Functional Imaging of Conditioned Fear in Conscious Rats

T. Garelick[1], J. King[1]

[1]Psychiatry, University of Massachusetts, Worcester, MA, United States

INTRODUCTION: Although much research has been dedicated to the characterization of fear conditioning in rodents, the vast majority of the studies have used aversive stimuli such as foot-shock rather than a fear or anxiety invoking stimulus. These studies utilized a predator odor namely 2,5-dihydro-2,4,5-trimethylthiazoline (TMT) extracted from fox feces, in a conditional fear paradigm and mapped this unique series of responses with functional MRI.

METHOD: Six young adult Sprague Dawley rats (250-350g) were conditioned using a Pavlovian conditioning paradigm to react fearfully to a discreet visual stimulus (a flashing high intensity LED). Animals were conditioned in a sound-attenuated chamber with constant airflow such that presentation of the TMT was limited only to the intended period of exposure. Three conditioning sessions (figure 1) were given to the animal over a period of three days using novel environments each day to ensure that the light, not the environment, acted as the conditional stimulus (CS). On the fourth day the animals were scanned using a 4.7 Tesla animal scanner (Bruker) with a paradigm that imitated the conditioning procedure. Control animals were given an identical procedure without the Unconditional Stimulus (CS-) for comparison. Heart rate, respiration rate, neuroendocrine markers, and behavioral markers were used to characterize and ensure the occurrence of conditioning prior to and during the imaging procedure.

Animals were placed in the imaging restrainer with 5% isoflurane anesthetic and allowed to completely regain consciousness before the imaging procedure began. Anatomical fast spin echo (FSE) images were acquired with the following parameters: TR = 2500ms, 90º flip angle, 8 echo trains, effective TE = 10 ms, matrix = 256 x 256, FOV = 2.56 cm^2, and fourteen 1.2-mm slices. Animals were subsequently imaged in a functional BOLD using an FSE sequence with the following parameters: TR = 2000ms (90º flip angle), 8 echo trains, effective TE = 7 ms, matrix = 64 x 64, FOV = 2.56x 2.56 cm, and fourteen 1.2-mm slices. fMRI Image analysis employed STIMULATE software (Strupp, 1996) to map event-related signal intensity changes in conservative ROIs based on the anatomical maps.

RESULTS: Heart rate and respiration rate data taken during the functional scan were compared to the functional time-courses of the BOLD intensities in each group. These time-courses have demonstrated that during the periods of light presentation, heart rates and respiration rates went up nearly 20% from baseline periods. Functional maps obtained from the BOLD analysis also suggested a broader and more intense neurological response in conditioned animals, especially in the amygdala, the hippocampus, and the frontal association cortex.

DISCUSSION: Our results suggest that TMT can support associative Pavlovian conditioning to a discrete stimulus such as a flashing light, and the utilization of predator odor in functional studies does provide a unique opportunity to assess the central features of conditioned fear to an ethologically relevant cue. Additionally, functional imaging of BOLD changes represents an effective method to elucidate specific mechanisms involved in cognitive and emotional aspects of the fear response. Taken together, the design to utilize TMT as a fear eliciting odor for functional imaging of the fear response has proven to be a highly effective methodology to explore conditioned fear.

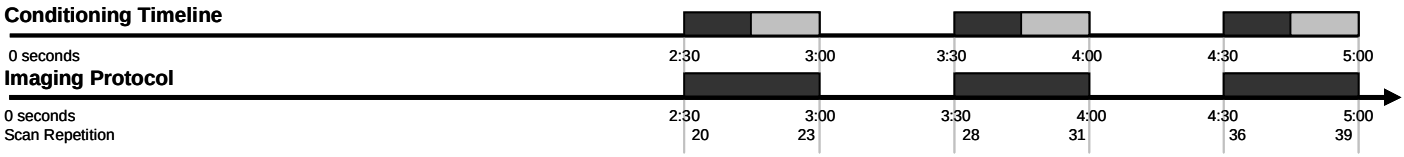

Figure 1: Animals were placed in the conditioning environment for five minutes. During the first two minutes and thirty seconds the animal was allowed to explore the environment and become accustomed to it. At 2:30, 3:30, and 4:30, the animals were presented with a flashing light for thirty seconds. During the latter fifteen seconds, TMT was pumped into the environment to pair it with the light. In the diagram above, the dark grey boxes represent the periods when the light is presented, while the light grey boxes show the times that the light and the fox scent were both presented.

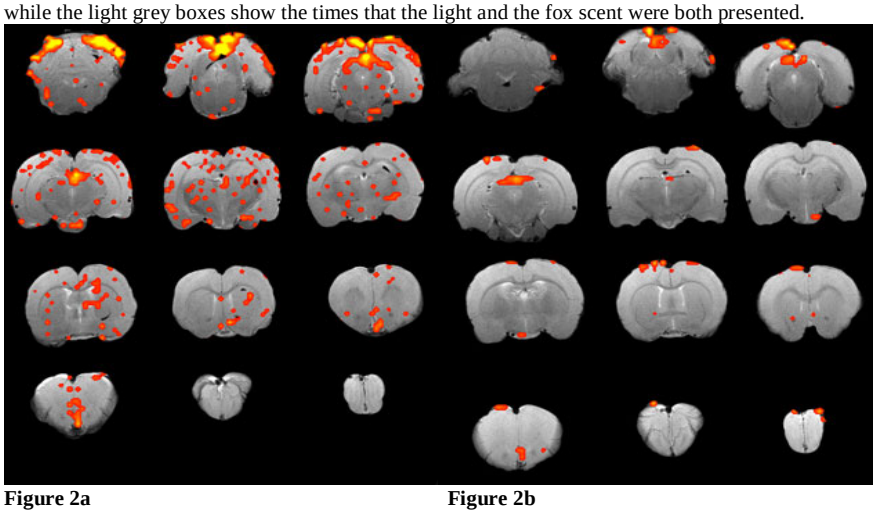

Figure 2a **Figure 2b**

Functional activation maps of BOLD intensity changes related to presentation of a light were overlaid on anatomical images. Figure 2a represents BOLD activation of a rat conditioned to fear the light while figure 2b represents an unconditioned control.

References:

1. Strupp JP. Stimulate: a GUI based FMRI analysis software package. Neuroimage., 1996; 3: S607