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Brain Stimulation Differentially Modulates Nociception and Inflammation in Aversive and Non-aversive Behavioral Conditions

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Abstract—Inflammation and pain are major clinical burdens contributing to multiple disorders and limiting the quality of life of patients. We previously reported that brain electrical stimulation can attenuate joint inflammation in experimental arthritis. Here, we report that non-aversive electrical stimulation of the locus coeruleus (LC), the paraventricular hypothalamic nucleus (PVN) or the ventrolateral column of the periaqueductal gray matter (vlPAG) decreases thermal pain sensitivity, knee inflammation and synovial neutrophilic infiltration in rats with intra-articular zymosan. We also analyzed the modulation of pain and inflammation during aversive neuronal stimulation, which produces defensive behavioral responses such as freezing immobility to avoid predator detection. Electrical stimulation with higher intensity to induce freezing immobility in rats further reduces pain but not inflammation. However, tonic immobility further reduces pain, knee inflammation and synovial neutrophilic infiltration in guinea pigs. The duration of the tonic immobility increases the control of pain and inflammation. These results reveal survival behavioral and neuromodulatory mechanisms conserved in different species to control pain and inflammation in aversive life-threatening conditions. Our results also suggest that activation of the LC, PVN, or vlPAG by non-invasive methods, such as physical exercise, meditation, psychological interventions or placebo treatments may reduce pain and joint inflammation in arthritis without inducing motor or behavioral alterations.

Key words: inflammation, pain, behavior, neuroimmunology, brain, stimulation.

INTRODUCTION

Chronic pain is one of the most common disabling factors contributing to cognitive impairments, morbidity, and mortality in multiple clinical disorders including arthritis (Hewlett et al., 2011; Upchurch and Kay, 2012; Boyden et al., 2016; Castañeda et al., 2016). The best current treatments for arthritis are based on disease-modifying anti-rheumatic drugs (DMARDs) that neutralize inflammatory cytokines such as TNF and thereby reduce inflammation and leucocytes activation (Ramiro et al., 2011; Upchurch and Kay, 2012). However, these treatments are very expensive and can induce severe side effects increasing the risk of infections and immunosuppression (Inanc and Direskeneli, 2006; Favalli et al., 2009; Ramiro et al., 2011; Inui and Koike, 2016).

Recent studies on alternative therapies to control inflammation showed a bidirectional interaction between nervous and the immune systems (Olofsson et al., 2012; Torres-Rosas et al., 2014; Bassi et al., 2015, 2017; Ulloa et al., 2017). Electrical nerve stimulation can represent a promising strategy to control inflammation without the effects of current pharmacological treatments (Olofsson et al., 2012; Bassi et al., 2015, 2017; Ulloa and Deitch, 2009). Clinical and experimental studies indicate that peripheral or central neural stimulation attenuates joint inflammation (Miao et al., 2003; Kox et al., 2014;

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Abbreviations: LC, locus coeruleus; EPM, elevated-plus maze; ES, electrical stimulation; IA, inter-aural; i.a, intra-articular; PVN, paraventricular hypothalamic nucleus; TI, tonic immobility; vlPAG, ventrolateral periaqueductal gray matter; VNS, vagus nerve stimulation.

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Bassi et al., 2015, 2017; Koopman et al., 2016) and pain sensitivity (Mayer and Liebeskind, 1974; Basbaum and Fields, 1984; Ren et al., 1988; de Luca et al., 2003; Busch et al., 2013) in diverse models of experimental arthritis. We recently reported that electrical stimulation of brain structures, including the locus coeruleus (LC) or the paraventricular nucleus (PVN), decreases joint inflammation without affecting rat behavior (Bassi et al., 2017). Other studies reported that stimulation of these brain areas can decrease nociception and pain sensitivity (Panksepp, 1971; Fuchs et al., 1985; West et al., 1993; Hickey et al., 2014), in aversive defensive responses such as that displayed by prey during a predator attack (Gallup, 1977; Yardley and Hilton, 1986; Coimbra et al., 2017). However, it is unknown whether the potential to control both pain and inflammation is due to different neuronal stimulation or different networks activate by defensive mechanisms in aversive conditions. Here, we analyzed whether neural stimulation of central areas regulating pain and behavior can modulate nociception and inflammation in conscious, non-anesthetized animals both in aversive and non-aversive conditions.

EXPERIMENTAL PROCEDURES

Animal experiments

Eighty eight male Wistar *Rattus norvegicus* (Rodentia, Muridae) (250–300 g) and 22 *Cavia porcellus* (Rodentia, Caviidae) (400–500 g) were obtained from the main Animal Facility of the Ribeirão Preto Medical School of the University of São Paulo, and housed at the animal facility in plastic cages (four in a cage) under a 12-h light/dark cycle (lights on at 7am) at 20 °C ± 1 °C. The animals had unrestricted access to food and tap water. The number of animals used was the minimum required to ensure reliability of the results, and every effort was made to minimize animal discomfort. All animals were anesthetized with a mixture of ketamine and xylazine (50 mg/kg and 10 mg/kg, respectively) administered into the right posterior calf muscle through a 30-G needle. The experimental protocols comply with the recommendations of the SBNeC (Brazilian Society of Neuroscience and Behaviour), the Ethical Principles of the National Council for Animal Experimentation Control (CONCEA) (Protocol 137/2013), the US National Institutes of Health Guide for The Care and Use of Laboratory Animals, and the Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals (Zimmerman, 1983).

Stereotaxic surgery

Anesthetized rats were placed in a stereotaxic frame (David Kopf, Tujunga, CA, USA) and underwent surgical implant of stainless steel bipolar electrodes or a guide cannula using coordinates extracted from Rat Brain in Stereotaxic Coordinates Atlas (Paxinos and Watson, 2007). The interaural line served as the reference for each plane and the upper incisor bar was set at 2.5 mm below the interaural line, so the skull was horizontal between the bregma and lambda. LC: anteroposterior = −1.04 mm, mediolateral = 0.9 mm, dorsoventral = 7.9 mm; PVN: anteroposterior = 7.1 mm, mediolateral = 0.2 mm, dorsoventral = 8.0 mm; ventrolateral PAG column (vlPAG): anteroposterior = −0.2 mm, mediolateral = 1.4 mm, dorsoventral = 4.2 mm. After surgery, electrodes or guide-cannulas were fixed to the skull with acrylic resin and two stainless steel screws. Then, all animals received an intramuscular injection (0.2 mL) of antibiotic Pentabiótico (0.5 mL/kg, Fort Dodge, Campinas, SP, Brazil), and analgesic flunixin meglumine (Banamine, 2.5 mg/kg, Schering-Plough, Cotia, SP, Brazil).

Brain nuclei stimulation in non-anesthetized rats

Seven to ten days after the stereotaxic surgery, the animals were individually placed in a circular arena (60 cm in diameter and 50 cm high) and the stimulation cable was connected to the bipolar electrode. Rats were allowed a 10-min period of free exploration of the experimental environment. Afterward, the LC, PVN or vlPAG was electrically stimulated for 2 min with a square wave stimulator (1M1C, AVS Project, São Carlos, SP, Brazil) as previously reported: LC: 20 Hz, 1 ms, 100 μA (Kannan et al., 1986); PVN: 20 Hz, 0.5 ms, 50 μA (Jones and Gebhart, 1989); vlPAG: 20 Hz, 1 ms, 50 μA (Fardin et al., 1984). Animals showed no alertness, freezing, escape behaviors, or seizure during these stimulations. For the tail-flick tests, we first determined the thermal pain threshold baseli.ne The animals were then stimulated and underwent the tail-flick. Twenty-four hours later, rats were subjected to the same stimulation and then to the elevated plus-maze test (Fig. 1J). The animals were re-stimulated for the immunological experiments at 24 h after the behavioral experiment (Fig. 1J). Sham-stimulated animals (control) did not receive electrical stimulation.

We also analyzed whether aversive brain stimulation in non-anesthetized rats induces freezing immobility and control inflammation. Freezing behavior, also called "attentive immobility", is a common adaptive defensive behavior characterized by physical immobility followed by neurovegetative responses to avoid predator detection (Gallup, 1977; Marks, 1987; Roelofs, 2017). To induce freezing immobility, rats were placed in the circular arena (60 cm in diameter and 50 cm high) and after 10-min of free exploration, the PVN, LC, or vlPAG was electrically stimulated for 2-min intervals with the electrical intensity beginning at 50 μA and increasing it at intervals of 10 μA to induce freezing immobility. Freezing behavior was defined as the production of physical immobility except for the respiration movements accompanied by at least two of the following responses: arching back, piloerection, defecation, micturition, exophthalmia and ear retraction during the period of brain stimulation as previously reported (Gallup, 1977). Then, the electrical stimulation was immediately stopped and the animals underwent the tail-flick nociceptive test (Fig. 6B). On the next day, animals were subjected to the same electrical stimulation followed by intra-articular injection of zymosan injection under anesthesia. Sham-stimulated animals (control) did not receive electrical stimulation.

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