHODS]. Enhanced magnitude and lower thresholds of the responses to stimulation of the arthritic knee and the noninflamed ankle were measured 6 h after induction of arthritis. The resulting upward shift of the stimulus-response functions suggests enhanced processing of mechanosensory, including nociceptive, information in the arthritis pain model.

ENHANCED PROCESSING OF MECHANOSENSORY, BUT NOT THERMORECEPTIVE, CUTANEOUS INPUTS. Similar to the processing of mechanosensory and mechano-nociceptive information from deep tissue, the responses of MR CeA neurons to mechanical stimulation of the skin were enhanced in the arthritis pain model. A series of von Frey monofilaments was used to apply cutaneous stimuli of innocuous and noxious intensity to the most responsive site of the receptive field, which was typically located on the lower back (see METHODS and Fig. 4, inset). Figure 4A shows that the stimulus-response relationships for mechanical stimuli were significantly enhanced 6 h after the induction of arthritis compared with those measured in the control period [P < 0.0001, n = 10 neurons, F(1,108) = 23.93, 2-way ANOVA followed by Bonferroni posttests].

The responses to thermal stimuli, however, did not change significantly in 7 of 7 MR neurons in the arthritis pain model. Figure 4B shows that the stimulus-response relationships for innocuous and noxious thermal stimuli (see METHODS) before and 6 h after induction of arthritis were not significantly different [P > 0.05, n = 7 neurons, F(1,204) = 2.29, 2-way ANOVA]. The differential changes in the processing of mechanosensory versus thermoreceptive information argue against a nonselective general increase of excitability of CeA neurons in the arthritis pain model.

EXPANSION OF MECHANO-RECEPTIVE FIELDS. MR CeA neurons with excitatory nociceptive input from the knee joint (n = 20) typically had large symmetrical receptive fields in the deep tissue of both hindlimbs (n = 12) or in all four extremities (n = 8). In the arthritis pain model, the receptive fields of all 12 MR neurons with original receptive fields confined to both hindlimbs increased to incorporate deep tissue of the forearm(s). In 5 of 8 MR neurons with a receptive field in all four extremities, the mechano-receptive field size increased to include the more distal parts of the extremities. In 8 of 11 neurons with input from the skin, the cutaneous mechano-receptive field expanded after the induction of arthritis, and new cutaneous receptive fields appeared in 2 of 9 neurons that did not have a detectable receptive field in the skin before arthritis. The size of the thermo-receptive fields did not change in 7 of 7 MR neurons.

Figure 5 illustrates the expansion of the receptive field of an individual MR CeA neuron. Before arthritis, this neuron responded to innocuous and noxious stimulation of the deep tissue in the hindlimbs (Fig. 5A, low- and high-threshold areas) and to noxious cutaneous stimuli applied to the the lower back and hip areas (Fig. 5B, high-threshold area). The total size of the receptive field in the deep tissue and skin increased and the threshold of some high-threshold areas of the receptive field decreased after induction of arthritis in one knee joint (arrows). Changes of receptive field size and threshold followed a biphasic time course. Expansion of receptive field size and
INDUCTION OF ARTHRITIS (Fig. 6, histograms (PSTH) of an individual MR CeA neuron. After input. Figure 6 shows original traces and poststimulus-time eral pons as a measure of increased sensitivity to a constant parabrachial input evoked by electrical stimulation in the lat-
we analyzed arthritis-evoked changes of their responses to innocuous and noxious mechanical stimulation of the skin (see METHODS). The size of the cutaneous receptive field increased and the threshold for evoking a response to cutaneous stimulation decreased 1 and 6 h after the induction of arthritis. Changes of deep tissue and cutaneous receptive field size and threshold persisted for the remainder of the experiment (11 h postinduction of arthritis).

REDUCTION OF RESPONSE THRESHOLD OCCURRED AS EARLY AS 1 h postinduction of arthritis. These changes partially reversed at 2 h and then resumed to reach their maximum after 5–6 h postinduction. Changes persisted for the remainder of the experiment (11 h postinduction).

INCREASED RESPONSIVENESS TO ORTHODROMIC ELECTRICAL STIMULATION IN THE LATERAL PONS. In 12 MR CeA neurons, we analyzed arthritis-evoked changes of their responses to parabrachial input evoked by electrical stimulation in the lateral pons as a measure of increased sensitivity to a constant input. Figure 6 shows original traces and poststimulus-time histograms (PSTH) of an individual MR CeA neuron. After induction of arthritis (Fig. 6, B–D), a larger number of action potentials were evoked by orthodromic electrical stimulation than before arthritis (Fig. 6A). PSTHs illustrate the responses to 10 successive stimulations. The stimulus intensity was set to twice the threshold intensity that evoked an orthodromic action potential in 50% of the trials in the control period before arthritis. Enhanced sensitivity to electrically evoked inputs was detected in 11 of 12 neurons (summarized for 12 MR neurons in Fig. 6E). The threshold (T) itself did not change nor did stimulation at this constant threshold intensity evoke more action potentials after arthritis. Suprathreshold stimulation (2 × T), however, evoked significantly more action potentials after induction of arthritis compared with control before arthritis [F(5,66) = 4.593, P < 0.01, 1-way ANOVA followed by Dunnett’s multiple comparison test]. Interestingly, the enhanced sensitivity to electrical stimulation, like the increase in background activity (see Fig. 2C), did not follow the biphasic time course observed for changes in responses to mechanical stimuli (Fig. 2, A and B) and changes in receptive field size and threshold (Fig. 5).

No significant changes of NS neurons in the arthritis pain model

The responses of NS neurons (n = 13) to mechanical, thermal, and electrical stimulation did not change significantly after induction of arthritis, at least not during the observation period in these experiments (≤12 h postinduction). Figure 7 shows an individual NS neuron recorded extracellularly in the CeA (Fig. 7C). Before arthritis, the neuron responded only to noxious mechanical stimulation of deep tissue in knee, ankle, paw, and forearm (Fig. 7A, bottom), but not to innocuous

FIG. 5. SIZE AND THRESHOLD OF THE RECEPTIVE FIELD OF A MR NEURON ARE ALTERED IN THE ARTHRITIS PAIN MODEL. A: deep tissue receptive field of the neuron recorded in the lateral capsular part of the CeA in the right hemisphere. Low- and high-threshold areas were determined from the neuron’s responses to mechanical stimuli of innocuous and noxious intensity (see METHODS). Note the expansion of the total receptive field 1 and 6 h after the induction of arthritis in the left knee. B: cutaneous receptive field of the same neuron. Thresholds were determined from the neuron’s responses to innocuous and noxious stimulation of the skin (see METHODS). The size of the cutaneous receptive field increased and the threshold for evoking a response to cutaneous stimulation decreased 1 and 6 h after the induction of arthritis. Changes of deep tissue and cutaneous receptive field size and threshold persisted for the remainder of the experiment (11 h postinduction of arthritis).

FIG. 6. ENHANCED SENSITIVITY OF A SINGLE MR NEURON IN THE CE A (LATERAL; CAPSULAR PART) TO ELECTRICAL STIMULATION IN THE EXTERNAL LATERAL PORTION OF THE PONTINE PARABRAHICAL AREA (SEE METHODS). THE THRESHOLD INTENSITY (T) FOR ORTHODROMIC ACTIVATION WAS 250 μA (150 μs). ACTION POTENTIALS SHOWN IN A–D WERE EVOKED ORTHODROMICALLY BY SUPRATHRESHOLD (2 × T) ELECTRICAL STIMULATION (500 μA; 150 μs). ASTERISKS IN A–D DENOTE STIMULUS ARTIFACTS. POSTSTIMULUS-TIME HISTOGRAMS (PSTH) ARE SHOWN ON THE RIGHT NEXT TO CORRESPONDING TRACES ON THE LEFT TO ILLUSTRATE THE INCREASED NUMBER OF ORTHODROMIC ACTION POTENTIALS IN THE ARTHRITIS PAIN MODEL. EACH PSTH SHOWS THE SUPERIMPOSED RESPONSES TO 10 SUCCESSIVE STIMULATIONS. ASTERISKS DENOTE STIMULUS ARTIFACTS. ANTIDROMIC ACTIVATION WAS RULED OUT BY THE (SLIGHTLY) VARYING LATENCIES OF EVOKED ACTION POTENTIALS AND THE INABILITY TO FOLLOW HIGH-FREQUENCY (333 Hz) STIMULATION (NOT SHOWN, BUT SEE FIG. 1F). E: TIME COURSE OF SENSITIZATION OF 12 CeA NEURONS TO ELECTRICAL STIMULATION WAS NOT BIPHASIC AS OBSERVED FOR CHANGES IN PERIPHERAL MECHANICAL STIMULATION (CF. FIG. 2, A AND B) BUT RATHER RESEMBLED THE PROGRESSIVELY INCREASING BACKGROUND ACTIVITY (CF. FIG. 2C). T REFERS TO THE THRESHOLD FOR ELECTRICALLY EVOKED ORTHODROMIC ACTION POTENTIALS IN 50% OF TRIALS. T REMAINED UNCHANGED THROUGHOUT THE EXPERIMENT. SYMBOLS INDICATE MEAN ± SE. *P < 0.05, **P < 0.001 (1-WAY ANOVA FOLLOWED BY DUNNETT’S POSTTESTS).
stimuli (top). The receptive field was bilateral and symmetrical. Neither background activity nor evoked responses changed after the induction of arthritis (Fig. 7B). Figure 7D summarizes the data for the population of NS neurons in this study. Stimulus-response relationships for mechanical stimulation of the knee in the innocuous (500–1,000 g/30 mm²) and noxious range (>1,500 g/30 mm²) were not significantly different in the arthritis pain model compared with control (P > 0.05, 2-way ANOVA, n = 13; Fig. 7D).

Sensitization of nonresponsive noSOM neurons in the arthritis pain model

In eight CeA neurons without a detectable somatic receptive field (noSOM neurons, see METHODS), background activity and effects of mechanical and thermal stimuli were monitored during the development of arthritis. In 5 of 8 noSOM neurons, background activity increased and evoked responses to mechanical, but not thermal, stimuli appeared after induction of arthritis. Figure 8 shows the sensitization of an individual noSOM neuron to innocuous and noxious mechanosensory stimuli (top). The receptive field was bilateral and symmetrical. Neither background activity nor evoked responses changed after the induction of arthritis (Fig. 7B). Figure 7D summarizes the data for the population of NS neurons in this study. Stimulus-response relationships for mechanical stimulation of the knee in the innocuous (500–1,000 g/30 mm²) and noxious range (>1,500 g/30 mm²) were not significantly different in the arthritis pain model compared with control (P > 0.05, 2-way ANOVA, n = 13; Fig. 7D).
inputs. The neuron was recorded extracellularly in the lateral capsular part of the CeA in the right hemisphere (see Fig. 8D). In a 3-h control period before arthritis, this neuron showed background activity but had no somatic receptive field in the deep tissue and skin (Fig. 8A). After induction of arthritis, background activity increased first (3 h postinduction, Fig. 8B), whereas evoked responses and a receptive field in and around the arthritic knee appeared only several hours after postinduction (5.5 h, Fig. 8C). Innocuous and noxious mechanical stimulation of the arthritic knee and adjacent tissue evoked prolonged responses (afterdischarges) for the remainder of the experiment (10 h postinduction). Responses to electrically evoked parabrachial input also increased in the five noSOM neurons that became sensitized in the arthritis pain model.

**Discussion**

This study is the first to address changes of nociceptive processing in amygdala neurons in a model of prolonged pain. The main findings of this study are as follows: MR neurons in the CeA develop increased responsiveness to nociceptive and nonnociceptive mechanosensory, but not thermoreceptive, inputs and to a constant afferent input evoked by electrical stimulation in the lateral pons. Enhancement of evoked responses to peripheral stimuli, reduction of response threshold, and expansion of receptive field size all occur with a characteristic biphasic time course, whereas background activity and electrically evoked responses increase monotonically. NS CeA neurons do not show any changes of responses to mechanical, thermal, and electrical stimulation in the arthritis pain model. Nonresponsive CeA neurons of the noSOM type without a somatic receptive field under control conditions show enhanced background activity and evoked responses to mechanonociceptive, but not thermoreceptive, inputs as well as to a constant electrically evoked afferent input in the arthritis pain model. Changes of noSOM neurons develop continuously and do not follow the biphasic time course that appears to be characteristic of MR neurons.

These differential changes (processing of mechanonociceptive inputs; sensitization of MR and noSOM neurons vs. NS neurons; biphasic time course vs. continuous changes) suggest that the sensitization of CeA neurons in the arthritis pain model is not simply the consequence of a generally increased excitability state in the amygdala and its networks but is input-specific and dependent on the response properties of a neuron under control conditions. Importantly, the fact that the response properties and receptive fields of NS neurons did not change for many hours after induction of arthritis also serves as a control for the changes observed in MR neurons. If changes in responsiveness and receptive fields of MR neurons after arthritis were due to variability of the animal state and/or recording situation, they would be expected to have occurred in NS neurons as well. Increased responses of MR neurons to mechanical, but not thermal, stimuli also argue against nonspecific changes. Furthermore, we went to great length to measure and monitor carefully a variety of parameters (body temperature, blood pressure, heart rate, ECG, CO₂ levels) to ensure the stability of the body environment of the animal and the recording situation (see METHODS). Finally, configuration, shape and height of each neuron’s action potentials were monitored and recorded continuously using a window discriminator and Spike2 software for on-line and off-line analysis (see METHODS). As in our previous long-term studies of individual neurons in the CNS (Neugebauer and Schaible 1990; Neugebauer et al. 1993–1996), we included in our analysis only those neurons whose spike configuration remained constant and could be clearly discriminated from background activity throughout the experiment, indicating that the activity of one neuron only and from the same one neuron was measured (see individual examples in Figs. 1, 3, 6, 7, and 8).

Interestingly, the changes in CeA neurons occurred with different time courses after induction of arthritis. Evoked responses of MR neurons to mechanical stimuli increased with a biphasic time course consisting of an early peak (1–1.5 h) and a later plateau phase (after 3–4 h) that persisted for the remainder of the experiment (several hours postinduction). Expansion of receptive field size and decrease of response threshold for mechanical stimuli also followed the biphasic time course. The first phase likely reflects the enhanced afferent input as a result of the sensitization of knee joint afferents and spinal dorsal horn neurons. In the arthritis pain model, changes of peripheral and spinal neurons start within 1–2 h postinduction and increase progressively to reach a maximum 4–6 h postinduction (Neugebauer and Schaible 1990; Neugebauer et al. 1989, 1993–1996; Schaible and Grubb 1993). Therefore the biphasic character of the time course of changes in CeA neurons may suggest additional, possibly intraamygdalar, mechanisms. This biphasic change in the amygdala in the arthritis pain model could be similar to the first and second phases of the formalin test, a model of prolonged inflammatory pain. The first phase represents acute nociception due to primary afferent activation, whereas the second phase includes an ensuing inflammatory response and reflects a combination of peripheral and central sensitization. The “interphase” in the formalin test is due to active inhibition (Henry et al. 1999). Likewise, the interphase in the amygdala in our arthritis pain model could involve active inhibitory mechanisms.

Background activity and electrically evoked orthodromic responses of CeA neurons increased continuously to reach a plateau of maximum changes after 4 h. Electrical stimulation creates a constant afferent input that is independent of the excitability state of the stimulated pathway as long as the stimulus threshold remains unchanged, which it did in our study. The unchanged threshold for orthodromic activation strongly suggests that the stimulation situation remained constant and, therefore it is the responses and excitability of the amygdala neurons that changed rather than the excitability of the stimulated tissue. The depolarization of stimulated cells would almost certainly affect the threshold and the responses evoked by threshold stimulation. Therefore the increased responses of MR neurons to electrical stimulation of projections from the lateral pons to the CeA strongly suggest that the excitability of amygdala neurons is enhanced in the arthritis pain model. Consequently, the second or plateau phase of the changes in the arthritis pain model would constitute sensitization of CeA neurons defined as increased sensitivity to a constant afferent input. Interestingly, evoked responses and receptive fields of nonresponsive noSOM neurons did not appear until this plateau phase started (after 3–4 h), suggesting that the sensitization of noSOM neurons may reflect and result from intraamygdalar excitability changes.

Presently, it is not known to what extent the amygdala...
changes reflect possible changes of neurons in the pontine parabrachial area (PB). PB neurons have not been studied in our kaolin/carrageenan-induced arthritis model. In a model of polyarthritis induced by injection of Mycobacterium butyricum into the tail, PB neurons from arthritic rats had higher background activity, increased responses to noxious mechanical stimuli, and decreased thresholds for mechanical stimuli (Matsumoto et al. 1996). Therefore at least part of the changes observed in CeA neurons in our study may be related to changes in PB neurons. However, the sensitization of CeA neurons is different from and in addition to any changes in PB neurons for the following reasons. 1) Responses of CeA neurons to electrical stimulation in the lateral pons, where the parabrachio-amygdaloid projections to the CeA originate, increased, whereas the threshold for electrical stimulation did not change, suggesting enhanced sensitivity of CeA neurons to a constant afferent input that is independent of the excitability state of PB neurons (see above). 2) Under normal conditions all PB neurons that participate in pain processing are NS neurons, which respond exclusively to noxious, but not innocuous, stimuli (Matsumoto et al. 1996). In our study, it is the MR neurons, but not NS neurons, that become sensitized in the arthritis model. MR neurons in the CeA receive both nociceptive inputs (from the PB) and nonnociceptive information (from thalamic nuclei and cortical areas through the lateral and basolateral amygdala). 3) No change of receptive field size was observed in PB neurons in arthritic rats (Matsumoto et al. 1996), whereas the majority of MR neurons in the amygdala showed expansion of receptive fields after induction of arthritis. 4) Magnitude and threshold of responses of MR neurons to thermal stimuli remained unchanged in the arthritis pain model in our study, whereas PB neurons showed a small but significant increase of the thermal threshold in arthritis (Matsumoto et al. 1996).

The spino-parabrachio-amygdaloid pathway arises from nociceptive-specific spinal lamina I neurons and provides purely nociceptive information to the CeA through nociceptive-specific PB neurons (Bernard and Bandler 1998; Bernard and Besson 1990; Bernard et al. 1993, 1996; Bourgeais et al. 2001b; Buritova et al. 1998; Gauriau and Bernard 2002; Jasmin et al. 1997). Somewhat surprisingly, it is not NS neurons, but MR and noSOM neurons that undergo changes of excitability and responsiveness in the arthritis pain model. The fact that the response properties of NS neurons in the CeA with input from the PB did not change in this study may suggest that input from this pathway is not sufficient for the pain-related sensitization of CeA neurons. The sensitization of MR neurons, however, which are activated by noxious as well as innocuous stimuli, suggests that other inputs than the nociceptive-specific inputs from the spino-parabrachio-amygdaloid pathway are necessary. These additional inputs consist of highly integrated polymodal, including nociceptive, information that reaches the CeA from thalamic nuclei, and cortical areas through the lateral (LA) and basolateral (BLA) amygdaloid nuclei (Bernard and Bandler 1998; Bourgeais et al. 2001b; Doron and LeDoux 1999; Gauriau and Bernard 2002; Herzog and Van Hoesen 1976; LeDoux 2000; LeDoux et al. 1990; Li et al. 1996; Linke et al. 1999; Pitkanen et al. 1995, 1997; Savander et al. 1995; Shi and Cassell 1998; Shi and Davis 1999; Smith et al. 2000; Stefanacci et al. 1992; see Discussion in Neugebauer and Li 2002). Another possible source of nociceptive input includes direct spino-amygdaloid projections (Burstein and Potrebic 1993; Newman et al. 1996; Wang et al. 1999), although their exact course and function have not been shown yet. The convergence and integration of nociceptive and polymodal inputs in CeA neurons as a requirement for pain-related sensitization of amygdala neurons would be consistent with the well-documented critical role of the amygdala in associative learning and memory to link sensory information and affective significance (Aggleton 2000; Bailey et al. 1999; Blair et al. 2001; Buchel and Dolan 2000; Davis 1998; Everitt et al. 1999; Gallagher and Chiba 1996; Holland and Gallagher 1999; LeDoux 2000; Maren 2001; Post et al. 1998; Rolls 2000).

This study is the first to demonstrate neuroplastic changes of nociceptive transmission in amygdala neurons in a model of prolonged pain. Consistent with the role of the amygdala in attaching emotional significance to sensory information (see previous paragraph), the sensitization of multireceptive CeA neurons in the arthritis pain model could play an important role in the integration of nociceptive and polymodal inputs and their evaluation in the context of prolonged pain. Recruitment of normally nonresponsive noSOM neurons would increase the gain of amygdala processing. The role of NS neurons may consist in attaching the label “nociceptive” to information processed in the CeA in prolonged pain states, thus distinguishing nociceptive from nonnociceptive inputs and preserving the pain-related context of altered amygdala processing.

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