Neural Basis of Unconditional Fear: Perfusion and Functional Imaging in Aging Rat Bed Nucleus of the Stria Terminalis

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Introduction

Fear, stress and anxiety form a trinity that can result in depression, disease and even death. To understand the neuronal, emotional and cognitive components of fear which underlies anxiety disorders, researchers have employed several methods, most directed at exploring the processes associated with fear conditioning. Although much of the progress has come from studies on fear conditioning, unconditioned fear remains relatively under-explored. Some authors argued that the Bed Nucleus of the Stria Terminalis (BNST), and not the amygdala, is responsible for the unconditioned emotional changes induced by corticotropin-releasing factor (CRF)[1]. The BNST is preferentially involved in mediating behavioral responses to unconditioned stress [2, 3]. The current study was designed to use novel perfusion and functional imaging techniques to explore the role of the BNST in the unconditional fear during aging. We hypothesize that any changes in BOLD fMRI signal between younger and older individuals in BNST may be due to vascular changes in normal aging.

Methods

Magnetic resonance imaging was performed on a Bruker 4.7 T/40 cm horizontal magnet (Billerica, MA) and a 20 G/ cm magnetic field gradient insert. The Perfusion study was conducted on four young (3-6 months) and four aged (18-24 months) Fischer 344 rats(300-400g) to elucidate the differences in resting cerebral blood flow (CBF)of normal aging. An actively decoupled surface coil (2.3 cm i.d.) was used for brain imaging and a neck coil [4] for perfusion labeling. Coil-to-coil electromagnetic interaction was actively decoupled. All images were acquired with a 2.56×2.56 cm^2 field of view and eight 1.5 mm slices. Anatomical images were acquired using the fast spin-echo pulse sequence (RARE) with TR = 2500 ms (90◦ flip angle), eight echo trains, effective TE = 10 ms, matrix = 128×128. CBF measurements were made using the continuous arterial spin-labeling technique [4] with four -segmented gradient-echo, echo-planar-imaging (EPI) acquisition. Paired images were acquired alternately—one with arterial spin-labeling and the other without spin-labeling (control). The EPI parameters were: data matrix = 128×128, TE = 14.4 ms, and TR=2s (90◦ flip angle).

The fMRI study was performed in the same age young and old rats as the perfusion study. A dual coil system consisting of a volume coil as transmitter and surface coil as receiver was used for awake rats' imaging. The coils were built into a composite unit that included a head and body restrainer used for imaging conscious animals (Insight Neuroimaging Systems, Worcester, MA). All images were acquired with a 3.0×3.0 cm^2 field of view and twelve 1.2 mm slices. High resolution multislice anatomical data sets were acquired (Fast spin echo, TR = 2.0s, effective TE =12ms, Matrix = 256 x 256.) at the beginning and end of each imaging session. Subtraction of these data sets confirmed there was no significant movement of the animal over the imaging session. The functional scan used Spin Echo EPI with a 64 x 64 matrix, TR=2s, TE=55ms, Number of Repetitions = 301 consisted of a two minute baseline followed by three minutes exposure to a predator odor TMT, 5-dihydro-2,4,5-trimethylthiazoline, extracted from fox feces/urine as an olfactory fear-eliciting cue in these studies to elicit unconditional fear. Finally, five additional minutes of data collection followed the intervention period to fully characterize the response to this stressful condition.

CBF measurements were analyzed using the imaging processing programs Matlab (math-Works, Natick, MA, USA) and STIMULATE software. BOLD activation maps were generated using a statistical threshold of $P < 0.05$. AFNI software was used to register, segment and analyze the data. Two-tail unpaired t -test was used to compare the parametric values.

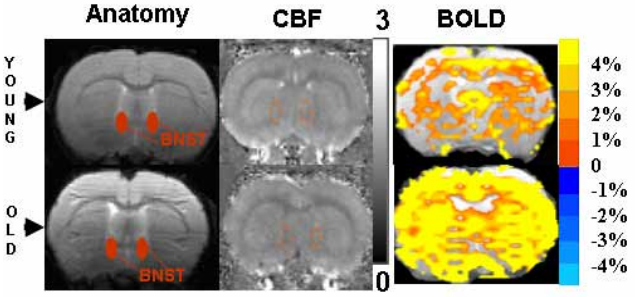

Figure 1

Results

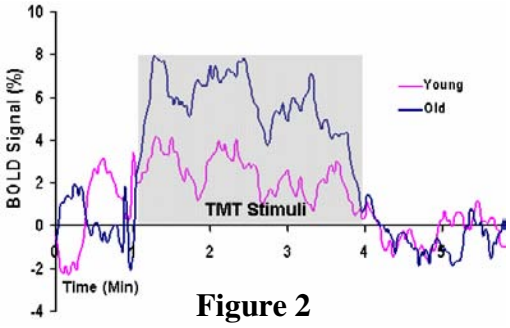

Figure 2

The results show the BNST is high active in response to the predator odor stimulus, and there are significant BOLD signal differences between the young and old rats ($P<0.05$). Meanwhile, the CBF values of young and old rats in the BNST were 1.6 ± 0.1 and 1.1 ± 0.1 ml/g/min, respectively. The CBF comparison between the young and old rats also shows significant differences ($p<0.01$). Figure 1 shows representative CBF and BOLD maps in the BNST of young and old rats. Figure 2 shows representative time course in responding to TMT in the BNST of awake young and old rats.

Discussion

This study demonstrates that it is feasible to address neural basis of unconditional fear using perfusion and spin echo EPI sequences. In addition aging effects on neurovascular coupling is also possible with this technique. Future work will perform simultaneous BOLD and CBF measurement to address these challenge.

References:

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