Manganese-Dependent Contrast Detection of Brain Activation in Response to Emotional Stimuli

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Introduction

Manganese is a calcium analog and an MR contrast agent. Manganese-dependent contrast has been used to non-invasively visualize anatomical details (Paulter MRM 1998, 2003) as well as calcium-dependent neural activities (Lin 1997, Duong 2000, Aoki 2002) at reasonably high spatiotemporal resolution. One unique property of Mn^{++} is that once it is internalized it is trapped intracellularly facilitating paradigms that can be designed under awake conditions and performed outside the magnet prior to fMRI techniques. However, functional maps obtained with Mn^{2+} can be confounded by basal neuronal activity and context dependant cues. This study was designed to examine the usefulness of this technique to explore the holistic response to a highly relevant emotional stimulus in an awake animal model. Since olfactory cues are particularly salient to animals we utilized two odors, one novel/neutral and the other novel/fear inducing as control and emotionally relevant stimuli, respectively, to explore the feasibility of using manganese- dependent contrast to visualize emotional processes. We hypothesize that adequate controls may facilitate the extraction of the relevant stimulus-specific responses,

Methods

Male Sprague-Dawley rats (300-400g) were anesthetized with 2%-isoflurane for surgery. Polyethylene catheters (PE-50) were placed in the femoral vein and the right common carotid artery (CCA). Mannitol could later be administered from the severed CCA toward the internal carotid artery (ICA) and the brain but not toward the heart. After surgery, isoflurane was discontinued and the animals were returned to cage awake. Rats were infused in the femoral vein with 120mM $MnCl_2$ at a rate of 2 ml/hr for a total of 30 minutes. Ten minutes after starting the infusion, a bolus of 20% mannitol (at 4 ^oC, dissolved in 0.1M PBS, pH 7.4) was given into the carotid artery at a concentration of 5 ml/Kg. Mannitol was injected at a constant rate over one minute to break the blood brain barrier (BBB). One minute after the mannitol injection, rats were presented with either fox scent (fear) or lemon scent (control) until the end of the infusion time.

After the infusion of $MnCl_2$, rats were secured in a MR compatible restrainer (Insight Neuroimaging Systems, Worcester, MA). All images were acquired using a 4.7T/40-cm horizontal magnet interfaced to a Paravision console (Bruker Medical Instruments, Billerica, MA). T1-weighted imaging was completed 45-50 minutes after the $MnCl_2$ infusion ended. T1-weighted (T1W) images were acquired using a gradient echo sequence (TR=300ms; TE=4.2ms; FOV=2.5x2.5cm; matrix=256x256; slice thickness=1.0mm; number of slices=18; averages=12).

STIMULATE software was used to determine the average signal intensity in various regions of interest (ROIs). In order to control for individual variation between rats, the values for each ROI were normalized to the left hemisphere, which served as a control since the BBB was disrupted mostly in the right hemisphere.

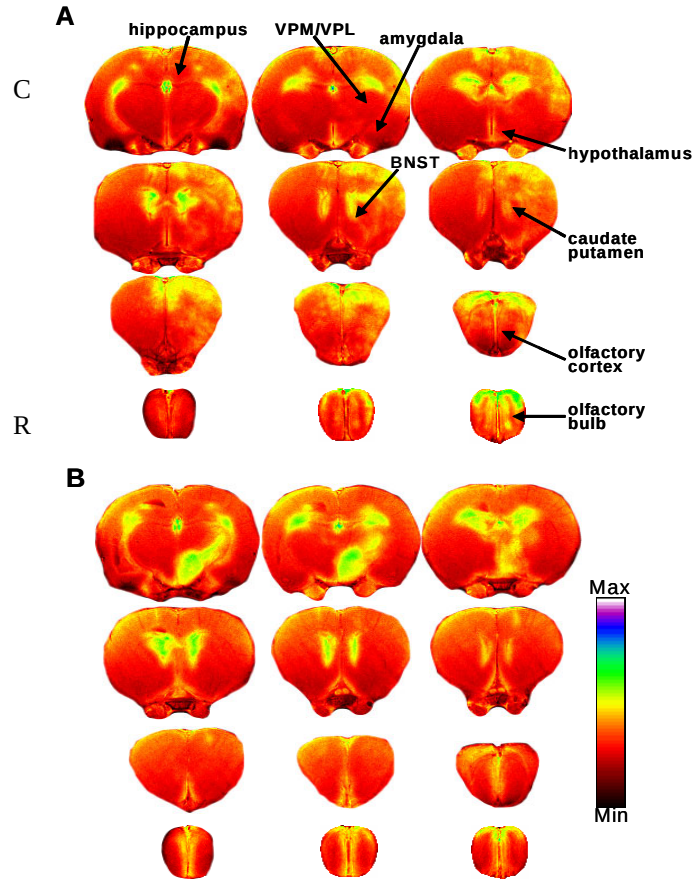

Figure 1. (A) Lemon scent activation and (B) Fox scent activation. Slices are consecutive and are labeled to indicate the most caudal (c) and rostral (r). VPM/VPL – ventral posterolateral/posteromedial thalamus, BNST – bed nucleus of stria terminalis.

Results

Figure 1A shows T1W images throughout the brain in a representative animal exposed to lemon scent along with the ROIs of interest. Figure 1B depicts T1W images throughout the brain in a representative animal exposed to fox scent. Areas in which the signal intensity increased significantly are shown in color. Comparisons between the control scent and the fox scent on the side ipsilateral to the infusion show significant signal differences in the hypothalamus and amygdala (Figure 2).

Discussion

These results indicate that lemon odor resulted in neuronal activation in the classical neuronal circuitry attributed to odor processing and novelty. Significant amygdala and hypothalamic activation in the presence of fox odor (fear–eliciting scent) corresponded to a response with high emotional valence. Therefore, the use of manganese-dependent contrast, in awake subjects, has the potential to delineate structures associated with emotional processes when highly relevant controls and reproducible context parameters are employed.

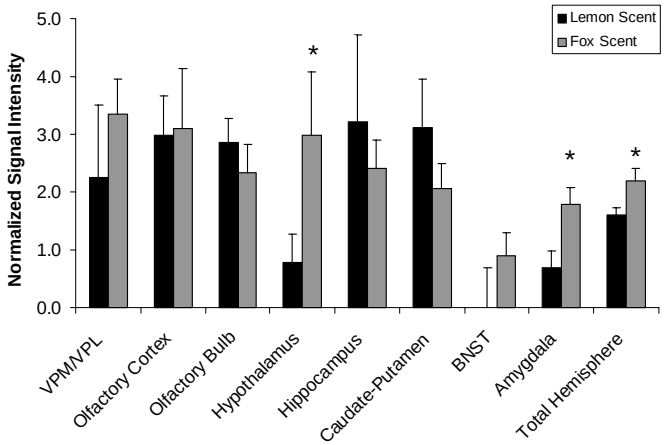

Figure 2. Normalized signal intensity (mean ± SEM, * $p<0.05$). VPM/VPL - ventral posterolateral/posteromedial thalamus, BNST – bed nucleus of stria terminalis.