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Changes on auditory physiology in response to the inactivation of amygdala nuclei in high anxiety rats expressing learned fear

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HIGHLIGHTS

- Humans exposed to fear stimuli have increased amplitude of the auditory-evoked potentials.
- Freezing is directly correlated to increases in the amplitude of auditory-evoked potentials.
- In anxious psychiatric patients bottom-up and top-down processes are conceivable to be impaired.
- The basolateral amygdala plays a role in the modulation of ascending auditory information.
- The central amygdala nuclei have no authority on the processing of auditory stimuli.

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ABSTRACT

The inferior colliculus (IC) is primarily involved in the processing of acoustic stimuli, including those emitted by prey and predators. The role of the central nucleus of the IC (CIC) in fear and anxiety has been suggested based on electrophysiological, behavioral and immunohistochemical studies. The reactivity of high-anxiety rats (HA) to diverse challenges is different from low-anxiety ones (LA). In humans and laboratory animals, pathological anxiety is often accompanied by heightened vigilance and alertness, hyperactivity of the amygdala (AM), and increased amplitude of the auditory evoked potentials (AEP) from the IC. This study aims to evaluate the influence of the inactivation of the central (CEA) and basolateral (BLA) nuclei of the amygdala, after local infusions of the full $GABA_A$ agonist muscimol (1 nmol/0.2 μl), on the AEP elicited in the CIC of rats tested under a learned fear state. Our results showed that both BLA and CEA inactivation change the expression of conditioned fear, in a paradigm using the context as the conditioned stimulus (CS). These changes are correlated to the innate anxiety levels of the animals. It is supposed that this shortcoming is in addition to the imbalance between the regulatory role of the top-down and bottom-up processes in the control of anxiety.

1. Introduction

The inferior colliculus (IC) lies on a crucial position in the primary auditory pathway [1]. The IC integrates input from brainstem nuclei, relaying information to the auditory thalamus and to nuclei at the sensorimotor interface, and creating selectiveness for various dimensions of relevant sounds [2]. This means that the IC modulates distinctly a broad range of affective auditory signals as, for example, the 22-kHz alarm calls [3]. The role of the central nucleus of the IC (CIC) in fear and anxiety has been suggested based on electrophysiological, behavioral and immunohistochemical studies. Electrical stimulation of the CIC induces defensive responses that mimic the fearful behavior elicited by environmental cues [4–6]. Moreover, rats are able to engage in tasks that decrease the aversiveness of CIC stimulation, exhibit increased CIC auditory-evoked potentials (AEP) in the presence of conditioned fear stimuli, and increased CIC Fos-immunolabeling when exposed to diverse emotional stressors [5–12].

Anxiety can be classified as a state (a "normal" pattern of response elicited in response to anxiety-provoking stimuli) or trait anxiety (a pathological condition in which the individual presents an innate predisposition to respond to innocuous stimuli or anxiety-evoking situations) [13–15]. In rodents, it was shown that the reactivity of high-anxious rats (HA) is different from low-anxious ones (LA) [16–18]. This variation could be due to innate physiological differences these

Abbreviations: LA, low-anxiety; HA, high-anxiety; IC, inferior colliculus; CIC, central nucleus of the inferior colliculus; AEP, auditory evoked potentials; GABA, γ-aminobutyric acid-A; EPM, elevated plus maze; BLA, basolateral amygdala complex; CEA, central nucleus of the amygdala.

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groups of animals exhibit in a series of brain regions that naturally modulates the expression of anxiety and fear-related behaviors as, for example, the amygdala. The amygdala is activated whenever the subject is faced with unconditioned and conditioned anxiety or fear-provoking stimuli, and this is not only in patients with anxiety disorders, but also in normal subjects [19]. In this context, it is well established that the "malfunctioning" of amygdala has been related to the generalized anxiety disorder [20,21].

Pathological fear and anxiety states are often accompanied by overt heightened vigilance and alertness [10], hyperactivity of the amygdala (AM) and the medial prefrontal cortex (mPFC) [22,23], and larger evoked potentials from the IC [10,24].

Taking into account the information above, the present study is a further attempt to looking at the amygdala as a probable regulator of the auditory evoked potentials (AEP) generated at the IC, the physiological component of the learned fear response.

Thus, this study aims to evaluate the influence of the central (CEA) and basolateral (BLA) nuclei of the amygdala on the AEP elicited in the CIC of rats tested under a conditioned fear-eliciting paradigm. BLA and CEA inactivation was accomplished through the local infusion of the full $GABA_A$ agonist muscimol (1 nmol/0.2 μl). Conditioned aversive stimuli were provided by a contextual fear-conditioning paradigm in which foot-shocks were used as unconditioned stimuli (US). Based on our previous assumptions, my hypothesis is that the chemical inactivation of BLA and CEA would change the physiological and behavioral components of conditioned fear, as revealed by recording the AEP and freezing behavior, respectively. It is supposed that this change could be dependent upon the levels of anxiety the animals present. In this context, in a previous study [31] it was showed that AEP magnitudes significantly correlated with the time spent in the open arms by HA and HA rats subjected to the EPM.

2. Materials and methods

2.1. Animals

Male Wistar rats (campus of Ribeirão Preto, University of São Paulo) weighing 250 ± 20 g at surgery were used in these experiments. The animals were given three days to habituate to the housing conditions in the Laboratory of Neuropsychopharmacology. They were maintained on a 12 h light/dark cycle. Food and water were available ad libitum. The experiments were performed in compliance with the recommendations of the Brazilian Society for Neuroscience and Behavior, which are in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. The number of animals used was the minimum required to ensure reliability of the results. Foot-shocks were applied in a current intensity of 0.4 mA, enough to be stressful but not to cause pain, as an effort to minimize the animal suffering.

2.2. Selection of low- (LA) and high- (HA) anxiety animals

The rats were separated as LA or HA according to their propensity to display high or low avoidance of the open arms in the EPM [15,25–27]. The EPM was constructed from dark plywood and had two open arms (50 × 10 cm), perpendicular to two closed arms of equal dimensions and surrounded by 40 cm high walls. The apparatus was elevated 50 cm from the floor [15,25–29]. A 1 cm wooden rim surrounded the open arms to prevent falls from the maze. The apparatus was located inside a room with constant background noise (50 dB). Behavior in the EPM was recorded by a video camera (Everfocus, Duarte, CA, USA) linked to a monitor. This device, located outside the experimental room, allowed the recordings to be analyzed later. Luminosity at the level of the open arms of the maze was 60 lx. Experimental sessions were conducted between 10:00 h and 18:00 h. Rats were placed individually in the center of the maze facing a closed arm and allowed 5 min of free exploration of the maze. An observer trained to measure conventional EPM parameters subsequently scored the videotapes. The behavioral categories were scored using Noldus software (Amsterdam, The Netherlands), which allowed the measurements of the number of entries into, and time spent onto both the open and closed arms of the maze. An arm entry or exit was defined as all four paws entering or exiting an arm, respectively. These data were used to calculate the percentage of open arm entries and percentage of time spent in the open arms. Each animal was tested once, and the measure of open arm time was used to assign animals to the HA and LA groups. The animals were ranked by their time spent on the open arms of the EPM in such a way that animals belonging to the 25% of the extremities, above or below the medians, were selected as rats with either LA or HA levels, respectively. The 50% of the animals that reached the 25% immediately above or below the median were discarded to be used in other studies. The apparatus was cleaned with 20% ethanol and water before each test. After exposure to the EPM, the animals were allocated to one of the two groups (HA or LA) and maintained in this condition throughout the experiments.

2.3. Surgery

Twenty-four hours after the EPM experiments, the animals were anesthetized with a 0.1 ml ketamine hydrochloride + 0.1 ml xylazine mixture (90/10 mg/kg), and mounted in a digital stereotaxic frame (Insight, São Paulo, Brazil). In order to access the AEP a cannula made from a stainless steel needle (24 gauges, 14 mm length) was implanted into the central nucleus of the left IC; regarding this point, results obtained in a previous study from our laboratory pointed out for the absence of hemispheric differences on the auditory evoked potential elicited by auditory stimuli, no matter the side of the stimulation [9]. Additionally, the same animal received a second cannula; this time oriented to the CEA or BLA. The upper incisor bar was set 2.5 mm below the interaural line, such that the skull was horizontal between bregma and lambda. For the CIC, the cannula was introduced vertically using the following coordinates, with bregma serving as the reference for each plane: anterior/posterior: −8.5 mm; medial/lateral: 1.5 mm; and dorsal/ventral: −4.0 mm. For the cannula inserted into the amygdala the coordinates used were: CEA—anterior/posterior −2.28 mm, medial/lateral ±4.2 mm, dorsal/ventral −8.2 mm; and BLA—anterior/posterior −2.28 mm, medial/lateral ±5.00 mm, dorsal/ventral −8.6 mm [30]. Cannulae were fixed to the skull by acrylic resin and three stainless steel screws. At the end of surgery, each animal received an intramuscular injection of a veterinary pentabiotic (120,000 UI, 0.2 ml) followed by an injection of the anti-inflammatory and analgesic drug Banamine (flunixin meglumine, 2.5 mg/kg). Afterward, each guide cannula was sealed with a stainless steel wire to protect it from blockage.

2.4. Drugs

CEA and BLA inactivation was conducted after a 5 day-period of recovery from surgery. Drug used was the selective $GABA_A$ agonist muscimol (1 nmol/0.2 μl; Sigma, St. Louis, MO, USA) dissolved in PBS shortly before intra-CEA or intra-BLA microinjections. The vehicle was also used as a control solution. The wait time for test sessions after drug injection was 15 min. The dose of muscimol used was based on previous studies [9,12,31–33]. Each animal received only one injection and was tested once.

2.5. Microinjection procedure

The animals were gently wrapped in a cloth and hand-held. A thin dental needle (outside diameter, 0.3 mm) was introduced through the guide cannula until its lower end was 3 mm below its tip. The