Enhanced pronociception by amygdaloid group I metabotropic glutamate receptors in nerve-injured animals

Osei B. Ansah[a], Leonor Gonçalves[a,b], Armando Almeida[b], Antti Pertovaara[a,*]

[a]*Institute of Biomedicine/Physiology, POB 63, University of Helsinki, 00014 Helsinki, Finland,*

[b]*Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, 4710 Braga, Portugal*

[*]Correspondending author. Fax: +358 9 191 25302

E-mail address: Antti.Pertovaara@helsinki.fi.

Peripheral neuropathy has been associated with structural and functional changes of the amygdala, a key player in emotions. Here we study whether peripheral neuropathy influences pain regulation by the amygdala. For this purpose, we determined discharge rates of presumably pro- and antinociceptive pain-regulatory neurons in the rostral ventromedial medulla (RVM) following microinjection of various glutamatergic compounds into the central nucleus of the amygdala. RVM neurons were recorded in pentobarbitone-anesthetized rats with a peripheral nerve injury or sham-operation. In a separate behavioral experiment, we determined whether the influence of amygdaloid administration of a glutamatergic compound on affective pain-related behavior, as assessed by an aversive place-conditioning test, is changed by neuropathy. While glutamate or an NMDA receptor antagonist in the amygdala failed to induce marked changes in discharge rates of RVM cells, amygdaloid administration of DHPG, a group I metabotropic glutamate receptor (mGluR) agonist acting on $mGluR_1$ and $mGluR_5$, increased discharge rates of presumably pronociceptive RVM ON-cells in nerve-injured but not sham-operated animals. This pronociceptive effect of DHPG was reversed by MPEP ($mGluR_5$ antagonist) and CPCCOEt ($mGluR_1$ antagonist). CHPG, an $mGluR_5$ agonist, failed to influence ON-cell activity and DHPG failed to influence activity of presumably antinociceptive RVM OFF-cells. Amygdaloid administration of DHPG increased and that of CPCCOEt decreased affective pain-related behavior in nerve-injured animals. The results suggest that following nerve injury, the amygdaloid group I mGluR, particularly subtype $mGluR_1$, has an enhanced pronociceptive effect providing a potential mechanism for emotional enhancement of pain in peripheral neuropathy.

Introduction

The amygdala is a major player in emotions (Phelps and LeDoux, 2005). It also receives ascending nociceptive signals (Bernard et al., 1996) and it has efferent projections to structures that are involved in pain modulation (e.g., Rizvi et al., 1991; Van Bockstaele et al., 1996). These findings, together with chemical or electrical stimulation and lesion studies (see below), indicate that the amygdala has a role in pain modulation. Interestingly, the role is dual one varying from antinociception (Helmstetter and Bellgowan, 1993; Helmstetter et al., 1998; Manning and Meyer, 1995; McGaraughty and Heinricher, 2002; Mena et al., 1995; Nandigama and Borszcz, 2003) to pronociception (Greenwood-Van Meerveld, 2001; Manning, 1998; Quin et al., 2003).

Sustained nociception produces synaptic plasticity in the amygdala. This has been shown in electrophysiological recordings performed in animals with inflammatory pain (Neugebauer et al., 2004; Neugebauer, 2006) and in control animals following tetanic stimulation of the parabrachial nucleus that relays nociceptive inputs to the amygdala (Lopez de Armentia and Sah, 2007). Peripheral nerve injuries may cause chronic neuropathic pain that is associated with plastic changes in pain-mediating (Woolf and Salter, 2006) and -regulating (Almeida et al., 2006; Pertovaara, 2000; Porreca et al., 2002) pathways. Recent studies indicate that peripheral nerve injury induces neural plasticity in the amygdala, as shown by increased postsynaptic currents evoked by ascending inputs (Ikeda et al., 2007) and generation of new amygdala neurons (Gonçalves et al., 2008). These findings still

leave open whether the pain regulatory role of the amygdala is changed by peripheral nerve injury.

In the present study we test a hypothesis that peripheral nerve injury influences pain regulation by the amygdala. Partial support for this hypothesis is provided by a recent finding showing that amygdaloid activation by glutamate suppressed presumably antinociceptive neurons in the noradrenergic locus coeruleus of nerve-injured animals (Viisanen and Pertovaara, 2007). To test further this hypothesis, we determined whether administration of glutamatergic compounds into the amygdala has a differential influence on discharge rates of putative pain-regulatory neurons in the rostroventromedial medulla (RVM) of nerve-injured versus sham-operated animals. For this purpose, we recorded discharge rates of presumably pronociceptive ON-cells and antinociceptive OFF-cells in the RVM (Fields et al., 2006). Moreover, we assessed whether the effect by amygdaloid administration of a glutamatergic compound on affective pain-related behavior in an aversive place-conditioning test is changed following peripheral nerve injury.

Materials and methods

The experiments were performed in adult, male Hannover-Wistar rats weighing 180-190 g at the beginning of the experiment (Harlan, Horst, The Netherlands). The experimental protocol was accepted by the Institutional Ethics Committee and the experiments were performed according to the guidelines of European Communities Council Directive of 24 November 1986 (86/609/EEC). All

efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

Techniques for producing neuropathy

The unilateral axotomy and ligation of the tibial and common peroneal nerves was performed under pentobarbitone anesthesia (50 mg/kg i.p.) as described in detail earlier (Decosterd and Woolf, 2000; Gonçalves et al., 2007). Briefly, the skin of the lateral surface of the thigh was incised and a section made directly through the biceps femoris muscle exposing the sciatic nerve and its three terminal branches. Following ligation and removing 2-4 mm of the distal nerve stumps of the tibial and common peroneal nerves, muscle and skin were closed in two layers. In sham-operated animals, the surgical procedure was identical, except that the tibial and common peroneal nerves were not ligated or sectioned. After the surgery, the animals were allowed to recover before the actual testing that was performed either one or eight weeks after the operation.

Behavioral verification of neuropathy

Development of hypersensitivity was verified behaviorally in animals habituated to the experimental conditions 1-2 h daily for 2 to 3 days. For assessment of tactile allodynia, the hind limb withdrawal threshold was determined stimulating the sural nerve area in the hind paw of the operated limb with monofilaments. The calibrated series of monofilaments used in this study produced forces ranging from 0.16 to 15 g (North Coast Medical, Inc. Morgan Hill, CA, USA. The monofilaments

were applied to the foot pad with increasing force until the rat withdrew its hind limb. The lowest force producing a withdrawal response was considered the threshold. The threshold for each hind paw of each rat was based on three separate measurements and the median of these values was considered to represent the threshold. Threshold values ≤1 g were considered to represent hypersensitivity. It should be noted that the currently used strain of rats delivered by Harlan (Horst, Netherlands) has an exceptionally low withdrawal threshold to monofilament stimulation in baseline (unoperated) condition: in ten unoperated control animals the lowest withdrawal threshold was only 4 g and therefore, the criterion for hypersensitivity was set to as low as ≤1 g in this study as we did earlier with the same strain of animals (Gonçalves et al., 2007).

Electrophysiological recordings

For electrophysiological recordings the anesthesia was induced by pentobarbitone at a dose of 50 mg/kg i.p. and the animal was placed in a standard stereotaxic frame according to the atlas of Paxinos and Watson (1998). Anesthesia was maintained by infusing pentobarbitone (15-20 mg/kg/h). The level of anesthesia was frequently monitored by observing the size of the pupils and by assessing withdrawal responses to noxious stimulation. When necessary, the infusion rate of pentobarbitone was adjusted to keep the level of anesthesia steady. Although a change in the level of anesthesia may significantly influence neuronal responses, anesthesia is not likely to explain differences among different experimental groups and drug treatments in the present study. This, because anesthesia was induced and maintained in an identical manner in all experimental conditions. The rats were spontaneously

breathing. A warming blanket was used to maintain body temperature within physiological range. Peripheral perfusion was checked by evaluating the color of ears and extremities. The skull was exposed and a hole drilled for placement of recording electrode in the RVM. The desired recording site in the RVM was 1.8-2.3 mm posterior from the ear bar, 0.4-0.9 mm lateral from the midline, and 8.9-10.7 mm ventral from the dura mater (Fig. 1).

Single neuron activity was recorded extracellularly with lacquer-coated tungsten electrodes (tip impedance 3-10 MΩ at 1 kHz) and then amplified and filtered using standard techniques. Data sampling was performed with a computer connected to a CED Micro 1401 interface and using Spike 2 software (Cambridge Electronic Design, Cambridge, U.K.).

Actual recordings did not start until the animal was under light anesthesia; i.e., the animals gave a brief withdrawal response to noxious pinch, but the pinch did not produce any longer lasting motor activity, nor did the animals have spontaneous limb movements. Neurons were classified based on their response to noxious pinch of the tail with a hemostatic clamp (Fig. 2). This stimulus was painful when applied to the finger of the experimenters. Neurons giving excitatory responses to pinch were considered ON-cells, those giving inhibitory responses were considered OFF-cells and neurons showing no or only a negligible (<10 %) change in their discharge rates as a response to pinch were considered NEUTRAL-cells. This classification scheme of medullary neurons was modified from that described by Fields and his co-workers (2006). A noteworthy difference is that we did not verify whether pinch-evoked responses of RVM neurons were associated with spinal reflex responses as in the original classification scheme (Fields et al., 2006). Therefore, the populations of ON- and OFF-cells in this study may not be identical with those in a study in which cells

are classified strictly according to the classification scheme of Fields and co-workers (2006). Our previous results suggest, however, that there is only little difference in the classification of RVM neurons whether or not spinal reflex responses are concurrently measured in lightly anesthetized animals (Pertovaara et al., 2001).

Intracerebral drug injections

The animals had a guide cannula for drug administrations into the amygdala ipsilateral to the spared nerve injury or sham-operated limb (left side), except for one group that had the cannula contralateral to the nerve injury (right side), and a control group that had the cannula in the hippocampus. Additionally, a group of animals tested in behavioral experiments only had a bilateral guide cannula for drug injections into the central nucleus of the amygdala. For placement of the guide cannula (26 gauge), the skull was exposed and a hole drilled for its placement. The desired injection site was in the central nucleus of the amygdala: 7.12 mm anterior from the ear bar, 3.40 mm lateral from the midline, and 8.00 mm ventral from the dura mater. A control injection site in a group of neuropathic animals was in the hippocampus, ipsilateral to nerve injury: 6.20 mm anterior from the ear bar, 1.00 mm lateral from the midline, and 3.20 mm ventral from the dura mater. The tip of the guide cannula was positioned 2 mm above the desired injection site. The cannula was fixed into the skull using a dental screw and dental cement. Drug administration to the brain and experimental protocols were performed one week after fixation of the guide cannula to the skull. When testing animals at the one-week postoperative time point, the guide cannula for amygdala injections was installed in the same operating session as sham or nerve surgery. When testing animals at the eight-week postoperative time point, the

guide cannula was installed in a separate session at least one week prior to electrophysiological recordings.

Drugs or saline control were microinjected into the amygdala through a 33-gauge stainless steel injection cannula inserted through and protruding 2 mm beyond the tip of the guide cannula. The microinjection was made using a 10 µl Hamilton syringe that was connected to the injection cannula by a length of a polyethylene (PE-10) tubing. The volume of injection was 0.5 µl. At this volume, the spread of the injected drugs within the brain was at least 1 mm (Myers, 1966). The efficacy of injection was monitored by watching the movement of a small air bubble through the tubing. The injection lasted 30 s and the injection cannula was left in place for an additional 30 s to minimize flow of the drug solution back up the injector track. At the completion of the experiment, the microinjection sites were histologically verified and plotted on a standardized section derived from the stereotaxic atlas of Paxinos and Watson (1998).

Course of the electrophysiological study

There were four groups of animals that were included in the electrophysiological study and that had a guide cannula for amygdala injections ipsilateral to nerve injury or sham operation: i) sham group tested one week after operation, ii) sham group tested eight weeks after operation, iii) SNI group tested one week after operation, iv) SNI group tested eight weeks after operation. Additionally, there was a fifth group of SNI animals tested one week after operation that had the guide cannula for amygdala injections contralateral to nerve injury, and a sixth group of animals with neuropathy of one week duration that had the guide cannula for drug

injections into a control site in the hippocampus ipsilateral to nerve injury. In each of these groups, neuropathic hypersensitivity was verified with the monofilament test (see above) before the start of the electrophysiological experiment.

After induction of anesthesia, the microelectrode was lowered to the RVM. After finding a single cell, it was first classified based on its response to noxious tail pinch (see above) and then its spontaneous activity was recorded for two to three minutes. Next, one of the studied drugs or saline control was administered in a varied order to the amygdala and the spontaneous activity was recorded for up to 30 min (except with glutamate and MK-801 for six min). One to two drug conditions were tested in one cell, and one to four cells were tested in each animal. The minimum interval to the next drug testing condition was 30 min following saline or glutamate, while it was 60 min following other drugs, except for MK-801 that was always the last drug tested in each animal. When attempting to reverse the effect induced by the glutamatergic agonist DHPG, the antagonist was injected into the amygdala immediately (MPEP) or 15 min (CPCCOEt) before the agonist. In the data analysis, the discharge rate before injection was compared with the discharge rate determined after the injection. This was done by subtracting the mean post-injection discharge frequency during one min at various time points following microinjection from the mean discharge frequency before microinjection; i.e., a positive value represents increase of activity in the RVM by amygdala injection, and vice versa.

Assessment of aversive avoidance behavior and its modulation by glutamatergic receptors of the amygdala

Place avoidance test was performed, as described earlier (LaBuda and Fuchs, 2000), to obtain a measure of affective pain induced by mechanical stimulation of the neuropathic hind paw. Before testing, the animals were habituated to the test conditions by spending one to two hours daily for two days in the test box. In the actual testing, the rat was placed within a Plexiglas chamber (60 x 30 x 30 cm; one half of which was painted black on the external surface) placed upon an elevated metal grid. The rats were placed over the midline of the chamber and stimulation of the plantar surface of the hind paw initiated with a 60 g monofilament once every 15 s for 30 min. When residing within the dark side of the chamber the injured or sham-operated hind paw was stimulated. Conversely, the non-operated hind paw was stimulated when residing within the light side of the chamber. Throughout the 30 min test period rats were allowed unrestricted movement throughout the chamber. The percent time spent in the light side of the chamber during the 30 min observation period was determined in each condition for each animal. It is assumed that the more aversive the mechanical stimulation of the hind paw, the more the animal spends time in the light side of the chamber; i.e., the place avoidance test is considered to assess affective-emotional pain behavior (LaBuda and Fuchs, 2000).

Three experimental groups of rats were tested in the place avoidance test: i) SNI animals with amygdaloid injections ipsilateral to the nerve injury ii) SNI animals with bilateral amygdaloid injections, iii) sham-operated animals with amygdaloid injections ipsilateral to the sham operation. In the bilateral treatment group, the drug conditions were saline, DHPG at the dose of 5 nmol or 10 nmol/amygdala (10 nmol or 20 nmol/animal, respectively), and CPCCOEt at the dose of 20 nmol or 40 nmol/amygdala (40 nmol or 80 nmol/animal/respectively). In the ipsilateral treatment groups, drug conditions were saline, 10 nmol of DHPG, or 40 nmol of CPCCOEt. In

all experimental conditions, drugs were administered into the amygdala immediately before the start of the place avoidance test. In each experimental group, each drug condition was assessed in a separate day, one to two weeks following nerve or sham injury. Each animal participated in three to four drug testing sessions, the interval in testing different drug conditions in one animal was at least two days. The order of testing different drug conditions was varied within the groups to avoid serial effects.

Drugs

(S)-3,5-Dihydroxyphenylglycine (DHPG; an $mGluR_1$ and $mGluR_5$ agonist), (RS)-2-Chloro-5-hydroxy (CHPG; an $mGluR_5$ agonist), 6-Methyl-2-(phenylethynyl)pyridine (MPEP; an $mGluR_5$ antagonist), (+)-MK-801 hydrogen maleate (MK-801; an NMDA-R antagonist) and glutamate were purchased from Sigma (St.Louis, MO) and 7-Hydroxyiminocyclopropan[b]chromen-1a-carboxylic acid ethyl ester (CPCCOEt; an $mGluR_1$ antagonist) was purchased from Tocris (Bristol, U.K.). Physiological saline (OrionPharma, Espoo, Finland) was used for control injections. Drugs were dissolved in saline, except for CPCCOEt that was dissolved in DMSO.

Previous studies indicate that DHPG and CPCCOEt at the currently used dose of 10 nmol have proved effective in activating group I mGluRs within the currently used observation period of 30 min following intracerebral administration in the rat (e.g., Kim et al., 2007; Renoldi et al., 2007). Previous studies indicate that the currently used doses of glutamate (50 nmol) and MK-801 (3 nmol) induce a significant antinociception (Zhuo and Gebhart, 1997) or antiallodynia (Pertovaara and Wei, 2003), respectively, following supraspinal microinjection. The maximum

antinociceptive effect induced by central injection of glutamate has been obtained within two min (Zhuo and Gebhart, 1997), whereas the maximum antiallodynic effect induced by central injection of MK-801 was reached within 15 min (Pertovaara and Wei, 2003). Thus, the currently used observation period of 6 min following the injection of glutamate in the amygdala was appropriate for detecting the maximum effect induced by glutamate but only a submaximal effect induced by MK-801.

At the completion of the study, an electrolytic lesion was made in the recording site, the animals were given a lethal dose of pentobarbitone and the brains removed for verification of recording and microinjection sites.

.

Statistics

Data are presented as mean $\pm$ S.E.M. One- or two-way ANOVA followed by Student-Newman-Keuls test or t-test (differences between two groups) were used for assessing differences between the experimental conditions. Grubb's test was used to exclude potential outliers (www.graphpad.com/quickcalcs/). $P<0.05$ was considered to represent a significant difference.

Results

Response characteristics of RVM neurons

Spontaneous activity of RVM ON-cells was significantly influenced by SNI ($F_{1,131}$=4.79, P=0.030; 2-w-ANOVA) and postoperative time point of testing ($F_{1,131}$=4.19, P=0.043; 2-w-ANOVA). *Post hoc* tests indicated that the spontaneous discharge rate of ON-cells was increased in the SNI group one week after nerve injury, while eight weeks following injury it was reduced to the same level as in sham controls (Fig. 3 a). Spontaneous activity of RVM OFF-cells was influenced by postoperative time point of testing ($F_{1,100}$=8.0, P=0.0057; 2-w-ANOVA), and this time-dependent effect varied with the experimental group (SNI versus sham; $F_{1,100}$=4.50, P=0.036; 2-w-ANOVA). Post hoc tests indicated that the discharge rate of OFF-cells was significantly decreased one week following nerve injury, while eight weeks following injury it was at the same level as in sham controls (Fig. 3 b). In the present sample of neurons, postoperative time point of testing (one versus eight weeks) had no significant influence on spontaneous discharge rates of ON- or OFF-cells in the sham control group (Fig. 3).

Discharge rates of RVM cells following amygdaloid administration of glutamatergic compounds

When studying influence of glutamatergic compounds on discharge rates of RVM cells, the studied compounds were microinjected at a volume of 0.5 µl into the amygdala ipsilateral to nerve injury or sham operation, except for one group in which

it was injected into the amygdala contralateral to nerve injury. Microinjection of DHPG, an agonist of group I metabotropic glutamate receptor (mGluR) subtypes $mGluR_1$ and $mGluR_5$, at a dose of 10 nmol produced a significant increase in the discharge rate of RVM ON-cells in the SNI group (Fig. 4). DHPG induced the maximum increase in the discharge rate of ON-cells within five min, and the increase was of equal magnitude one and eight weeks following nerve injury (Fig. 5 a,b). The increase in the discharge rate of RVM ON-cells by 10 nmol of DHPG was of equal magnitude following its microinjection into the amygdala ipsi- (n=4) as contralateral (n=14) to the nerve injury in animals that were operated eight weeks before time point of testing (F=0.05; 2-w-ANOVA; not shown). CHPG, an $mGluR_5$ agonist (10 nmol), failed to influence the discharge rate of RVM ON-cells in nerve-injured animals (Fig. 5 b). The DHPG-induced increase of ON-cell activity in nerve-injured animals was completely reversed by pretreatment of the amygdala with MPEP, an $mGluR_5$ antagonist, at a dose of 50 nmol that produced no significant effect when administered alone (Fig. 5 c). The DHPG-induced increase of ON-cell activity in nerve-injured animals was also completely reversed by pretreatment of the amygdala with CPCCOEt, an $mGluR_1$ antagonist, at a dose (40 nmol) that failed to produce a change in ON-cell discharge rate when administered alone (Fig. 5 d). Administration of DHPG at the dose of 10 nmol into a control site, the hippocampus, failed to produce any significant change on the discharge rate of five ON-cells (not shown) in neuropathic animals, while in one ON-cell DHPG administration into the hippocampus was followed at a 15 min post-injection time point by a sudden increase of the discharge rate from the pre-injection baseline rate by 384 % (not shown). According to Grubb's test, the RVM ON-cell with a sudden increase in the discharge rate 15 min following hippocampal injection of DHPG was a significant outlier

($P<0.05$) and therefore, it was not included in the statistical assessment of the over-all effect.

DHPG (10 nmol) in the amygdala failed to influence OFF-cell activity in the SNI group, independent of postoperative time point (Fig. 6 a). Moreover, DHPG (10 nmol) in the amygdala had no significant effect on discharge rates of ON- or OFF-cells in the sham group (Fig. 6 b).

Microinjections of glutamate at a dose of 50 nmol or MK-801, an NMDA receptor (NMDA-R) antagonist, at a dose of 3 nmol failed to produce significant changes in the discharge rates of ON- or OFF-cells in the SNI or sham group (Fig. 7).

Affective pain-related behavior following amygdaloid administration of DHPG or CPCCOEt

A behavioral place avoidance paradigm was used to assess whether amygdaloid administration of DHPG or CPCCOEt influences aversive quality of mechanical stimulation of the neuropathic hind paw. In sham-operated animals, mechanical stimulation of the operated hind paw induced no or negligible avoidance behavior, independent whether saline or DHPG (10 nmol) was injected into the ipsilateral amygdala (Fig. 8). In nerve-injured animals, mechanical stimulation of the neuropathic hind paw induced a marked avoidance behavior (as revealed by increased time spent in light) that was increased by administration of DHPG into the amygdala: while the increase of avoidance behavior induced by ipsilateral injection of 10 nmol of DHPG was short of significance, bilateral administration of DHPG produced a dose-related increase in place avoidance ($F_{2,14}=8.7$, $P<0.01$; 1-w-ANOVA) that was significant at a dose of 10 nmol of DHPG/amygdala, corresponding to 20 nmol of

DHPG/animal (Fig. 8 a). In contrast, avoidance behavior was reduced by administration of CPCCOEt in the amygdala: while the decrease of avoidance behavior was short of significance following ipsilateral administration of 40 nmol of CPCCOEt, bilateral administration of CPCCOEt produced a dose-related decrease in place avoidance ($F_{2,10}$=4.86, P<0.04; 1-w-ANOVA) that was significant at a dose of 40 nmol of CPCCOEt/amygdala (Fig. 8 b).

Discussion

In the present study, amygdaloid administration of DHPG, an $mGluR_{1/5}$ agonist, increased the discharge rate of presumably pronociceptive ON-cells in the RVM of nerve-injured but not sham-operated animals. This enhanced pronociceptive effect by was at least due to action on the amygdaloid $mGluR_1$, since DHPG, an agonist of the $mGluR_1$ and $mGluR_5$, but not CHPG, an $mGluR_5$ agonist, increased discharge rates of RVM ON-cells and this DHPG-induced effect was reversed by CPCCOEt, an $mGluR_1$ antagonist. However, since MPEP, an $mGluR_5$ antagonist, applied at a high dose also reversed the DHPG-induced increase of the ON-cell discharge rate, we cannot exclude contribution of the $mGluR_5$ to the DHPG-induced pronociception. Administration of DHPG into a control site, the hippocampus, failed to produce a change in the discharge rate of RVM ON-cells in neuropathic animals. Amygdaloid administration of DHPG also failed to influence discharge rates of presumably antinociceptive OFF-cells of the RVM indicating that the nerve injury-induced change was selective for the pronociceptive cell type. Since amygdaloid

administration of NMDA-R or group I mGluR antagonists alone failed to influence discharge rates of pro- or antinociceptive RVM cells, the amygdaloid NMDA-R or group I mGluRs may not contribute to tonic maintenance of neuropathic pain and hypersensitivity.

In behavioral experiments of the present study, affective pain of nerve-injured animals was increased by amygdaloid administration of DHPG and decreased by CPCCOEt. Since the aversive place-conditioning test used in assessing affective pain-related behavior (LaBuda and Fuchs, 2000) provides an emotional challenge putatively activating the amygdala, the decrease of affective pain by amygdaloid administration of an $mGluR_1$ antagonist is in line with the hypothesis that emotions processed by the amygdala may enhance pain in nerve-injured animals, due to action on the amygdaloid $mGluR_1$.

Interaction between the amygdala and pain

Psychophysical studies suggest that emotions presumably processed by the amygdala produce significant changes in human pain reactivity (Craig, 2006). The direction of the change has varied from pain facilitation in anxious subjects to suppression of pain sensitivity in subjects with intense fear (Rhudy and Meagher, 2000). In line with this, previous studies in non-neuropathic animals have shown that the amygdala has a dual role in regulation of nociception varying from pronociception to antinociception (see the Introduction). There is accumulating evidence indicating that sustained pain induces plastic changes in the amygdala (Neugebauer, 2006). Pain-induced neural plasticity of the amygdala may influence its pain regulatory action as indicated by a recent study showing that activation of the extracellular signal-

regulated kinase in the amygdala contributes to inflammatory hypersensitivity (Carrasquillo and Gereau, 2007). In addition to inflammation, peripheral neuropathy induces neural plasticity in the amygdala as shown by the findings that postsynaptic currents evoked by ascending inputs in the central amygdala were enhanced (Ikeda et al., 2007) and new amygdala neurons were generated (Gonçalves et al., 2008) following peripheral nerve injury. While the enhanced synaptic responses to ascending signals indicate that the relay of pain-related signals to the amygdala is facilitated in neuropathy (Ikeda et al., 2007), these previous results still left open whether the amygdala-induced pain regulation is changed by peripheral nerve injury.

In neuropathic animals, not only pain processing within the amygdala (Ikeda et al., 2007) but also pain regulatory influence of the amygdala may be changed as suggested by the following findings. Amygdaloid administration of a $GABA_A$ receptor agonist suppressed aversive pain-related behavior and hypersensitive spinal reflex responses in nerve-injured animals (Pedersen et al., 2007). This behavioral finding suggests that the amygdala may contribute to regulation of neuropathic hypersensitivity, possibly through action on descending pathways relaying in the RVM. Also, amygdaloid administration of glutamate suppressed presumably antinociceptive neurons in the noradrenergic locus coeruleus of nerve-injured but not sham-operated animals suggesting that activation of the amygdala may have a pronociceptive effect in peripheral neuropathy (Viisanen and Pertovaara, 2007). In line with this, the present results suggest that activation of the group I mGluR in the amygdala of nerve-injured animals promotes activity of presumably pronociceptive neurons in the RVM. Together these results suggest that the amygdala-induced pain modulation is changed in neuropathy. In neuropathic animals, activation of the

amygdala may promote hypersensitivity by, at least, two different types of actions on brain regulatory nuclei of the brainstem: by facilitating pronociceptive neurons in the RVM and by inhibiting antinociceptive neurons in the locus coeruleus. While the pronociceptive influence by the amygdala may be partly tonic in peripheral neuropathy (Pedersen et al., 2007), the present results with amygdaloid administrations of specific receptor antagonists alone suggest that the amygdaloid NMDA-R or group I mGluR do not explain the tonic pronociceptive action of the amygdala.

Interestingly, while the enhancement of synaptic currents evoked by afferent inputs to the amygdala occurred predominantly in the central nucleus of the amygdala contralateral to peripheral nerve injury (Ikeda et al., 2007), the pronociceptive action of DHPG on the RVM was obtained following its amygdaloid administration ipsi- as well as contralateral to peripheral nerve injury. This result is in line with the earlier finding that activation of the amygdala ipsilateral to nerve injury attenuated presumably antinociceptive locus coeruleus neurons (Viisanen and Pertovaara, 2007) and it also fits the finding that the amygdala receives ascending nociceptive inputs from the ipsi- as well as contralateral body half (Bernard et al., 1996). It may be proposed that the enhancement of contralateral afferent inputs in the amygdala (Ikeda et al., 2007) and the pronociceptive change in the amygdala-induced pain regulatory effect that was observed also ipsilateral to nerve injury (Viisanen and Pertovaara, 2007; the present results) have, at least partly, different underlying mechanisms.

Glutamatergic receptor types sensitized in the amygdala

Earlier results indicate that in arthritis, the responses of multireceptive neurons in the central nucleus of the amygdala are sensitized to administration of DHPG, an $mGluR_1$ and $mGluR_5$ agonist but not to CHPG, an $mGluR_5$ agonist (Li and Neugebauer, 2004b; Neugebauer et al., 2003). This finding suggests that the amygdaloid $mGluR_1$ plays a role in arthritic pain-related sensitization (Li and Neugebauer, 2004b; Neugebauer et al., 2003). The present results on the amygdaloid influence on the RVM and affective pain-related behavior extend this earlier finding by showing that a change in the function of the amygdaloid group I mGluR, and particularly the subtype $mGluR_1$, may contribute to pain-related sensitization in peripheral neuropathy as well as in arthritis. In contrast, while the NMDA-R contributes to pain-related sensitization of amygdala neurons in arthritis (Li and Neugebauer, 2004a), a recent study indicated that sensitization of amygdala neurons in peripheral neuropathy is not dependent on the NMDA-R (Ikeda et al., 2007). In line with this, amygdaloid administration of an NMDA-R antagonist failed to induce a change in the discharge rate of RVM neurons in nerve-injured animals of the present study. While studies performed in non-neuropathic animals have provided evidence suggesting that the central nucleus of the amygdala plays a significant role in promoting affective pain behavior induced by noxious visceral stimulation, its lesion has attenuated place aversion induced by noxious cutaneous stimulation, too (Tanimoto et al., 2003), which finding is in line with the present results. It should be noted, however, that the currently administered injection volume of 0.5 µl into the central nucleus of the amygdala is likely to spread also to immediately adjacent areas (Myers, 1966), particularly other amygdaloid subnuclei. Therefore, we cannot exclude the possibility that the group I mGluRs e.g. in the basolateral nucleus of the amygdala contribute to the DHPG-induced pronociceptive effect in the present study. In a

distant control site, the hippocampus, DHPG, however, had no pronociceptive effect indicating that the pronociceptive effect was region-specific.

Influence of the amygdala on discharge rates of RVM cells

An earlier study showed that microinjection of morphine into the basolateral nucleus of the amygdala produced antinociception that was accompanied by a decrease in the discharge rate of RVM ON-cells and an increase in the discharge rate of RVM OFF-cells, while morphine in the central or lateral nuclei of the amygdala failed to influence pain-related behavior or discharge rates of RVM ON- or OFF-cells (McGaraughty and Heinricher, 2002). Since the main amygdaloid output nucleus is the central nucleus receiving convergent information from various amygdaloid nuclei (Pitkänen et al., 1997), the antinociceptive effect by morphine in the basolateral nucleus was likely to be relayed to the RVM via the amygdaloid central nucleus. In the present study, DHPG was microinjected into the central nucleus of the amygdala, although the results do not allow excluding that group I mGluRs in adjacent amygdaloid subnuclei contributed to its pronociceptive effect. Together, earlier (McGaraughty and Heinricher, 2002) and the present results indicate that the amygdala has an influence on neuronal discharge rates in the RVM, a major pain regulatory region (Gebhart, 2004). The pain-regulatory effect originating in or relaying through the amygdala may have been mediated to the RVM directly (Hermann et al., 1997) or indirectly through the periaqueductal gray (Rizzi et al., 1991). Moreover, efferent pathways other than those projecting directly or indirectly to the RVM may contribute to pain regulation by the amygdala. It should also be noted that the increased affective pain response to mechanical stimulation of the

neuropathic limb in the aversive place-conditioning task by the amygdaloid $mGluR_1$ may reflect increased afferent barrage evoked by the mechanical test stimulus due to increased RVM ON-cell activity, a distinct mechanism facilitating affective pain, or both.

Spontaneous discharge rate of RVM cells following nerve injury

Increase in the ongoing discharge rate of RVM ON-cells and a decrease in the ongoing discharge rate of RVM OFF-cells have been associated with sustained hyperalgesia induced by inflammation (Kincaid et al., 2006). The cumulative results of the present and our preceding study (Gonçalves et al., 2007) support the hypothesis that an increased spontaneous discharge rate of RVM ON-cells and a decreased discharge rate of RVM OFF-cells contribute to maintenance of hypersensitivity also during the early phase (first week) of peripheral neuropathy in animals with SNI. During the late phase (eighth week) of neuropathy, the discharge rates of RVM ON- and OFF-cells were returned to control levels indicating that an abnormality in the ongoing discharge rate of RVM ON- or OFF-cells may not explain hypersensitivity induced by SNI. The return of the ongoing discharge rate to control levels during the late phase of SNI-induced neuropathy, however, does not exclude the possibility that the RVM ON- or OFF-cells contributed to maintenance of hypersensitivity through a change in the synaptic efficacy of their efferent connections. A change in the ongoing discharge rates of RVM ON- and OFF-cells is not pathognomic for the early phase of peripheral neuropathy in all experimental models as indicated by observations made in animals with ligation of the spinal nerves (Carlson et al., 2007; Pertovaara et al., 2001) or chronic constriction of the sciatic nerve (Luukko and Pertovaara, 1993).

Additionally, peripheral nerve injury may induce enhanced responses of RVM cells to peripheral stimulation that potentially contributes to abnormal feedback regulation of ascending nociceptive signals (Carlson et al., 2007; Golçalves et al., 2007).

Conclusions

Present results indicate that group I mGluRs in the amygdala may promote nociception in nerve-injured animals. This was shown by increased excitation of pronociceptive RVM ON-cells following amygdaloid administration of a group I mGluR agonist in neuropathic animals. Since the amygdala has a key role in processing emotions and since the administration of glutamatergic compounds into the amygdala potentially mimicks emotional activation of the amygdala, it may be proposed that activation of the amygdaloid group I mGluR, provides a possible mechanism for emotional enhancement of nerve injury-related hypersensitivity and pain. This proposal is supported by the behavioral finding indicating that amygdaloid administration of an $mGluR_{1/5}$ agonist increased and that of an $mGluR_1$ antagonist decreased affective pain-related behavior when the animal was exposed to an emotional challenge provided by the aversive place-conditioning test.

Acknowledgments

This work was supported by the Academy of Finland and the Sigrid Jusélius Foundation, Helsinki, Finland, and the Portuguese Foundation for Science and Technology and the Gulbenkian Foundation, Lisbon, Portugal.

References

Almeida, A., Leite-Almeida, H., Tavares, I., 2006. Medullary control of nociceptive transmission: reciprocal dual communication with the spinal cord. Drug Discov. Today Dis. Mech. 3, 305-312.

Bernard, J.F., Bester, H., Besson, J.M., 1996. Involvement of the spino-parabrachio-amygdaloid and –hypothalamic pathways in the autonomic and affective emotional aspects of pain. Prog. Brain Res. 107, 243-255.

Carlson, J.D., Maire, J.J., Martenson, M.E., Heinricher, M.M., 2007. Sensitization of pain-modulating neurons in the rostral ventromedial medulla after peripheral nerve injury. J. Neurosci. 27, 13222-13231.

Carrasquillo, Y., Gereau, R.W. 4th, 2007. Activation of the extracellular signal-related kinase in the amygdala modulates pain perception. J. Neurosci. 27, 1543-1551.

Craig, K.D., 2006. Emotions and psychobiology. In: McMahon, S.B., Koltzenburg, M. (Eds.), Wall and Melzack's Textbook of Pain, 5th ed. Elsevier, China, pp. 231-239.

Decosterd, I., Woolf, C.J., 2000. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. Pain 87, 149-158.

Fields, H.L., Basbaum, A.I., Heinricher, M.M., 2006. Central nervous system mechanisms of pain modulation. In: McMahon, S.B. & Koltzenburg, M. (eds) Wall and Melzack's Textbook of Pain, 5th ed., Elsevier, China, pp. 125-142.

Gebhart, G.F., 2004. Descending modulation of pain. Neurosci. Biobehav. Rev. 27, 729-737.

Gonçalves, L., Almeida, A., Pertovaara, A., 2007. Pronociceptive changes in response properties of rostroventromedial medullary neurons in a rat model of peripheral neuropathy. Eur. J. Neurosci. 26, 2188-2195.

Gonçalves, L., Silva, R., Pinto-Ribeiro, F., Pêgo, J.M., Bessa, J.M., Pertovaara, A., Sousa, N., Almeida, A., 2008. Neuropathic pain is associated with depressive behaviour and induces neuroplastivcity in the amygdala of the rat. Exp. Neurol. 213, 48-56.

Greenwood-Van Meerveld, B., Gibson, M., Gunder, W., Shepard, J., Foreman, R., Myers, D., 2001. Stereotaxic delivery of corticosterone to the amygdala modulates colonic sensitivity in rats. Brain Res. 893, 135-142.

Han, J.S., Neugebauer, V., 2005. mGluR1 and mGluR5 antagonists in the amygdala inhibit different components of audible and ultrasonic vocalizations in a model of arthritic pain. Pain 113, 211-222.

Helmstetter, F.J., Bellgowan, P.S., 1993. Lesions of the amygdala block conditional hypoalgesia on the tail flick test. Brain Res. 612, 253-257.

Helmstetter, F.J., Tershner, S.A., Poore, L.H., Bellgowan, P.S., 1998. Antinociception following opioid stimulation of the basolateral amygdala is expressed through the periaqueductal gray and rostral ventromedial medulla. Brain Res. 779, 104-118.

Hermann, D., Luppi, P., Peyron, C., Hinckel, P., Jouvet, M., 1997. Afferent projections to the rat nuclei raphe magnus, raphe pallidus, and reticularis gigantocellularis pars alpha demonstrated by iontophoretic application of choleratoxin. J. Chem. Neuroanat. 13, 1-21.

Ikeda, R., Takahashi, Y., Inoue, K., Kato, F., 2007. NMDA receptor-independent synaptic plasticity in the central amygdala in the rat model of neuropathic pain. Pain 127, 161-172.

Ji, G., Neugebauer, V., 2008. Pro- and anti-nociceptive effects of corticotrophin-releasing factor (CRF) in central-amygdala neurons are mediated through different receptors. J. Neurophysiol. 99, 1201-1212.

Kim, J., Lee, S., Park, H., Song, B., Hong, I., Geum, D., Shin, K., Choi, S., 2007. Blockade of amygdala metabotropic glutamate receptor subtype 1 impairs fear extinction. Biochem. Biophys. Res. Comm. 355, 188-193.

Kincaid, W., Neubert, M.J., Xu, M., Kim, C.J., Heinricher, M.M., 2006. Role of medullary pain facilitating neurons in secondary thermal hyperalgesia. J. Neurophysiol. 95, 33-41.

LaBuda, C.J., Fuchs, P.N., 2000. A behavioral test paradigm to measure the aversive quality of inflammatory and neuropathic pain in rats. Exp. Neurol. 163, 490-494.

Li, W., Neugebauer, V., 2004a. Block of NMDA and non-NMDA receptor activation results in reduced background and evoked activity of central amygdala neurons in a model of arthritic pain. Pain 110, 112-122.

Li, W., Neugebauer, V., 2004b. Differential roles of mGluR1 and mGluR5 in brief and prolonged nociceptive processing in central amygdala neurons. J. Neurophysiol. 91, 13-24.

Lopez de Armentia, M., Sah, P., 2007. Bidirectional synaptic plasticity at nociceptive afferents in the rat central amygdala. J. Physiol. (Lond.) 581, 961-970.

Luukko, M., Pertovaara, A., 1993. Influence of an experimental peripheral mononeuropathy on the responses of medial bulboreticular neurons to noxious

skin stimulation and the modulation of the responses by an α_2-adrenoceptor agonist in the rat. Exp. Neurol. 124, 390-394.

Manning, B.H., 1998. A lateralized deficit in morphine antinociception after unilateral inactivation of the central amygdala. J. Neurosci. 18, 9453-9470.

Manning, B.H., Mayer, D.J., 1995. The central nucleus of the amygdala contributes to the production of morphine antinociception in the rat tail-flick test. J. Neurosci. 15, 8199-8213.

McGaraughty, S., Heinricher, M.M., 2002. Microinjection of morphine into various amygdaloid nuclei differentially affects nociceptive responsiveness and RVM neuronal activity. Pain 96, 153-162.

Mena, N.B., Mathur, R., Nayar, U., 1995. Amygdaloid involvement in pain. Indian J. Physiol. Pharmacol. 39, 339-346.

Myers, R.D., 1966. Injection of solutions into cerebral tissue: relation between volume and diffusion. Physiol. Behav. 1, 171-174.

Nandigama, P., Borszcz, G.S., 2003. Affective analgesia following the administration of morphine into the amygdala of rats. Brain Res. 959, 343-354.

Neugebauer, V., 2006. Subcortical processing of nociceptive information: basal ganglia and amygdala. In: Cervero, F., Jensen, T.S. (Eds.), Handbook of Clinical Neurology, vol. 81, Elsevier, Amsterdam, pp. 141-158.

Neugebauer, V., Li, W., Bird, G.C., Han, J.S., 2004. The amygdala and persistent pain. Neuroscientist 10, 221-234.

Neugebauer, V., Li, W., Bird, G.C., Bhave, G., Gereau, R.W. 4th, 2003. Synaptic plasticity in the amygdala in a model of arthritic pain: differential roles of metabotropic glutamate receptors 1 and 5. J. Neurosci. 23, 52-63.

Paxinos, G., Watson, C., 1998. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.

Pedersen, L.H., Scheel-Kruger, J., Blackburn-Munro, G., 2007. Amygdala GABA-A receptor involvement in mediating sensory-dicriminative and affective-motivational pain responses in a rat model of peripheral nerve injury. Pain 127, 17-26.

Pertovaara, A., 2000. Plasticity in descending pain modulatory systems. Prog. Brain Res. 129, 231-242.

Pertovaara, A., Wei, H., 2003. A dissociative change in the efficacy of supraspinal versus spinal morphine in the neuropathic rat. Pain 101, 237-250.

Pertovaara, A., Keski-Vakkuri, U., Kalmari, J., Wei, H., Panula, P., 2001. Response properties of neurons in the rostroventromedial medulla of neuropathic rats: attempted modulation of responses by [1DMe]NPYF, a neuropeptide FF analogue. Neuroscience 105, 457-468.

Phelps, E.A., LeDoux, J.E., 2005. Contributions of the amygdala to emotion processing: from animal models to human behavior. Neuron 48, 175-187.

Pitkänen, A., Savander, V., LeDoux, J.E., 1997. Organization of intra-amygdaloid circuitries in the rat: an emerging framework for understanding functions of the amygdala. Trends Neurosci. 20, 517-523.

Porreca, F., Ossipov, M.H., Gebhart, G.H., 2002. Chronic pain and medullary descending facilitation. Trends Neurosci. 25, 319-325.

Quin, C., Greenwood-Van Meerveld, B., Myers, D.A., Foreman, R.D., 2003. Corticosterone acts directly at the amygdala to alter spinal neuronal activity in response to colorectal distension. J. Neurophysiol. 89, 1343-1352.

Renoldi, G., Calcagno, E., Borsini, F., Invernizzi, R.W., 2007. Stimulation of group I mGlu receptors in the ventrotegmental area enhances extracellular dopamine in the rat medial prefrontal cortex. J. Neurochem. 100, 1658-1666.

Rhudy, J.L., Meagher, M.W., 2000. Fear and anxiety: divergent effects on human pain thresholds. Pain 84, 65-75.

Rhudy, J.L., Meagher, M.W., 2003. Negative affect: effects on an evaluative measure of human pain. Pain 104, 617-626.

Rizvi, T.A., Ennis, M., Behbehani, M.M., Shipley, M.T., 1991. Connections between the central nucleus of the amygdala and the midbrain periaqueductal gray: topography and reciprocity. J. Comp. Neurol. 303, 121-131.

Tanimoto, S., Nakagawa, T., Yamauchi, Y., Minami, M., Satoh, M., 2003. Differential contributions of the basolateral and central nuclei of the amygdala in the negative affective component of chemical somatic and visceral pains in rats. Eur. J. Neurosci. 18, 2343-2350.

Urban, M.O., Hama, A.T., Bradbury, M., Anderson, J., Varney, M.A., Bristow, L., 2003. Role of metabotropic glutamate receptor subtype 5 (mGluR5) in the maintenance of cold hypersensitivity following a peripheral mononeuropathy in the rat. Neuropharmacology 44, 983-993.

Van Bockstaele, E.J., Chan, J., Pickel, V.M., 1996. Input from central nucleus of the amygdala efferents to pericoerulear dendrites, some of which contain tyrosine hydroxylase immunoreactivity. J. Neurosci. Res. 45, 289-302.

Viisanen, H., Pertovaara, A., 2007. Influence of peripheral nerve injury on response properties of locus coeruleus neurons and coeruleospinal antinociception in the rat. Neuroscience 146, 1785-1794.

Woolf, C.J., Salter, M.W., 2006. Plasticity and pain: role of the dorsal horn. In: McMahon, S.B., Koltzenburg, M. (Eds), Wall and Melzack's Textbook of Pain, 5th ed., Elsevier, China, pp. 91-106.

Zhuo, M., Gebhart, G.F., 1997. Biphasic modulation of spinal nociceptive transmission from the medullary raphe nuclei in the rat. J. Neurophysiol. 78, 746-758.

Legends for Figures

Figure 1. Microelectrode recording sites in the RVM (left column) and microinjection sites in the amygdala (right column). The upper row shows an example of an electrolytic lesion made by the recording electrode in the RVM (left) and the track of the injection cannula in the amygdala (right). The rectangle and the circle in the schematic graphs of the lower row indicate the dorsolateral extent of areas, across several anteroposterior sections, in which the tips of medullary recording electrodes and amygdaloid microinjection cannulae were located, respectively. RVM=rostroventromedial medulla, CeA=central nucleus of the amygdala, Rmg=raphe magnus

Figure 2. Examples of an RVM ON-cell (upper graph) and OFF-cell (lower graph) response to noxious pinch of the tail in a neuropathic animal. P_P indicates the duration of noxious tail pinch. The horizontal bars indicating tail pinch represent 5 s and the vertical calibration bars for the peristimulus time histograms represent 50 imp/s. The insets show the shapes of the action potential.

Figure 3. Spontaneous discharge rates of RVM ON-cells (a) and OFF-cells (b) in nerve-injured (SNI) and sham-operated animals. 1 and 8 wk (weeks) refer to the postoperative time point of testing. *$P<0.05$ (reference: the corresponding value in the

sham-operated group at the same postoperative time point). +P<0.05, +++P<0.005 (Student-Newman-Keuls test; reference: the corresponding value at an earlier postoperative time point). Error bars represent S.E.M. (n=19-29)

Figure 4. Influence of DHPG (10 nmol; left column) or saline (right column) injection into the amygdala on the discharge rate of an RVM ON-cell in a neuropathic animal one week following the nerve injury. Time point of testing before and after the injection is shown above each row. The horizontal calibration bar represents 10 s and the vertical one 50 impulses/s.

Figure 5. Discharge rates of RVM ON-cells in nerve-injured (SNI) animals following administration of group I metabotropic glutamatergic compounds into the amygdala ipsilateral to the nerve injury. (a) Time course of the effect by DHPG (10 nmol), an $mGluR_1$ and $mGluR_5$ agonist. (b) The effect by DHPG versus CHPG (10 nmol), an $mGluR_5$ agonist. (c) Reversal of the DHPG-induced increase in the discharge rate by MPEP (50 nmol), an $mGluR_5$ antagonist. (d) Reversal of the DHPG-induced increase in the discharge rate by CPCCOEt (40 nmol), an $mGluR_1$ antagonist.

1 and 8 wk (weeks) refer to the postoperative time point of testing. Sal=saline. MPEP=MPEP alone, CPCCO=CPCCOEt alone, +MPEP or +CPCCO=MPEP or CPCCOEt co-administered with DHPG. *P<0.05,**P<0.01, ***P<0.005 (Student-Newman-Keuls test; In a, reference is the corresponding pre-injection value. In b, reference is the saline group. In c and d, reference is the DHPG group). Error bars represent S.E.M. (n_{sal}=16, n_{DHPG_1wk}=8, n_{DHPG_8wk}=14, n_{CHPG}=4, n_{MPEP}=5, n_{+MPEP}=6,

n_{CPCCO}=10, n_{+CPCCO}=8). 100% (dotted horizontal line) represents the pre-injection discharge rate.

Figure 6. (a) Discharge rates of RVM OFF-cells in nerve-injured (SNI) animals following administration of saline (Sal) or DHPG (10 nmol), an $mGluR_1$ and $mGluR_5$ agonist, into the amygdala. (b) Discharge rates of RVM ON- and OFF-cells in sham-operated animals following amygdaloid administration of saline or DHPG.

1 and 8 wk (weeks) refer to the postoperative time point of testing. Error bars represent S.E.M. (In a, n_{Sal}=6, n_{DHPG_1wk}=9, n_{DHPG_8wk}=6. In b, n_{Sal}=8, $n_{DHPG\_ON\text{-}cell}$=8, $n_{DHPG\_OFF\text{-}cell}$=5). 100% represents the pre-injection discharge rate.

Figure 7. Discharge rates of RVM cells following administration of glutamate (50 nmol) or MK-801 (3 nmol), an NMDA-R antagonist, into the amygdala. (a) ON-cells in nerve-injured (SNI) animals. (b) OFF-cells in nerve-injured animals. (c) ON- and OFF-cells in sham-operated animals.

1 and 8 wk (weeks) refer to the postoperative time point of testing. Sal=saline Error bars represent S.E.M. (n=6-10). 100% represents the pre-injection discharge rate.

Figure 8. Behavior in aversive place-conditioning test following administration DHPG, an $mGluR_1$ and $mGluR_5$ agonist (a), or CPCCOEt, an $mGluR_1$ antagonist (b), into the amygdala in nerve-injured (SNI) or sham-operated animals.

An increase in time spent in light is considered to reflect an increase in affective pain induced by monofilament stimulation of the hind paw. ipsi=

amygdaloid injection was performed only ipsilateral to the nerve injury/sham operation, bilat= amygdaloid injection was performed bilaterally. In the Y-axis, doses represent the dose/side; e.g., the dose 10 nmol represents 10 nmol/animal with ipsilateral injections and 20 nmol/animal with bilateral injections. *$p<0.05$ (Student-Newman-Keuls test; reference: the corresponding saline condition). Error bars represent S.E.M. (In a, $n_{SNI\text{-}ipsi}=9$, $n_{SNI\text{-}bilat}=5$, $n_{Sham\text{-}ipsi}=5$. In b, $n_{SNI\text{-}ipsi}=4$, $n_{SNI\text{-}bilat}=4$, $n_{Sham\text{-}ipsi}=5$).

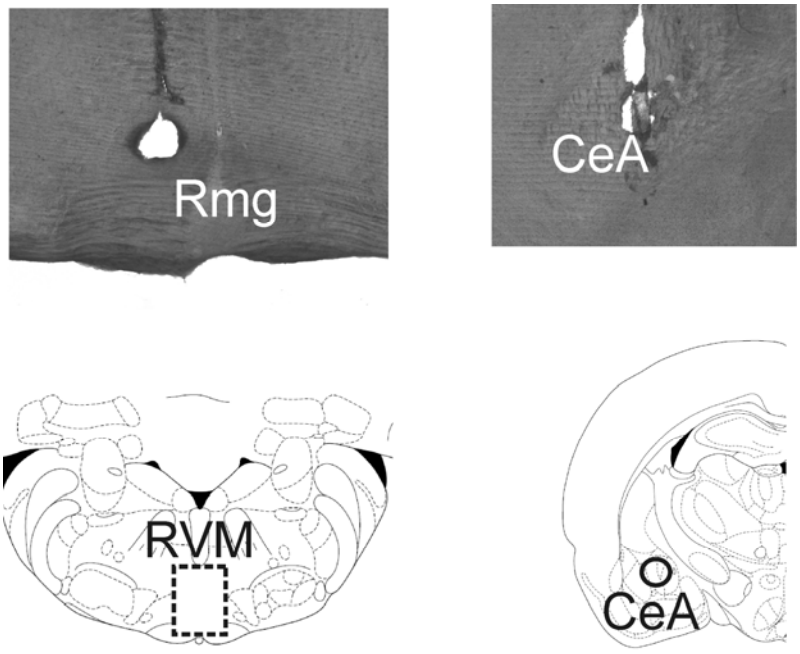

Fig. 1

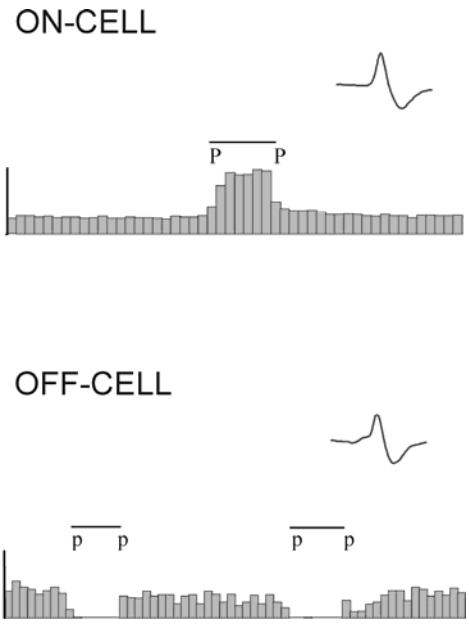

Fig. 2

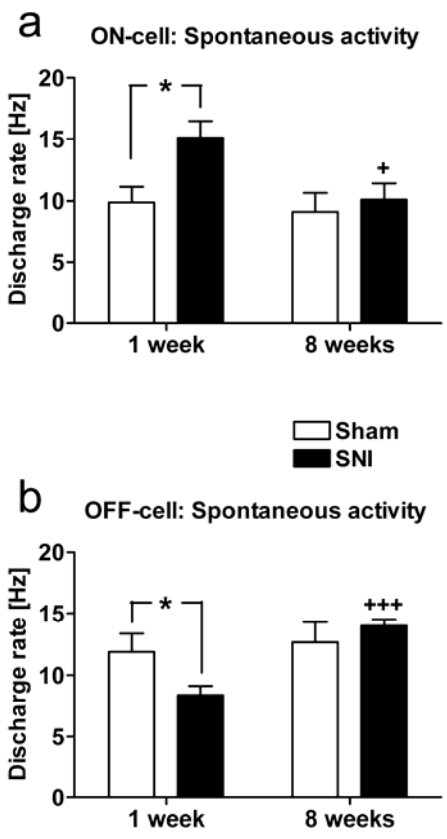
a
ON-cell: Spontaneous activity
Discharge rate [Hz]
20
15
10
5
0
*
+
1 week
8 weeks
Sham
SNI
b
OFF-cell: Spontaneous activity
Discharge rate [Hz]
20
15
10
5
0
*
+++
1 week
8 weeks

Fig. 3

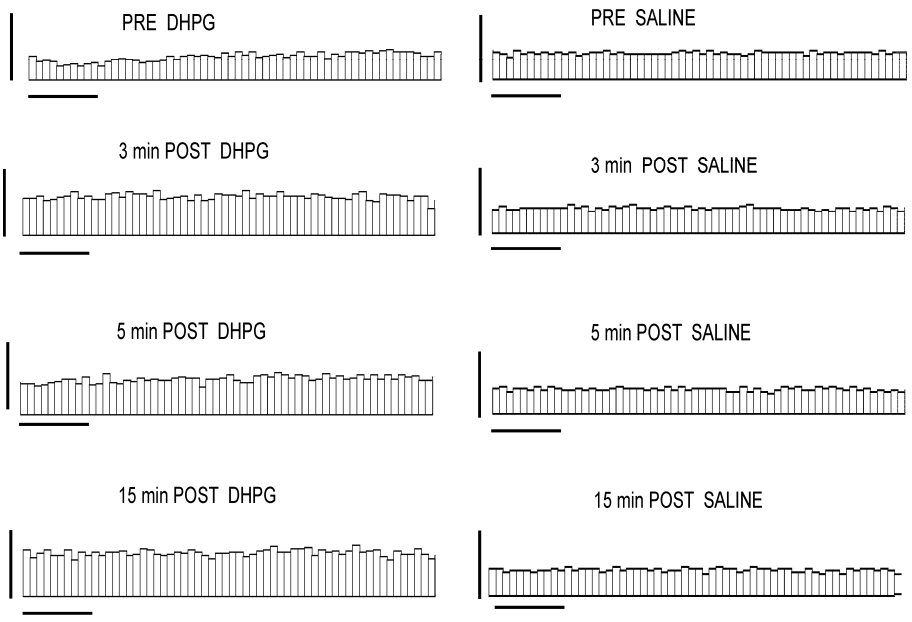
PRE DHPG
PRE SALINE
3 min POST DHPG
3 min POST SALINE
5 min POST DHPG
5 min POST SALINE
15 min POST DHPG
15 min POST SALINE

Fig. 4

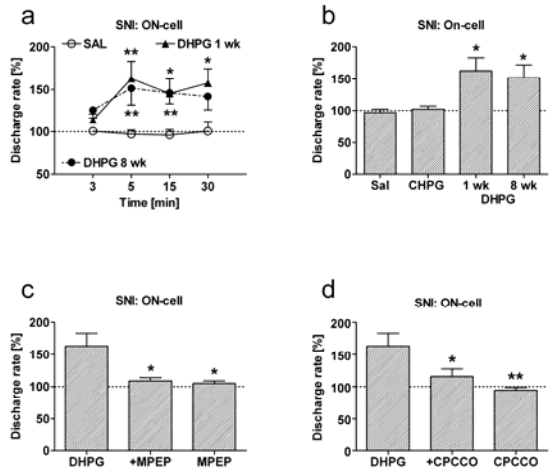
a
SNI: ON-cell
SAL
DHPG 1 wk
DHPG 8 wk
Discharge rate [%]
Time [min]
b
SNI: On-cell
Sal
CHPG
1 wk
8 wk
DHPG
c
SNI: ON-cell
DHPG
+MPEP
MPEP
d
SNI: ON-cell
DHPG
+CPCCO
CPCCO

Fig. 5

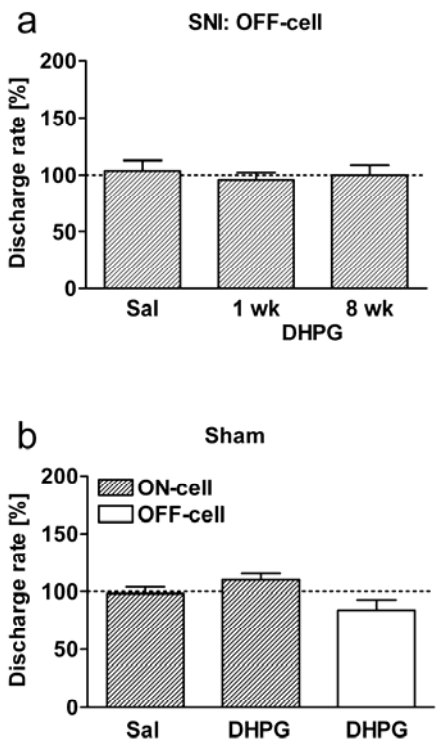
a
SNI: OFF-cell
Discharge rate [%]
200
150
100
50
0
Sal
1 wk
8 wk
DHPG
b
Sham
ON-cell
OFF-cell
Discharge rate [%]
200
150
100
50
0
Sal
DHPG
DHPG

Fig. 6

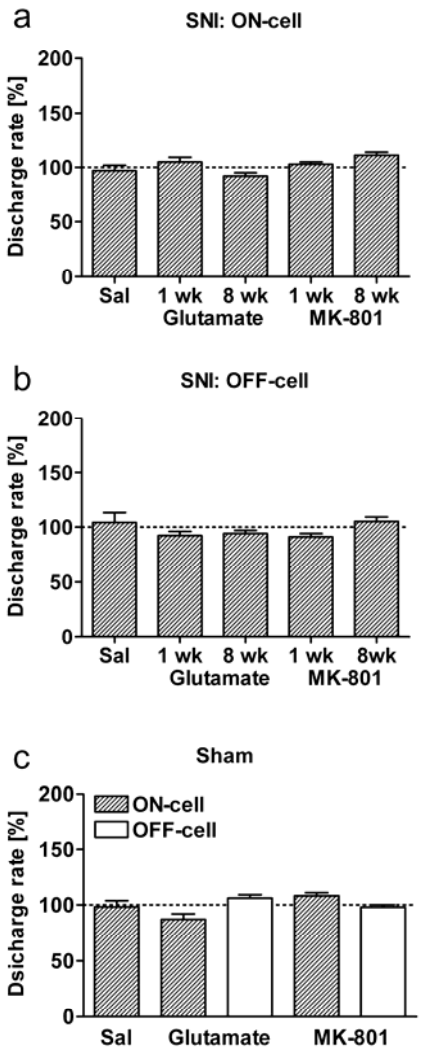
a
SNI: ON-cell
Discharge rate [%]
200
150
100
50
0
Sal
1 wk
8 wk
1 wk
8 wk
Glutamate
MK-801
b
SNI: OFF-cell
Discharge rate [%]
200
150
100
50
0
Sal
1 wk
8 wk
1 wk
8wk
Glutamate
MK-801
c
Sham
ON-cell
OFF-cell
Dsicharge rate [%]
200
150
100
50
0
Sal
Glutamate
MK-801

Fig. 7

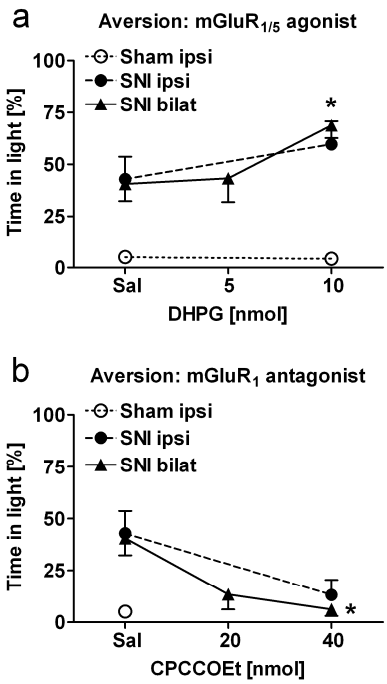
a
Aversion: mGluR1/5 agonist
Sham ipsi
SNI ipsi
SNI bilat
Time in light [%]
100
75
50
25
0
*
Sal
5
10
DHPG [nmol]
b
Aversion: mGluR1 antagonist
Sham ipsi
SNI ipsi
SNI bilat
Time in light [%]
100
75
50
25
0
*
Sal
20
40
CPCCOEt [nmol]

Fig. 8