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# GABAergic neurons in the medullary raphé possess network independent chemosensitivity *in situ*

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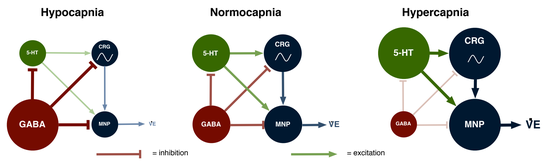
The identity and location of central pH/CO2 sensitive chemoreceptors are not fully understood. Serotonin (5-HT) and γ-aminobutyric acid (GABA) synthesizing neurons in the medullary raphé have demonstrated intrinsic chemosensitivity *in vitro*. This evidence forms the basis for our "push-pull" model of raphé contributions to chemosensitivity. We have previously shown that CO2-stimulated 5-HT neurons occur in the medullary raphé *in situ*. Here, we test the hypothesis that the medullary raphé contains GABA synthesizing CO2-inhibited neurons that retain their chemosensitivity after pharmacological blockade of major fast synaptic inputs. To assess chemosensitivity, we record extracellular single neuron discharge during normocapnic and hypercapnic conditions within the medullary raphé of the unanesthetized juvenile rat *in situ* perfused decerebrate brainstem preparation. Network dependence of chemosensitivity is assessed by application of antagonists for AMPA, NMDA, glycine, and GABAa receptors that disrupt fast-synaptic network properties. Juxtacellular labeling and immunohistochemistry establish neurotransmitter phenotypes of recorded neurons. Results support independence of CO2-inhibited GABA neuron chemosensitivity from fast synaptic inputs. Supported by NIH 5NS041069-06A1.

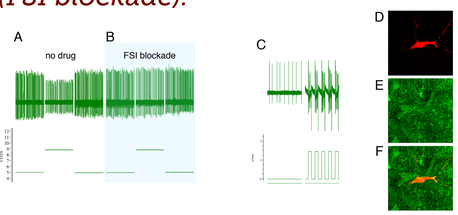
## Question

Serotonin (5-HT) and γ-aminobutyric acid (GABA) synthesizing neurons from the rat medullary raphé express intrinsic sensitivity to changes in pH/acidosis in vitro, but their chemosensitivity in vivo is debated.




- We propose a "Push-Pull" model of raphé contributions to central chemosensitivity.
- $CO_2$-stimulated raphé 5-HT neurons activate central rhythm generators (CRG) and/or motor neuron pools (MNP) to enhance ventilation (V̇E).
- $CO_2$-inhibited raphé GABA neurons deactivate CRG and/or MNP to attenuate V̇E.
- Ventilation is stimulated by $CO_2$ both through activation of 5-HT neurons, and disinhibition resulting from deactivation of GABA neurons (after Corcoran et al. 2008).
- Earlier we have shown $CO_2$-stimulated 5-HT neurons.
- The current project is aimed at identifying chemosensitive GABA neurons predicted by our model, and assessing network independence of their chemosensitivity.

***We test the hypothesis that the medullary raphé contains CO2-inhibited GABAergic neurons that retain their chemosensitivity after pharmacological blockade of major fast synaptic inputs (FSI blockade).***

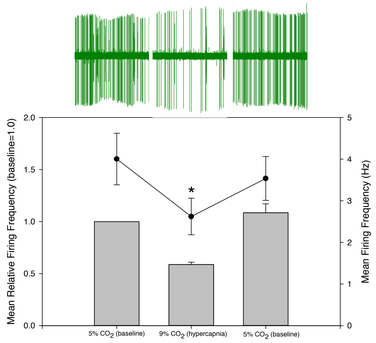



Spontaneously active single neurons in medullary raphé were recorded with glass capillary electrodes before, during, and after 5 minute hypercapnic challenges (A) in an unanesthetized juvenile rat in situ perfused decerebrate brainstem preparation (P20-P30; 60-150 g male albino rats; Paton 1996). Protocols were performed before(A) and after (B) bath application of agents that disrupt fast synaptic network properties; CNQX, CPP, strychnine, and bicuculline (antagonists for AMPA, NMDA, glycine, and GABAa receptors, respectively; Peña et al. 2004). We used juxtacellular labeling (C-D; Pinault 1996) of recorded neurons and subsequent immunohistochemistry for the GABA synthesizing enzyme glutamic acid decarboxylase (GAD67; E) to identify electrophysiologically characterized $CO_2$-inhibited cells as GABAergic (F).

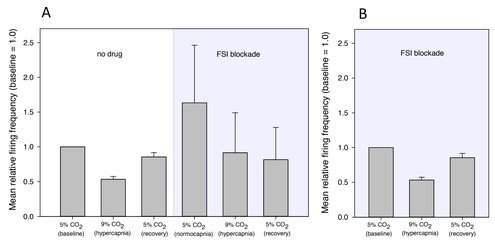
## $CO_2$-inhibited cells

**$CO_2$-inhibited neurons occur in the raphé.**

The top tracings illustrate a $CO_2$-inhibited cell. Mean firing frequencies of these cells were 4.0 Hz at initial baseline, reduced to 2.6 Hz with hypercapnia ($P < 0.001$) and recovered upon return to baseline (line plot). Overall, normalized firing frequencies reduced by 41% with hypercapnia in these cells (histograms); N=63 individual neurons.




## Fast synaptic blockade



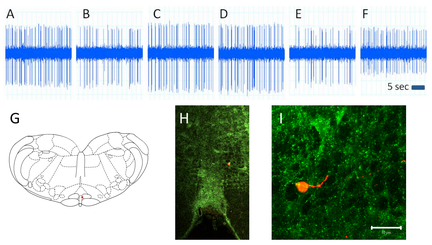

**$CO_2$-inhibited neurons retain chemosensitivity with FSI blockade.**

Histograms show average relative firing frequencies (normalized to initial baseline) of $CO_2$-inhibited cells before and during FSI blockade (A; N=6 individual neurons). Figure B illustrates average relative firing frequencies (normalized to 5% CO2 firing levels) of individual neurons recorded during FSI blockade (N=15).

## GABAergic $CO_2$-inhibited cells are independently sensitive

Here, a $CO_2$-inhibited raphé neuron firing at 4.5 Hz at 5% $CO_2$ (A), decreased by 60% (to 1.8 Hz) at 9% $CO_2$ (B). Firing frequency recovered (C; 4.0Hz) and was unchanged by FSI blockade (D; 4.1 Hz). Cell firing was decreased again by hypercapnia under continued FSI blockade (E; 2.0 Hz at 9% $CO_2$) and recovered with a return to 5% $CO_2$ (F; 3.0Hz). A coronal section (G: Paxinos and Watson, 1998) shows the location of the biotinamide filled (red) cell within raphé magnus (H, 10x). Higher magnification image (I, 40x) reveals a population of GAD67-immunoreactive GABA cells (green). The recorded cell, is colocalized (orange) with GAD67 immunoreactivity, positively identifying this network-independent $CO_2$-inhibited GABA neuron.

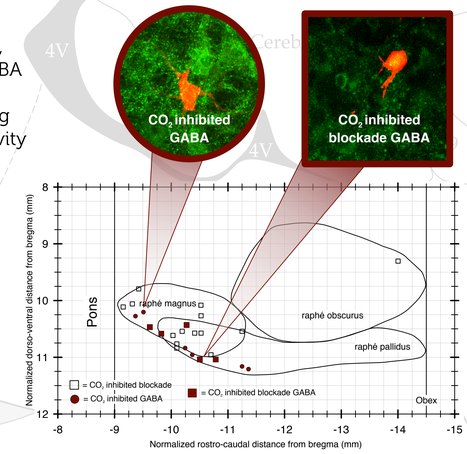
**Single-unit recordings demonstrate $CO_2$-inhibited GABA neurons that retain chemosensitivity with FSI blockade.**




**CO2-inhibited neurons in the rat medullary raphé are GABAergic. Their chemosensitivity is independent of fast synaptic input.**

We show $CO_2$-inhibited GABA neurons in the medullary raphé that support our "push-pull" model. Under FSI blockade conditions, neuron chemosensitivity persists. All labeled $CO_2$-inhibited neurons were GABAergic.

Our $CO_2$-inhibited cells, (including GABA neurons and those retaining chemosensitivity after FSI blockade) are clustered in the rostral medullary raphé




$CO_2$-inhibited neurons occur in the medullary raphé.

$CO_2$-inhibited cells remain so under FSI blockade.

$CO_2$-inhibited cells are identified as GABAergic.

These $CO_2$-inhibited neurons are clustered in the rostral and ventral medullary raphé.

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