Behavioural Brain Research 321 (2017) 193–200

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Deep brain stimulation of the dorsal raphe inhibits avoidance and escape reactions and activates forebrain regions related to the modulation of anxiety/panic

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HIGHLIGHTS

- DBS applied to the DRD is anxiolytic.
- DRB applied to the lwDR is panicolytic.
- DBS applied to the DRD increases Fos-ir in forebrain anxiety-related regions.

ARTICLE INFO

Article history:
Received 23 September 2016
Received in revised form
11 November 2016
Accepted 17 November 2016
Available online 27 December 2016

Keywords:
Dorsal raphe subnuclei
Deep brain stimulation
Anxiety
Elevated T-maze
Fos immunohistochemistry

ABSTRACT

One of the main neurochemical systems associated with anxiety/panic is the serotonergic system originating from the dorsal raphe nucleus (DR). Previous evidence suggests that the DR is composed of distinct subpopulations of neurons, both morphologically and functionally distinct. It seems that mainly the dorsal region of the DR (DRD) regulates anxiety-related reactions, while lateral wings DR (lwDR) serotonin (5-HT) neurons inhibit panic-related responses. In this study we used the technique of deep brain stimulation (DBS) to investigate the role played by the DRD and lwDR in defense. Male Wistar rats were submitted to high-frequency stimulation (100 μA, 100 Hz) in one of the two DR regions for 1 h and immediately after tested in the avoidance or escape tasks of the elevated T-maze (ETM). In clinical terms, these responses have been related to generalized anxiety and panic disorder, respectively. After being submitted to the ETM, animals were placed in an open field for locomotor activity assessment. An additional group of rats was submitted to DBS of the DRD or the lwDR and used for quantification of c-Fos immunoreactive (Fos-ir) neurons in brain regions related to the modulation of defense. Results showed that stimulation of the DRD decreased avoidance latencies, an anxiolytic-like effect. DRD stimulation also led to increases in Fos-ir in the medial amygdala, lateral septum and cingulate cortex. DBS applied to the lwDR increased escape latencies, a panicolytic-like effect. This data highlights the importance of raphe topography and the potential benefit of the DBS technique for the treatment of anxiety-related disorders.

1. Introduction

Anxiety disorders are comprised of distinct subtypes of pathological conditions, with differences in their symptoms and their response to treatment [1]. Antidepressants are first option pharmacological compounds used in clinical settings to alleviate the symptoms of anxiety-related disorders [2]. These drugs act through the facilitation of monoamine neurotransmission, in particular of serotonin (5-HT) [2–6].

Approximately 80% of the serotonergic projections to forebrain regions arise from the dorsal raphe nucleus (DR) [7], located in the brain stem. During the last years, however, a growing body of evidence suggests that this nucleus is not homogeneous, but composed of subpopulations of serotonergic and non-serotonergic neurons, both morphologically and neurochemically distinct [7–11]. Evidence also suggests that the DR subnuclei differ in functional terms, in particular regard-

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http://dx.doi.org/10.1016/j.bbr.2016.11.054
0166-4328/

ing stress/anxiety modulation. It has been shown, for instance, that anxiety-provoking stimuli/situations, such as social defeat and anxiogenic drugs/neuropeptides, increase Fos and tryptophan hydroxylase immunoreactivity, mainly in the mid-rostral and caudal regions of the dorsal DR (DRD) [12,13]. On the other hand, exposure of rats to an elevated concentration of carbon dioxide, which precipitates panic attacks in panic disorder patients [14], induces Fos immunoreactivity in neurons located in particular within the lateral wings of the DR (lwDR) [15]. These physiological differences are most likely related to the distinct intrinsic excitability to stressors presented by the two subnuclei [16,17].

Deep Brain Stimulation (DBS) is a surgical technique that has been increasingly used during the last decades [18]. It consists of the administration of high-frequency electrical stimuli to specific brain regions, leading to a process that has been called depolarization block. This process is characterized by a state in which cells undergo depolarization with an almost complete abolishment of spontaneous action potentials [19,20]. It has also been postulated that GABA release from presynaptic terminals or from local interneurons may contribute to functional target inactivation [21]. Additionally, a third mechanism, which might explain the effects of DBS, is the activation of fibers of passage in the vicinity of the electrodes [22].

Although DBS has been successfully used for the treatment in particular of movement disorders [18], some evidence suggests that it may be useful for the treatment of other types of brain disorders, including psychiatric conditions such as depression and anxiety [20,23]. With respect to this last remark, previous studies have shown that DBS applied to the lateral amygdala induces anxiolytic-like effects in the probe burying test and elevated plus-maze [24]. Also, DBS applied to the ventral striatum, dorsal to the anterior commissure, reduces the number of freezing events in a fear-conditioning paradigm [25]. Similar results have also been found in experiments with DBS applied to the hippocampus or to the pre-frontal cortex [26–29]. Furthermore, a clinical review aimed to summarize published evidence on the outcomes of anxiety and depressive symptoms in Parkinson's disease patients following DBS applied to the subthalamic nucleus showed that both seem to improve in the short-term, although the results may vary depending on the protocol adopted by the study [30]. Regardless of this positive evidence and the fact that 5-HT seems to be a critical neurotransmitter involved in the modulation of anxiety, to our knowledge no previous study with DBS has focused on the raphe nuclei.

Taking the above observations into account, the purpose of the present study was to investigate the effects of DBS applied to the DRD and lwDR of male Wistar rats in the avoidance and escape tasks of the elevated T-maze (experiment 1). These responses have been related to different anxiety subtypes found in clinical settings, generalized anxiety and panic disorder, respectively [31–34]. All animals were tested in an open field after the ETM for locomotor activity assessment. To better understand the mechanisms of action of the DBS technique in the DR, an additional group of animals was used for the quantification of c-Fos immunoreactive (Fos-ir) neurons in brain regions related to the modulation of defense after being submitted to DBS of the DRD and lwDR (experiment 2).

2. Materials and methods

2.1. Subjects

Seventy-six Male Wistar rats (CEDEME, Universidade Federal de São Paulo, Campus Santos, Brazil), weighing 280–320 g at the beginning of the experiment, were used. Sixteen animals were discarded due to electrode misplacement. Animals were housed in groups of 5–6 per cage until surgery. After surgery, they were housed in pairs in Plexiglas-walled cages until testing. Room temperature was controlled ($22 \pm 1\,^{\circ}C$) and a light-dark cycle was maintained on a 12-h on-off cycle (0700–1900 h lights on). Food and water were available throughout the experiments. The study was approved by the Ethical Committee for Animal Research of the Federal University of São Paulo (number 8474291013) and was performed in compliance with the recommendations of the Brazilian Society of Neuroscience and Behavior, which are based on the conditions stated in the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 1996).

2.2. Surgery for electrode placement

One week after their arrival in the laboratory, animals were submitted to a surgical procedure for the implantation of the electrode (Thomas Recording, Germany). The electrodes had the following specifications: Fiber material: quartz glass insulated platinum/tungsten fiber; Fiber outer diameter: 200 μm; tip shape: only ground; tip coating: Iridium-Oxide; Length: 7.5 mm.

Each animal was anaesthetized with an IP injection of ketamine hydrochloride (80 mg/kg; Agribrands, Brazil) and xylazine (10 mg/kg; Agribrands, Brazil) and fixed to a stereotaxic frame (David Kopf, USA). The level of anesthesia was monitored by frequently checking response to tail pinch, and ketamine + xylazine were supplemented as necessary to maintain the depth of anesthesia.

Electrodes were inserted into the brain through a hole drilled in the skull above the DR, following the coordinates from the atlas of Paxinos and Watson [35]: DRD: AP: 7.80 mm from Bregma; ML: 2.1 mm; DV: 6.7 mm; at an angle of 14°) lwDR: 7.80 mm from Bregma; ML: 1.8 mm; DV: 6.4 mm; at an angle of 17°). After implantation, they were attached to the skull by means of acrylic resin and two stainless steel screws.

To prevent infection, at the end of the surgery all animals were injected (IM) with a 0.2 ml of pentabiotic preparation (Pentabiotico Veterinário Pequeno Porte; Forte Dodge, Brazil). In addition, flunixin meglumine (Schering–Plough, Brazil; 3 mg/kg), a drug with analgesic, antipyretic and anti-inflammatory properties, was administered subcutaneously for post-surgery analgesia.

The animals were left undisturbed in their home cages for 7 days after the surgery, except for normal handling for cage cleaning, and monitoring of signs of postoperative pain or behavioral alterations.

2.3. Apparatus

The DBS procedure was carried out in a bowl shaped cage (Round bottom bowl, model MD1500—Bioanalytical Systems, USA; height = 35 cm, top and base diameter = 40 and 25 cm, respectively). Electrical stimuli were generated by a sine-wave stimulator (Multi-Channel System, Germany). The implanted electrode was connected to the stimulator by means of an electromechanical swivel and a flexible cable, allowing ample movement of the animal inside the experimental cage.

The elevated T-maze was made of wood and had three arms of equal dimensions (50 cm × 12 cm). One of the arms was enclosed by 40 cm high walls and was oriented perpendicularly to two opposed open arms. The whole apparatus was elevated 50 cm above the floor. To avoid falls, the open arms were surrounded by a 1-cm high Plexiglas rim.

An open field, composed of a round arena (60 × 60 cm), with the floor divided into 12 parts and walls 50 cm high, was used to evaluate locomotor activity.