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Cooperative interaction between the basolateral amygdala and ventral tegmental area modulates the consolidation of inhibitory avoidance memory

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ABSTRACT

The aim of the current study was to examine the existence of a cooperative interaction between the basolateral nucleus of amygdala (BLA) and the ventral tegmental area (VTA) in inhibitory avoidance task. The BLA and the VTA regions of adult male Wistar rats were simultaneously cannulated and memory consolidation was measured in a step-through type inhibitory avoidance apparatus. Post-training microinjection of muscimol, a potent GABA-A receptor agonist (0.01–0.02 μg/rat), into the VTA impaired memory in a dose-dependent manner. Post-training intra-BLA microinjection of NMDA (0.02–0.04 μg/rat), 5 min before the intra-VTA injection of muscimol (0.02 μg/rat), attenuated muscimol-induced memory impairment. Microinjection of a NMDA receptor antagonist, D-AP5 (0.02–0.06 μg/rat) into the BLA inhibited NMDA effect on the memory impairment induced by intra-VTA microinjection of muscimol. On the other hand, post-training intra-BLA microinjection of muscimol (0.02–0.04 μg/rat) dose-dependently decreased step-through latency, indicating an impairing effect on memory. This impairing effect was however significantly attenuated by intra-VTA microinjection of NMDA (0.01–0.03 μg/rat). Intra-VTA microinjection of D-AP5 (0.02–0.08 μg/rat), 5 min prior to NMDA injection, inhibited NMDA response on the impairing effect induced by intra-BLA microinjection of muscimol. It should be considered that post-training microinjection of the same doses of NMDA or D-AP5 into the BLA or the VTA alone had no effect on memory consolidation. The data suggest that the relationship between the BLA and the VTA in mediating memory consolidation in inhibitory avoidance learning may be dependent on a cooperative interaction between the glutamatergic and GABAergic systems via NMDA and GABA-A receptors.

1. Introduction

Inhibitory avoidance (IA) task is a common simple test which has been widely used in behavioral and pharmacological studies of learning and memory processes (Izquierdo et al., 2006). The findings of these studies have revealed that the basolateral nucleus of the amygdala (BLA) is highly involved in modulating memory for inhibitory avoidance training so that intra-BLA injections of different drugs and neurotransmitter agents impair or enhance memory in this task (for a review see McGaugh, 2004; McGaugh et al., 2002). However, it is established that the BLA does not work alone in the modulation of memory and perform its role via potent interactions with other cortical and limbic structures such as the hippocampus (McIntyre et al., 2003, 2005; Phelps, 2004; Roozendaal et al., 1999; Tsoory et al., 2008), medial septum (Spanis et al., 1999), entorhinal cortex (Roesler et al., 2002) and nucleus accumbens (Roozendaal et al., 2001) all of which are involved in memory. Another brain site that has recently been considered in the formation of avoidance memory is the ventral tegmental area (VTA). The VTA is a main source of dopamine in the brain and has mostly been considered as a reward structure (Wise, 2004). Mahmoodi et al. (2011) showed that inactivation of the VTA by lidocaine administration disrupts different stages of memory in inhibitory avoidance task. Moreover, the VTA is functionally connected to the hippocampus through the VTA–hippocampus loop (Lisman and Grace, 2005). Also, our laboratory has recently shown that the VTA can regulate hippocampal memory formation via the mechanism that is mediated by the interaction between the dopamine and γ-aminobutyric acid (GABA)-A receptors (Nazari-Serenjeh et al., 2011).

The BLA and the VTA have been suggested to be the major components of limbic circuit which have anatomical connection to each other. The BLA innervates the VTA via the indirect glutamatergic outputs while the VTA sends direct dopaminergic projections to the BLA (Haber and Fudge, 1997; Jackson and Moghaddam, 2001; Phillips et al., 2003). It seems that the involvement of these two

Abbreviations: ANOVA, analysis of variance; BLA, basolateral amygdala; D-AP5, D-(−)-2-amino-5-phosphonopentanoic acid; GABA, γ-aminobutyric acid; IA, inhibitory avoidance; LTP, long term potentiation; mPFC, medial prefrontal cortex; NMDA, N-methyl-D-aspartate; SEM, standard error of mean; VTA, ventral tegmental area.

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brain structures in the processing of learning functions at the same time may be due to the existence of neural circuits between them (Alvarez and Ruarte, 2004). Previous electrophysiological evidence has shown that the lesion of the VTA decreases BLA-dentate gyrus-induced LTP which indicates that the VTA function is critical for memory-modulatory effect of the BLA (Abe et al., 2009). Moreover, Packard and Cahill (2001) suggested that midbrain dopamine neurons may act as a pathway for amygdala modulation of memory. Glutamate is a major excitatory neurotransmitter in the central nervous system that has stimulatory effect (Riedel et al., 2003), while GABA, as an inhibitory neurotransmitter, has disturbing effect on memory formation (Myhrer, 2003). Both glutamate and GABA receptors are however involved in learning and memory processes which are mediated by the BLA and the VTA (Ahmadi et al., 2007; Ferreira et al., 2005; McGaugh et al., 1996). It is important to note that the BLA and the VTA receive important glutamatergic inputs (Geisler et al., 2007; Likhtik et al., 2005) and glutamate release may be important for the regulation of neuronal activity in these structures (Geisler and Wise, 2008; Grace and Rosenkranz, 2002). Considering that the glutamate N-methyl-D-aspartic acid (NMDA) receptors regulate the effect of spatial working memory induced by muscimol, a GABA-A receptor agonist, (Saito et al., 2010) and with regard to the existence of anatomical connections between the BLA and the VTA, the aim of the present study was to examine the existence of a possible cooperative interaction between the NMDA and GABA-A receptors of the BLA and the VTA in inhibitory avoidance learning.

2. Methods

2.1. Animals

Adult male Wistar rats (Pasteur Institute, Tehran, Iran) weighing 220–250 g at the time of surgery were used in this study. Animals were housed four per cage in a temperature-controlled environment (22 °C) under a 12-h light/12-h dark cycle (lights on at 07:00 h) and had free access to food and water. Experiments were carried out during the light portion of the cycle between 10:00 a.m. and 2:00 p.m. Each animal was used once only. All procedures for the treatment of animals were approved by the Research and Ethics Committee of the School of Biology, University of Tehran and were done in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publications no. 80-23). Moreover, all efforts were made to minimize the number of animals used and their suffering.

2.2. Surgical and infusion procedures

Rats were fully anesthetized intraperitonealy with a ketamine–xylazine mixture (100 mg/kg and 5 mg/kg, respectively). The skull was fixed in a stereotaxic frame and two steel guide cannulae (22 gauge) were implanted unilaterally in the right hemisphere, aimed at 1 mm above the VTA and BLA according to the atlas of Paxinos and Watson (2007). Coordinates for the VTA were anterocaudal: −5.8 mm; lateral: ±0.7 mm; and vertical: 8 mm and for the BLA were: anterocaudal: −2.8 mm; lateral: ±4.8 mm; and vertical: 8.5 mm. The cannulae were affixed to the skull with two jewelers screw and acrylic dental cement. To prevent clogging, stainless steel stylets (27 gauge) were placed in the guide cannulae until the animals were given the drug injection. The rats were allowed 1 week to recover from the surgical procedure.

For microinfusions, stylets were removed from the guide cannulae and replaced by 27-gauge injection needle (1 mm below the tip of the guide cannulae). The injection needle was attached with a polyethylene tube to a 2-μl Hamilton syringe. The VTA and the BLA region were injected with a 0.2 μl and 0.3 μl solution over a 60-s period respectively. To allow diffusion of the drug, the injection needles were retained within the cannulae for an additional 60 s after drug infusion and then the stylets were reinserted into the guide cannulae.

2.3. Drugs

The drugs used in the present study included muscimol (Tocris, UK), N-methyl-D-aspartate (NMDA) and D-AP5 [D-(−)-2-amino-5-phosphonopentanoic acid] (Tocris, Bristol, UK). All drugs were dissolved in sterile 0.9% saline. Control animals received saline.

2.4. Inhibitory avoidance apparatus

To test the memory consolidation, a two-compartment step-through inhibitory avoidance apparatus was used. Two compartments had the same size (20 cm×20 cm×30 cm) and were separated by a guillotine door (7 cm×9 cm) in the middle of the dividing wall. The walls and floor of one compartment consisted of white opaque resin (light compartment) and the walls of the other compartment were dark but its floor was made of stainless steel grids (2.5 mm in diameter, separated by a distance of 1 cm) and was connected to insulated stimulator so that an intermittent electric shock (50 Hz, 3 s, 1 mA) could be delivered when a rat entered the compartment.

2.5. Behavioral testing

2.5.1. Training phase

The animals were allowed to habituate in the experimental room for 1 h before the experiments. Then, each animal was gently placed in the brightly lit compartment of the apparatus; after five seconds the guillotine door was opened and the animal was allowed to enter the dark compartment. The latency with which the animal crossed into the dark compartment was recorded. Animals that waited more than 100 s to cross to the dark compartment were eliminated from the experiments. Once the animal crossed with all four paws to the next compartment, the guillotine door was closed and the rat was taken into its home cage. After 30 min, the trial was repeated for each subject as in the acquisition trial except that as soon as the animal crossed to the dark compartment, the door was closed and a foot shock (50 Hz, 1 mA and 3 s) was immediately delivered to the grid floor. After 20 s, the animal was removed from the apparatus and placed temporarily into its home cage. Two minutes later, the animal was retested in the same way as in the previous trials and a successful acquisition of IA memory was recorded for animals with 120 s latency to enter into the dark compartment. Otherwise, when the animal entered the dark compartment (before 120 s) a second time, the door was closed and the animal received the shock again. All animals were learned with maximum 3 trials. We previously reported that the number of trials to acquisition among the groups of rats used in the multiple-trial method was not significant and thus confirmed their uniformity (Darbandi et al., 2008; Rezayof et al., 2009). After retesting and successful acquisition, the animal was removed from the apparatus and immediately received post-training injection of drugs. The aim of all experiments with post-training drug microinjection was to evaluate the effects of drugs on IA memory consolidation.

2.5.2. Testing phase

Retrieval test was done 24 h after training. Each animal was gently placed in the light compartment; after 5 s the guillotine door was opened and the step-through latency with which the animal crossed to the dark compartment with all four paws was recorded. The testing process ended when the animal entered the dark compartment or waited in the light compartment for 300 s. During testing sessions no electric shock was given. The step-through latency was considered as an index of the inhibitory avoidance memory consolidation.