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BRAIN RESEARCH

Research Report

Fos-Tau-LacZ mice reveal sex differences in brainstem c-*fos* activation in response to mild carbon dioxide exposure

Mary Melissa Niblock[a,*], *Hong Gao*[b], *Aihua Li*[b], *Elizabeth Carney Jeffress*[a], *Mark Murphy*[c], *Eugene Edward Nattie*[b]

[a]*Biology Department and Neuroscience Program, Dickinson College, Carlisle, PA, USA*
[b]*Physiology Department, Dartmouth Medical School, Lebanon, NH, USA*
[c]*Department of Anatomy and Cell Biology, University of Melbourne, Australia*

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ABSTRACT

There are sex differences in the neurochemistry of brainstem nuclei that participate in the control of breathing as well as sex differences in respiratory responses to hypoxia. Central chemoreception refers to the detection within the brain of minute changes in carbon dioxide (CO_2) levels and the subsequent modulation of breathing. Putative central chemoreceptor sites are widespread and include cells located near the ventral surface of the brainstem in the retrotrapezoid nucleus (RTN), in the medullary midline raphe nuclei, and, more dorsally in the medulla, in the nucleus of the solitary tract and in the locus caeruleus at the pontomedullary junction as well as in the fastigial nucleus of the cerebellum. In this study, we ask if the cells that respond to CO_2 differ between the sexes. We used a transgenic mouse with a c-*fos* promoter driven *tau-lacZ* reporter construct (FTL) to map the locations of cells in the mouse brainstem and cerebellum that responded to exposure of mice of both sexes to 5% CO_2 or room air (control). X-gal (5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside) histochemical staining to detect the beta-galactosidase enzyme produced staining in the brains of mice of both sexes in all of the previously identified putative chemoreceptor sites, with the exception of the fastigial nucleus. Notably, the male RTN region contained significantly more x-gal-labeled cells than the female RTN region. In addition to new observations regarding potential sex differences in the retrotrapezoid region, we found the FTL mouse to be a useful tool for identifying cells that respond to the exposure of the whole animal to relatively low concentrations of CO_2.

1. Introduction

Numerous regions of the vertebrate brain have been shown to exhibit sexual dimorphism, ranging from large-scale anatomical differences between the sexes, such as differences in cortical thickness (Sowell et al., 2007), to more subtle neurochemical differences, such as the levels of neurotransmitters or neurotransmitter receptors present in certain nuclei (for a review, McCarthy et al., 1997). Even though many of the anatomical and neurochemical sex differences in the brain are associated with reproductive behavioral differences between the sexes, such as the larger vocal control nuclei in male songbirds, who sing to attract their mates (Nottebohm and Arnold, 1976), and the pronounced neurochemical differences

* *Corresponding author*. Fax: +1 717 245 1995.
E-mail address: niblockm@dickinson.edu (M.M. Niblock).

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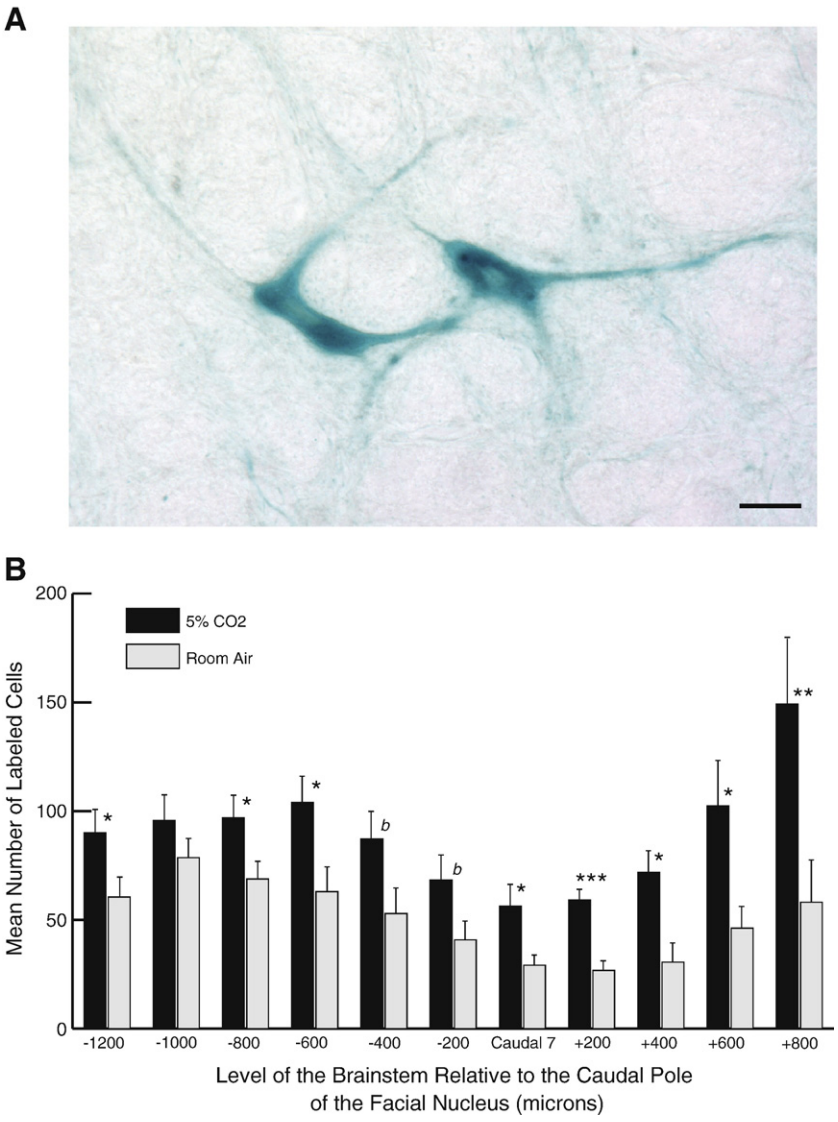

Fig. 1 – Labeled cells in the Fos-Tau-LacZ mouse brainstem following exposure to 5% CO_2 or room air. (A) Histochemical detection of the tau-beta-galactosidase reporter produced a blue reaction product in the axons, dendrites, and cell bodies of activated neurons, but the dark blue reaction product was relatively sparse in their nuclei. These two cells are examples from the inter-raphe-retrotrapezoid region (scale bar = 10 μm). (B) This graph illustrates the mean number of labeled cells at each level of the brainstem in animals exposed to 5% CO_2 (black) or room air (gray). There were significantly more labeled cells at most levels of the brainstem following exposure to 5% carbon dioxide than there were following exposure to room air (*$p < 0.05$, **$p < 0.01$, *b* = borderline significance; $p = 0.059$ for −400 and $p = 0.056$ for −200; bars are standard errors).

among these nuclei (Bottjer et al., 1997), there is no reason to assume that sex differences in the brain are limited to neural substrates that underlie mating behaviors (Cahill, 2006). Sex differences could exist in the neural circuitry underlying a behavior as fundamental as breathing. In fact, a number of studies have reported neurochemical sex differences in respiratory-related nuclei, including the phrenic nucleus (Behan et al., 2003), the hypoglossal nucleus (Behan et al., 2003; Schlenker and Hansen, 2007; Barker et al., 2009), the nucleus of the solitary tract (Schlenker and Hansen, 2007), and the brainstem noradrenergic cell groups (Pequignot et al., 1997). In addition, sex differences in the respiratory responses to hypoxic challenges (Tatsumi et al., 1991; Mortola and Saiki, 1996), to hypercapnic challenges in serotonin transporter knockout mice (Li and Nattie, 2008), and age-related sex differences in responses to hypoxic and hypercapnic challenges in rats (Schlenker and Goldman, 1985; Wenninger et al., 2009) have been reported. All of these data suggest that sex may be a very important, although frequently overlooked, component of respiratory control and modulation. The data related to the hypercapnic response and the nuclei that participate in that response are especially interesting given the disproportionate number of males affected by the sudden infant death syndrome and sleep apnea, two disorders thought to be related to deficits in central chemoreceptor function.