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# Integrity of the dorsolateral periaqueductal grey is essential for the fight-or-flight response, but not the respiratory component of a defense reaction

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ABSTRACT

Periaqueductal grey is believed to be one of the key structures of the central respiratory stress network. Previous studies established that stimulation of the periaqueductal grey, especially its dorsolateral division (dlPAG), evokes tachypnea as well as increases in other autonomic parameters and motor activity. We investigated the effects of blockade of the dlPAG with $GABA_A$ agonist muscimol on respiration during stress and presentation of brief alerting stimuli in conscious unrestrained rats. We found that integrity of the dlPAG is not essential for stress-induced increase in basal/resting respiratory rate or for generation of respiratory responses to brief alerting stimuli. However, blockade of the dlPAG reduced the amount of motor activity and concomitant high-frequency respiratory activity during restraint and the first 5 min of novelty stress. We conclude that the integrity of the dlPAG is not essential for generation of respiratory component of the defense reaction, but it mediates expression of the fight-or-flight response including its respiratory component.

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## 1. Introduction

Respiration is a very sensitive index of autonomic activation and arousal. Most of the research in respiratory neurophysiology has focused on investigating ponto-medullary rhythmogenic and homeostatic mechanisms (see Dutschmann and Dick, 2012; Feldman et al., 2013; Smith et al., 2013 for reviews), while suprapontine mechanisms that coordinate stress-induced respiratory activation are still poorly understood. Previous studies by our laboratory described relative contributions of various structures of the central autonomic pathway to respiratory stress-induced activation in conscious rats. Inhibition of the dorsomedial hypothalamus, a major center of autonomic integration, almost completely abolished generation of respiratory responses to brief and prolonged stressors of various intensities (Bondarenko et al., 2015). Blockade of the prelimbic prefrontal cortex inhibited responses to prolonged, but not brief, high and low intensity stimuli (Bondarenko et al., 2014a). Both of these structures have projections to the dorsolateral periaqueductal grey (dlPAG; Dampney et al., 2013). Activation of the PAG with an excitatory amino acid evoked significant respiratory responses in anesthetized rats with the strongest responses evoked from the dlPAG (Iigaya et al., 2010). Similarly, activation of this area evoked significant tachypneic responses in the in situ preparation (Farmer et al., 2014). Lastly, exposure to a predator increases Fos immunoreactivity in the PAG with the strongest activation in the dlPAG subregion (Canteras and Goto, 1999). These findings suggest that this area may be involved in stress-induced modulation of respiratory rhythm. Dorsolateral PAG is believed to be one of the key areas of central mechanisms that mediate defensive autonomic responses, including respiratory responses (Dampney et al., 2013; Subramanian and Holstege, 2014). However, the exact contribution of this area to generation of respiratory responses to stress has never been examined.

Arousing and stressful stimuli can module respiration in different ways, depending on their properties. Brief arousing stimuli, such as acoustic stimuli, evoke transient changes in respiratory rate, proportional to the stimulus intensity (Bondarenko et al., 2014b). Prolonged stimuli and stressors, such as novelty stress and restraint, elevate the basal respiratory rate and also evoke periods of high respiratory rate associated with motor activity (e.g., sniffing the new environment, exploring the new environment, struggling against the restrainer) (Kabir et al., 2010). The current study aims to investigate the effects of pharmacological inhibition of the dlPAG by a $GABA_A$ agonist muscimol on different types of respiratory

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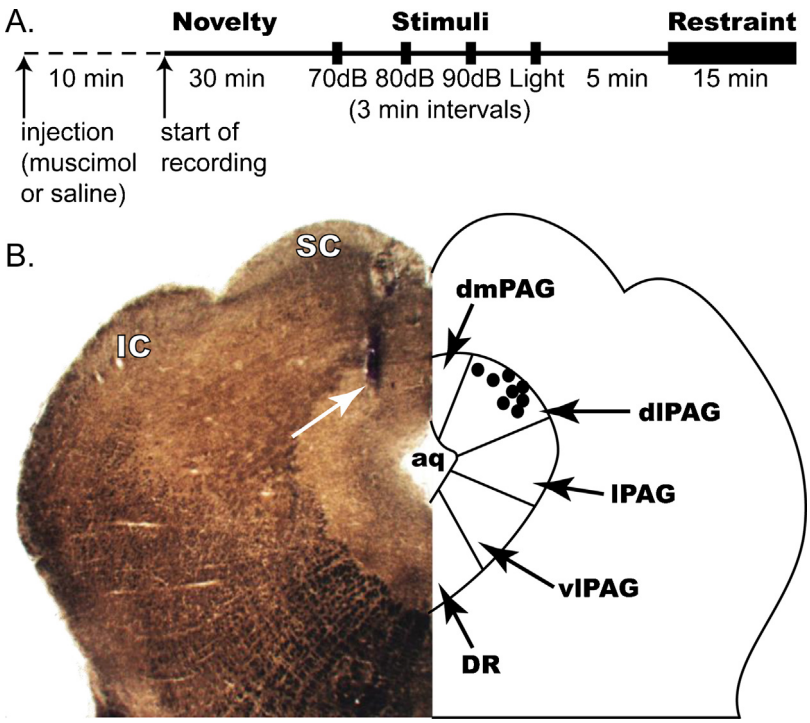


**Fig. 1.** (A) Summary of the experimental protocol of the current study. 8 adult rats completed the protocol twice in a counter-balanced design—once with microinjection of muscimol (100 pmol/100 nl/side) into the dorsolateral periaqueductal grey and once with saline microinjection. (B) Histological verification of microinjection sites into the dorsolateral periaqueductal grey. Left side of the picture displays a coronal section of brain of one of the rats in the current experiment; the white arrowhead points to a microinjection site. The right side of the picture displays centers of successful microinjection sites (black circles). Abbreviations: aq, cerebral aqueduct; dlPAG, dorsolateral periaqueductal grey; dmPAG, dorsomedial periaqueductal grey; DR, dorsal raphe; IC, inferior colliculus; lPAG, lateral periaqueductal grey; SC, superior colliculus; vlPAG, ventrolateral periaqueductal grey.

responses to short and prolonged stimuli of various intensities in conscious unrestrained rats.

## 2. Methods

### 2.1. Animals

Eight male adult Wistar rats (300 ± 50 g) were obtained from Animal Care of the Federal University of Paraiba. For the duration of the experimental protocol animals were single housed and kept on a reverse 12 h dark–light cycle (lights on at 1900). Animals were provided with food and water ad libitum. All experimental procedures were approved by Animal Use and Experimentation Committee at Federal University of Paraiba (Ethics protocol #305/14 (CEUA/UFPB)), and were in accordance with the Brazilian Federal law #11.794/08 and the EU Directive 2010/63/EU for animal experiments.

### 2.2. Experimental protocol

Under ketamine/xylazine anesthesia animals were surgically implanted with bilateral guide cannulas targeting the dorsolateral periaqueductal grey (dlPAG: −7.8 mm caudal, 4.5 mm ventral, 0.6 mm lateral from bregma; Paxinos and Watson, 2007). Baytril and Ibuprofen were used as an antibiotic and an analgesic during post-operative care. Animals were allowed to recover for 7 days before being subjected to respiratory assessment.

In a randomized counter-balanced design, at least 48 h apart, animals completed the recording session twice–once after a microinjection of $GABA_A$ agonist muscimol (100 pmol in 100 nl per side; purchased from Sigma–Aldrich, USA) to the dlPAG and once after a microinjection of the equal volume of saline. For the recording session animals were placed inside a plethysmographic chamber (Perspex cylinder, I.D.: 95 mm, length: 260 mm, volume: 1.8 l, wall thickness: 3 mm) with animal bedding placed on the bottom of the chamber and constant illumination of 20 lux. The chamber was fitted with a removable lid on one side and was constantly flushed with air at the flow rate of 3 l/min. The output flow line made of polyethylene tubing (O.D.: 1.45 mm, I.D.: 0.75 mm) was divided into two lines using a T-connector. One end (10 cm) was attached to the differential pressure amplifier (model 24PC01SMT, Honeywell Sensing and Control, Golden Valley, MN), while the other end (60 cm long) was open to the room air. Each respiratory cycle of an animal inside the chamber corresponded to a brief change of pressure inside the cylinder due to a difference of temperature between the inhaled and exhaled air. The rate of such fluctuations provides a reliable assessment of respiratory rate.

The dose of muscimol (100 pmol/100 nl/side) used in this study is smaller than the one used in our previous studies (Bondarenko et al., 2015, 2014a,b). We have chosen this dose to limit the effect of muscimol to the target area as the target area in this study is considerably smaller. Since the effect of this smaller dose is presumably shorter, we have modified the experimental protocol, so that each recording session was approximately 1 h long. Each recording session consisted of a 30-min novelty stress (the first 30 min after being placed into the chamber), followed by presentation of 3 acoustic stimuli (70, 80 and 90 dB, 500 ms white noise) and light stimulus (2000 lux, 30 s) with 3-min inter-stimulus interval and a 15-min restraint stress. All acoustic stimuli were presented from a generic speaker placed immediately next to the plethysmograph. These stimuli were presented during periods of no locomotor activity (as assessed by the a piezo-electric pulse transducer (MLT1010/D, ADInstruments, Sydney, Australia) placed under the plethysmograph) with an inter-stimulus interval of 5-min. All stimuli were presented when animals were awake and quiet and when their breathing was slow (<150 cycles per

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