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Enhanced serotonin and mesolimbic dopamine transmissions in a rat model of neuropathic pain

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ABSTRACT

In humans, affective consequences of neuropathic pain, ranging from depression to anxiety and anhedonia, severely impair quality of life and are a major disease burden, often requiring specific medications. Depressive- and anxiety-like behaviors have also been observed in animal models of peripheral nerve injury. Dysfunctions in central nervous system monoamine transmission have been hypothesized to underlie depressive and anxiety disorders in neuropathic pain. To assess whether these neurons display early changes in their activity that in the long-term might lead to chronicization, maladaptive plasticity and affective consequences, we carried out *in vivo* extracellular single unit recordings from serotonin neurons in the dorsal raphe nucleus (DRN) and from dopamine neurons in ventral tegmental area (VTA) in the spared nerve injury (SNI) model of neuropathic pain in rats. Extracellular dopamine levels and the expression of dopamine D1, D2 receptors and tyrosine hydroxylase (TH) were measured in the nucleus accumbens. We report that, two weeks following peripheral nerve injury, discharge rate of serotonin DRN neurons and burst firing of VTA dopamine cells are enhanced, when compared with sham-operated animals. We also observed higher extracellular dopamine levels and reduced expression of D2, but not D1, receptors and TH in the nucleus accumbens.

Our study confirms that peripheral neuropathy induces changes in the serotonin and dopamine systems that might be the early result of chronic maladaptation to persistent pain. The allostatic activation of these neural systems, which mirrors that already described as a consequence of stress, might lead to depression and anxiety previously observed in neuropathic animals but also an attempt to cope positively with the negative experience.

1. Introduction

Neuropathic pain is caused by a lesion or damage to peripheral or central nervous system, and is characterized by hyperactivity of nociceptive afferents unrelated to the application of a stimulus, resulting in the clinical manifestations of allodynia and hyperalgesia (Baron, 2006; Treede et al., 2008). Moreover, increased peripheral sensory nerve activity induces multiple transsynaptic modifications that extend to the central nervous system (Baliki et al., 2006; Baron, 2006; Woolf, 2004; Yalcin et al., 2014, 2011). Indeed, chronic maladaptive plasticity underlies emotional, affective and cognitive symptoms of neuropathic pain syndrome, i.e. anxiety, anhedonia and depression (Baliki et al., 2006;

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Neugebauer et al., 2009; Oluigbo et al., 2012; Wang et al., 2011; Yalcin et al., 2014).

Molecular and functional studies on humans and animal models have shown that several brain areas, such as the periaqueductal grey (Baliki et al., 2014; Palazzo et al., 2012), the anterior cingulate cortex (Barthas et al., 2015; Rainville et al., 1997), the prefrontal cortex (Metz et al., 2009; Zhang et al., 2004) and the thalamus (Baliki et al., 2014; Henderson et al., 2013) are involved in pain processing.

Recent progress has also been made to unveil neural activity changes in response to acute noxious stimuli in brainstem areas, such as dorsal raphe nucleus (DRN) (Schweimer and Ungless, 2010), ventral tegmental area (VTA) (Brischoux et al., 2009; Ungless et al., 2004) and rostromedial tegmental nucleus (RMTg) (Jhou et al., 2009a; Lecca et al., 2011), as well as limbic structures (Hess et al., 2011; Nakamura et al., 2010; Onozawa et al., 2011). Moreover, DRN (Palazzo et al., 2006), hippocampus (Mutso et al., 2012; Ren et al., 2011b), amygdala (Neugebauer et al., 2004; Ren et al., 2011a) and ventral striatum (Austin et al., 2010; Baliki et al., 2014; Goffer et al., 2013) have been shown to be involved in neuropathic pain, as a possible substrate for the emotional-affective component of chronic pain syndromes.

Given the complex experience of pain and frequent inefficacy of treatments, neuropathic pain is considered a major clinical problem (Oluigbo et al., 2012). Despite efforts, the identification of the multiple neurobiological mechanisms responsible for diverse facets of neuropathic pain is still a challenge, representing a goal toward a rational approach for new therapies.

Elucidating the role of neural circuits for mood and motivation, such as DRN serotonin (5-HT) and VTA dopamine (DA) will help developing targeted therapies against the negative affective states associated with chronic pain. In fact, early changes in neuronal activity might be responsible for the initiation of phenomena of long-term plasticity that might lead to pain chronicization and induce maladaptive consequences responsible for affective disorders. To address this issue, we took advantage of *in vivo* extracellular single unit recordings to study whether and how DRN, VTA and RMTg neurons activity is modulated in the rat spared nerve injury (SNI) model of neuropathic pain. We further studied DA transmission by measuring extracellular DA levels, the expression of tyrosine hydroxylase (TH), the rate-limiting enzyme in DA synthesis, and DA D1 and D2 receptors in the nucleus accumbens (NAc) shell.

2. Materials and methods

2.1. Animals

All procedures were performed in accordance with the EEC Council Directive (219/1990 and 220/1990). Experimental procedures were approved by the Animal Ethics Committees of the University of Cagliari and Second University of Naples and by the Italian Ministry of Health (protocol n. 99-2014-B). Male Sprague Dawley rats (Harlan, Italy) weighing 250–400 g were used. We made all efforts to minimize pain and suffering, and to reduce the number of animals used. Animals were housed in groups of three to six per cage in standard conditions of temperature (21 ± 1 °C) and humidity (60%) on a 12 h light/dark cycle with food and water available *ad libitum*.

2.2. Spared nerve injury (SNI)

Neuropathic pain was induced in adult rats following the SNI procedures described by Decosterd and Woolf (2000). Under isoflurane anaesthesia (4% induction; 1.5% maintenance), the left hind leg sciatic nerve was exposed at the level of trifurcation into the sural, tibial, and common peroneal nerves. The tibial and common peroneal nerves were tightly ligated with 5.0 silk and severed, leaving the sural nerve intact. Control rats underwent a sham surgery with the left sciatic nerve exposed without further manipulation. After recovery, rats were housed separately (naïve, sham and SNI) in groups of 3–5 individuals.

2.3. Measurement of mechanical allodynia

Before and after surgery, tactile sensitivity of the operated hind paws was assessed from the withdrawal responses to mechanical stimulation with von Frey filaments. Rats were placed on a wire grid floor and allowed for habituation to the environment for 5 min. Filaments of varying forces were applied for 3–5 s to the plantar surface of the hind paw. Quick withdrawal or licking of the paw during the application of the stimulus was considered a positive response, and response threshold (in grams) was calculated according to Chaplan et al. (1994).

2.4. In vivo electrophysiology

On day 7 or 14 following the surgery, rats were anaesthetized with urethane (1.3 g/kg, i.p.) and placed in the stereotaxic apparatus (Kopf, Tujunga, CA, USA) with their body temperature maintained at 37 ± 1 °C by a heating pad. Thereafter, the scalp was retracted, and one burr hole was drilled above the area selected for the experiments, contralateral to the operated hind paw, according to the stereotaxic rat brain atlas of Paxinos and Watson (2007). Single unit activity of neurons was extracellularly recorded with glass micropipettes filled with 2% Pontamine sky blue dissolved in 0.5 M sodium acetate.

Individual action potentials were amplified (Neurolog System, Digitimer, Hertfordshire, UK) and displayed on a digital storage oscilloscope (TDS 3012, Tektronics, Marlow, UK). Experiments were sampled on line and off line with Spike2 software (Cambridge Electronic Design, Cambridge, UK) by a computer connected to CED 1401 interface (Cambridge Electronic Design, Cambridge, UK).

To estimate the cell population spontaneous activity, the electrode was passed within each area in 6–9 predetermined tracks separated by 200 μm and the total number of active cells encountered in each area was divided by the number of tracks. Paw pinch was delivered by a hand-driven forceps exerting a force comprised between 80 and 100 g/mm^2

At the end of recording sessions, DC current (15 mA for 5 min) was passed through the recording micropipette in order to eject Pontamine sky blue for marking the recording site. Brains were then rapidly removed and were frozen in isopentane cooled to −40 °C. The position of the electrodes was microscopically identified on serial 60 μm sections stained with Neutral Red.

2.4.1. Experiments in the DRN

Within the DRN (AP, -7.5 mm from bregma; LM, 0.0–0.1 mm from midline; V, 5.0–6.0 mm to cortical surface) putative 5-HT neurons were isolated (bandpass filter 0.1–10.000 Hz) and identified according to previously published criteria (Sawyer et al., 1985; Schweimer and Ungless, 2010): a 2–5 ms broad action potential with positive–negative or positive–negative–positive deflections, and a spontaneous either regular or irregular firing pattern (0.1–9.0 Hz). Firing regularity was assessed by analyzing the coefficient of variation (CV) and confirmed by qualitative inspection of the autocorrelogramms.

2.4.2. Experiments in the VTA

Within the VTA (AP, −6.0 mm from bregma; L, 0.4–0.6 mm from midline; V, 7.0–8.0 mm from cortical surface), single putative DA