European Neuropsychopharmacology (2014) 24, 996–1003

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SHORT COMMUNICATION

Pain exacerbates chronic mild stress-induced changes in noradrenergic transmission in rats

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Received 26 September 2013; received in revised form 2 January 2014; accepted 11 January 2014

KEYWORDS
Depression;
Stress;
Pain;
Comorbidity;
Locus coeruleus;
Tyrosine hydroxylase

Abstract
Depression can influence pain and vice versa, yet the biological mechanisms underlying how one influences the pathophysiology of the other remains unclear. Dysregulation of locus coeruleus-noradrenergic transmission is implicated in both conditions, although it is not known whether this effect is exacerbated in cases of co-morbid depression and chronic pain. We studied locus coeruleus activity using immunofluorescence and electrophysiological approaches in rats subjected to unpredictable chronic mild stress (CMS, an experimental model of depression) and/or chronic constriction injury (CCI, a model of chronic neuropathic pain) for 2 weeks. CCI alone had no effect on any of the locus coeruleus parameters studied, while CMS led to a slight reduction in the electrophysiological activity of the locus coeruleus. Furthermore, CMS was associated with an increase in the number of tyrosine hydroxylase-positive cells in the locus coeruleus, although they were smaller in size. Interestingly, these effects of CMS were exacerbated when combined with CCI, even though no changes in the α2-adrenoreceptors or the noradrenaline transporter were observed in any group. Together, these findings suggest that CMS triggers several modifications in locus coeruleus-noradrenergic transmission that are exacerbated by co-morbid chronic pain.

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http://dx.doi.org/10.1016/j.euroneuro.2014.01.011

1. Introduction

Depression and chronic pain frequently co-exist, and they can mutually exacerbate one another, worsening the patient's prognosis and treatment response (Bair et al., 2004; Karp et al., 2005). It is unclear whether depression and chronic pain are causally related, or whether diagnosis with one condition implies a predisposition to the other. We previously investigated the complex relationship between these conditions in animal models of depression, pain, and co-morbid depression and pain (Bravo et al., 2012). In rats subjected to chronic mild stress (CMS), which induces depressive-like behavior, we observed minimal changes in sensorial pain perception. Moreover, depressive-like behavior in these animals was unaffected by chronic constriction injury (CCI), a commonly used model of neuropathic pain. However, we observed increased avoidance of painful stimuli in animals subjected to CMS. Moreover, we subsequently demonstrated that in rats with neuropathic pain, social stress exacerbates aversion to painful experiences through a locus coeruleus (LC)-related mechanism (Bravo et al., 2013). Moreover, LC impairment coincides with the onset of anxio-depressive-like symptoms in rats suffering chronic neuropathic pain (Alba-Delgado et al., 2013). Together, these findings point to a key role of the LC in the interaction between chronic pain and depression.

The LC is the main site of noradrenergic cell bodies in the CNS. Descending projections from the LC to the spinal cord block nociceptive inputs, while projections ascending to the forebrain regulate emotional responses (Haidkind et al., 2003). Although the involvement of the LC in both pain and depression has been well established, its role in comorbid pain and depression remains unclear. Accordingly, we have addressed this issue by analyzing LC activity in an animal model of co-morbid depression and chronic neuropathic pain.

2. Experimental procedures

2.1. Animals and experimental design

Experimental procedures were approved by the Committee for Animal Experimentation at the University of Cadiz in accordance with governmental guidelines and they complied with the International Association for the Study of Pain ethical guidelines (Zimmermann, 1983). After a period of habituation in standard conditions, male Sprague-Dawley rats (280–300 g) were subjected to chronic stress and/or neuropathic pain for 2 weeks (Figure 1A). Neuropathic pain was induced by chronic constriction injury (CCI) of the common left sciatic nerve (Figure 1B: (Bennett and Xie, 1988; Berrocoso and Mico, 2007). In sham-operated rats, an identical dissection was performed but the sciatic nerve was not ligated. To induce a depressive-like state, animals were individually and continuously subjected to CMS (Figure 1C) in sessions lasting 10–14 h (day and night). Control animals were not subjected to any stress.

2.2. Behavioral tests

At the end of the habituation period and throughout the experimental phase, sensory pain was evaluated in all the experimental groups. Mechanical allodynia was measured using the von Frey test (Ugo Basile, Italy) by applying a vertical force to the paw, with a cut-off at 50 g (Berrocoso et al., 2011). The latency of paw withdrawal was recorded. Cold allodynia was measured using the acetone test, whereby a 100 μl drop of acetone was applied to the center of the hindpaw with a pipette, and the response was graded to a four-point scale: 0, no response; 1, paw withdrawal; 2, repeated paw flicking; 3, paw licking. The mean score value was calculated according to: (summation score value of each trials/number of total trials) (Bravo et al., 2012).

Behavioral despair was evaluated in one set of animals at the end of the experimental period using the modified forced swimming test (mFST). Rats were placed for 15 min in Plexiglas cylinders filled with 30 cm of water (pre-test session), and again for 5 min 24 h later (test session). The animal's behavior (immobility, swimming or climbing) was assessed throughout the test session (Bravo et al., 2012; Detke et al., 1995). Administration of the noradrenaline reuptake inhibitor, desipramine (20 mg/kg, i.p.; Sigma-Aldrich, USA), was used as a positive control in a parallel group and it was administered 23.5, 5 and 1 h before testing.

2.3. Electrophysiology

Single-unit extracellular recordings of LC neurons were obtained as described in Supplementary material (Alba-Delgado et al., 2012; Berrocoso and Mico, 2007).

2.4. Western blot

Rats were sacrificed by administering an overdose of chloral hydrate, and the LC was removed bilaterally to assess the presence of the noradrenaline transporter (NAT) (Supplementary material). Protein expression was detected using a LI-COR Odyssey® two-channel quantitative fluorescence imaging system (Bonsai Advanced Techonologies, Spain) and the digital images were analyzed by densitometry using ImageJ software (National Institutes of Health, USA).

2.5. Immunohistochemistry

Tyrosine hydroxylase (TH) immunohistochemistry of LC sections was performed as described in Supplementary material. The fluorescent signal was visualized on an Olympus BX60 microscope equipped with a U-MNU filter system and coupled to Olympus DP71 camera (Olympus, USA). TH-immunoreactive (TH-IR) cells were counted manually in an average of 6 LC sections per animal (n=4–5 rats per group). To detect changes in soma size, the mean soma area per group was calculated in 7–9 randomly selected TH-IR cells per section. Each TH-IR soma was outlined manually using a computer mouse and the cross-sectional area was calculated with ImageJ software.

2.6. Statistics

The data are represented as the mean ± SEM. The results were analyzed using either an unpaired Student t test or two- or three-way analysis of variance (ANOVA), with or without repeated measures, as appropriate. All post-hoc analyses were carried out using a Bonferroni post-hoc test. Burst incidence was analyzed using Fisher's exact test. The differences were considered significant at $p<0.05$.

3. Results

We first performed a behavioral analyses of each experimental groups and in agreement with our previous findings (Bravo et al., 2012) no differences in mechanical or cold allodynia in the injured hind-paw were observed between the two groups subjected to neuropathic pain (CCI-control and CCI-CMS; Figure 1D and F). In both CMS groups (sham-CMS and CCI-CMS), we detected cold but not mechanical