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Research report

Relevance of dorsomedial hypothalamus, dorsomedial division of the ventromedial hypothalamus and the dorsal periaqueductal gray matter in the organization of freezing or oriented and non-oriented escape emotional behaviors

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HIGHLIGHTS

- 6 nmol NMDA in dmVMH elicited alertness, freezing and oriented escape behavior.
- 9 nmol NMDA in DMH elicited mainly alertness and oriented escape behavior.
- 9 nmol NMDA in dmVMH elicited alertness, freezing and oriented/non-oriented escape.
- 1, 3 and 6 nmol NMDA in dPAG elicited freezing behavior.
- 3 and 6 nmol NMDA in dPAG elicited explosive escape and long-lasting freezing.
- Mainly dPAG plays a role in panic attack-like behaviors.

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ABSTRACT

Electrical stimulation of the periaqueductal gray matter and ventromedial hypothalamus in humans showed the involvement of both these structures in panic attacks. The aim of this work was to make clear the role of dorsal periaqueductal gray (dPAG) matter, dorsomedial hypothalamus (DMH) and the dorsomedial part of the ventromedial hypothalamus (dmVMH) in panic attack-like behaviors. DMH, dmVMH and dPAG of Wistar rats were treated with *N*-methyl- D-aspartic acid (NMDA) at different doses. The rodents were then kept in a polygonal arena with a burrow to record panic attack-like responses and oriented defensive behaviors. In dmVMH, 6 nmol of NMDA elicited alertness, freezing and oriented escape. The same set of behaviors was elicited by DMH neurons when stimulated by 9 nmol of NMDA. Treatment of dmVMH with 9 nmol of NMDA elicited typical explosive behaviors followed by freezing and oriented behaviors. The stimulation of the dPAG with NMDA at different doses provoked alertness and freezing (1 nmol) or alertness, freezing, tail twitching, explosive behavior and oriented escape (3 nmol), and explosive behavior followed by long-lasting freezing (6 nmol). These data suggest that mainly dPAG plays a role in panic attack-like behaviors that resemble panic syndrome in humans. However, hypothalamic nuclei like dmVMH that mainly elicits oriented escape, can also produce explosive reaction when stimulated with 9 nmol NMDA, whereas, DMH plays a role in coordinating defensive behaviors.

1. Introduction

Panic disorder is a very common mental disorder characterized by periodic occurrence of panic attacks. It is defined by a distinct time-period of extreme fear, mainly followed by significant changes in the behaviors. Its common symptoms are increase in heart rate,

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sweating, shivering, dyspnea, chest pain, vomiting, fainting, craziness, chill or hot and fear of dying that occur suddenly and reach to its peak point within 10 min [1].

The treatment of this mental disorder really needs better understanding of its neurochemical and neural substrates. Nashold et al. reported for the first time in humans that the electrical stimulation of the dorsal periaqueductal gray matter (dPAG) and other midbrain tectum structures produce autonomic changes and feelings of fear that are the symptoms of panic attacks [2]. Later on, the prior findings were confirmed in animal model by electrically stimulating dPAG that induced explosive escape responses [3,4]. Additionally, pharmacological studies also confirmed that dPAG is an important structure that has a role in organizing defensive behaviors [5,6]. Microinjection of excitatory amino acids like glutamate and *N*-methyl-D-aspartic acid (NMDA) in dPAG induced defensive immobility (freezing) and flight response [7,8]. Considering vigorous attempts of escape, preceded by defensive immobility, followed by autonomic reactions like tachycardia, high blood pressure, and dyspnea, these non-oriented/explosive innate-fear induced responses are commonly related to panic attacks [9,10]. Based on these symptoms some authors suggest that dPAG is a key structure that organizes panic-related responses in humans [11,12]. Interestingly, in normal human volunteers, the neuroimaging data correlated the facial anger and fear expressions with PAG activation [13].

Along PAG, the hypothalamus, a diencephalic structure, is also implicated in organizing defensive behaviors [14,15]. Among several hypothalamic nuclei, anterior hypothalamus, dorsal premammillary hypothalamic nucleus and the dorsomedial division of the ventromedial nucleus (dmVMH) constitute antipredatory diencephalic system [16]. The dorsomedial hypothalamus (DMH) is one of the hypothalamic nuclei that plays a critical role in organizing autonomic, reproductive and fear-induced behavioral responses [17,18]. The microinjection of bicuculline (a $GABA_A$ receptor antagonist) in the DMH caused panic attack-like behaviors in laboratory animals, such as tachycardia, tachypnea, increase in arterial blood pressure and anxiety [19] and escape behaviors [20,21]. With this view, the chronic infusion of L-allylglycine (a GABA synthesis inhibitor) by minipumps into the DMH of rats elicited behavioral responses to lactate infusion similar to panic disorders in humans [22]. This investigation shows the interaction between GABAergic inhibition and glutamate mediated-excitation that control lactate responses in DMH [23]. These evidences suggest that DMH plays a critical role in "panic-related neural circuit" [24].

The electrical stimulation of the ventromedial hypothalamus (VMH) causes panic attack like responses in different species (rats and cats) [25,26]. Rise in Fos-protein expression has been found during exposure of rats to predator in the dmVMH, and discrete chemical lesions of medial hypothalamic nuclei decrease the anti-predator behavioral reactions [27]. This nucleus of the hypothalamus is believed to be the critical part of the neuronal network that constitutes medial hypothalamic defensive system, organizing innate defensive behavioral reactions in threatening situations [16,27–29]. Likewise laboratory animals, the electrical stimulation of the VMH also caused panic attacks in humans [30]. Recently it has been showed that dmVMH and central division of the ventromedial hypothalamus (cVMH) neurons that express steroidogenic factor 1, when optogenetically activated, elicit freezing, running, jumps, rearing to the walls and escape to the hidden place [31]. Other nuclei of the hypothalamus such as lateral hypothalamus [32], anterior hypothalamus [31], and posterior hypothalamus [20] are also involved in organizing defensive behaviors and have been implicated in the neuroanatomy of panic disorder. Additionally, the inferior colliculus (IC) [33,34,35] and the superior colliculus (SC) [36,37] also play a role in the elaboration of instinctive fear-induced defensive behaviors. Amygdalar region is also reported in the development of innate defensive reactions [38].

However the pattern of defensive responses organized either by dorsal midbrain structures or by diencephalic structures is not yet clear. The aim of this study was to investigate the precise contributions of dPAG and ventral hypothalamic nuclei (DMH and dmVMH) in freezing or oriented and non-oriented escape behaviors associated with panic attacks.

2. Materials and methods

2.1. Ethical approval

All experiments were approved by the Commission of Ethics in Animal Experimentation of the FMRP-USP (proc. 192/2009) which abides by the ethical principles in animal research adopted by the Brazilian College of Animal Experimentation (COBEA).

2.2. Animals

Male Wistar rats (*Rattus norvegicus,* Rodentia, Muridae), weighing 220–290 g (n = 7–8 per group), from the animal facility of Ribeirão Preto Medical School of the University of São Paulo (FMRP-USP) were used. They were kept four per cage in the experimental room prior to the stereotaxic surgery, with free access to water and food, on a 12 h light/dark cycle at 22–23 °C.

2.3. Surgical procedure

Animals were anaesthetized with 92 mg/kg ketamine (Ketamine Agener, *União Química Farmacêutica Nacional*, Brazil) and 9.2 mg/kg xylazine (Dopaser®, Hertape/Calier, Juatuba, Minas Gerais, Brazil). After giving anesthesia the animals were fixed in a stereotaxic frame (David Kopf, Tujunga, California, USA). A stainless steel guide-cannula (outer diameter 0.6 mm, inner diameter 0.4 mm) was implanted in the diencephalon, targeting the DMH or dmVMH nuclei and in the dorsal mesencephalon, targeting the dPAG. The upper incisor bar was set at 3.3 mm below the interaural line, such that the skull was horizontal between bregma and lambda. The guide-cannula was vertically introduced into the DMH or dmVMH using the following coordinates, with bregma serving as a reference: AP = −1.92 mm, ML = −0.3 mm, DV = −8.2 for DMH and −8.3 mm for dmVMH. Coordinates used for implanting cannula in dPAG were, AP = −6.4 mm, ML = −0.7 mm and DV = −4.2 mm. Stereotaxic coordinates for cannula implantation were selected from the rat brain stereotaxic atlas [39].

The guide-cannula was fixed with the skull using acrylic resin and one stainless steel screw and remained 1 mm above the site of drug injection to avoid lesions at the structure of interest. Each guide cannula was sealed with a stainless steel wire to protect it from obstruction. At the end of the surgery, each rodent was treated with an intramuscular injection of penicillin G-Benzathine (120,000 UI; 0.2 mL) followed by intramuscular injection of the analgesic and anti-inflammatory flunixin meglumine (2.5 mg/kg).

2.4. Experimental procedure

All the experiments were performed in a quite environment. To reduce the stress during experiment, after surgery the animals were kept in a group for habituation in the experimental polygonal arena for almost three days (72 h). The polygonal arena was made of a semi-transparent acrylic quadrangular enclosure (172 cm in length, 56 cm in height and 72 cm in width) with the inner wall surface covered with a light-reflector film that provides 80% light reflection to minimize the visual contact of the animal with the