# Exploring Laryngeal Effects of Dorsolateral Periaqueductal Grey Stimulation in Anesthetized Rats: implications for c-Fos and FOXP2 Expression in the Nucleus Ambiguus Subdivisions.

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**ABSTRACT**

**Background**

Stimulation of the Periaqueductal Gray matter (PAG) and nucleus retroambiguus (nRA) evokes vocalization (1). The nRA serves as a key target for converting passive into active expiration by modulating the activity of laryngeal motoneurons located in the nucleus ambiguus (nA) (2). Previously, we have demonstrated the involvement of rostral and ventral pontine structures in altering laryngeal caliber (3). Furthermore, a heightened expression of the FOXP2 protein (a transcription factor closely linked to vocalization) has been observed in both mesencephalic (PAG) and pontine regions implicated in cardiorespiratory control (4).

**Objectives**

To investigate the potential interactions between the dlPAG and laryngeal motor neurons of the nA assessing the double staining patterns of c-Fos/FoxP2 protein immunoreactivity (c-Fos-ir/Fox-P2-ir) and Tyrosine Hydroxylase (TH) across the rostro-caudal extent of the nA.

**Methods**

Experimental studies were carried out with non-inbred male rats (n=10), SPF, Sprague-Dawley (250-300 g) housed under standard conditions. Animals were anesthetized with sodium pentobarbitone (60 mg/kg i.p., initial dose, supplemented 2mg/ kg, i.v., as necessary). The pattern of immunohistochemical and immunofluorescence staining for c-Fos and FOXP2 protein immunoreactivity (c-Fos-ir/FOXP2-ir) were examined throughout the rostrocaudal extent of the nA region during electrical stimulation of the dlPAG (1 millisecond pulses, ranging from 30-50 µA, at a frequency of 100 Hertz for a duration of 5 seconds). Guanethidine (10 milligrams per kilogram intravenous) was administered to suppress sympathetically mediated cardiovascular responses. Only data from animals in which the histology showed that the microelectrodes were positioned within the dlPAG were used for statistical procedures.

**Results**

Stimulation of the dlPAG induced a specific increase in c-Fos-ir, with ipsilateral predominance in the somatas of both the loose ($p<0.05$) and compact formation ($p<0.01$) within the nA. Furthermore, we could confirm the bilateral expression of FoxP2 across all domains within the nA.

**Conclusions**

Our study contributes with new data on the role of the mesencephalic neuronal circuits in the control mechanisms of subglottic pressure and laryngeal activity.

**Ethical approval**

All experimental protocols were performed in accordance with the recommendations of the European Union directive (2010/63/EU) for animal care and experimental procedures. The experiments were approved by the Ethical Committee for Animal Research of the University of Malaga and the Junta de Andalucía.

**Keywords**

dlPAG, Nucleus Ambiguus, Laryngeal Motoneurons, c-Fos, FOXP2

**References:**

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