

Microinfusion of nefazodone into the basolateral nucleus of the amygdala enhances defensive behavior induced by NMDA stimulation of the inferior colliculus

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Abstract

The inferior colliculus is notably associated with defensive behavior. Electrical or pharmacological stimulation of the inferior colliculus induces aversive reactions such as running and jumping. Lesion of the basolateral nucleus of the amygdala decreases the threshold of aversive reactions induced by electrical stimulation of the inferior colliculus. The present work examined the influence of microinjections of nefazodone, a serotonin (5-HT₂) antagonist, into the basolateral nucleus of amygdala on aversive reactions induced by *N*-methyl-D-aspartate (NMDA) microinjected into the inferior colliculus. Rats implanted with cannulae in the inferior colliculus and in the basolateral nucleus of the amygdala were submitted to the open-field test where defensive behaviors were observed. Results indicated that microinjection of nefazodone into the basolateral nucleus of the amygdala increases aversive responses induced by NMDA injections into the inferior colliculus. This result suggests that the inferior colliculus and the basolateral nucleus of the amygdala have a functional relationship on the neural circuitry of defensive behavior. Moreover, 5-HT₂ receptors located at the basolateral nucleus of the amygdala seem to play an inhibitory role on defensive behaviors induced by inferior colliculus stimulation. © 2000 Elsevier Science Inc. All rights reserved.

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1. Introduction

Immunohistochemical and behavioral results indicate that the inferior colliculus (IC) is a critical structure involved in the neural circuitry of defensive behavior [1,25,27]. Electrical or chemical stimulation of the IC produces clear-cut aversive-like behaviors, such as running and jumping [2–4,15–17]. Several neurotransmitter systems within the IC appear to mediate the occurrence of these defensive behaviors. For example, microinjections of bicuculline [2], midazolam [17] or *N*-methyl-D-aspartate (NMDA, [3]) into the IC produces aversive reactions while high doses of morphine enhance defensive behavior induced by electrical stimulation of the IC [4]. On the other hand, microinjections of alpha-methyl-5-hydroxytryptamine, a 5-HT₂ antagonist [16], or low doses of morphine [4] decrease aversive reactions induced by IC electrical stimulation.

Another structure that has been associated with defensive behavior is the amygdaloid complex [8,11,23,24]. Lesions

in several nuclei composing this structure (i.e., central, lateral, and basolateral) disrupt innate or learned defensive-related behavior, whereas stimulation produces clear-cut aversive responses (see [23] for a review). There are some reports indicating that the IC and the amygdaloid complex have important functional connections related to auditory conditioning [12]. It is likely that the amygdaloid complex combines auditory information, relayed from the IC, involved in recognition of threatening stimuli. Anatomically, the amygdaloid complex receives information from all sensory modalities via association cortex and/or directly from the thalamus [29].

The purpose of the present study was to investigate the possible relationship between the IC and the amygdaloid complex in the generation of aversively motivated behavior. Previous reports have shown that neurotoxic lesions of the basolateral nucleus of the amygdala (BLA) decrease the aversive response threshold evoked by progressive increments of electric stimulation of the IC [15]. However, it is still unclear which neurotransmitter might mediate this effect. There are evidences indicating that serotonergic terminals located in the BLA are involved in aversive reaction [9]. Furthermore, the BLA has a high a concentration of

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5-HT₂ receptors [21,22,32]. Therefore, it is possible that 5-HT₂ terminals located in the BLA may play a role on the aversive reactions induced by stimulation of the IC. The present experiment investigated the effect of microinjections of the 5-HT₂ antagonist nefazodone (0.2 µg/0.5 µL) into the BLA on defensive behaviors induced by NMDA (10 µg/0.5 µL) stimulation of the IC. The NMDA and nefazodone doses were chosen according to previous report ([3], for the NMDA stimulation of the IC) as well as pilot results from our laboratory (nefazodone microinjections into the BLA).

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 250–300 g from the PUC-Rio, Psychology Department vivarium were employed as subjects. Room temperature was controlled, and light–dark cycle was maintained on a 12-h on–off cycle (0700–1900 h lights on). Animals were reared in groups of six. After surgery, they were kept individually housed and given food and water ad lib.

2.2. Surgery

Animals were anaesthetized with thionembutal (45 mg/kg, i.p.), fixed in stereotaxic frame and lidocaine (20 mg/mL) was injected locally. The upper incisor bar was set at 3.3 mm below the interaural line such that the skull was horizontal between bregma and lambda. Each animal was implanted with a unilateral cannula made of stainless steel (26 ga Plastic One Inc., Roanoke, VA) aimed at the IC, using the following coordinates, with the lambda serving as the reference for each plane according to the Paxinos and Watson atlas [20]: anteroposterior (AP) = –1.5 mm; mediolateral (ML) = 1.5 mm; and dorsoventral (DV) = 5.0 mm. The second cannula was aimed at the BLA. Taking bregma as the reference point, the coordinates for the BLA were: AP = –2.3 mm, ML = 5.3, DV = 8.0 mm. Cannula implanted in the IC and BLA were always placed in the same side of the brain. At the end of the surgery, each guide cannula was sealed with a stainless steel wire to protect it from congestion.

2.3. Procedure

Experiment started 1 week after surgery. Half of the animals were microinjected with 0.5 µL of nefazodone dissolved in saline (0.2 µg/0.5 µL) into the BLA. The other half was microinjected with the same volume of saline. Drug was made fresh on the day of the experiment. Drug microinfusion was made by an internal cannula (22 ga, Plastic One), which was introduced and lowered to 0.5 mm below the guide cannula. The injection cannula was connected to a 10-µL Hamilton syringe via PE tubing. The Hamilton syringe was mounted on an injection pump set up to deliver 1 µL of drug over 3 min. Following the end of the injection,

the internal cannula was held inside the brain for 1 min to prevent drug backup. As soon as the microinjection was over, the animal was placed into a squared wooden arena (60 × 60 × 60 cm) with the floor divided in 12 squares and observed for 10 min. Animal's locomotor activity was employed as a measure of running, a defensive behavior generally observed during NMDA stimulation of the IC, and was defined as the number of squares the rat crossed with his four paws. Occurrence of jumping was also observed, and was registered when the animal present four paws on the air. After the initial 10-min baseline, all the animals were taken off the arena. Half of the animals injected with nefazodone or saline into the BLA were microinjected with 0.5 µL of NMDA dissolved in saline (10 µg/0.5 µL) into the IC. The other half was microinfused with saline into the IC. Therefore, the experiment contained four groups according to a 2 × 2 factorial design. The group NEF-NMDA was infused with nefazodone into the BLA and NMDA into the IC; group NEF-SAL was infused with nefazodone into the BLA and saline into the IC; group SAL-NMDA received an infusion of saline into the BLA and an infusion of NMDA into the IC; group SAL-SAL was infused with saline into the BLA and IC. After the second infusion procedure, all the animals were returned to the arena for 40 min. Locomotor activity and jumping behavior were recorded throughout the time.

2.4. Histology

At the end of the experiment, animals were sacrificed with an overdose of thionembutal and perfused intracardially with saline followed by formalin solution (10%). The cannulae were taken off and the brains were removed and placed in a 10% formalin solution. Three days later the brains were frozen and serial 50-µm brain sections were cut using a microtome and stained with cresyl violet to localize cannula positions.

3. Results

Histological examination of the slides indicated that most of the cannula tips aimed at the IC and BLA were located within these nuclei. Data from rats with injection sites located outside the IC or BLA were removed from the study. The final size of each group was: NEF-NMDA = 9; NEF-SAL = 9; SAL-NMDA = 9; SAL-SAL = 7. Figure 1 presents a composite of the internal cannula tip locations aimed at the IC. Most of the cannula tips were located within the central nucleus of the IC. A few animals had the cannula located in the dorsal cortex of the IC (two animals) or in the external cortex of the IC (one animal). However, no differences in defensive behavior induced by NMDA stimulation of the IC was found among animals with cannula located among the different nuclei within the IC. Figure 2 presents a composite of the internal cannula tip locations aimed at the BLA. As can be observed from Fig. 2, all the cannula tips were located within this nucleus.

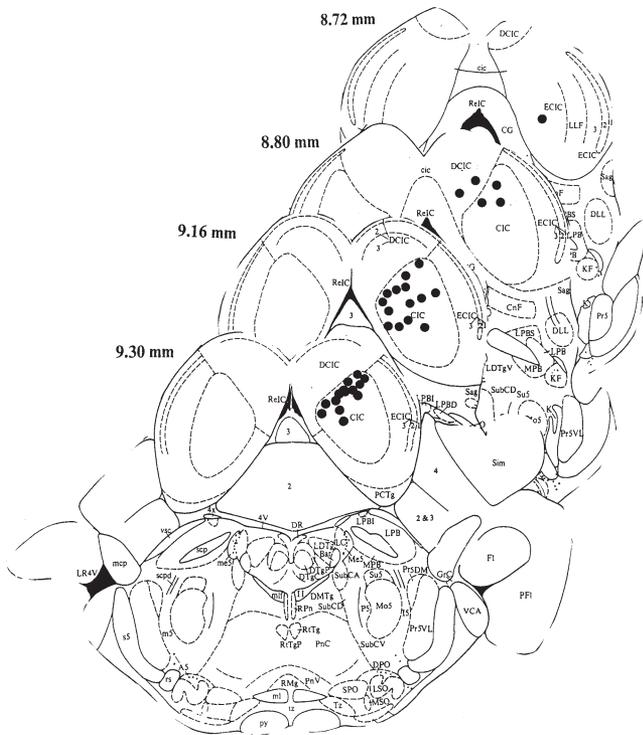


Fig. 1. Composite of internal cannula locations aimed at the IC. With the reference to Paxinos and Watson atlas [20], the numbers on the left side of each plate indicates the distance from bregma.

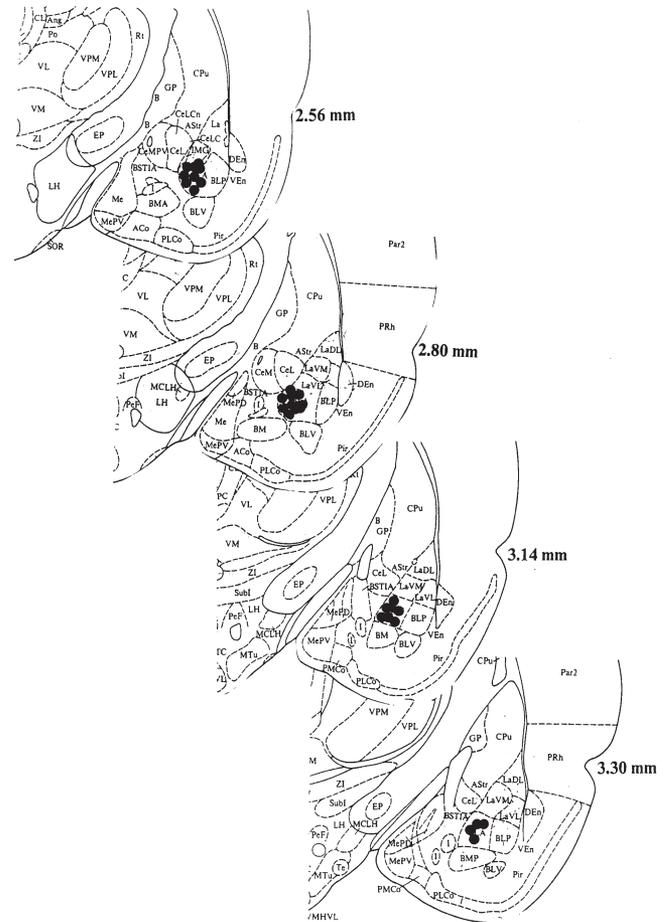


Fig. 2. Composite of internal cannula locations aimed at the BLA. With the reference to Paxinos and Watson atlas [20], the numbers on the right side of each plate indicates the distance from bregma.

Behavioral results are presented as means \pm SEM of the 10 min after the infusion of nefazodone or saline into the BLA (PRE-period) and four periods of 10 min after the infusion of NMDA or saline into the IC. Figure 3 presents the mean amount of locomotor activity during the experiment. No differences among groups were found during PRE-period, indicating that microinjections of nefazodone into the BLA did not cause any effect on animal's locomotor activity. A two-way ANOVA of the four 10-min periods after the NMDA or saline microinjections into the IC found a main effect of drug manipulation in the IC during 10-, 20-, and 30-min periods, $F(1, 41) = 9.59, p < 0.01$; $F(1, 41) = 5.58, p < 0.02$; $F(1, 41) = 5.95, p < 0.02$, respectively. Finally, an interaction between BLA and IC was detected in the 30-min period, $F(1, 38) = 4.35, p < 0.05$. A *t*-test post hoc comparison indicated that nefazodone microinjection in the BLA did not cause any effect on locomotor activity among animals that received saline microinjections in the IC all over the experiment (all $p > 0.1$, when compared with animals microinjected with saline into the BLA or into the IC). In contrast, NMDA microinjections into the IC increased locomotor activity during the 10-min period, regardless if the animals were microinjected with saline, $t(22) = 2.84; p < 0.01$, or nefazodone, $t(16) = 2.19; p < 0.05$, into the BLA. However, nefazodone microinjections into the BLA drastically enhanced the NMDA effect during the 20- and 30-min

periods, $t(16) = 2.14; p < 0.05$; $t(16) = 2.33; p < 0.05$, respectively. No differences were found during the 40-min period.

Figure 4 illustrates the mean of occurrence of jumping behavior that animals displayed during the experiment. None of the animals jumped during the baseline period. Moreover, the animals microinjected with saline in the IC did not exhibit any jumping throughout the period of testing. Therefore, a nonparametric statistic was employed to analyze this behavioral output. The Kruskal–Wallis test analysis revealed the same result pattern observed with crossing behavior. NMDA microinjections in the IC induced jumping during the 10-min period, regardless of whether the animals were microinjected with saline, $H(1) = 5.44; p < 0.02$, or nefazodone, $H(1) = 6.33; p < 0.02$, into the BLA. Moreover, nefazodone microinjections into the BLA enhanced jumping behavior during the 20- and 30-min periods, $H(1) = 4.76; p < 0.03$; $H(1) = 3.36; p < 0.06$, respectively. No differences were found during the 40-min period.

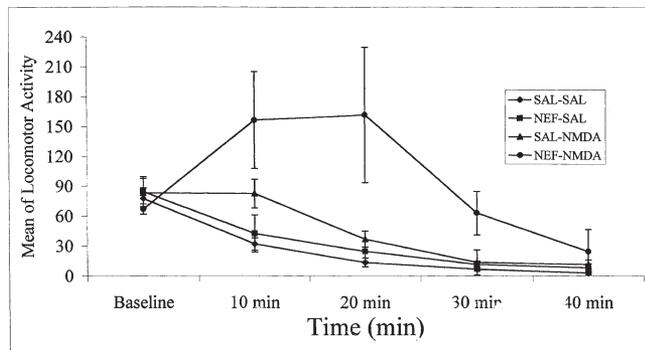


Fig. 3. Mean of locomotor activity (\pm SEM) during the experiment. The baseline refers to a 10-min period after saline (SAL) or nefazodone (NEF) microinjection into the BLA. The remaining 40 min refers to a period after saline (SAL) or NMDA microinjection into the IC.

4. Discussion

Electrical or chemical stimulation of the IC results in a well-defined defensive reactions patterns such as running and jumping [2–4,15–17]. The amygdaloid complex seems to mediate these aversive reactions to IC stimulation because lesions of the BLA decrease the aversive threshold to progressive increments of electrical stimulation of the IC [15]. The main purpose of the present work was to investigate the BLA neurochemical mediation of the aversive effect induced by IC stimulation. Present results indicate that microinjections of nefazodone, a 5-HT₂ antagonist, into the BLA increased running and jumping defensive responses to NMDA stimulation of the IC. These results are in accordance with previous report that suggest that participation of BLA 5-HT₂ receptors on aversive behavior [5], and indicate that IC and BLA have an important relationship in the neural circuitry of defensive behavior.

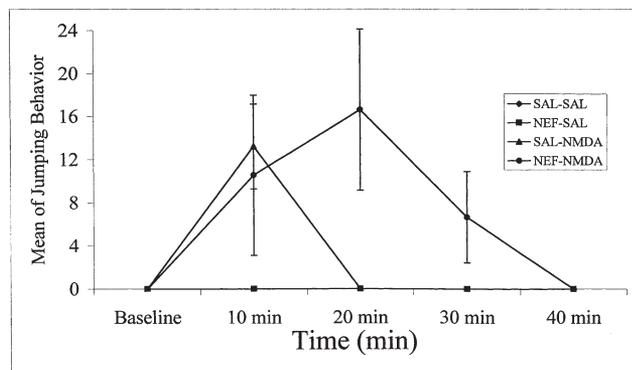


Fig. 4. Mean of occurrence of jumping behavior (\pm SEM) during the experiment. The baseline refers to a 10-min period after saline (SAL) or nefazodone (NEF) microinjection into the BLA. The remaining 40 min refers to a period after saline (SAL) or NMDA microinjection into the IC.

Previous results from our laboratory revealed that neurotoxic lesions of central nucleus of the amygdala (CNA) increases the thresholds of aversive responses elicited by electric stimulation in IC, whereas lesion of BLA decreases the threshold of these responses [15]. These results indicate that these two nuclei have opposite roles on the defensive reactions induced by IC stimulation. This dual role of the amygdaloid complex has also been observed in the regulation of stress-induced ulceration. Lesions of the CNA reduce stomach erosion induced by physical restraint, while lesions in the BLA increases the severity of gastric ulceration caused by stressors [7]. Some evidences suggest that the CNA receives inhibitory projection from BLA [12,29] and disruption of BLA activity may amplify fear, whereas enhancement of the inhibitory mechanisms of the BLA attenuates these aversive responses. Actually, some findings suggest that the anxiolytic effects of benzodiazepines are mediated by the BLA [19,28].

Our results indicated that aversive behavior induced by NMDA stimulation of the IC can be enhanced by microinfusions of nefazodone into the BLA. This finding follows our previous results with neurotoxic lesions, and suggests that 5-HT₂ receptors located at the BLA have an inhibitory role on defensive behavior. The present experiment did not attempt to investigate the neurochemical activity of the CNA involved in defensive behavior. However, it has been reported that 5-HT₂ antagonists microinjected into the CNA promoted a release of punished behavior [26]. Therefore, 5-HT₂ receptors within the amygdaloid complex seem to modulate defensive behavior differently. Decreased 5-HT₂ activity in the BLA enhances defensive reaction (present experiment), while decreased 5-HT₂ activity of the CNA releases aversive behavior [26]. Although it is unclear how 5-HT₂ receptors located in the BLA and CNA might have opposite effects on defensive behavior, it is possible that 5-HT₂ receptors can have different modulatory activity on neurotransmission release within different amygdaloid nuclei.

Finally, the modulatory effect of 5-HT₂ receptors located at the BLA on defensive behavior is not related to fibers originating from the IC, because there is no direct projection from the IC to the amygdaloid complex. Amygdaloid complex receives auditory information from the IC through the medial geniculate body [29]. Besides the main projection of the IC to the medial geniculate body, the IC also sends afferences to the dorsal raphe nucleus (DRN, [10]). This might be a significant connection for the present study because the major serotonergic input to the amygdaloid complex comes from the DRN [13,18,31]. In fact, chemical stimulation of the DRN with kainic acid induces serotonin release in the BLA [30]. Moreover, the DRN is notably associated with aversive behavior [5,6,14]. Therefore, the DRN might be an important structure for the functional relationship between the IC and the amygdaloid complex involved on aversive behavior triggered by IC stimulation and modulated by 5-HT₂ receptors within the BLA.

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