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Research report

Effect of acute and subchronic stress on electrical activity of basolateral amygdala neurons in conditioned place preference paradigm: An electrophysiological study

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ABSTRACT

The basolateral amygdala (BLA) plays a critical role in the neural circuitry of stress and mediates the effects of stress on memory related processes. Moreover, this area has an important role in drug-seeking and relapse of approach behavior to drug-associated cues. Therefore, in the present study, we aimed to investigate the effects of acute and subchronic stress in saline- and/or morphine-treated rats in conditioned place preference paradigm on the neural activity in the BLA. Male Wistar rats were divided into two saline- and morphine-treated supergroups. Each supergroup contained control, acute stress (AS) and subchronic stress (SS) groups. In all of the groups, conditioned place preference paradigm was done and thereinafter the spontaneous firing activity was recorded by in vivo single unit recording for 20 min. Results showed that in saline-and/or morphine-treated animals, both AS and SS increased neural activity of projection neurons and this increase in morphine-treated animals was more considerable than that of saline-treated animals. Besides, firing rate of interneurons in both supergroups decreased during AS and SS. Decrease of interneurons activity after application of SS in morphine-treated animals was more than that of saline-treated animals. These finding revealed that both of AS and SS increased firing rate of projection neurons but decreased neural activity of interneurons in the BLA. However, effect of AS and SS on the firing rate of BLA neurons in morphine-treated animals was more remarkable than that of saline-treated animals.

1. Introduction

The basolateral amygdala (BLA) has been shown to play a pivotal role in the neural circuitry of stress and mediates the effects of stress on memory related processes [1,2]. This region has a critical role in fear, anxiety and activation of the hypothalamus-pituitary-adrenal axis which results in the release of glucocorticoid hormones (main hormones involved in stress) [3,4]. It was established that chronic stress elicits structural changes such as increased dendritic branching and increased number of spines in BLA projection neurons [5,6]. Besides, previous evidence revealed that glucocorticoids enhance the excitability of principal BLA cells [7] and systemic injections of glucocorticoids enhance baseline responses in BLA in a dose-dependent manner [2]. Moreover, Rosenkranz et al. clarified that chronic stress increased excitability of BLA projection neurons recorded in vivo [8] and Cui et al. suggested that exposure to a single prolonged stress paradigm followed by a 7 days recovery period identified increases in total dendritic length and branch points of BLA pyramidal neurons [1].

On the other hand, there are numerous studies indicating that the BLA mediates stimulus-reward learning that is required for a conditioned stimulus to gain incentive motivational and conditioned reinforcing value [9]. Electrophysiological studies have revealed that BLA neurons are responsive to reward-predictive cues [10]. Inactivation or lesions of the BLA impairs acquisition of cocaine-seeking behavior and conditioned place preference (CPP) in rats, demonstrating a critical role for the BLA during opiate-related learning and memory [9,11]. In addition, the BLA has a critical role in drug-seeking, relapse and also the extinction of approach behavior to drug-associated cues [9,12]. A substantial literature indicates that the role of amygdala in processing rewarding aspects of stimuli may occur via functional relations between the BLA and the mesoaccumbens dopamine system [12]. In the other words, mesolimbic dopamine pathway from the ventral tegmental area to the nucleus accumbens mediates the rewarding effects of opiates and the BLA is connected anatomically with this pathway [13,14]. The ventral tegmental area, nucleus accumbens, and BLA form a functionally interconnected network that is critical for processing the primary

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rewarding effects of opiates as well as opiate-related memory [11]. Indeed, the functional connectivity of the BLA-nucleus accumbens pathway is necessary for animals to respond on a second order conditioning task with a natural or drug reinforce [10]. Therefore, in this study, we tried to determine the effects of morphine-CPP or stress alone to alternate firing rate of BLA neurons. Then, we estimated the effect of simultaneous application of stress and morphine-CPP on these alterations. In other words, we aimed to compare changes in firing rate during simultaneous application of stress and morphine-CPP than application of morphine or stress alone.

2. Materials and methods

2.1. Animals

Male Wistar rats (Pasteur Institute, Tehran, Iran), weighting 230–290 g were used in this study. The animals were randomly kept in groups of three with free access to chow and tap water. The vivarium was maintained at 12:12 h light/dark cycle and controlled temperature (23 ± 1 °C). All procedures were performed according to the Guide for the care and use of laboratory animals (National Institutes of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

2.2. Behavioral test

2.2.1. Conditioning apparatus and paradigm

The rewarding properties of morphine were investigated using a CPP paradigm. Place conditioning boxes were made up of Plexiglas with two sides (30 cm × 30 cm × 40 cm) which were different in shading and texture. Compartment A was white with black horizontal stripes 2 cm wide on its walls and also had a net-like floor. Compartment B was black with vertical white stripes, 2 cm wide with a smooth floor. The third compartment, C, was a red tunnel (30 cm × 15 cm × 40 cm). It protruded from the rear of the two other large compartments and connected their entrances. In this apparatus, rats showed no consistent preference for either large compartments (A and B), which is in support of our unbiased CPP paradigm. It took place in the following manner.

2.2.1.1. Pre-conditioning phase. On day 1, each rat was placed separately into the apparatus for 10 min, with free access to all compartments. Time spent in each compartment and the rat's movements and time spent in each compartment were recorded (pre-test day). Animals were then randomly assigned to one of the two compartments for place conditioning.

2.2.1.2. Conditioning phase. This phase consisted of a 3-day schedule of conditioning sessions. The conditioning training was carried out twice a day each for 30 min with an interval of 6 h for saline- and morphine (5 mg/kg, morphine sulfate; Temad Co., Tehran, Iran) pairing in an alternated morning–afternoon design. In this phase, animals received three trials in which they experienced the effects of the drugs while confined to one compartment for 30 min, and three trials in which they experienced the effects of saline while confined to the other compartment by closing the removable gate.

2.2.1.3. Post-conditioning phase. This phase was carried out on day 5th (the preference test day). For testing, the removable wall was raised and rat could access the entire apparatus for a 10-min period. The mean time spent for each rat in both compartments was recorded by a 3CCD camera (Panasonic Inc., Japan) and analyzed using the Ethovision software (Version 7), a video tracking system for automation of behavioral experiments (Noldus Information Technology, the Netherlands). In order to calculate the conditioning score as the preference criteria, the time spent in the drug-paired place minus the time spent in saline-paired place was calculated [15].

2.2.2. Forced swim stress

The forced swim stress (FSS) was performed in a Plexiglas tank (50 cm height × 30 cm diameter) filled with 35 cm depth of water (24 ± 27 °C). Each rat was forced to swim individually for 6 min once a day. For induction of acute stress (AS), the animals received FSS for 6 min just one day but, in subchronic stress (SS) groups, the animals received FSS for 6 min once a day for 3 consecutive days.

2.3. Experimental procedures

Male Wistar rats were divided into two supergroups, saline- and morphine-treated animals. Each supergroup consisted of control, acute stress (AS) and subchronic stress (SS) groups. All of the groups passed CPP stages according to the CPP protocol (Electronic supplementary Fig. A). In the morphine-treated animals, the control group was treated with morphine (5 mg/kg) during the conditioning phase, while the AS group received morphine during the conditioning phase and also FSS on the test day (post-conditioning phase) just 10 min before behavioral test (Electronic supplementary Fig. B), and in the SS group, animals received FSS during the conditioning phase for 10 min before each morphine injection during the acquisition period (Electronic supplementary Fig. C). In the saline-treated animals, the control group received saline (1 ml/kg) instead of morphine (5 mg/kg) during the conditioning phase, the AS group received saline during the conditioning phase and FSS on the test day 10 min before the behavioral test. The SS group received FSS in a 3-days schedule during the conditioning phase, 10 min before saline injection once a day. Conditioning score was calculated for each rat on the test day.

For the measurement of neural firing rate in the BLA, on the fifth day after behavioral test (as mentioned above), the neural activity was measured by in vivo single unit recording for 20 min.

2.4. Electrical recording and data acquisition

Animals were deeply anesthetized with urethane (1.5 g/kg, intraperitoneally, with supplemental doses as required; Sigma–Aldrich, Germany). Then, tracheotomy was done in order to prevent suffocation and the animals were placed in a stereotaxic instrument (Stoelting; USA). The body temperature was preserved for the entire experiment using a heating pad (Int. Biomedical Inc., USA). Extracellular recording from individual neurons was obtained with tungsten microelectrode (shaft diameter 127 μm, tip exposure 1–3 μm, tip impedance 5 MΩ; Harvard Apparatus, Holliston, MA). Microelectrode was stereotaxically advanced into the BLA (AP = 2.8 mm, La t = ± 4.6 mm and DV = 8.7 mm) according to rat brain atlas [16]. Spike signals which were received from neurons by a preamplifier, were amplified by a differential amplifier (DAM-80, WPI, USA; ×10000 gain; 300 Hz and 10 kHz for low and high filters, respectively) and digitalized at 50 kHz sampling rate and 12-bit voltage resolution using a data acquisition system (D3109; WSI, Tehran, Iran). All-or-none spike events were detected using a window discriminator (W3205; WSI, Tehran, Iran) based on the spike amplitude. The spike frequencies were counted and displayed online in time bins of 1000 ms over the entire recording period by online-sorter software (Spike; ScienceBeam, Tehran, Iran). Data of the discriminated spikes as well as the whole data streams (including undetected spikes and background activities) of all recordings was saved on a computer device for later offline analyses. In these experiments, time setting for data collection was 1800sec with 1000 ms bin size as a file which was saved continuously during experiment in hard disk. Upon completion of the recording, a negative DC current of 50 μA was applied for 15 s through the recording electrode to electrically mark the recording site for later histological verifications.