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Research report

# Neural segregation of Fos-protein distribution in the brain following freezing and escape behaviors induced by injections of either glutamate or NMDA into the dorsal periaqueductal gray of rats

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**Abstract**

Freezing and escape responses induced by gradual increases in the intensity of the electrical current applied to dorsal regions of the periaqueductal gray (dPAG) cause a distinct pattern of Fos distribution in the brain. From these studies, it has been suggested that a pathway involving the dPAG itself, dorsomedial hypothalamus and the cuneiform nucleus (CnF) would mediate responses to immediate danger and another one involving the amygdala and ventrolateral periaqueductal gray (vlPAG) would mediate cue-elicited responses. As electrical stimulation activates body cells and fibers of passage the need of studies with chemical stimulation of only post-synaptic fibers of the dPAG is obvious. To examine further this issue we measured Fos protein expression in brain areas activated by stimulation of the dPAG with glutamate (5 nmol/0.2 μL) and *N*-methyl-D-aspartate (NMDA) at doses that provoke either freezing (4 nmol/0.2 μL) or escape (7 nmol/0.2 μL) responses, respectively. The results showed that glutamate-induced freezing caused a selective increase in Fos expression in the superior and inferior colliculi as well as in the laterodorsal nucleus of the thalamus. On the other hand, NMDA-induced escape led to widespread increases in Fos labeling in almost all structures studied. Differently from glutamate, NMDA at doses provoking freezing caused significant increase of Fos labeling in the dPAG and CnF. Therefore, the present data support the notion that freezing behavior induced by activation of either non-NMDA or NMDA receptors in the dorsolateral periaqueductal gray (dlPAG) is neurally segregated: glutamate activates only structures that are mainly involved in the sensorial processing and NMDA-induced freezing structures involved in the motor output of defensive behavior. Therefore, the freezing elicited by the activation of non-NMDA receptors seem to be related to the acquisition of aversive information, whereas that resulting from the activation of NMDA receptors could serve as a preparatory response for flight.


*Theme:* Neural Basis of Behavior
*Topic:* Stress

*Keywords:* Freezing; Escape; Glutamate; NMDA; Dorsal periaqueductal gray matter; Fos-protein

*Abbreviations:* AntH, anterior hypothalamus; BLA, basolateral amygdaloid nucleus; CeA, central amygdaloid nucleus; Cg, cingulated cortex; CnF, cuneiform nucleus; Contra, contralateral; dlPAG, dorsolateral periaqueductal gray; DmH, dorsomedial hypothalamus; dmPAG, dorsomedial periaqueductal gray; dPAG, dorsal periaqueductal gray; DRN, dorsal raphe nucleus; EAA, excitatory amino acids; IC, central inferior colliculus; Ipsi, ipsilateral; LC, *locus coeruleus*; LDDM, laterodorsal nucleus of thalamus, dorsomedial part; LDVL, laterodorsal nucleus of thalamus, ventrolateral part; LH, lateral hypothalamus; lPAG, lateral periaqueductal gray; MeA, medial amygdaloid nucleus; MRN, median raphe nucleus; NMDA, *N*-methyl-D-aspartate; PMd, dorsal premammillary nucleus; PrL, prelimbic cortex; PVN, paraventricular hypothalamic nucleus; PVT, paraventricular nucleus of the thalamus; SC, superior colliculus; VMHdm, ventromedial hypothalamic nucleus, dorsomedial part; vlPAG, ventrolateral periaqueductal gray

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## 1. Introduction

When animals face dangerous situations they freeze or escape when a way out is not or is available, respectively [8]. Gradual increases in the intensity of the electrical stimulation of the dorsal aspects of the periaqueductal gray (dPAG) also causes freezing followed by intense and uncoordinated behavioral activation, characterized by turnings, runnings and jumping [10,13,14]. From clinical reports that human beings freeze during panic attacks it has been suggested that freezing behavior in animal models of anxiety may be better isomorphic to panic than flight [9]. Moreover, freezing and escape are negatively correlated, suggesting a competition between fear motor systems [43]. For this reason, some studies have been conducted aimed at the identification of the neural organization of freezing and escape induced by electrical stimulation of the midbrain tectum [13,41,57]. From these studies it has been shown that rats predominantly displaying freezing behavior had preferential neural activity in the dorsomedial (dmPAG) and ventrolateral (vlPAG), amygdala and entorhinal cortex, whereas rats predominantly displaying flight had more activity in the dorsolateral (dlPAG), lateral (lPAG), ventromedial hypothalamus (VMH), dorsal premammillary nucleus (PMd) and cuneiform nucleus (CnF). Electrical stimulation of a given structure has the disadvantage of exciting not only perikarya but also axons and fibers of passage [4]. Besides, human studies on Parkinson disease have reported that brain stimulation could result finally in inhibition of brain activity [42,46].

To circumvent this problem the use of chemicals that stimulate only post-synaptic receptors is preferable in the studies on the organization of neural circuits responsible for defensive behaviors. From these studies it has sorted out that defensive behavior elicited by chemical stimulation of the dPAG is supposed to be mediated by a number of neurotransmitters and neuromodulators. Among them, it has been shown that excitatory amino acids (EAA) may play an important role in the production of defensive behaviors [4,6,12]. Indeed, all types of glutamate receptors are significantly present in the dPAG [2]. Moreover, microinjections of low doses of glutamate (5 nmol) into the midbrain tectum cause a short lasting freezing behavior, while a sustained escape reactions interspersed with freezing are usually seen following similar injections of *N*-methyl-D-aspartate (NMDA, 7 nmol) [18,39]. Glutamate seems to act initially at AMPA/kainate receptors that possess rapid activation and inactivation times, whereas the NMDA receptors are activated slowly [7,18,29]. Consequently, it has been suggested that AMPA/kainate and NMDA receptors could mediate freezing and escape behaviors, respectively, as consequence of EAA locally applied into the midbrain tectum [44,45,47]. The neural substrates of aversion in the midbrain tectum mediated by EAA and/or other neurotransmitters are supposed to be under the tonic inhibitory control of GABAergic mechanisms [13,14,31].

The disclosure of Fos distribution in the encephalon from stimulation of dPAG with agents that activate only post-synaptic neurons for producing freezing or escape behaviors has special interest in the context of bringing a contribution to our knowledge on the neural circuits triggered by different fear conditions. In this study we go one step ahead and measured Fos distribution in serial sections of the brain following dlPAG stimulation with glutamate and NMDA in rats placed in an arena. As described previously NMDA injections into the dlPAG cause escape behavior interspersed with freezing, a lower dose of this agent that caused only freezing (4 nmol) was also tested in the present study to see whether or not Fos-protein distribution in this case could be different from that obtained with glutamate-induced freezing. Saline-injected animals were exposed to the same procedure in order to control for novelty of the exposure and locomotor activity. We have chosen to concentrate on those structures with higher chance of being involved in the neural circuits of defensive behavior, i.e. those that when electrically stimulated also give rise to defensive responses, such as the amygdala and medial hypothalamus, and the cuneiform nucleus [25,27,35,50]. We also focused our attention on structures that are sources of afferents to PAG such as the medial frontal and cingulate cortices or those structures that show increased Fos expression when rats are submitted to anxiogenic procedures and have also been implicated in the mediation of affective responses [3,34,40], namely, cingulate (Cg) and prelimbic (PrL) areas [34], central amygdaloid nucleus (CeA) [48], ventromedial (dorsomedial part, VMHdm) and dorsal premammillary (PMd) nuclei of the hypothalamus [15], cuneiform nucleus (CnF) [49], inferior colliculus (IC) [11,41,57], *locus coeruleus* (LC) [32] and the longitudinal columns of the PAG since they have distinct roles in the organization of the defense reaction [5,20–22].

## 2. Materials and methods

### 2.1. Animals

Naive male Wistar rats weighing 230–250 g were used. Animals were kept under controlled temperature (22 ± 2 °C) and a 12-h light/12-h dark cycle (lights on at 07:00 h). They were housed in two per cage and had free access to food and water throughout the experiment. The experiments were conducted between 8:00 and 14:00 p.m. The experiments were performed in compliance with the recommendations of SBNeC (Brazilian Society of Neuroscience and Behavior), which are based on the US National Institutes of Health Guide for Care and Use of Laboratory Animals.

### 2.2. Surgery

The animals were anaesthetized with tribromoethanol (250 mg/kg, i.p.) and fixed in a stereotaxic frame (David