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

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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, 2004; 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 and Rummans, 2002). 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).

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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 5 min. The rats were then

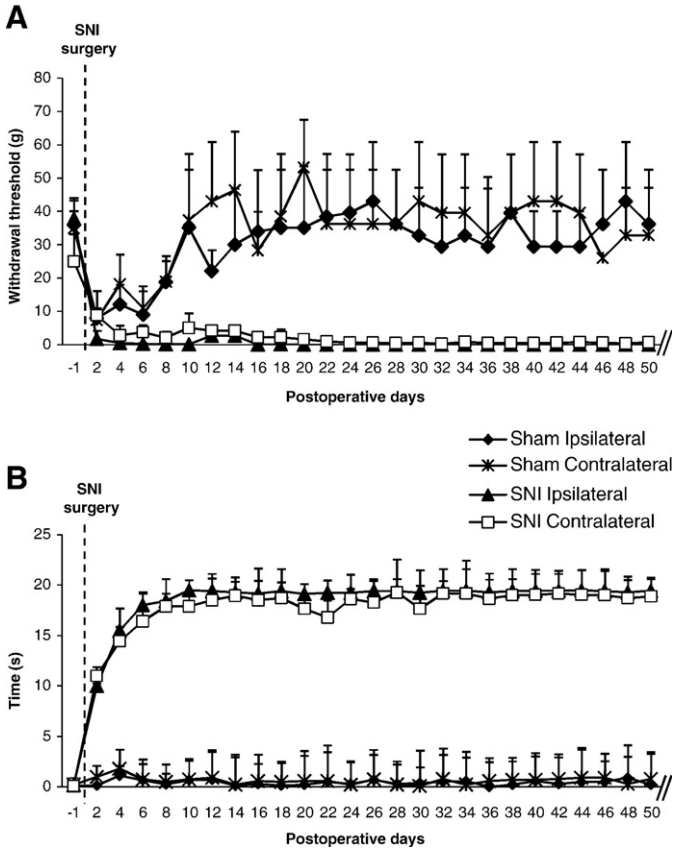

Fig. 1. Mechanical allodynia assessed by von Frey filaments (A) and mechanical hyperalgesia assessed by the pin-prick test (B) before and after surgery in SNI and Sham groups (dotted line indicates the day of SNI surgery). (A) Note that the pre-surgery threshold to von Frey filaments was similar in both 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.