Hemispheric Lateralization of Pain Processing by Amygdala Neurons

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Ji G, Neugebauer V. Hemispheric lateralization of pain processing by amygdala neurons. J Neurophysiol 102: 2253–2264, 2009. First published July 22, 2009; doi:10.1152/jn.00166.2009. Recent biochemical and behavioral data suggest right-hemispheric lateralization of amygdala functions in pain. Our previous electrophysiological studies showed pain-related neuroplasticity in the latero-capsular division of the central nucleus of the amygdala (CeLC) in the right brain hemisphere. Here we determined differences in the processing of pain-related signals in right versus left CeLC neurons. Individual CeLC neurons were recorded extracellularly before and after induction of an arthritis pain state in anesthetized rats. Brief innocuous and noxious test stimuli were applied to peripheral tissues ipsi- and contralateral to the recording site. A monoarthritic was induced in the ipsi- or contralateral knee by intraarticular injections of kaolin and carrageenan. Under normal conditions, CeLC neurons in the left amygdala had smaller receptive fields than those in the right, but the magnitude of background and evoked activity was not significantly different. After arthritis induction, neurons in the right, but not left, CeLC developed increased background activity and evoked responses, irrespective of the location of the arthritis (ipsi- or contralateral to the recording site). A protein kinase A (PKA) inhibitor decreased the activity of right CeLC neurons after arthritis induction but had no effect in the left amygdala. Forskolin, however, increased the activity of left and right CeLC neurons under normal conditions. The results show for the first time laterality of pain-related electrophysiological activity changes in individual amygdala neurons. Whereas both left and right amygdala neurons receive nociceptive inputs and can become sensitized in principle, a yet unknown mechanism prevents PKA activation and pain-related changes in the left amygdala.

Introduction

Hemispheric lateralization in emotional processing is now well documented, but it remains to be determined if brain asymmetries are based on right hemispheric dominance, positive versus negative valence, appetitive approach versus defensive withdrawal, or behavioral activation versus inhibition systems (Atchley et al. 2003; Davidson et al. 2004; Demaree et al. 2005; Stephan et al. 2007). Hemispheric specialization for emotions involves not only the cerebral cortex but also subcortical areas such as the amygdala, a key player in emotion (Adolphs 2002; Davidson 2002; Maren 2005; Pare et al. 2004; Phelps and Ledoux 2005).

Lateralization of amygdala function in emotional processing has been suggested to depend on valence, gender, and other factors such as level of awareness, actuality of experience, and temporal activation dynamics. Predominant activation or involvement of the right amygdala in aversive behavior and negative emotions was found in animal models (Baker and Kim 2004; Coleman-Mesches and McGaugh 1995a,b; Coleman-Mesches et al. 1996; Lalumiere and McGaugh 2005) and in humans (Angrilli et al. 1996; Canli et al. 1998; Funayama et al. 2001; LaBar et al. 1998; Lee et al. 2004; Yoshimura et al. 2008). There is also evidence to suggest the preferential involvement of the right amygdala in emotional responses and emotional memory in men and of the left amygdala in women (see Cahill 2006 for review). The underlying principle of hemispheric lateralization of amygdala function in emotions remains unclear and needs to be determined for different emotions and conditions.

Pain has a strong emotional-affective component. The amygdala plays a critical role in the emotional response to pain and in pain modulation (Carrasquillo and Gereau 2007; Fields 2004; Gauriau and Bernard 2004; Heinricher and McGarvaughty 1999; Ikeda et al. 2007; Neugebauer et al. 2004, 2006; Pedersen et al. 2007; Rhudy and Meagher 2001). Our previous studies focused on the right amygdala and showed central sensitization and synaptic plasticity in neurons of the latero-capsular division of the central nucleus (CeLC) in an animal model of arthritis pain (Bird et al. 2005; Fu and Neugebauer 2008; Han et al. 2005b; Ji and Neugebauer 2007; Neugebauer and Li 2003; Neugebauer et al. 2003). The localized arthritis was induced in the contralateral (left) knee only. It remains to be determined if neuronal changes depend on the side of injury (ipsi- or contralateral knee) and if they occur in the left amygdala as well.

This question is important because recent studies showed that pain is associated with biochemical changes predominantly in the right amygdala. Pain-related ERK activation was observed in the right but not left CeLC, irrespective of the side of a formalin injection in the hind paw (Carrasquillo and Gereau 2007, 2008). Accordingly, blockade of ERK activation in the right but not left CeLC significantly decreased formalin-induced mechanical hypersensitivity in both the injected and the uninjured contralateral hind paw (Carrasquillo and Gereau 2007, 2008).

Evidence for pain-related lateralization is sparse and controversial. Psychophysical studies have suggested a functional asymmetry toward the right hemisphere for pain perception based on higher pain ratings for stimuli applied to the left side, independently of handedness (Lugo et al. 2002; Mersey and Watson 1979; Schiff and Gagliese 1994). Other studies found no such difference in pain sensation (Coghill et al. 2001; Hall et al. 1981; Seltzer et al. 1992). More direct evidence for right hemispheric lateralization in pain comes from a neuroimaging (PET) study that observed right lateralized activation of several brain areas, regardless of the side of peripheral stimulation (Coghill et al. 2001). Patients with chronic complex regional pain syndrome (CRPS) showed signs of gray matter atrophy in the right hemisphere but decreased white matter connectivity in the left (Geha et al. 2008). Right amygdala activation was seen
in an fMRI study in response to painful visceral (gastric) stimulation (Lu et al. 2004).

The present study tested the hypothesis that functional properties (responsiveness) of neurons in the right but not left CeLC are altered in a pain state, suggesting right-hemispheric lateralization of pain processing in a subcortical brain area. We also sought to determine if the lack of pain-related functional changes in left CeLC neurons correlates with failure to activate PKA in these neurons and if CeLC neurons in both hemispheres are capable of PKA-mediated sensitization. Our previous studies identified PKA activation as a critical mechanism of pain-related sensitization and plasticity in the amygdala (Bird et al. 2005; Fu et al. 2008; Ji and Neugebauer 2008). The present electrophysiological study is the first to show hemispheric differences in the responsiveness of individual amygdala neurons to noxious stimuli in a pain model. The results further suggest that PKA activation is necessary and sufficient for increased responsiveness of CeLC neurons but does not occur in the left CeLC in the arthritis pain model.

METHODS

Adult male Sprague–Dawley rats (250–350 g) were housed in a temperature-controlled room and maintained on a 12-h day/night cycle. Water and food were available without restriction. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Texas Medical Branch and conform to the guidelines of the International Association for the Study of Pain and of the National Institutes of Health.

Animal preparation and anesthesia

On the day of the electrophysiological experiment, the animal was 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 of anesthesia and for fluid support (3–4 ml·kg−1·h−1 lactated Ringer solution, administered intravenously). Depth of anesthesia was assessed by testing the corneal blink, hind-paw withdrawal, and tail-pinch reflexes and by continuously monitoring the end-tidal CO2 levels (kept at 4.0 ± 0.2%), heart rate, electrocardiogram (ECG) and breathing patterns. Core body temperature was 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 stroke/min). Constant levels of anesthesia were maintained by continuous intravenous infusion of pentobarbital (15 mg·kg−1·h−1). An individual CeLC neuron was identified by its background activity and by its responses to brief mechanical stimuli applied to the ipsi- and contralateral knee with a calibrated forceps (see Mechanical stimuli). Spike size and configuration were continuously monitored on the storage oscilloscopes and with the use of Spike2 software. Spikes were detected and recorded by the waveform signal that crossed a trigger level and matched a preset shape or template that was created for the individual neuron at the beginning of the recording period. Included in this study were only those neurons the spike configuration of which remained constant (matching the template) and could be clearly discriminated from activity in the background throughout the experiment, indicating that the activity of one and the same neuron was measured.

Receptive fields

Neurons were selected that had a receptive field in the knee. Size and thresholds of the receptive fields in deep tissue and skin were mapped using graded mechanical stimuli of innocuous and noxious intensities (see Mechanical stimuli). Cutaneous input was distinguished from deep tissue input by selective stimulation of skin folds gently raised from the underlying deep tissue. Mechanical stimuli were considered to activate deep tissue (joints and muscles) if the stimulation of overlying skin evoked no or a clearly distinct response. The focus of this study was on the processing of nociceptive information from the deep tissue. Standard diagrams of the rat body were used to record the location and size of the receptive field.

Mechanical stimuli

Mechanical stimuli were applied to the deep tissue by means of a forceps equipped with a force transducer the calibrated output of which was amplified, digitized, and recorded on a Pentium PC for on- and off-line analysis. Stimulus-response functions were generated using brief (15 s) graded mechanical test stimuli of increasing intensities (100, 500, 1,000, 1,500, and 2,000 g/30 mm2) at 15 s interstimulus intervals. Stimulus intensities of 100 and 500 g/30 mm2 applied to the knee and other deep tissue are considered innocuous because they do not evoke hind limb withdrawal reflexes in awake rats and are not felt to be painful when tested on the experimenters. An intensity of 1,000 g/30 mm2 represents a firm but nonpainful stimulus that does not evoke a hind limb withdrawal reflex. Pressure stimuli >1,500 g/30 mm2 are noxious because they evoke hind limb withdrawal reflexes and vocalizations in awake rats and are distinctly painful when applied to the experimenters (Han et al. 2005a,b; Han and Neugebauer 2005; Ji et al. 2007). Background activity before stimulation was subtracted from the total response during stimulation to calculate the net response evoked by a particular stimulus.

Classification and response thresholds

All neurons selected for this study were multireceptive (MR) neurons according to our classification of CeLC neurons with deep tissue input (Han et al. 2005b; Ji and Neugebauer 2007; Li and Neugebauer 2004a,b; 2006; Neugebauer and Li 2003), long-term extracellular recordings were made from single neurons in the CeLC with glass-insulated carbon filament electrodes (4–6 MΩ) using the following stereotaxic coordinates (Paxinos and Watson 1998): 2.1–2.8 mm caudal to bregma; 3.8–4.5 mm lateral to midline; depth 7–9 mm. The recorded signals were amplified and displayed on analog and digital storage oscilloscopes. Signals were also fed into a window discriminator the output of which was processed by an interface (CED 1401 Plus) connected to a Pentium 4 PC. Spike2 software (CED, version 4) was used to create peristimulus rate histograms on-line and to store and analyze digital records of single-unit activity off-line.

Identification of amygdala neurons

An individual CeLC neuron was identified by its background activity and by its responses to brief mechanical stimuli applied to the ipsi- and contralateral knee with a calibrated forceps (see Mechanical stimuli). Spike size and configuration were continuously monitored on the storage oscilloscopes and with the use of Spike2 software. Spikes were detected and recorded by the waveform signal that crossed a trigger level and matched a preset shape or template that was created for the individual neuron at the beginning of the recording period. Included in this study were only those neurons the spike configuration of which remained constant (matching the template) and could be clearly discriminated from activity in the background throughout the experiment, indicating that the activity of one and the same neuron was measured.

Electrophysiological recording

As described previously (Han et al. 2005b; Ji and Neugebauer 2007; Li and Neugebauer 2004a,b, 2006; Neugebauer and Li 2003), the recorded signals were amplified and displayed on analog and digital storage oscilloscopes.
more strongly activated by noxious stimuli (>1500 g/30 mm²). Mechanical threshold was defined as the minimum stimulus intensity that evoked an excitatory response (spike frequency higher than the upper 95% confidence interval of background activity).

**Experimental protocol**

In each experiment, one CeLC neuron was recorded in the left or right CeLC before and for several hours after arthritis induction in the ipsi- or contralateral knee joint (see Arthritis). Background activity, evoked responses, and receptive-field size were measured repeatedly before and after induction of arthritis and before and during drug administration into the CeLC (see Drugs). Background activity was recorded for >10 min to calculate means ± SE and 95% confidence intervals (CI; GraphPad Prism 3.0). Before arthritis induction and during the development of arthritis, mechanical test stimuli were applied to the knee and other deep tissue in the receptive field at regular intervals of ~30 min. Before and during drug applications, intervals between the test stimuli were 5–10 min. Number of stimulations was kept at a minimum to avoid any “sensitization” that might be produced by repeated stimulation. Sufficiently long control periods were used to establish consistent baseline responses before arthritis induction and drug application. A paired paradigm was used to determine arthritis pain-related changes. Rather than comparing neuronal activity in arthritis with saline-injected control groups, each neuron served as its own control and was recorded continuously before and after arthritis induction in the same animal. A previous study found no difference between saline-injected and untreated normal rats on synaptic transmission and excitability in CeLC neurons (Neugebauer et al. 2003).

**Arthritis**

Arthritis was induced as described in detail previously (Neugebauer et al. 2007; Schaible and Schmidt 1990). A kaolin suspension (4%, 100 μl) was slowly injected into the knee cavity through the patellar ligament with the use of a syringe and needle (1 ml, 25 gauge, 5/8 in). After repetitive flexions and extensions of the knee for 15 min, a carrageenan solution (2%, 100 μl) was injected into the knee joint 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 the inflammation persists for weeks (Neugebauer et al. 2007).

**Drugs and drug administration by microdialysis**

KT5720, a potent and selective membrane-permeable PKA inhibitor (Bird et al. 2005; Cabell and Audesirk 1993), and forskolin, a membrane-permeable activator of adenylyl cyclase (Awad et al. 1983; Laurezza et al. 1989), were purchased from Tocris Bioscience, Ellisville, MO. Drugs were administered into the CeLC by microdialysis at a rate of 5 μl/min for 15 min. Several hours before the start of the electrophysiological recordings a microdialysis probe (CMA11; CMA/Microdialysis; membrane diameter: 250 μm; membrane length: 1 mm) was positioned stereotaxically in the left or right CeLC, using the following coordinates: 1.6 mm caudal to bregma; 4.0 mm lateral to midline; depth of tip 9.0 mm (Han et al. 2005b; Jia and Neugebauer 2007; Li and Neugebauer 2004a,b, 2006). Using PE-50 tubing, the microdialysis probe was connected to an infusion pump (Harvard) and perfused with artificial cerebrospinal fluid (ACSF) containing (in mM) 125.0 NaCl, 2.6 KCl, 2.5 NaH2PO4, 1.3 CaCl2, 0.9 MgCl2, 21.0 NaHCO3, and 3.5 glucose; oxygenated and equilibrated to pH = 7.4. Before the recordings, ACSF was pumped through the fiber for >1 h to establish equilibrium in the tissue. ACSF was present throughout the experiment and also served as a vehicle control.

KT5720 and forskolin were dissolved in ACSF on the day of the experiment at a concentration of 100 times lower than in the microdialysis probe as a result of the concentration gradient across the dialysis membrane and diffusion in the tissue (Fu et al. 2008; Jia and Neugebauer 2008). The numbers given in this article refer to the drug concentrations in the microdialysis fiber.

**Histology**

At the end of each experiment, the recording site in the CeLC 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-sectioned at 50 μm. 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 adapted from Paxinos and Watson (1998) (see Fig. 1). The positions of the microdialysis probes in the CeLC were also verified histologically (not shown; they were virtually identical and the same stereotaxic coordinates were used in every experiment).

**Data analysis**

Extracellularly recorded single-unit action potentials were analyzed off-line from peristimulus rate histograms using Spike2 software (CED, version 4). Responses to mechanical stimuli were measured and expressed as spikes per second (Hz). Background activity was subtracted from the total activity during the stimulus to obtain the “net” stimulus-evoked activity. A two-way ANOVA with Tukey posttests (SigmaStat 3.1) was used to evaluate statistically the effect of lateralization (left vs. right amygdala) and treatment (arthritis vs. normal) on neuronal activity (data in Fig. 3). A paired t-test was used to compare in four different experimental paradigms (left CeLC/right knee; left CeLC/lefthand; right CeLC/lefthand; right CeLC/riighthand; data in Fig. 4) each neuron’s activity in arthritis with prearthrits normal control values (paired experimental paradigm; Prism 3.0, GraphPad Software). A paired t-test was also used to determine significant differences of neuronal activity before and during drug administration (Prism 3.0, GraphPad Software). Statistical analysis was performed on raw data (firing rate measured as spikes per second). Statistical significance was accepted at the level P < 0.05.

For quantification of the receptive field size, the body map was divided into 21 different areas. A score of 1 was assigned to each area that was part of the total receptive field. Addition of the scores yielded a value for the total receptive field of a neuron. Averaged values for left versus right amygdala neurons were compared using a Mann-Whitney U test (unpaired experimental paradigm; Prism 3.0, GraphPad Software). Averaged values for normal versus arthritis state were compared using a Wilcoxon signed-rank test (paired experimental paradigm; Prism 3.0, GraphPad Software). For multiple comparisons in nonparametric tests, the alpha level was adjusted and statistical significance accepted at the level P < 0.025.

**Results**

Extracellular single-unit recordings were made from 17 neurons in the left and 15 neurons in the right CeLC of anesthetized adult male rats (Fig. 1). Only one neuron was recorded in each rat. Neurons were selected that had a receptive field in the knee joint as in our previous studies. Neurons were multireceptive (MR, see Classification) and responded more strongly to brief noxious than innocuous stimuli applied to the knee and other parts of the receptive field. In this study, we included only MR neurons because our previous studies showed that they consistently and reliably become sensitized in the arthritis pain model (Han et al. 2005b; Jia and Neugebauer 2007; Li and Neugebauer 2004a,b, 2006; Neugebauer and Li 2003) and are believed to integrate
nociceptive and affective information (Neugebauer 2006; Neugebauer et al. 2004). Continuous recordings before and after arthritis induction were made from 11 neurons in the left and 9 neurons in the right CeLC. The remaining neurons (6 in the left and 6 in the right CeLC) were only recorded under normal conditions to determine the effects of forskolin alone and in the presence of a PKA inhibitor (KT5720).

Properties of left and right CeLC neurons under normal conditions

RECEPTIVE FIELD SIZE. All neurons were activated by mechanical stimulation (compression) of the knee joint, which served as the search stimulus. The receptive field of neurons in the left CeLC ($n = 17$) was smaller than that of neurons in the right CeLC ($n = 15$; see Fig. 2). Receptive fields of left CeLC neurons were either confined to the contralateral hindlimb ($n = 9$) or included an additional high-threshold receptive field in the ipsilateral hindlimb ($n = 8$; Fig. 2B, normal). In contrast, receptive fields of right CeLC neurons were always ($n = 15$) bilateral and symmetrical in the deep tissue of the hindlimbs and tail; some of these neurons ($n = 8$) had additional receptive fields in the forepaws and trunk (Fig. 2A, normal).

In an attempt to quantify the differences, we divided the body map into 21 different sectors (see Fig. 2). The total number of areas that contained part of the receptive field of a neuron was calculated and averaged for neurons in the left and for those in the right CeLC. The comparison (Fig. 2C) showed that the average receptive field size of right CeLC neurons was significantly larger than that of left CeLC neurons under normal conditions ($P < 0.0005$, Mann-Whitney $U$ test).

BACKGROUND ACTIVITY AND EVOKED RESPONSES. No evidence of lateralization was found for background activity and responses to innocuous and noxious stimuli under normal conditions. Figure 3 shows the background activity and evoked
responses of individual CeLC neurons in the left (A) and right (B) CeLC before arthritis induction (and changes after arthritis; see Properties of left and right CeLC neurons in the arthritis pain model). The averaged values for the samples of left (n = 11) and right (n = 9) CeLC neurons under normal conditions are shown in Fig. 3C. The analysis only includes neurons that were recorded before and after arthritis induction to allow the direct comparison. Statistical analysis revealed no significant differences between left and right CeLC neurons under normal conditions (P > 0.05, Tukey test).
Properties of left and right CeLC neurons in the arthritis pain model

RECEPTIVE FIELD SIZE. After the induction of a knee joint arthritis (see METHODS) the size of the receptive field of neurons in the right CeLC expanded (Fig. 2A). This change was observed in the majority of right CeLC neurons (6 of 9 neurons); the receptive fields of the remaining right CeLC neurons covered the whole body before arthritis and no apparent increase was detected. In contrast, the receptive field size of neurons in the left CeLC did not change (Fig. 2B). The quantitative analysis of the receptive field size on the body map (Fig. 2C) also revealed significant increases for right \( (P < 0.025) \) but not left \( (P > 0.05) \) CeLC neurons in the arthritis pain model (Wilcoxon signed-rank test). The receptive field size of right CeLC neurons was significantly greater than that of left CeLC neurons \( (P < 0.0005, \text{Mann-Whitney } U \text{ test}) \). The analysis only included neurons that were recorded before and after arthritis induction to allow the direct comparison of the receptive field size.

BACKGROUND ACTIVITY AND EVOKED RESPONSES. Activity of right but not left CeLC neurons increased in the arthritis pain model. Figure 2B shows an individual example of a neuron in the right CeLC. In agreement with our previous studies (Han et al. 2005b; Ji and Neugebauer 2007; Li and Neugebauer 2004a,b, 2006; Neugebauer and Li 2003), background activity and responses to innocuous and noxious stimulation of the knee (see Mechanical stimuli) increased after arthritis induction and reached a plateau at \( 4-5 \) h. In contrast, background activity and evoked responses of a neuron in the left CeLC did not change for several hours after arthritis induction (Fig. 3A). In both cases, arthritis was induced in the knee contralateral to the recording site because our previous studies showed sensitization of right CeLC neurons when arthritis was induced in the contralateral (left) knee (Han et al. 2005b; Ji and Neugebauer 2007; Li and Neugebauer 2004a,b, 2006; Neugebauer and Li 2003). Both neurons were recorded continuously before and after arthritis induction.

Figure 3C shows significant right-hemispheric lateralization of arthritis pain-related changes. The activity of left \((n = 11)\) versus right \((n = 9)\) CeLC neurons are compared under normal conditions and in the arthritis pain model. Two-way ANOVA revealed significant main effects of lateralization on background activity \( [P < 0.05, F(1,36) = 5.06] \) and on responses to innocuous \( [P < 0.01, F(1,36) = 8.28] \) and noxious \( [P < 0.05, F(1,36) = 5.76] \) stimuli. Tukey posttests showed significant differences between left and right CeLC neurons in the arthritis pain model (background, \( P < 0.05 \); innocuous, \( P < 0.01 \); noxious, \( P < 0.01 \)) but not under normal conditions \( (P > 0.05) \). Two-way ANOVA also revealed significant main effects of treatment on the responses to innocuous \( [P < 0.05, F(1,36) = 4.24] \) and noxious \( (P < 0.05, \text{F}(1,36) = 4.24) \).
Importantly, the differential effects of arthritis pain on right and left CeLC neurons were independent of the side of the monoarthritis. Significant (P < 0.05, paired t-test performed on raw data) changes of background and evoked activity were observed in right CeLC neurons after arthritis was induced in the contralateral (left) knee (n = 5 neurons; Fig. 4C) or ipsilateral (right) knee (n = 4 neurons; D). Left CeLC neurons showed no changes after arthritis was induced either in the right knee (n = 5; A) or left knee (n = 6; B). These results suggest that pain-related lateralization is independent of the side of arthritis.

Effects of a PKA inhibitor

Our previous studies showed an important contribution of PKA to central sensitization and synaptic plasticity in the right CeLC (Bird et al. 2005; Fu et al. 2008). Here we addressed the hypothesis that lack of PKA activation in the left CeLC (Bird et al. 2005; Fu et al. 2008). Here we addressed the hypothesis that lack of PKA activation in the left CeLC (Bird et al. 2005; Fu et al. 2008). Here we addressed the hypothesis that lack of PKA activation in the left CeLC (Bird et al. 2005; Fu et al. 2008). Here we addressed the hypothesis that lack of PKA activation in the left CeLC contributed to pain-related right-hemispheric lateralization. A selective PKA inhibitor (KT5720, KT; 100 μM, concentration in the microdialysis fiber; 15 min; see Drugs and drug administration by microdialysis) was administered into the CeLC 5–6 h postinduction of arthritis. The positions of the microdialysis probes in the CeLC were verified histologically. Administration of KT5720 into the left CeLC had no effect on CeLC neurons in the left hemisphere. An individual example is shown in Fig. 5A. Figure 5B summarizes the lack of effect of KT5720 on background and evoked activity in the sample of left CeLC neurons (n = 5) after arthritis induction in the contralateral knee. Administration of KT5720 into the right CeLC significantly (P < 0.05, paired t-test) inhibited the increased activity of neurons in the right CeLC (n = 5; see individual example in Fig. 5C and summary of data in D). The data suggest that PKA is not activated endogenously in the left CeLC in the arthritis pain model.

Effects of forskolin

Next we sought to determine if the exogenous activation of signal transduction pathways could sensitize neurons in the left CeLC. A widely used cell-permeable activator of adenylyl cyclase (forskolin, 1 mM, concentration in microdialysis fiber; 15 min) was administered into the left or right CeLC under normal conditions (no arthritis). The positions of the microdialysis probes in the CeLC were verified histologically. Forskolin increased background activity and evoked responses of left and right CeLC neurons. The receptive field size did not change. Figure 6 shows individual neurons in the left (A) and right (C) CeLC and summarizes the significant effects of forskolin on the sample of neurons in the left (n = 4, B) and right (n = 4, D) CeLC. In the presence of a PKA inhibitor (KT5720, 100 μM, concentration in the microdialysis fiber; 15 min), forskolin had no significant effect (P > 0.05, compared with predrug control values; paired t-test;

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**Left CeLC**

A. Arthritis contralateral

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**Right CeLC**

C. Arthritis contralateral

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D. Arthritis ipsilateral

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*P < 0.05 (paired t-test comparing values in arthritis with prearthritis values under normal conditions; statistical analysis was performed on raw data).
DISCUSSION

The key findings of this study are as follows. Unlike CeLC neurons in the right amygdala, neurons in the left CeLC do not develop increased responsiveness in a rodent model of arthritis pain. This hemispheric lateralization is independent of the side of the peripheral injury (ipsi- or contralateral to the recording site). No significant difference was found in the magnitude of the responses of left and right CeLC neurons to brief physiological noxious stimuli under normal conditions, indicating that individual inputs have comparable effects on neurons in the left and right CeLC.

The smaller receptive field size of left compared with right CeLC neurons may suggest the tonic control of effective inputs. The contribution of PKA in the right but not left amygdala to pain-related changes is in agreement with the findings of another group (Carrasquillo and Gereau 2007, 2008) that pain-related lateralization involves differences in the endogenous activation of signaling pathways in the CeLC. Importantly, the exogenous activation of intracellular effectors in the left or right CeLC by forskolin produced activity changes that resembled those observed in the arthritis pain model. The results suggest that PKA activation is necessary and sufficient for increased responsiveness of CeLC neurons but does not occur in the left CeLC in the arthritis pain model.
**Left CeLC**

![Graph A](image)

**Right CeLC**

![Graph C](image)

unknown mechanism prevents their activation in the arthritis pain model. This result is novel and significant because there has been little evidence for hemispheric lateralization of brain functions related to pain (see Introduction). Two recent biochemical and behavioral studies (Carrasquillo and Gereau 2007, 2008) were the first to show lateralization of amygdala function in pain. Activation of the MAP kinase ERK was observed in the right, but not left, CeLC in the formalin pain model. Conversely, blockade of ERK activation in the right, but not the left, CeLC inhibited pain behavior. Results from the present study suggest that hemispheric lateralization is not restricted to the function of a single molecule (ERK) but also involves PKA and possibly other effectors. Failure to activate these signaling pathways appears to prevent the left CeLC from developing pain-related activity increases, therefore contributing to right-hemispheric lateralization.

A consequence of pain-related changes in the right, but not left CeLC, would be “asymmetric” output from the amygdala to target structures such as the periaqueductal gray (PAG) (Heinricher and McGaraughty 1999; Neugebauer et al. 2004; Rizvi et al. 1991; Shipley et al. 1991; Tracey and Mantyh 2007). The PAG is an important brain stem center for the descending modulation of pain and other behaviors (Heinricher and McGaraughty 1999; Mason 2005; Tracey and Mantyh 2007). Importantly, increased neural transmission in this largely ipsilateral output pathway during stress-induced anxiety was observed in the right but not left hemisphere (Adamec et al. 2005a,b). Consistent with this finding, pain behavior was modified by manipulating ERK activation in the right but not left CeLC (Carrasquillo and Gereau 2007, 2008).

Relatively few studies have specifically addressed or described lateralization of amygdala function in rodents. Predominant activation or involvement of the right amygdala was found in aversively motivated learning and memory (Coleman-Mesches and McGaugh 1995a,b; Coleman-Mesches et al. 1996; Lalumiere and McGaugh 2005) and contextual fear memory.
conditioning (Baker and Kim 2004) in rats. In humans, right hemispheric lateralization of amygdala function was associated with negative emotions (Angrilli et al. 1996; Canli et al. 1998; Funayama et al. 2001; Lee et al. 2004; Yoshimura et al. 2008), fear extinction (LaBar et al. 1998), subconscious emotional learning (Morris et al. 1998), rapid automatic stimulus detection and response (Costafreda et al. 2008; Sergerie et al. 2008), and pain (Lu et al. 2004). Positive emotions tend to be lateralized to the left amygdala in humans (Canli et al. 1998; Lee et al. 2004; Yoshimura et al. 2008). However, greater right than left amygdala activation was reported for viewing happy faces and greater left amygdala activation for fearful faces (Hardee et al. 2008; Killgore and Yurgelun-Todd 2001). The left rather than right amygdala has been implicated in perceived or anticipated but not actually experienced fear (Funayama et al. 2001; Phelps et al. 2001). Sex-related hemispheric differences of amygdala activation include the preferential involvement of the right amygdala in emotional responses and emotional memory in men and of the left amygdala in women (see Cahill 2006 for review). Sex-related differences, however, may be valence dependent because they were observed for happy but not fearful faces (Killgore and Yurgelun-Todd 2001). These data strongly support the concept of hemispheric lateralization of amygdala function in emotions, but the underlying principle and mechanisms remain to be determined.

Pain-related right hemispherical lateralization of amygdala function is consistent with the predominant activation or involvement of the right amygdala in emotions. One study suggested that left sided pain, experimental or chronic, produced greater “emotional disturbance” and anxiety (Schiff and Gagliese 1994). Pain has a negative emotional-affective component and is closely related to anxiety and depression (Gallagher and Verma 2004; Grachev et al. 2001; Rhudy and Meagher 2003; Tracey and Mantyh 2007), but the role of hemispheric lateralization in this relationship remains to be determined.

Possible mechanisms of pain-related lateralization of amygdala function include differences in nociceptive inputs, neuronal properties, and control by other brain areas. The amygdala receives nociceptive information through anatomically and functionally distinct lines of input (Braz et al. 2005; Neugebauer 2006; Neugebauer et al. 2004). Purely nociceptive information reaches the CeLC directly from the spinal cord and brain stem (parabrachial area), thus bypassing the thalamus (Bernard and Besson 1990; Cliffer et al. 1991; Gauriau and Bernard 2004). Polymodal sensory, including nociceptive, inputs from thalamus (posterior areas) and cortex (insula and association cortices) reach primarily the lateral amygdala (Pare et al. 2004; Phelps and Ledoux 2005; Shi and Davis 1999). Associative processing in the lateral-basolateral amygdala network generates affect-related information that is transmitted to the central nucleus, a major output nucleus for amygdala functions (Maren 2005; Pare et al. 2004; Phelps and Ledoux 2005). The present study shows that CeLC neurons on the left and right respond to brief innocuous and noxious stimuli with similar magnitude under normal conditions, arguing against a major difference in nociceptive and nonnociceptive inputs to the CeLC.

Differences in the neuronal populations of the left and right CeLC are unlikely to account for lateralized amygdala functions. The two principal types of CeLC neurons are nociceptive-specific (NS) neurons, which receive exclusively nociceptive input, and multireceptive (MR) neurons, which respond to innocuous and noxious stimuli and integrate nociceptive signals with affective information from the lateral-basolateral circuitry (Neugebauer 2006; Neugebauer et al. 2004). MR neurons, but not NS neurons, undergo central sensitization in the arthritis pain model (Neugebauer and Li 2003). The present study did not systematically analyze the proportion of NS and MR neurons, but MR neurons were identified in the left CeLC and did not show the pain-related changes of MR neurons in the right CeLC.

Biochemical and pharmacological data suggest that lateralized amygdala function in pain involves differences in the activation of cellular signaling pathways (ERK, Carrasquillo and Greer 2007, 2008; PKA, present study). The effects of forskolin in the present study show that effector systems can be activated in left CeLC neurons and produce increased neuronal activity. These data suggest that a yet unknown mechanism prevents the pain-related endogenous activation of signaling mechanisms and sensitization of left CeLC neurons. The smaller receptive field sizes of neurons in the left compared with the right CeLC also argues for the presence of a control mechanism of effective inputs and cellular effectors. Expansion of the receptive fields of CNS neurons is well documented in the arthritis pain model and is generally taken as evidence for central sensitization that renders normally ineffective inputs functional (Neugebauer and Li 2003; Neugebauer and Schaible 1990).

The difference in receptive field size between left and right CeLC neurons and the lack of pain-related changes in left CeLC neurons may suggest a tonic inhibitory mechanism, which could involve the well-known cortical control of amygdala functions (see Banks et al. 2007). The amygdala is reciprocally connected with prefrontal cortical areas (see Ghasshghai et al. 2007) that exert a top-down inhibitory influence on the amygdala (Carmichael and Price 1995; McDonald et al. 1996; Quirk and Beer 2006; Rosenkranz and Grace 2002). Inverse coupling of prefrontal cortex and amygdala was observed in imaging studies in humans in association with aversive and other emotional stimuli, possibly representing a neural system for cognitive regulation of emotions (Kim et al. 2003; Ochsner et al. 2002; Urry et al. 2006; but see Banks et al. 2007). Pertinent to the current study, greater left than right prefrontal cortical electroencephalographic (EEG) activity predicted an attenuated physiological response to aversive stimuli (Jackson et al. 2003).

In conclusion, the present study demonstrates differences in the processing of nociceptive information by neurons in the left versus right amygdala in a model of arthritic pain. Pain-related sensitization of left CeLC neurons is prevented by a mechanism that remains to be determined but may involve prefrontal cortical inhibition of the amygdala. Right hemispheric lateralization of pain processing in the amygdala is consistent with a predominant role of the right amygdala in negative emotions.

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