Simultaneous assessment of CO₂ sensitivity in the respiratory network and its neurons

Introduction

Tadpoles exhibit bimodal breathing; a combination of buccal and lung breaths. Breathing is controlled by a central neural network that is sensitive to changes in central CO₂. The respiratory neural network can be isolated as the en bloc brainstem preparation and remains active under physiologically relevant conditions for over 48h. Thus, the isolated tadpole brainstem preparation is a powerful tool for investigating neural control of breathing in vertebrates.

Aims

To simultaneously record respiratory-related activity from cranial nerves and single neurons
To label the recorded neuron
To characterize and quantify central chemosensitivity in the brainstem

Methods

Whole-Nerve Recording
• The tadpole brainstem was isolated in a recording chamber and bathed in artificial cerebrospinal fluid (aCSF). Cranial nerve 5 or 7 was drawn into a suction electrode, which recorded whole-nerve respiratory activity.

Extracellular Recording
• A micro-electrode filled with 5% biotinamide was vertically inserted into the brainstem proximal to CN 5 or 10 or in the medullary raphe. Single-neuron activity was recorded and correlated with whole-nerve activity to determine its respiratory relatedness.

Treatments
• The brainstem is bathed in normocapnia (1.5% CO₂) or hypercapnia (5% CO₂) aCSF.

Simultaneous Recordings

• Single-neuron and whole-nerve recordings are made simultaneously in vitro
• Neuron firing that is coincident with whole-nerve activity indicates respiratory-relatedness of the cell

Figures

Chemosensitive Sites
• Single-neuron recordings were made in rostral (A & B) and caudal chemosensitive sites and the raphe (C)
• Firing of respiratory-related neurons (A & B) exhibits synchrony to lung and/or buccal bursts
• CO₂-sensitive neurons, respiratory-related and unrelated, were found in all three chemosensitive sites

CO₂ Sensitivity
• CO₂-sensitive neurons changed firing frequency and pattern in response to hypercapnia
• CO₂-induced changes included increases and decreases (see right) in frequency
• CO₂-induced changes in pattern included increases (see right) and decreases in synchronicity

Juxtapacellular Labeling

Neurons are entrained with juxtapacellum application of current pulses that induces electroporation of the cell membrane, and facilitates uptake of neurobiotin ejected from the microelectrode during the current pulse.

Tissue is fixed in 4% paraformaldehyde and then sliced into 60μm sections. Sections are stained with a streptavidin dye which binds to biotin, and imaged to visualize the recorded neuron.

References


Acknowledgements

This work was funded by the National Science Foundation 0310123-G.