Identifying raphé respiratory chemosensory amplifiers *in situ.*

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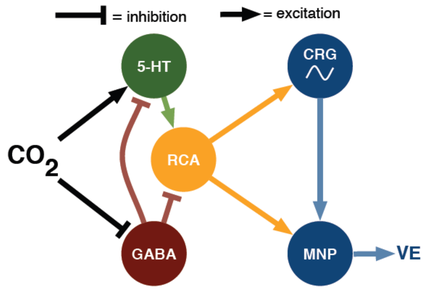
Introduction

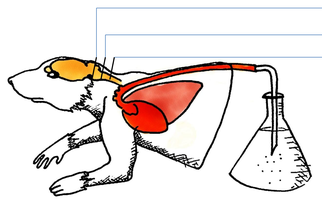


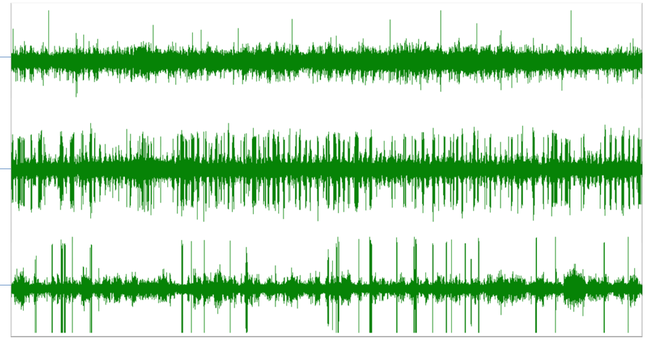
We test the hypothesis that chemosensitivity of medullary raphé interneurons is due to intra-network input from 5-HT and GABA neurons.

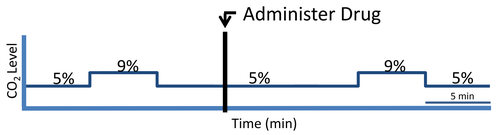
Serotonin (5-HT) and γ-aminobutyric acid (GABA) synthesizing neurons from the rat medullary raphé express intrinsic sensitivity to changes in pH/acidosis *in vitro* but their role *in vivo* is debated.

- We propose a “push–pull network" model of raphé contributions to central chemosensitivity.
- CO_2-stimulated raphé 5-HT neurons and CO_2-inhibited raphé GABA neurons synapse with "raphé chemosensory amplifier" (RCA) interneurons.
- RCA interneurons function as an amplifier to activate central rhythm generators (CRG) and/or motor neuron pools (MNP) to enhance ventilation (VE).
- 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).

Methods







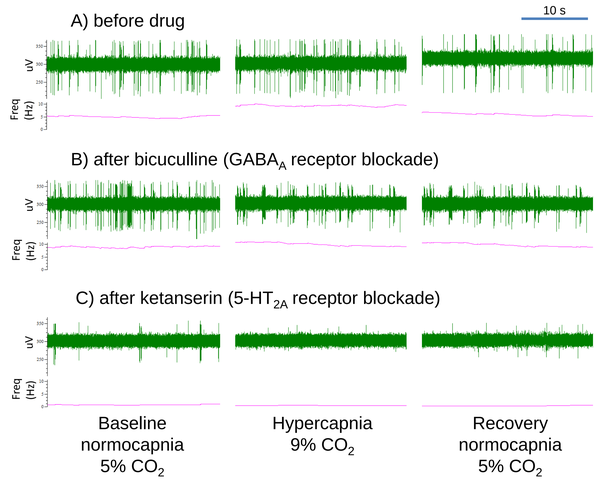
Spontaneously active neurons in the medullary raphé were recorded with sharp tungsten electrodes before, during, and after 5 minute hypercapnic challenges of the unanesthetized juvenile rat *in situ* perfused decerebrate brainstem preparation (P20-P30; 60-150g male albino rats; Paton 1996).

Neurons were recorded under normo- and hypercapnic conditions (5% and 9% CO_2 respectively). If the cell was CO_2 –stimulated, bicuculline ($GABA_A$ receptor antagonist) was applied in the perfusate, and the gas challenge was repeated. Subsequently, ketanserin (5-HT_{2A} receptor antagonist) was applied, and a final gas challenge was performed.

Activity of CO_2–stimulated rat medullary raphé RCA interneurons is mediated by intra-network inputs from serotonergic and GABAergic neurons.

Results

Representative tracings of a single spontaneously active CO_2-stimulated RCA neuron:



RCA neuron chemosensitivity is $GABA_A$ receptor-mediated and RCA excitatory drive is 5-HT_{2A} receptor-mediated

Without drug (A), the firing rate increased from 5 Hz during normocapnia (5% CO_2) to 9 Hz during hypercapnia (9% CO_2), and recovered to 5 Hz with a return to normocapnia, identifying this as a chemosensitive cell.

After bicuculline treatment (B), the baseline normocapnic firing rate increased to an average of 9 Hz. The firing rate did not change significantly with hypercapnia. $GABA_A$ receptor blockade resulted in both disinhibition and loss of chemosensitivity.

After ketanserin treatment (C), the baseline firing rate decreased significantly, and continued to decrease steadily throughout gas challenges until the average firing rate was <1 Hz.

Conclusions

- We find CO_2-stimulated RCA neurons in medullary raphé.
- Blockade of $GABA_A$ receptors abrogates RCA neuron chemosensitivity.
- Blockade of $GABA_A$ receptors also increases RCA neuron baseline firing rate, indicative of removal of tonic inhibitory input from GABA neurons.
- Blockade of 5-HT_{2A} receptors decreases RCA neuron baseline firing rate, indicative of removal of tonic excitatory input from 5-HT neurons.
- $GABA_A$ receptor input is necessary for RCA neuron chemosensitivity and 5-HT_{2A} receptor input is necessary for RCA tonic drive.
- These results support our “push-pull network” model of raphé contributions to central chemosensitivity.

References

Corcoran AE, Richerson GB, Harris MB. 2008. Both serotonergic and GABAergic neurons contribute to central chemosensitivity in a perfused rat brainstem. Soc. Neuroscience abstracts.

Paton JF. 1996. A working heart-brainstem preparation of the mouse. J Neurosci Methods 65(1):63-8.

Acknowledgements

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