Experimental Neurology xxx (2008) xxx-xxx

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Neuropathic pain is associated with depressive behaviour and induces neuroplasticity in the amygdala of the rat

Leonor Gonçalves [a,b], Rui Silva [a], Filipa Pinto-Ribeiro [a], José M. Pêgo [a], João M. Bessa [a], Antti Pertovaara [b], Nuno Sousa [a], Armando Almeida [a,*]

[a] Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[b] Department of Physiology, Institute of Biomedicine, University of Helsinki, Helsinki, Finland

ARTICLE INFO

Article history:
Received 22 February 2008
Revised 24 April 2008
Accepted 28 April 2008
Available online xxxx

Keywords:
Neuropathic pain
Amygdala
Adult neurogenesis
Depressive-like behaviour

ABSTRACT

Chronic pain is associated with the development of affective disorders but the underlying mechanisms are not fully understood. Changes in brain centres implicated in both emotional and pain processing are likely to be critical in the interplay of pain control and affective emotional behaviour. In the present study, we assessed emotional behaviour and performed a structural analysis of the amygdala (AMY) in neuropathic rats after two months of hyperalgesia and allodynia, induced by the spared nerve injury model (SNI). When compared with Sham-controls, SNI animals displayed signs of depressive-like behaviour. In addition, we found an increased amygdalar volume in SNI rats. No alterations were found in the dendritic arborizations of AMY neurons but, surprisingly, the amygdalar hypertrophy was associated with an increased cell proliferation [bromodeoxyuridine (BrdU)-positive cells] in the central (CeA) and basolateral (BLA) amygdaloid nuclei. The phenotypic analysis of the newly-acquired cells revealed that they co-label for neuronal markers (BrdU+NeuN and BrdU+Calbindin), but not for differentiated glial cells (BrdU+glial fibrillary acidic protein).
We demonstrate that neuropathic pain promotes generation of new neurons in the AMY. Given the established role of the AMY in emotional behaviour, we propose that these neuroplastic changes might contribute for the development of depressive-like symptoms that are usually present in prolonged pain syndromes in humans.

Introduction

Pain is a multidimensional experience with sensitive–discriminative and motivational–affective dimensions (Anand and Craig, 1996). Persistent pain, including chronic pain syndromes (Tal and Bennett, 1994), is a common condition associated to a wide spectrum of disorders including cancer, inflammation and neuropathic pain. Neuropathic pain (NP) is caused by a primary lesion or dysfunction of the nervous tissue (Merskey and Bogduk, 1994) and results in prolonged hyperalgesia, allodynia and spontaneous pain (Devor, 2006). NP results from a process of peripheral and central sensitization that generates an enhanced transmission of nociceptive input to the brain (Gao et al., 2005; Ren and Dubner, 1996), which may impair the endogenous supraspinal pain control system (Danziger et al., 2001; Kauppila et al., 1998; Pertovaara, 2000; Rasmussen et al., 2004; Tal and Bennett, 1994).

The amygdala (AMY) is a central component of the limbic system and plays a crucial role in behavioural responses to emotional stimuli (Davis and Whalen, 2001; Han and Neugebauer, ~~2001~~; Neugebauer and Li, 1992). Moreover, the AMY is deeply involved in processing the emotional component of pain, probably through a modulatory role upon major supraspinal pain control centres (SPCC) (Manning and Mayer, 1995; Manning, 1998; Manning et al., 2001). On the other hand, it is possible that neuroplasticity in higher centres controlling SPCC may contribute to alterations in the fine control of pain. In fact, an imbalance between inhibiting and facilitating descending modulation of nociceptive transmission may underlie, at least in part, the development of chronic pain (Almeida et al., 2006; Lima and Almeida, 2002; Pertovaara, 2000; Porreca et al., 2002; Schaible et al., 1991). Accordingly, arthritic and neuropathic pain enhance synaptic transmission of nociceptive-specific input to the AMY (Han and Neugebauer, 2004; Neugebauer and Li, 1992; Neugebauer et al., 2003), which reinforces the potential role of AMY in SPCC alterations resulting from prolonged pain syndromes.

Chronic pain induces mood disorders, including depression and anxiety (Rasmussen, ~~2004~~). In addition, the adverseness of pain is amplified or reduced depending on the emotional environment (Merskey, 1965), and conditions of increased anxiety (Rhudy and Meagher, 2000) and depression (Merskey, 1965; Willoughby et al., 2002; Zelman et al., 1991) are usually associated with decreased pain tolerance. This vicious circle may trigger, or even result from, neuronal changes in the limbic system. Accordingly, imaging studies indicate that gross structural changes may occur in the AMY in situations of major depression (Altshuler et al., 2005; Bremner et al., 2000; Frodl et al., 2002; Strakowski et al., 1999; Tebartz van Elst et al., 2000).

* Corresponding author.
E-mail address: aalmeida@ecsaude.uminho.pt (A. Almeida).
URL: http://www.ecsaude.uminho.pt/icvs/domains/neurc/index.htm (A. Almeida).

0014-4886/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2008.04.043

As a rationale for the present study, we hypothesized that chronic pain induces emotional disturbances that are associated with neuroplasticity of the amygdaloid complex. To assess this hypothesis, we performed behavioural, stereological and immunocytochemical analysis during or after the induction of a two month neuropathy following the model of Decosterd and Woolf (2000). Part of the present results have already been published in abstract form (Gonçalves et al., 2006).

Materials and methods

Animals

All procedures were performed on adult (200–250 g, 55–65 days) male Wistar–Han rats. Animals were housed under standard laboratory conditions (12 h light cycle; 22 °C, 55% humidity; food and water available *ad libitum*). Experiments were conducted in accordance with local regulations, European Union Directive 86/609/EEC, NIH guidelines on animal care experimentation and IASP ethical guidelines for pain experimentation on awaken animals (Zimmermann, 1983). Sixty animals were divided in two main experimental groups of 30 rats each: spared nerve injury (SNI) and sham operated (Sham). A set of rats (n=18 each group) received one injection of the cell proliferation marker bromodeoxyuridine (BrdU; Miller and Nowakowski, 1988), 50 mg/kg body weight, i.p. (Sigma, St. Louis, MO) for three consecutive days before their death (see below), two months after SNI induction or Sham surgery.

Spared nerve injury surgery

The SNI model of chronic neuropathic pain included an axotomy and ligation of two of the three peripheral ramifications of the sciatic nerve, the tibial and common peroneal nerves and leaving the sural nerve intact, as described elsewhere (Decosterd and Woolf, 2000). The animals were lightly anesthetized with pentobarbital 0.5% (Eutasil, Ceva Saúde Animal, Portugal). The common peroneal and tibial nerves were tight-ligated with 5.0 silk and sectioned distal to the ligation, removing 2–4 mm of the distal nerve stump. Great care was taken to avoid any contact with or stretching of the intact sural nerve. Muscle and skin were closed in two layers. Sham-controls involved exposure of the sciatic nerve and its branches without performing any manipulation.

Nociceptive tests

Nociceptive tests were performed in all animals a day before and two days after the surgery procedure, followed by testing every two days then forward, during the two months of experimental period. Both the ipsilateral (right hind paw) and the contralateral hind paw were tested in order to evaluate the presence of "mirror pain", described elsewhere as present in neuropathic pain pathologies (Tal and Bennett, 1994).

Mechanical allodynia

Animals were placed on an elevated wire grid and the lateral plantar surface of the paw stimulated with a series of ascending force von Frey monofilaments. The nociceptive threshold was taken as the lowest force that evoked a brisk withdrawal response to one of five repetitive stimuli (Tal and Bennett, 1994).

Mechanical hyperalgesia

With the animals on the elevated grid, a pin-prick test was performed using a safety pin. The lateral part of the plantar surface of the paw was briefly stimulated at intensity sufficient to touch but not penetrate the skin (Decosterd et al., 1998). The duration of paw withdrawal was measured, with an arbitrary minimal time of 0.5 seconds (s) (for the brief normal response) and maximal cut-off of 20 s (Tal and Bennett, 1994).

Assessment of emotional behaviour

All behavioural tests were performed five days preceding animal sacrifice during light period (9am to 6pm) in a restricted group of animals (n=18 each group).

Anxiety-like behaviour — elevated plus-maze test (EPM)

Anxiety-like behaviour was evaluated in the EPM test through an apparatus consisting of two open and two closed arms (50.8× 10.2×40.6 cm each arm) (MedAssociates Inc., St. Albans, Vermont, USA). Each rat was placed in the centre of the elevated plus-maze facing one of the open arms, and the time spent (s) in the open or closed arms was recorded during a 5-min test period (Mesquita et al., 2006; Sousa et al., 2006). The elevated plus-maze was carefully cleaned with 10% ethanol before each animal was placed on the equipment.

Depressive-like behaviour — forced-swimming test (FST)

The test was performed as in the original method described elsewhere (Porsolt et al., 1977, 1978). On day 1 (conditioning, pre-test session), rats were individually placed in a clear Plexiglass cylinder (29 cm in diameter and 50 cm in height) containing 30 cm of water (25+0.5 °C) and left to swim for ~~15~~ min. The rats were then removed from water and towel-dried, placed under a heating lamp

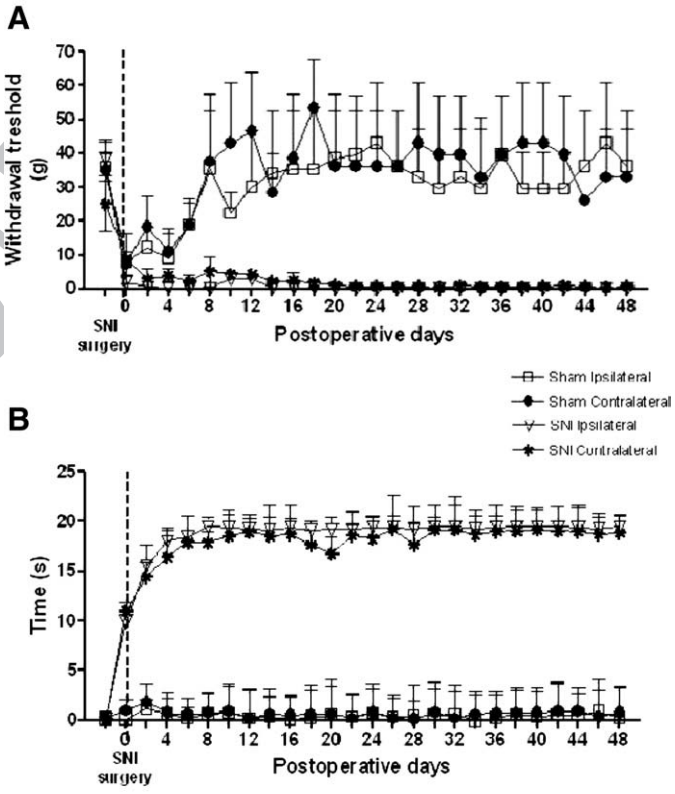

Fig. 1. Mechanical allodynia assessed by von Frey filaments (A) and mechanical hyperalgesia assessed by the pin-prick test (B) before and after surgery in SNI and Sham groups (dotted line indicates the day of SNI surgery). (A) Note that the pre-surgery threshold to von Frey filaments was similar in both SNI and Sham groups and in both hind paws; after surgery, the withdrawal threshold of the SNI group decreased within 24 h and remained low until the end of the 2 month experimental period. In Sham animals, the withdrawal threshold to von Frey filaments was decreased during the first postoperative days but returned to baseline values. (B) In the pin-prick test, SNI animals showed a strong hyperalgesia from the first postoperative day onwards, whereas Sham animals showed no hyperalgesia. The symbols and error bars represent mean+S.D.

Please cite this article as: Gonçalves, L., et al., Neuropathic pain is associated with depressive behaviour and induces neuroplasticity in the amygdala of the rat, Exp. Neurol. (2008), doi:10.1016/j.expneurol.2008.04.043

for 5 min, and finally returned to their home cage. Twenty-four hours later, the rats were tested under the same conditions for 5 min (test session). Rats were judged to be immobile when both hind legs were not moving, and the rat was slightly bent forward (Mesquita et al., 2006).

Locomotion and exploratory behaviour — open field test (OF)

Motor activity and exploratory behaviour were evaluated by placing the rat into an infrared photobeam controlled open field activity test chamber in a brightly illuminated (white light) room. Animals were tested for 10 min in an arena (43.2 cm×43.2 cm transparent acrylic walls and white floor) (MedAssociates Inc., St. Albans, Vermont) that was divided into a central and a peripheral zone. The time spent by each animal in the central and peripheral (residual) zone and its vertical activity (rearings) were the parameters evaluated in this test (Mesquita et al., 2006). Environmental odours were removed with 10% ethanol solution.

Tissue preparation

Both the SNI and Sham groups were divided as follows: i) in the first group (n=6 each), designated to stereological analysis, the animals were anaesthetized with pentobarbital and perfused with 4% paraformaldehyde (PFA), the brains were removed, embedded in 2-hydroxyethyl glycol methacrylate, serially sectioned in a microtome at 30 µm and stained with Giemsa; ii) in the second group (n=6 each), designated to 3D-morphologycal analyses of dendritic arborization of AMY neurons, the animals were anesthetized with pentobarbital, perfused with saline and the brains were removed and processed for posterior staining following the Golgi-Cox method (Gibb and Kolb, 1998) and slicing in a vibratome at 100 µm; iii) in the third group (n=18 each), processed for immunocytochemistry for detection of BrdU, GFAP (glial fibrillary acidic protein), NeuN (neuronal nuclei) and Calb (Calbindin), the animals were decapitated, the brains dissected, frozen in liquid nitrogen and sectioned in a cryostat (−14 °C).

Stereological procedures

The amygdaloid complex was subdivided in its nuclear components as in Paxinos and Watson (1998): central (CeA), lateral (La), basolateral anterior (BLA) and posterior (BLP), basomedial anterior (BMA) and posterior (BMP) nuclei. The nuclei volume and cell number estimation in AMY nuclei in every 8th section stained with Giemsa was obtained through the Cavalieri's principle and optical fractionator methods using the Stereoinvestigator software (MicroBrightField, Inc., Williston, VT, USA).

3D-morphologycal analysis of dendrites

The brain sections stained with the Golgi-Cox method were observed at the optical microscope and multipolar and bipolar AMY neurons completely and perfectly stained (Cerqueira et al., 2007) were considered for further analysis using the Neurolucida software (MicroBrightField, Inc., Williston, VT, USA). The dendrites and spines of 6 AMY neurons per animal were drawn.

Immunohistochemical procedures

All quantifications of markers for cell division and neuronal fate were performed in the AMY. Positive controls for histochemical reactions were confirmed by analysing the subgranular zone (SGZ) of the hippocampus, since neuronal proliferation is known to occur in this area (Gould et al., 1999a). As negative controls of immunocytochemical reactions, the primary antibody was not included in the protocol of each reaction; no specific immunoreaction was observed following negative controls.

BrdU immunohistochemistry and quantification of BrdU-labelled cells

Bromodeoxyuridine (BrdU; an analogue of thymidine, incorporated into the newly synthesized DNA of replicating cells) incorporation was detected by immunocytochemistry on every 8th serial brain

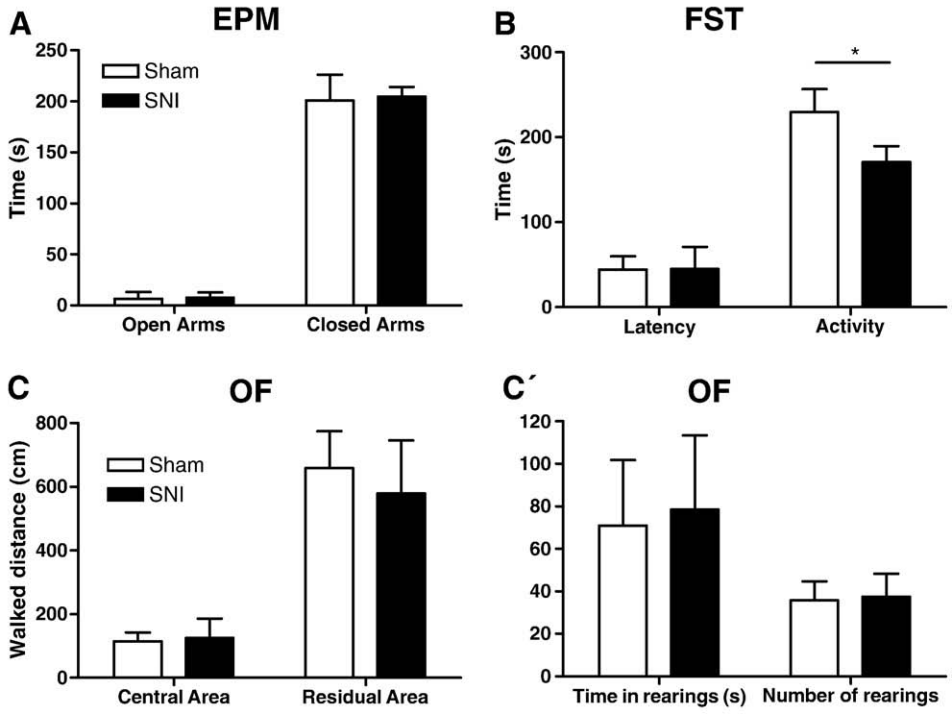

Fig. 2. Performance of SNI and Sham groups during behavioural tests. No differences were observed between the two groups in the EPM test (A), neither in the time spent in the open or closed arms. In the FST (B), the time of activity was lower in ~~the~~ SNI animal, which indicates the presence of depressive-like behaviour. No differences were observed for the OF test (C, C′) in any of the parameters evaluated.

Please cite this article as: Gonçalves, L., et al., Neuropathic pain is associated with depressive behaviour and induces neuroplasticity in the amygdala of the rat, Exp. Neurol. (2008), doi:10.1016/j.expneurol.2008.04.043

section containing the amygdaloid complex. Briefly, sections were fixed in 4% PFA for 30 minutes (min), permeabilized for 10 min in a solution containing 0.2% Triton X-100 in Tris buffer saline (TBS) after a 3×3 min wash in TBS, heated during 20 min in citrate buffer 0.1 M following a 3×3 min wash and acidified in HCl 2 M for 30 min after rinsing in distillated water. Endogenous peroxidase activity was blocked with 3% H2O2 in TBS for 10 min after a 3×3 min wash in TBS, followed by immersion in 4% bovine serum albumin (BSA) in TBS for 30 min (to block non-specific staining) after a 3×3 min wash. After another 3×3 min wash in TBS, the tissue was incubated overnight with a primary monoclonal anti-BrdU antibody raised in mouse (1:50, Dako, Glostrup, DK) and stained cells were detected using a universal detection system (BioGenex, San Ramon, CA, USA) and diaminobenzidine (DAB 0.025% and H2O2 0.5% in Tris–HCl 0.05M pH 7.2), after a 3×2 min wash in TBS and a 1×3 min wash in Tris–HCl, followed by counterstaining with haematoxylin. BrdU-positive cells were counted throughout the entire AMY area.

Immunofluorescence and quantification of double-labelled cells

Double-staining immunofluorescent reactions were performed in order to reveal three different groups: (i) BrdU and GFAP (glial fibrillary acidic protein; a marker of astrocyte glial cells; Reeves et al., 1989), (ii) BrdU and NeuN (protein expressed exclusively in mature neurons; Mullen et al., 1992) and (iii) BrdU and Calb (Calbindin; a calcium-binding protein present in functional mature neurons Meguro et al., 2004). The following primary antibody dilutions were used: rat anti-BrdU (1:500, Accurrate, Westbury, MA), mouse anti-GFAP (1:500, Dako Glostrup, Denmark), mouse anti-NeuN (1:500, Chemicon International, Temecula, CA, USA) and rabbit anti-Calb (1:200, Chemicon International, Temecula, CA, USA). The initial protocol procedure (until the primary antibody incubation) was the same in the first three groups and similar to that described above for revealing BrdU. The following specific procedures for each double-staining method are explained briefly and separately for each group.

Brain sections were mounted in slides with Vectashield (Vector Laboratories, Burlingame, CA, USA) to delay fluorescence decay, and observed two days later in a fluorescence microscope. Data were confirmed posteriorly using confocal microscopy (Olympus FluoViewTM FV1000, OLYMPUS).

i) BrdU and GFAP

After overnight incubation with the primary antibody anti-BrdU raised in rat, sections were washed 3×2 min in TBS and then incubated with a fluorescent Alexa 568 secondary antibody (goat anti-rat, 1:200; Molecular Probes, Eugene, OR) for 1 h. Following a 3×3 min wash in TBS, sections were incubated during 3 h with the primary antibody mouse anti-GFAP, followed by the fluorescent Alexa 488 secondary antibody (goat anti-mouse, 1:100, Molecular Probes, Eugene, OR) for 1 h. The sections were finally washed 2×2 min in TBS and 2 min in distillate water before being mounted in slides.

ii) BrdU and NeuN

Sections were incubated overnight with the primary antibody anti-BrdU raised in rat followed by the fluorescent Alexa 568 secondary antibody (goat anti-rat, 1:200; Molecular Probes, Eugene, OR) for 1 h, after a 3×3 min wash in TBS. Then, sections were immersed for 3 h with the primary antibody anti-NeuN raised in mouse (1:500) and washed 3×3 min. Subsequently, they were incubated with biotinylated secondary antibody anti-mouse (1:200) for 1 h and, after a 3×3 min wash, incubated with Alexa Streptavidine 488 (1:100, Molecular Probes, Eugene, OR) for one final hour. The sections were washed in TBS and distillate water as above and mounted in slides.

iii) BrdU and Calb

Sections were incubated overnight with the rat anti-BrdU and mouse anti-Calb primary antibodies. In the next day, after a 3×3 min wash in TBS sections were firstly incubated with fluorescent Alexa 568 (goat anti-mouse, 1:200) secondary antibody for 1 h and then with fluorescent Alexa 488 (~~goat anti-rat~~, 1:200; Molecular Probes, Eugene, OR) secondary antibody, after a 3×3 min wash. The sections were washed in TBS and distillate water and mounted in slides.

Statistic analysis

For the analysis of baseline thresholds of SNI/Sham and ipsi/contralateral hind paws in the von Frey and pin-prick tests, one-way analysis of variance (ANOVA) was performed. Considering that in the

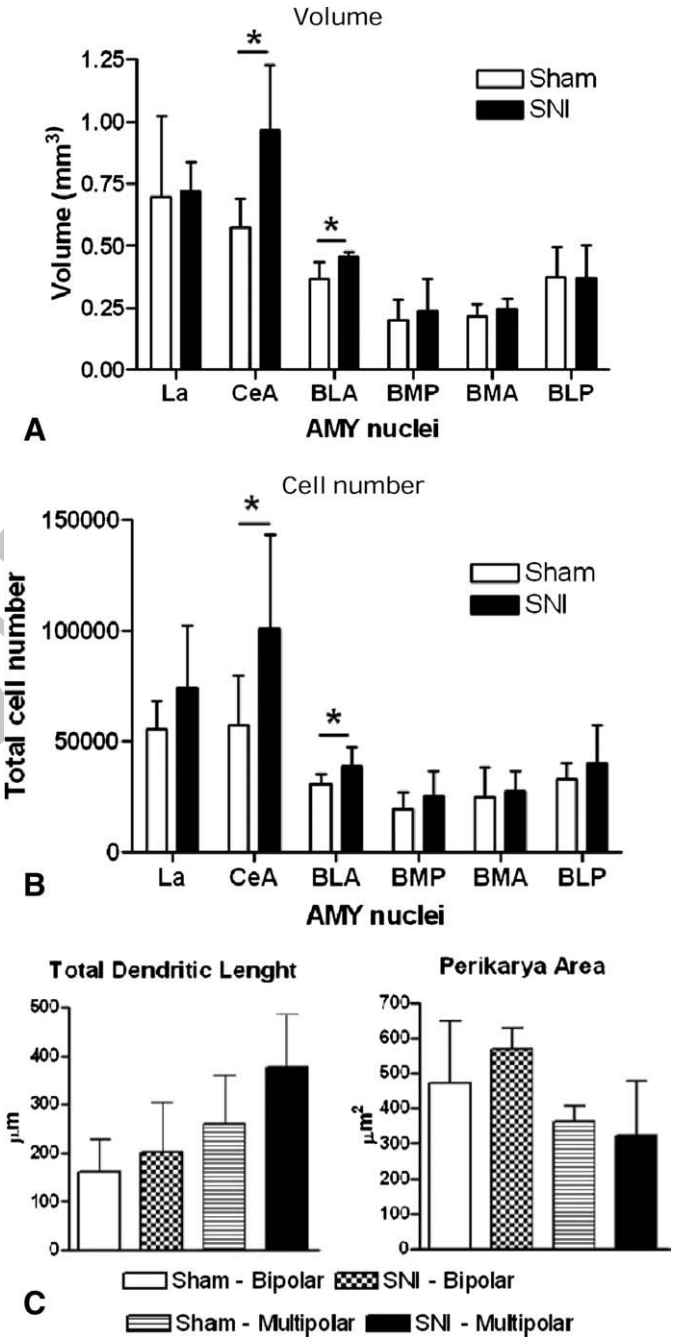

Fig. 3. Morphological analysis of AMY nuclei. (A) Volumes of different AMY nuclei were higher in neuropathic animals when compared to Sham, with differences being statistically significant for the CeA and BLA nuclei. (B) Cell number is also higher in all amygdalar nuclei of SNI animals, with differences being significant again in the CeA and BLA nuclei. (C) Structural analysis through Golgi-Cox method showed no differences in cell body volume and dendrite length of AMY neurons between SNI and Sham groups.

Please cite this article as: Gonçalves, L., et al., Neuropathic pain is associated with depressive behaviour and induces neuroplasticity in the amygdala of the rat, Exp. Neurol. (2008), doi:10.1016/j.expneurol.2008.04.043

rest of this study only comparisons between two groups were performed, the ~~s~~Student's *t*-test was used to analyse the results of all tests and procedures. The results were considered to be statistically different when $p < 0.05$. Data are presented as mean ± standard deviation.

Results

The spared nerve injury model induces hypersensitivity for at least 2 months

Assessment of mechanical allodynia and hyperalgesia using, respectively, von Frey filament and pin-prick tests, were performed twice before the SNI surgery (baseline measurements) and every two days afterwards (during a two month period). Both neuropathic (SNI group) and sham-control (Sham group) animals presented a similar baseline withdrawal threshold measured by von Frey filaments (SNI: ipsilateral 38 ± 6.1 g, contralateral 25 ± 8.2 g; Sham: ipsilateral 36 ± 7.3 g, contralateral 35 ± 5.1; Fig. 1A). A bilateral decrease in nociceptive threshold was observed in neuropathic animals within 24 h after surgery. This threshold decrease reached the level of 0–5 g ~~five~~ days after the surgery, a value that remained constant until the end of the two month experimental period. These data showed that the SNI group developed and maintained a strong mechanical allodynia in both hind paws, as a consequence of the surgery. On the contrary, nociceptive threshold in Sham animals decreased slightly with the sham surgery, returning to baseline values within a week, never reaching thresholds as low as those presented by SNI animals (Fig. 1A). In what concerns the pin-prick test, the baseline duration of hyperalgesic behaviour was less than 1 s in all animals, and there were no differences between groups (SNI: ipsilateral 0.17 ± 0.17 s, contralateral 0.11 ± 0.8 s; Sham: ipsilateral 0.13 ± 1.11 s, contralateral 0.2 ± 0.2; Fig. 1B). Within 24 h from the surgery, SNI animals reached the maximal duration of hyperalgesic behaviour in both hind paws (20 s) whereas no changes were observed in Sham animals (Fig. 1B). These data showed that the SNI group developed and maintained a clear hyperalgesic state during virtually the entire experimental period. In summary, data on pain-related behaviour demonstrated that SNI animals developed a clear neuropathy that extended throughout the complete experimental period.

Neuropathic animals develop a depressive-like behaviour but do not display signs of increased anxiety

Emotional behaviour was assessed seven weeks after the surgery. EPM was performed to evaluate anxious behaviour, FST to assess depressive-like behaviour and the OF test to determine locomotion and exploratory behaviour (Mesquita et al., 2006). In the EPM, no differences were found in the behavioural responses between SNI and

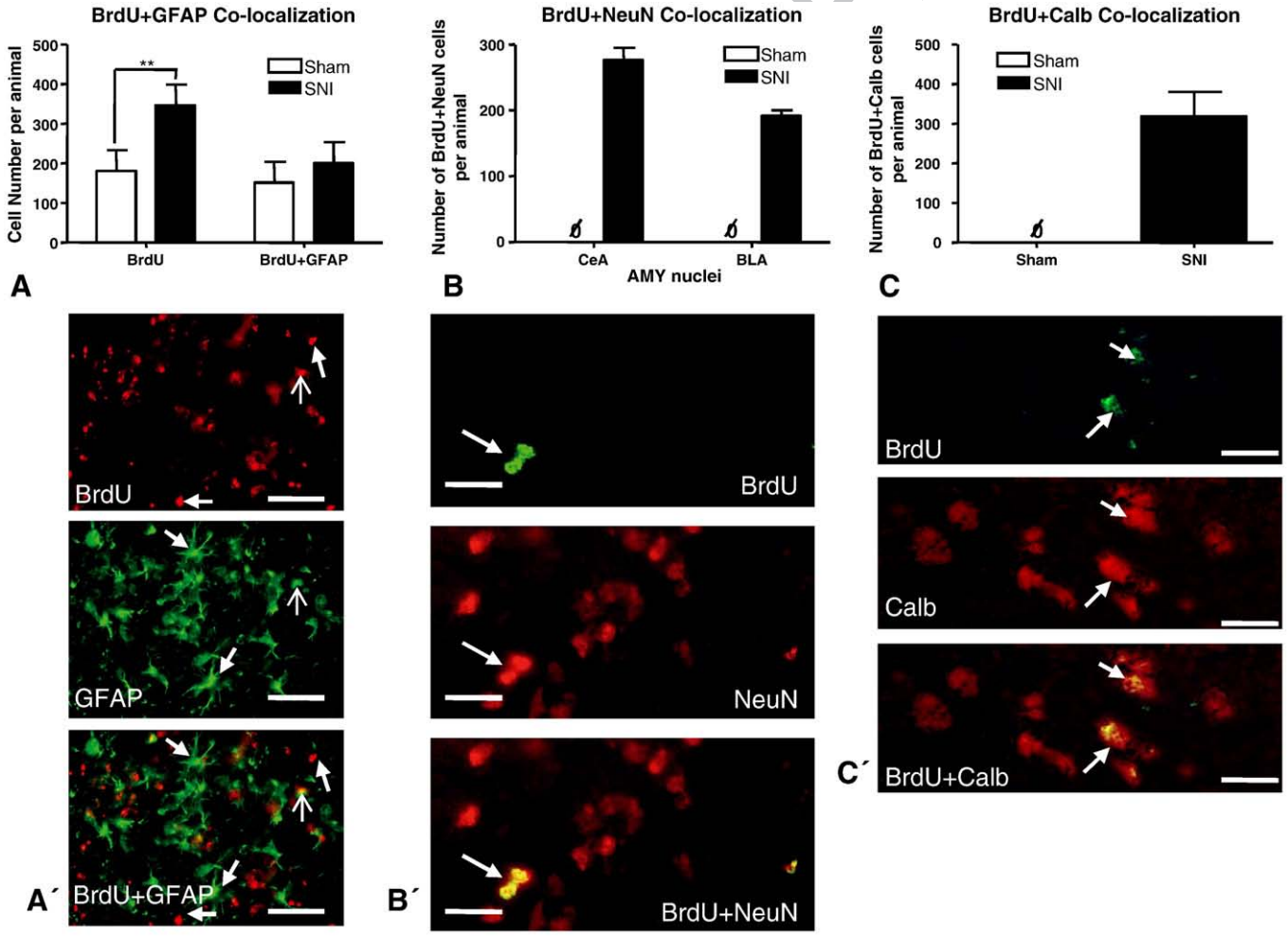

Fig. 4. Cell fate resulting from amygdalar neuroplasticity. (A) The number of cells that were BrdU-positive was significantly superior in SNI animals, but no differences were observed in the number of BrdU+GFAP double-labelled cells between SNI and Sham groups. (A′) Representative images of GFAP, BrdU and GFAP+BrdU (double-stained)-positive cells. (B) BrdU+NeuN double-labelled cells were present only in AMY nuclei, being absent in Sham animals. (B′) Representative images of BrdU, NeuN and NeuN+BrdU double-stained cells. (C) Calb+BrdU double-stained cells were also present only in neuropathic animals. (C′) Representative images of BrdU, Calb and Calb+BrdU double-stained cells. Magnification bar: 60 μm (A′), 20 μm (B′, C′).

Please cite this article as: Gonçalves, L., et al., Neuropathic pain is associated with depressive behaviour and induces neuroplasticity in the amygdala of the rat, Exp. Neurol. (2008), doi:10.1016/j.expneurol.2008.04.043

Sham groups (Fig. 2A), thereby showing that the anxiety levels were unaltered by induction of SNI. On the other hand, the FST revealed significant differences between experimental groups (Fig. 2B): while Sham animals were active for 230±27 s, SNI animals only tried to escape/swim for 180±38 s ($p=0.012$), which indicates the presence of a learned helplessness (depressive-like) behaviour in neuropathic animals. Since FST test includes movement of the paws and neuropathic animals are hyperalgesic and allodynic in both ipsilateral and contralateral hind paws, the OF test was performed in order to validate the FST test. This test revealed that the SNI group had no differences in the locomotion ability when compared with Sham group and it also revealed that the number of rearings (an indicator of exploratory behaviour) did not differ between experimental groups (Figs. 2C,C′). The absence of differences in the time spent in central vs. peripheral part of the OF arena also indicates the absence of altered anxiety behaviour in neuropathic animals. In summary, these behavioural studies demonstrate that a 2 month neuropathy induced a depressive-like, but not anxious-like, behaviour.

Volume and cell number are increased in amygdaloid nuclei

After animal perfusion, 6 brains of each experimental group were prepared for stereological analysis and other 6 SNI and Sham brains were processed for tri-dimensional morphological analysis. For stereological analysis the AMY was divided in 6 nuclei (Paxinos and Watson, 1998): central (CeA), lateral (La), basolateral anterior (BLA) and posterior (BLP), basomedial anterior (BMA) and posterior (BMP). We found a general increase in the volume of all these nuclei in SNI neuropathic animals, with a significant increase being observed in CeA ($p=0.02$) and BLA ($p=0.019$) nuclei (Fig. 3A). In order to determine the causes for these structural changes of AMY, we analysed potential alterations in cell numbers and cellular volumes. SNI neuropathic animals showed a general increase in the number of cells in all AMY nuclei, with a significant difference being present again in CeA ($p=0.015$) and BLA ($p=0.016$) nuclei (Fig. 3B). On the contrary, 3D-morphological analysis revealed no significant differences in dendritic lengths (Fig. 3C) or perikarya areas (Fig. 3D) between neuropathic and Sham animals, both in bipolar and multipolar AMY neurons. Taken together, these results indicate that the significant increase observed in CeA and BLA nuclear volumes of SNI animals was due, at least in part, to an increase in cell numbers.

Newborn neurons contribute to increased cell numbers in AMY

Rats received one injection of the cell proliferation marker bromodeoxyuridine (BrdU) in the three consecutive days before their sacrifice. The aim of this procedure was to determine if cell proliferation was responsible for the higher number of cells observed in the CeA and BLA nuclei in SNI animals. Immunohistochemistry revealed the presence of BrdU-positive cells in the AMY of both SNI and Sham groups, but with significantly higher numbers in neuropathic animals ($p=0.001$; Fig. 4A). In order to identify the phenotype of these newly-acquired cells, two different double-staining immunohistochemistry reactions were performed: BrdU+

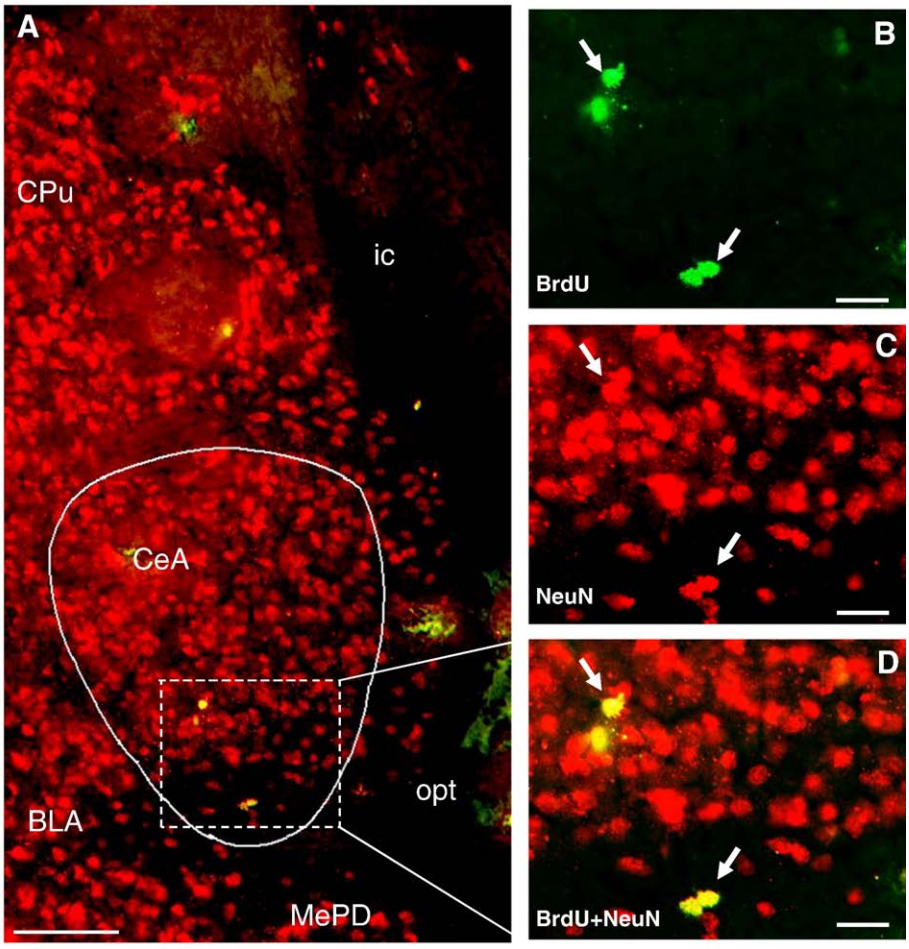

Fig. 5. (A–D) Microphotograph showing examples of BrdU+NeuN double-labelled cells in the CeA. The rectangle in micrograph A is magnified in figures B–D; the border of CeA nucleus is outlined by a continuous line. CPu — caudate putamen (striatum); MePD — medial amygdaloid nucleus, posterodorsal part; ic — internal capsule; opt — optic tract. Magnification bar: 100 μm (A), 20 μm (B–F).

Please cite this article as: Gonçalves, L., et al., Neuropathic pain is associated with depressive behaviour and induces neuroplasticity in the amygdala of the rat, Exp. Neurol. (2008), doi:10.1016/j.expneurol.2008.04.043

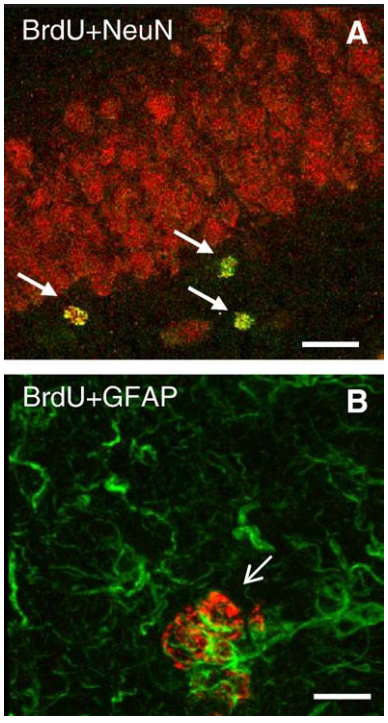

Fig. 6. Examples of BrdU+NeuN (A) and BrdU+GFAP (B) double-labelled cells (arrows) obtained in positive-control sections from the subgranular zone of the dentate gyrus of the hippocampus. Magnification bar: 20 μm (A), 10 μm (B).

glial fibrillary acidic protein marker (GFAP) and BrdU+post-mitotic neuronal marker (NeuN). The number of BrdU+GFAP-positive cells was similar between the SNI and Sham groups. On the other hand, BrdU+NeuN double-labelled cells were observed only in the SNI group; interestingly, they were mainly located in the CeA and BLA nuclei (Figs. 4B, B′, 5). These findings indicate the presence of newly proliferating neurons in the AMY after prolonged SNI, as further demonstrated by the presence of BrdU+Calbindin-positive cells in the AMY of neuropathic animals (Fig. 4C,C′). Positive control sections obtained from the subgranular zone of the hippocampal dentate gyrus showed the presence of both BrdU+NeuN and BrdU+GFAP double-labelled cells (Fig. 6).

In summary, data demonstrate not only that recently-divided newborn neurons are formed in the AMY of chronic pain animals, but also that these neurons reach a physiologically mature (i.e., functional) state.

Discussion

After two months of neuropathic pain, SNI animals exhibited signs of sustained persistent pain associated with a significant depressive-like behaviour. At the CNS level, a structural reorganization of the amygdaloid complex was observed that was associated with a significant increase in the volume of the basolateral (BLA) and central (CeA) AMY nuclei. The volume increase was due to an increased number of AMY cells, and not to hypertrophy of dendrites or perikarya of amygdalar neurons. The present study is the first demonstrating cell proliferation in a limbic area, as a result of chronic neuropathic pain. Earlier, only electrophysiological studies have shown chronic pain-related neuroplasticity of AMY neurons in persistent arthritis, visceral pain (Han and Neugebauer, 2004) or neuropathy (Ikeda et al., 2007). Moreover, this is the first study demonstrating that chronic pain results in depressive-like behaviour associated with neuroplasticity in a major brain centre implicated in the control of both emotions and pain.

Changes in emotional behaviour and neuroplasticity in the AMY

Morphological plasticity in the AMY was previously suggested in cases of prolonged emotional disturbance, as shown by increased AMY volumes measured by structural magnetic resonance in patients with depression and anxiety (Frodl et al., 2002; Tebartz van Elst et al., 2000). Clinical data also reveal that prolonged pain conditions are associated with a high incidence of emotional disorders, including anxiety and depression (Rasmussen et al., 2004). Herein, we show that in the rat, a two month neuropathy resulted also in a depressive-like behaviour measured by the forced-swimming test (FST), but no alterations in anxiety levels were detected in the elevated plus-maze and open field tests. We also show that increased immobility time in the FST should not be ascribed to motor impairments as there were no changes in locomotor activity and exploratory behavior. As in humans, SNI neuropathy associated with emotional alterations may result from, or contribute to, the structural changes observed in the AMY. It has been proposed that the increase in AMY volume observed in depressive patients was a consequence of the continuous prolonged activation of this area (Frodl et al., 2002). Following the same rationale, the present increase in AMY volume may result from the continuous flow of nociceptive information into AMY regions receiving sensory information (including the BLA) and the consequent prolonged activity of AMY neurons triggering the appropriate response action (CeA is the main effector of AMY). Especially relevant is the increase in the CeA volume, as its latero-capsular part is defined as the 'nociceptive amygdala' due to its high content in neurons implicated in nociceptive processing (Bernard et al., 1996; Neugebauer and Li, 1992; Neugebauer et al., 2004).

The volume increase in the AMY after two months of neuropathic pain may have resulted from one or various different processes: cell size (soma and dendritic size) increase, cell number (neurons or glial cells) increase, or increased extracellular volume. However, subsequent analysis revealed that the increased volume of the AMY in SNI animals could not be ascribed to cell size variations, but rather to an increase in cell number. Interestingly, such increase in cell numbers was confirmed by the observation of newly proliferating cells in AMY nuclei of SNI animals. Although the presence of newborn neurons in the adult brain of mammals is considered to be restricted to two areas, the subgranular zone (SGZ) of the hippocampus and the subventricular zone (SVZ) (Doetsch et al., 1997; Gould et al., 1999b; Kempermann and Gage, 2000), the possibility of neurogenesis in the AMY has already been raised in a study showing evidence for the presence of newly generated neurons in the AMY of adult primates, at basal conditions (Bernier et al., 2002). The results of double-immunoreactions (BrdU+NeuN) performed in the present study demonstrate that a significant number of these newly-born cells undergo a neuronal phenotype. Thus, the genesis of newborn neurons is responsible, at least in part, for the increase in cell number underlying the increase of volume observed in the AMY of SNI animals. In contrast, the number of cells stained simultaneously for markers of cell proliferation (BrdU) and glia (GFAP) revealed no additional glial cell proliferation in the AMY following SNI induction; this indicates that SNI results only in additional neuronal proliferation, with a similar basal rate of astrocyte cell division being common to both Sham and SNI animals.

Neurogenesis and the AMY

Our observation of NeuN and BrdU co-localization in AMY cells indicate that newly generated cells reached neuronal maturation in the amygdaloid complex. This is in accordance with the time points for expression of neuronal differentiation markers described by Kempermann et al. (2004) and Steiner et al. (2004): in the hippocampus of adult mice NeuN expression becomes higher than immature-neuron markers 3 days after cell division. Additionally, the presence of BrdU+Calb double-labelled neurons in the AMY confirms the maturation and

Please cite this article as: Gonçalves, L., et al., Neuropathic pain is associated with depressive behaviour and induces neuroplasticity in the amygdala of the rat, Exp. Neurol. (2008), doi:10.1016/j.expneurol.2008.04.043

phenotypical differentiation of newborn neurons in definitive AMY of SNI animals.

Whether these newly-born cells observed in the AMY of SNI rats result from local progenitor cells or migrate from adjacent neurogenic regions is still not known. However, several studies have shown that besides the normal migration of proliferative cells from the SVZ to the olfactory bulb (through the rostral migratory stream, RMS) or from the SGZ to other areas of the DG, they can migrate from the SVZ to injured areas of the brain (Iwai et al., 2003; Parent et al., 2002; Van Kampen et al., 2004). Therefore, it is possible that the new neurons here observed have their origin in SVZ progenitor cells that, through migration, reached the amygdaloid complex following the prolonged pain syndrome induced by the SNI model. Supporting this hypothesis, post-natal neurogenesis in the SVZ and SGZ can be regulated positively through the enhancement of the survival of newly generated cells and negatively through the down regulation of cell proliferation (Gould and Gross, 2002) following different stimuli (Jin et al., 2001). On the other hand, a growing amount of evidence supports the notion that the CNS itself is not as static as once believed: BrdU-positive cells were shown to be present in several regions of the adult CNS currently thought to be mitotically quiescent (Rietze et al., 2000); studies report that neurogenesis is prone to occur in other areas of adult mammals, like the neocortex (Gould et al., 1999a; Takemura, 2005), the striatum (Van Kampen et al., 2004; Bedard et al., 2006), the substantia nigra (Yoshimi et al., 2005) and the amygdala itself (Bernier et al., 2002). Taking into account these data, it should not be excluded the possibility that neural stem cells could be present in the AMY and proliferate following the prolonged neuropathy resulting from the SNI model. Further experimental procedures must be performed to elucidate this issue.

Roles of AMY in pain and emotional processing

Several data implicate the AMY in pain modulation, as shown by changes in pain tolerance induced by AMY manipulation (Manning, 1998). Moreover, the AMY has a role in both pain inhibition and pain facilitation (Manning and Mayer, 1995; Manning et al., 2001; Tershner and Helmstetter, 2000). This dual effect may result from direct AMY projections to brainstem areas implicated in both descending antinociception and pronociception (Almeida et al., 1999; Bouhassira et al., 1992; Porreca et al., 2002). As a balance between descending inhibiting (antinociceptive) and facilitating (pronociceptive) actions upon spinal nociceptive transmission can contribute to the normal control of pain perception (Lima and Almeida, 2002; Pertovaara, 2000; Porreca et al., 2002; Ren and Dubner, 1996; Schaible et al., 1991), the AMY may have a crucial role as a higher centre modulating the brainstem pain centres responsible for the fine regulation of the spinal nociceptive transmission. Thus, it is possible that the here observed amygdalar neuroplasticity may contribute not only to emotional changes but also to alterations in nociception. In support of this hypothesis, volume changes of AMY were already shown in imaging studies of patients with a major depression (Drevets, 2000) and changes in synaptic function of nociceptive AMY neurons have been described in sustained pain conditions (Han and Neugebauer, 2004; Ikeda et al., 2007). Additionally, the neuronal proliferation observed in AMY areas involved in afferent (BLA) and efferent (CeA) nociceptive processing may disrupt fine neuronal networks between high brain centres, which provide a structural basis for deregulation of emotional behaviour.

Conclusion

In conclusion, this study shows that besides mechanical hyperalgesia and allodynia, animals subjected to the SNI model of neuropathic pain during a two month period developed a depressive-like behaviour associated with an increased volume of AMY nuclei that results from cell proliferation. Importantly, this is the first study providing evidence for the presence of newly-born cells in the amygdaloid complex as a consequence of a sustained chronic (neuropathic) pain condition. We hypothesize that these neuroplastic changes of the AMY could be associated with the development of depressive-like behaviour in neuropathic animals. Nonetheless, future studies on the origin of newborn neurons and their integration in the pre-existing synaptic network should be performed in order to determine the relevance of this phenomenon.

~~Uncited references~~ Q5

~~Aliashkevich et al., 2003~~
~~Almeida et al., 2002~~
~~Coutaux et al., 2005~~
~~Gage, 2002~~
~~Gage et al., 1998~~
~~Kuhn et al., 1996~~
~~Kumar et al., 2005~~
~~LeDoux et al., 1990~~
~~McEwen, 2003~~
~~McQuay et al., 1996~~
~~Mini et al., 1995~~
~~Reynolds and Weiss, 1992~~
~~Shim et al., 2005~~
~~Taub, 1982~~
~~Vanegas and Schaible, 2004~~
~~Watkins and Maier, 2002~~
~~Weiss et al., 1996~~
~~Zhao et al., 2003~~

Acknowledgments

This study was supported by Fundação para a Ciência e Tecnologia (FCT) Project no. POCTI/NSE/46399/2002, FEDER and Fundação Calouste Gulbenkian Project no. 74551. Leonor Gonçalves is a PhD Fellow supported by FCT.

References

Aliashkevich, A.F., Yilmazer-Hanke, D., Van Roost, D., Mundhenk, B., Schramm, J., Blumcke, I., 2003. Cellular pathology of amygdala neurons in human temporal lobe epilepsy. Acta Neuropathol. (Berl) 106, 99–106.

Almeida, A., Størkson, R., Lima, D., Hole, K., Tjølsen, A., 1999. The medullary dorsal reticular nucleus facilitates pain behaviour induced by formalin in the rat. Eur. J. Neurosci. 11, 110–122.

Almeida, A., Cobos, A., Tavares, I., Lima, D., 2002. Brain afferents to the medullary dorsal reticular nucleus: a retrograde and anterograde tracing study in the rat. Eur. J. Neurosci. 16, 81–95.

Almeida, A., Leite-Almeida, H., Tavares, I., 2006. Medullary control of nociceptive transmission: reciprocal dual communication with the spinal cord drug discovery today: disease mechanisms, 3, pp. 305–312.

Altshuler, L., Bookheimer, S., Proenza, M.A., Townsend, J., Sabb, F., Firestine, A., Bartzokis, G., Mintz, J., Mazziotta, J., Cohen, M.S., 2005. Increased amygdala activation during mania: a functional magnetic resonance magnetic resonance imaging study. Am. J. Psychiatry 162, 1211–1213.

Anand, K.J., Craig, K.D., 1996. New perspectives on the definition of pain. Pain 70, 209–211.

Bedard, A., Gravel, C., Parent, A., 2006. Chemical characterization of newly generated neurons in the striatum of adult primates. Exp. Brain Res. 170, 501–512.

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.

Bernier, P.J., Bedard, A., Vinet, J., Levesque, M., Parent, A., 2002. Newly generated neurons in the amygdala and adjoining cortex of adult primates. Proc. Natl. Acad. Sci. U. S. A. 99, 11464–11469.

Bouhassira, D., Villanueva, L., Le Bars, D., 1992. Effects of systemic morphine on diffuse noxious inhibitory controls: role of the periaqueductal grey. Eur. J. Pharmacol. 216, 149–156.

Bremner, J.D., Narayan, M., Anderson, E.R., Eric, R., Staib, L.H., Miller, H.L., Charney, D.S., 2000. Hippocampal volume reduction in major depression. Am. J. Psychiatry 157, 115–117.

Cerqueira, J.J., Taipa, R., Uylings, H.B., Almeida, O.F., Sousa, N., 2007. Specific configuration of dendritic degeneration in pyramidal neurons of the medial prefrontal cortex induced by differing corticosteroid regimens. Cereb. Cortex 17, 1998–2006.

Please cite this article as: Gonçalves, L., et al., Neuropathic pain is associated with depressive behaviour and induces neuroplasticity in the amygdala of the rat, Exp. Neurol. (2008), doi:10.1016/j.expneurol.2008.04.043

Coutaux, A., Adam, F., Willer, J.C., Le Bars, D., 2005. Hyperalgesia and allodynia: peripheral mechanisms. Jt. bone spine 72, 359–371.

Danziger, N., Weil-Fugazza, J., Le Bars, D., Bouhassira, D., 2001. Stage-dependent changes in the modulation of spinal nociceptive neuronal activity during the course of inflammation. Eur. J. Neurosci. 13, 230–240.

Davis, M., Whalen, P.J., 2001. The amygdala: vigilance and emotion. Mol. Psychiatry 6, 13–34.

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

Decosterd, I., Buchser, E., Gilliard, N., Saydoff, J., Zurn, A.D., Aebischer, P., 1998. Intrathecal implants of bovine chromaffin cells alleviate mechanical allodynia in a rat model of neuropathic pain. Pain 76, 159–166.

Devor, M., 2006. Sodium channels and mechanisms of neuropathic pain. J. Pain 7 (Suppl 1), S3–S12 Review.

Doetsch, F., Garcia-Verdugo, J.M., Alvarez-Buylla, A., 1997. Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. J. Neurosci. 17, 5046–5061.

Drevets, W.C., 2000. Neuroimaging studies of mood disorders. Biol. Psychiatry 48, 813–829.

Frodl, T., Meisenzahl, E., Zetzsche, T., Bottlender, R., Born, C., Groll, C., Jäger, M., Leinsinger, G., Hahn, K., Möller, H.-J., 2002. Enlargement of the amygdala in patients with a first episode of major depression. Biol. Psychiatry 51, 708–714.

Gao, X., Kim, H.K., Chung, J.M., Chung, K., 2005. Enhancement of NMDA receptor phosphorylation of the spinal dorsal horn and nucleus gracilis neurons in neuropathic rats. Pain 116, 62–72.

Gage, F.H., 2002. Neurogenesis in the adult brain. J. Neurosci. 22, 612–613.

Gage, F.H., Kempermann, G., Palmer, T.D., Peterson, D.A., Ray, J., 1998. Multipotent progenitor cells in the adult dentate gyrus. J. Neurobiol. 36, 294-266.

Gibb, R., Kolb, B., 1998. A method for vibratome sectioning of Golgi-Cox stained whole rat brain. J. Neurosci. Methods 79, 1–4.

Gonçalves, L., Silva, R., Pinto-Ribeiro, F., Pego, J.M., Bessa, J.M., Pertovaara, A., Sousa, N., Almeida, A., 2006. Chronic neuropathic pain induces neurogenesis in the rat amygdala and is associated with altered emotional behavior. Society Neurosci Abstr, No 443.17, Abstract Viewer/Itinerary Planner.

Gould, E., Gross, C.G., 2002. Neurogenesis in adult mammals: some progress and problems. J. Neurosci. 22, 619–623.

Gould, E., Reeves, A.J., Graziano, M.S., Gross, C.G., 1999a. Neurogenesis in the neocortex of adult primates. Science 286, 548–552.

Gould, E., Reeves, A.J., Fallah, M., Tanapat, P., Gross, C.G., 1999b. Hippocampal neurogenesis in adult Old World primates. Proc. Natl. Acad. Sci. U. S. A. 96, 5263–5267.

Han, J.S., Neugebauer, V., 2004. Synaptic plasticity in the amygdala in a visceral pain model in rats. Neurosci. Lett. 361, 254–257.

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.

Iwai, M., Sato, K., Kamada, H., Omori, N., Nagano, I., Shoji, M., Abe, K., 2003. Temporal profile of stem cell division, migration, and differentiation from subventricular zone to the olfactory bulb after transient forebrain ischemia in gerbils. J. Cereb. Blood Flow Metab. 23, 331–341.

Jin, K., Minami, M., Lan, J.Q., Mao, X.O., Batteur, S., Simon, R.P., Greenberg, D.A., 2001. Neurogenesis in the dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. Proc. Natl. Acad. Sci. U. S. A. 98, 4710–4715.

Kauppila, T., Xu, X.J., Yu, W., Wiesenfeld-Hallin, Z., 1998. Dextromethorphan potentiates the effect of morphine in rats with peripheral neuropathy. Neuroreport 9, 1071–1074.

Kempermann, G., Gage, F.H., 2000. Neurogenesis in the adult hippocampus. Novartis Found. Symp. 231, 220–235 discussion 235–241, 302–306.

Kempermann, G., Jessberger, S., Steiner, B., Kronenberger, G., 2004. Milestones of neuronal development in the adult hippocampus. Trends Neurosci. 27, 447–552.

Kuhn, H.G., Dickinson, A.H., Gage, F.H., 1996. Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J. Neurosci. 16, 2027–2033.

Kumar, A.M., Solano, M.P., Fernandez, J.B., Kumar, M., 2005. Adrenocortical response to ovine corticotropin-releasing hormone in young men: cortisol measurement in matched samples of saliva and plasma. Horm. Res. 64, 55–60.

LeDoux, J.E., Cicchetti, P., Xagoraris, A., Romanski, L.M., 1990. The lateral amygdaloid nucleus: sensory interface of the amygdala in fear conditioning. J. Neurosci. 10, 1062–1069.

Lima, D., Almeida, A., 2002. The medullary dorsal reticular nucleus as a pronociceptive centre of the pain control system. Prog. Neurobiol. 66, 81–108.

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.

Manning, B.H., Merin, N.M., Meng, I.D., Amaral, D.G., 2001. Reduction in opioid- and cannabinoid-induced antinociception in rhesus monkeys after bilateral lesions of the amygdaloid complex. J. Neurosci. 21, 8238–8246.

McEwen, B.S., 2003. Mood disorders and allostatic load. Biol. Psychiatry 54, 200–207.

McQuay, H.J., Tramèr, M., Nye, B.A., Carroll, D., Wiffenb, P.J., Moore, R.A., 1996. A systematic review of antidepressants in neuropathic pain. Pain 68, 217–227.

Meguro, R., Lu, J., Gavrilovici, C., Poulter, M.O., 2004. Static, transient and permanent organization of GABA receptor expression in calbindin-positive interneurons in response to amygdala kindled seizures. J. Neurochem. 91, 144–154.

Merskey, H., 1965. Psychiatric patients with persistent pain. J. Psychosom. Res. 9, 299–309.

Merskey, H., Bogduk, N. (Eds.), 1994. Classification of Chronic Pain: Descriptions of Chronic Pain Syndromes and Definitions of Pain Terms, 2nd ed. IASP Press, Seattle.

Mesquita, A.R., Tavares, H.B., Silva, R., Sousa, N., 2006. Febrile convulsions in developing rats induce a hyperanxious phenotype later in life. Epilepsy Behav. 9, 401–406.

Miller, M.W., Nowakowski, R.S., 1988. Use of bromodeoxyuridine-immunohistochemistry to examine the proliferation, migration and time of origin of cells in the central nervous system. Brain Res. 457, 44–52.

Mini, A., Rau, H., Montoya, P., Palomba, D., Birbaumer, N., 1995. Baroreceptor cortical effects, emotions and pain. Int. J. Psychophysiol. 19, 67–77.

Mullen, R.J., Buck, C.R., Smith, A.M., 1992. NeuN, a neuronal specific nuclear protein in vertebrates. Development 116, 201–211.

Neugebauer, V., Li, W., 1992. Processing of nociceptive mechanical and thermal information in central amygdala neurons with knee-joint input. J. Neurophysiol. 87, 103–112.

Neugebauer, V., Li, W., Bird, G.C., Bhave, G., Gereau IV, R.W., 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.

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

Parent, J.M., Vexler, Z.S., Gong, C., Derugin, N., Ferriero, D.M., 2002. Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. Ann. Neurol. 52, 802–813.

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

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

Porsolt, R.D., Bertin, A., Jalfre, M., 1977. Behavioural despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. 229, 327–336.

Porsolt, R.D., Anton, G., Blavet, N., Jalfre, M., 1978. Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur. J. Pharmacol. 47, 379–391.

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

Rasmussen, P.V., Sindrup, S.H., Jensen, T.S., Bach, F.W., 2004. Symptoms and signs in patients with suspected neuropathic pain. Pain 110, 461–469.

Reeves, S., Helman, L., Allison, A., Israel, M., 1989. Molecular cloning and primary structure of human glial fibrillary acidic protein. Proc. Natl. Acad. Sci. 86, 5178–5182.

Rietze, R., Poulin, P., Weiss, S., 2000. Mitotically active cells that generate neurons and astrocytes are present in multiple regions of the adult mouse hippocampus. J. Comp. Neurol. 424, 397–408.

Ren, K., Dubner, R., 1996. Enhanced descending modulation of nociception in rats with persistent hindpaw inflammation. J. Neurophysiol. 76, 3025–3037.

Reynolds, B.A., Weiss, S., 1992. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255, 1707–1710.

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

Schaible, H.G., Neugebauer, V., Cervero, F., Schmidt, R.F., 1991. Changes in tonic descending inhibition of spinal neurons with articular input during the development of acute arthritis in the cat. J. Neurophysiol. 66, 1021–1032.

Shim, B., Kim, D.W., Kim, B.H., Nam, T.S., Leem, J.W., Chung, J.M., 2005. Mechanical and heat sensitization of cutaneous nociceptors in rats with experimental peripheral neuropathy. Neuroscience 132, 193–201.

Sousa, N., Almeida, O.F.X., Wotjak, C.T., 2006. A hitchhiker's guide to behavioral analysis in laboratory. Rodents Genes. Brain Behav. 5 (Suppl. 2), 5–24.

Steiner, B., Kronenberg, G., Jessberger, S., Brandt, M.D., Reuter, K., Kempermann, G., 2004. Differential regulation of gliogenesis in the context of adult hippocampal neurogenesis in mice. Glia 46, 41–52.

Strakowski, S.M., DelBello, M.P., Sax, K.W., Zimmerman, M.E., Shear, P.K., Hawkins, J.M., Larson, E.R., 1999. Brain magnetic resonance imaging of structural abnormalities in bipolar disorder. Arch. Gen. Psychiatry 56, 254–260.

Takemura, N.U., 2005. Evidence for neurogenesis within the white matter beneath the temporal neocortex of the adult rat brain. Neuroscience 134, 121–132.

Tal, M., Bennett, G.J., 1994. Extra-territorial pain in rats with a peripheral mononeuropathy: mechano-hyperalgesia and mechano-allodynia in the territory of an uninjured nerve. Pain 57, 375–382.

Taub, A., 1982. Opioid analgesic in the treatment of chronic intractable pain of non-neoplastic origin. In: Kitahata, L.M., Colllins, J.G. (Eds.), Narcotic Analgesics in Anesthesiology. Williams & Wilkins, Baltimore/London, pp. 199–208.

Tebartz van Elst, L., Woermann, F., Lemieux, L., Trimble, M.R., 2000. Increased amygdala volumes in female and depressed humans. A quantitative magnetic resonance imaging study. Neurosci. Lett. 281, 103–106.

Tershner, S.A., Helmstetter, F.J., 2000. Antinociception produced by mu opioid receptor activation in the amygdala is partly dependent on activation of mu opioid and neurotensin receptors in the ventral periaqueductal gray. Brain Res. 865, 17–26.

Vanegas, H., Schaible, H.G., 2004. Descending control of persistent pain: inhibitory or facilitatory? Brain Res. Rev. 46, 295–309.

Van Kampen, J.M., Hagg, T., Robertson, H.A., 2004. Induction of neurogenesis in the adult rat subventricular zone and neostriatum following dopamine D receptor stimulation. Eur. J. Neurosci. 19, 2377–2387.

Watkins, L.R., Maier, S.F., 2002. Beyond neurons: evidence that immune and glial cells contribute to pathological pain states. Physiol. Rev. 82, 981–1011.

Weiss, S., Reynolds, B.A., Vescovi, A.L., Morshead, C., Craig, C.G., van der Kooy, D., 1996. Is there a neuronal stem cell in the mammalian forebrain? Trends Neurosci. 19, 387–393.

Willoughby, S.G., Hailey, B.J., Mulkana, S., Rowe, J., 2002. The effect of laboratory-induced depressed mood state on responses to pain. Behav. Med. 28, 23–31.

Please cite this article as: Gonçalves, L., et al., Neuropathic pain is associated with depressive behaviour and induces neuroplasticity in the amygdala of the rat, Exp. Neurol. (2008), doi:10.1016/j.expneurol.2008.04.043

774 Yoshimi, K., Ren, Y.R., Seki, T., Yamada, M., Ooizumi, H., Onodera, M., Saito, Y., Murayama, S., Okano, H., Mizuno, Y., Mochizuki, H., 2005. Possibility for neurogenesis in substantia nigra of Parkinsonian brain. Ann. Neurol. 58, 31–40.
777 Zelman, D.C., Howland, E.W., Nichols, S.N., Cleeland, C.S., 1991. The effects of induced mood on laboratory pain. Pain 46, 105–111.
779 Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16, 109–110.
781 Zhao, M., Momma, S., Delfani, K., Carlen, M., Cassidy, R.M., Johansson, C.B., Brismar, H., Shupliakov, O., Frisen, J., Janson, A.M., 2003. Evidence for neurogenesis in the adult mammalian substantia nigra. Proc. Natl. Acad. Sci. U. S. A. 00, 7925–7930.

Please cite this article as: Gonçalves, L., et al., Neuropathic pain is associated with depressive behaviour and induces neuroplasticity in the amygdala of the rat, Exp. Neurol. (2008), doi:10.1016/j.expneurol.2008.04.043