Activation of Neural Projections from the Amygdala and Agranular Insular Cortex during Tracheal Obstruction in the Rat using ME-MRI

Andrea Vovk, Ph.D. (Physiological Sciences, CVM); Paul W. Davenport, Ph.D. (Physiological Sciences, College of Veterinary Med.); Gary Blaskowski (AMRIS, MBI); Arthur Edison, Ph.D. (AMRIS, MBI)

Introduction

Airway obstruction produces a distressing respiratory sensation that is common to many respiratory diseases such as emphysema and asthma. Elucidating the neural mechanisms involved in producing distress to respiratory sensations may lead to better treatment strategies for patients with respiratory diseases. Recent studies in humans and preliminary data from our laboratory have shown activation in several limbic areas to respiratory stimuli that produce distress. Of particular interest are the amygdala and the insular cortex as they both receive visceral sensory information and play and important role in generating fear, anxiety and other aversive behaviors. We have developed a model of transient tracheal obstruction in rats that elicits escape behaviors typical of the fear response. The purpose of this project was to investigate the role of the amygdala and insular cortex in generating aversive responses to distressing respiratory stimuli. Specifically, we assessed activation of the neural projections from the central nucleus of the amygdala and the agranular insular during transient tracheal obstruction in the rat. Given that the amygdala and insular cortex are highly integrated with other cortical structures and based on our preliminary data that show activations in the amygdala and the insular cortex during tracheal obstruction, we hypothesized that neural projections from the amygdala and insular cortex will be activated during tracheal obstruction, and that these neural projections will travel to other limbic structures. To accomplish this, we used a novel technique called manganese enhanced MRI (ME-MRI). Briefly, manganese enters activated neurons through voltage gated calcium channels. Accumulation of manganese in activated neural structures is then visualized using MRI.

Experimental

Tracheal Obstruction. A 3 cm tube was used to tracheostomize six male Sprague-Dawley rats and sutured into place. The airway was obstructed by placing a finger over the opening of the tube. The protocol is described below.
ME-MRI. 50 uL of 100mM MnCl2 solution was injected into the central nucleus of the amygdala in five anaesthetized rats and in the agranular insula in one anesthetized rat. The trachea was occluded for 10 breaths every minute for 5 min, and unoccluded for 5 min. This 10 min protocol was repeated for an hour, for a total of six 10 min protocols. After 1 hr of rest, the anaesthetized rat was positioned in an MRI-compatible stereotaxic head holder and placed into an 11 T magnet (Bruker Inc.). A T1-weighted 3D fast low-angle shot (FLASH) sequence was used to image the neural tracks activated from the central nucleus of the amygdala and the agranular insular cortex during tracheal obstruction (TR: 25 ms, TE: 4 ms, FA: 30, Matrix: 256 x 256, inplane resolution: 0.16 x 0.19, slice thickness: 0.156 mm). Images were analyzed using NeuroLens (Mac). Activated neural tracts and its anticipated projection site were identified using the Paxinos & Watson rat brain atlas.

Results and Discussion

We identified activated neural projections from the central nucleus of the amygdala (Fig 1). The presence of activated neural projections verifies the role of the amygdala in respiratory sensation to tracheal obstruction, thus supporting our hypothesis. The structure to which this neural track is projecting to may be the periaqueductal grey, an area previously shown to be activated by respiratory stimuli. To verify the target structure in future studies, we will increase time between stimulus presentation and imaging to allow Mn2+ to diffuse further down the activated neural tracks to its target. Projections from the agranular insula appeared to travel toward the hypothalamus.

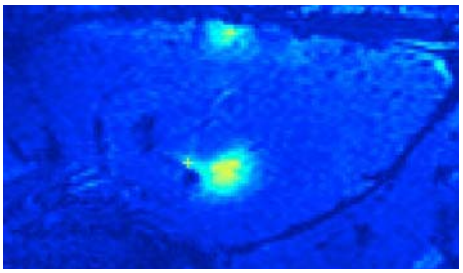

Fig 1. Sagittal view of the rat brain using a blue filter to enhance manganese contrast. An activated neural tract from the amygdala travelling caudally is shown.

Conclusions

This is a novel method of track tracing that allows for only activated neural tracts to be visualized. Traditional track tracing techniques identify all possible neural tracts and cannot distinguish between activated and non-activated tracks. Tracheal obstruction in the rat activate neurons projecting from the amygdala to other limbic structures involved in generating aversive responses to distressing respiratory sensations.

Acknowledgements

This work was supported by the National High Magnetic Field Laboratory.