# Orientation maps in ferret visual cortex measured by multi-slice fMRI

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### Introduction

High-resolution fMRI conducted in single slice in cats has revealed orientation maps that co-register tightly with maps observed by optical imaging [1,2]. The use of single slice, however, limits the ability to observe maps in three-dimension. Ferret is a useful animal model for studying neural plasticity, particularly interactions between neural feature maps [3,4]. Here we use multi-slice fMRI to acquire visual orientation maps in ferrets for the first time, and study the distribution of orientation-specific responses across the layers of cortical gray matter.

#### Methods

Animal preparation. Adult ferret (male, 1.2-1.5 kg) was intubated and artificially respired, and monitored for expired  $CO_2$  level (caponography) and rectal temperature. Anesthesia was maintained by IV-administered sufentanil and low level (typical < 0.6%) of isoflurane, and ferret was paralyzed with vecuronium (0.25mg/kg/hr). Ferret was placed in a custom-designed cradle with earbars and nosebar for stabilization. Visual stimuli were projected to an opaque screen anterior to the ferret (distance to eyes: 8 cm; spanned ~ 60° visual angle). After each imaging session (~ 5 hours), anesthesia was reversed and animal was closely monitored until complete recovery.

*MRI protocols.* A coil pocket made of dental cement was glued to the skull dorsal to visual cortex in a minor surgery. Ferrets were allowed for recovery from the surgery before the first imaging sessions were conducted. The coil pocket, inserted with a small tunable surface coil (14 mm diameter) during imaging, ensured maximal coverage of visual cortex and consistent coil intensity pattern. The small coil, in return, ensured high SNR and minimal field of view (and thus high spatial resolution). We acquired 3-4 trans-axial slices (FOV 15 x 15 mm, 2-shot, effective resolution 250 x 250 µm, thickness 500 µm) or sagittal slices (FOV 13.2 x 9.6 mm, 1-shot, eff. resolution 240 x 200 µm, thickness 800 µm) using gradient-echo EPI sequences (TE 18 ms, TR 1500-2000 ms). RARE (spin-echo) anatomy images were acquired at identical slice locations.

*Stimuli and data analysis.* We used repeated cycles of phase-encoded stimuli to map orientation; each cycle (60 s) comprised moving gratings (0.1 cpd, 2 Hz) that changed orientation at 3°/s. Using temporal Fourier analysis, the stimulus-correlated signal component (60 s/cycle) was isolated and its temporal phase encoded the orientation that elicited maximal BOLD response. This orientation was rendered in pseudocolor maps (Fig.1).

## Results

Figure 1 shows the orientation maps measured in axial-like slices parallel to the cortical surface near V1. In the cortical regions near V1, these maps are qualitatively similar to maps observed by optical imaging in previous studies [3,4]. Quantitative analysis (not shown) suggest that the maps are similar in both anisotropy and the pinwheel patterns. Both split-half test and time series scrambling showed that the maps are statistically significant. In separate sessions using sagittal slices orthogonal to the cortical surface, we measured both the orientation-specific response and the general visual "on/off" response. The later was measured by comparing all the oriented gratings ("on") against a blank screen ("off") in a block design, and represents the sum of orientation-specific and non-specific responses. Figure 2 shows the laminar profiles for these two types of responses. The total "on/off" responses is stronger toward superficial layers, in agreement with previous studies [5]. In contrast, the orientation-specific response is relatively uniform across the gray matter depths.





Figure 1. In axial-like slices parallel to cortical surface of V1, visual stimulus orientation that elicits maximum BOLD response is measured voxel-by-voxel and summarized into pseudocolor orientation maps ( $0^{\circ}$  –180°, see legend in up-right corner). Dashed black lines demarcate the regions estimated to be near V1, based on anatomical landmarks.

Figure 2. Laminar profiles, i.e., BOLD amplitudes as functions of cortical depth, are shown for responses to all stimuli ("on") compared against blank ("off"), and orientation-specific responses.

#### Discussion

We have shown that multi-slice fMRI (gradient-echo BOLD, without contrast agent) can measure the visual orientation maps in anesthetized ferret at different cortical depths. The pinwheel features and spatial pattern in these maps are similar to optical imaging results. Whereas the orientation-specific responses are relatively uniform in amplitude across cortical layers, the non-specific visual responses are stronger near superficial layers. Previous research suggested that the laminar bias in on/off visual responses arises from the draining artifacts of large blood vessels near the pia [5]. Here, the absence of laminar bias in orientation-specific responses suggests that the large blood vessels and their draining artifacts contribute little to the orientation-specific responses and the orientation maps.

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