Functional MRI of the Olfactory System in Awake and Anesthetized Dogs

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Introduction

While much is known about the canine olfactory system at the cellular and behavioral levels, little work has been done at the cognitive level, which is an important and largely unexplored link in the odorant detection network of dogs. On the other hand, functional MRI (fMRI) of the olfactory system has been performed in humans and other animals such as monkeys [1-3]. In dogs, previous imaging has largely been on audio or visual processing [4]. Canines are used extensively for detecting odors in both civilian and national security contexts. Therefore, in order to bridge the knowledge gap of the dog olfactory system at a cognitive level, we developed and tested an experimental setup for controlled delivery of odorant stimulus to both awake and lightly anesthetized dogs. We have demonstrated the feasibility of obtaining functional MRI data from the brains of lightly anesthetized and awake dogs receiving odorant stimulus. Further, we compared the effects of low and high concentrations of odorant and the effect of anesthesia.

Methods

T2*-weighted functional images were acquired using a single-shot gradient-recalled echo-planar imaging (EPI) sequence for BOLD contrast on a Siemens 3T scanner. 18 axial slices of 3 mm thickness, and 200 volumes were acquired using the following parameters: TR=1000 ms, TE=29 ms, FOV=220 mm, FA=90°, in-plane resolution 3×3 mm^2, and in-plane matrix 64×64. One dog in awake state was scanned twice two weeks apart, each including 4 independent runs, and 5 anethetized dogs were scanned with 4 runs for each. Dogs were sedated with xylazine (1.1 mg/kg, im.) and lightly anesthetized with ketamine HCL (11mg/kg,im.). For awake imaging, dogs were trained to move to the correct position within the scanner, lie down, insert and correctly position their heads within a human knee coil, and remain still for the required duration of imaging using positive reinforcement behavior shaping procedures. A conditioned positive reinforcer (clicker) and a target stick repertoire were established to shape the desired responses using a mock-up of the coil and scanner table while concurrently desensitizing the dogs to noises associated with imaging using recordings of scanner operations. Each run consisted of 5 blocked trials of 10s duration, with a fixed interval of 30s between the end of one block and the start of the next. The anatomical images were acquired with MPRAGE for overlay and localization. The odorant was ethyl butyrate, eugenol, and (+) and (–) carvone in water with concentrations of 0.16 mM and 0.016 mM each. Head space vapor mixtures of these odorants were delivered by a computer controlled device. An interlinked trigger system ensured timing synchrony among the fMRI, and odorant delivery. The functional MRI data obtained from each of the dogs was corrected for motion using rigid-body realignment, resliced, normalized to the anatomical of one of the dogs, spatially smoothed, detrended and input into a general linear model (GLM). Time and dispersion derivatives were included in the design matrix in order to account for the variability of HRF. In order to find the brain regions modulated by the odorant concentration, we included parametric modulators in the GLM.

Results and Discussion

Fig.1 and Fig.2 illustrate the regions showing more activity at high concentration as compared to low concentration of odorant in anesthetized and awake dogs, respectively. For the first time to our knowledge, fMRI of olfactory system was performed with anesthetized and awake dogs. Comparison of activation maps of sedated and conscious dogs shows dramatic differences. The activity of the awake dog is primary concentrated in olfactory bulb and a part of frontal cortex, while the anesthetized dogs show responses in structures such as piriform cortex, hypothalamic nucleus, and midbrain tegmentum. A significant difference in the neural response of anesthetized and awake dogs is consistent with electrophysiological results on activity of the olfactory bulb mitral cells obtained for anesthetized and awake rodents [5]. They demonstrated that odor-elicited changes in mitral cell firing rate were larger and more frequently observed in the anesthetized than in the awake condition. The intensity of odor perception I, as a function of odorant concentration C, can be described by the Weber-Fechner law: $I=a\times lnC+b$, where I is the perceived psychological intensity, a is the Weber-Fechner coefficient, and b is a constant. The experimental values of a and b measured for many odorants are approximately equal to 1.3 and 0.5, respectively. We previously demonstrated that a 10-fold increase in odorant concentration resulted in 2.5-fold increase of the rodent electro-olfactogram (EOG) signal [6]. If we assume that amplitudes of EOG signals elicited by various odorants *in vitro* correlate with perception of odor intensity [7], the change in perception due to a 10-fold increase in odorant concentration is equal to $1.3\times ln(10)\approx 3$. This expected increase in odor perception is in agreement with the parametric increase of fMRI activation observed in this work.

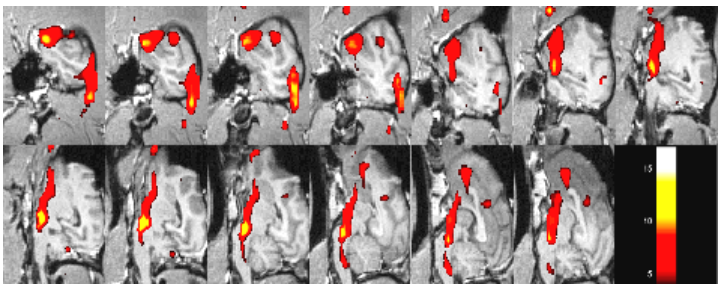

Figure 1.Sequential sagittal slices showing group activation for anesthetized dogs. Activated regions had significantly higher response for high concentration (0.016mmol) as compared to low concentration (0.016mM) of odorants.

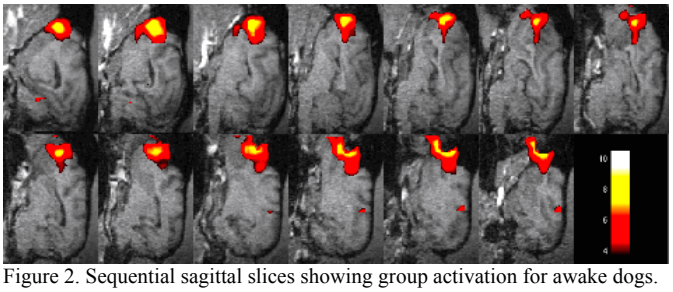

Figure 2. Sequential sagittal slices showing group activation for awake dogs. Activated regions had significantly higher response for high concentration (0.016mmol) as compared to low concentration (0.016mM) of odorants.

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