BOLD-fMRI study of Effect of Dark-rearing on Postnatal Visual Development

J. S. Cheng[1,2], K. C. Chan[1,2], I. Y. Zhou[1,2], M. M. Cheung[1,2], C. Lau[1,2], and E. X. Wu[1,2]

[1]Laboratory of Biomedical Imaging and Signal Processing, The University of Hong Kong, Hong Kong SAR, China, People's Republic of, [2]Department of Electrial and Electronic Engineering, The University of Hong Kong, Hong Kong SAR, China, People's Republic of

INTRODUCTION: Environmental inputs play a significant role in visual system development. However, current studies on the effects of visual input deprivation (dark-rearing) mainly use histological, electrophysiological, and optical imaging techniques [1-3]. Histology can only be examined *ex vivo* and electrical recordings sample a limited portion of the brain. Optical imaging offers high spatial resolution but limited depth penetration. Blood oxygenation level-dependent fMRI (BOLD-fMRI) is a non-invasive functional imaging technique that offers large field of view and depth penetration, which makes it suitable for studying the effects of dark-rearing on visual system development. In this study, we compare the BOLD responses in visual cortex (VC), superior colliculus (SC), and lateral geniculate nucleus (LGN) in normally reared and dark-reared rats following visual stimulation.

MATERIALS AND METHODS: Animal Preparation: 14 Sprague-Dawley rats were employed and divided into 2 groups. For the dark-reared group (DR, n=7), a pregnant mother rat was kept in a dark cage 2 days before parturition. The seven newborns were then kept in the same dark cage from birth for 6 weeks. For the normal-rearing group (Norm, n=7), 7 neonatal rats were kept in regular 12 hour/12 hour light/dark cycle for 6 weeks from birth. Newborns were weaned from their mothers at postnatal day (P) 21. **MRI Protocol:** All MRI measurements were acquired in a 7T Bruker scanner. The animals inhaled isoflurane anesthesia (3% induction and 1.5% maintenance) and were kept warm with circulating water at 37°C. BOLD functional MR images were acquired with a single-shot gradient-echo echo planar imaging (GE-EPI), with FOV=32 ×32 mm^2, matrix resolution= 64 ×64, slice thickness=1.0mm, number of slice =6 and TR/TE=1000/20ms. A T2W RARE image was acquired with the same geometry plan as GE-EPI, with TR/TE = 4200/12ms and matrix resolution= 256 ×256. **Stimulation Protocol:** Two optic fibers with green LEDs at the distal end were placed 0.5 cm in front of both eyes of the rat. A standard boxcar paradigm with a 40s off period followed by four on/off cycles (20s on/40s off) was used. During the 'on' period, the LEDs were flashed at 1 Hz with duration of 50ms. Three to five sets of data were acquired for each rat. **Data Analysis:** All GE-EPI data were co-registered. Model-driven cross-correlation analysis was performed using STIMULATE software with a correlation threshold of 0.15 defining active voxels. ROIs were drawn based on the rat atlas[4]. Data was presented as mean ± standard error of mean.

RESULTS: Typical BOLD activation maps are shown in Fig.1. For the normal group, SC and LGN showed higher correlation coefficient than VC, which is consistent with previous studies [5, 6]. This is also true for the DR group. In Fig.2, SC exhibited a stronger BOLD signal change compared to LGN and VC by 0.14%±0.06% and 0.42%±0.08% respectively in the normal group, and by 0.10%±0.04% and 0.62%±0.04% respectively in the DR group. The BOLD signal changes in SC and LGN showed no significant difference between the DR and normal groups ($p>0.05$), while the BOLD percentage change in VC was significantly smaller in the DR group than the normal group ($p<0.05$).

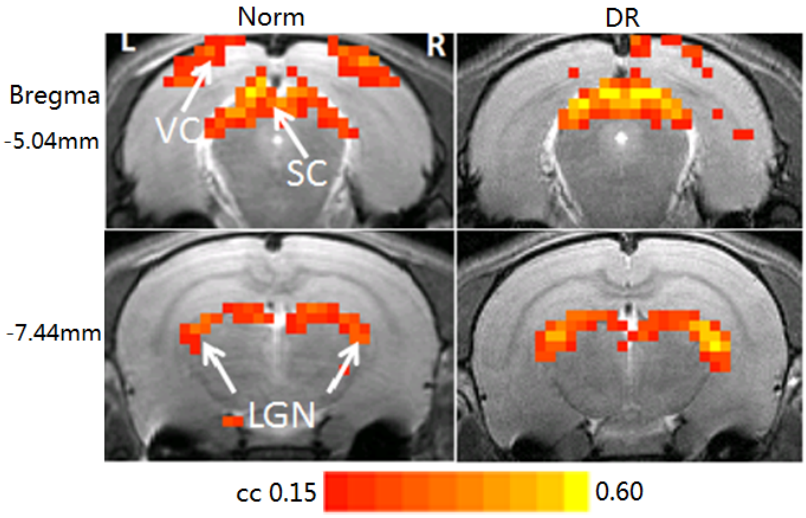

Fig. 1: Typical BOLD activation maps overlaid on T2W RARE images of both normally-reared (Norm) and dark-reared (DR) groups. Color bar indicates the cross-correlation coefficients of the activated voxels.

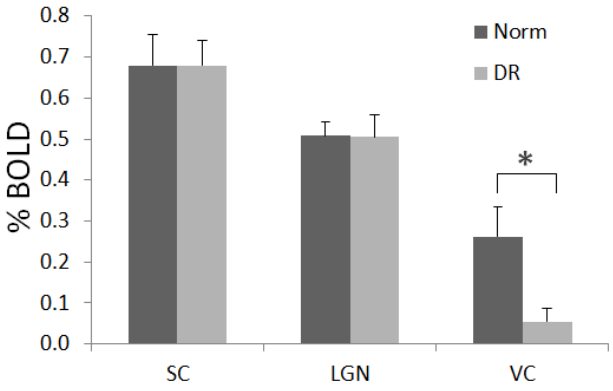

Fig. 2: Comparison of BOLD signal change (% BOLD) in the SC, LGN and VC in both groups. (Two-tailed unpaired t-test: *$p<0.05$)

DISCUSSIONS AND CONCLUSION: The result of this study demonstrated for the first time an in vivo approach for simultaneously assessing the functional developmental changes in rat cortical and subcortical visual system, and found that the VC's BOLD response is reduced by dark-rearing ($p<0.05$) while that of the subcortical visual nuclei (SC and LGN) are less affected. This might be related to electrophysiological findings in DR rats which showed fewer cells in the VC were excited by visual stimuli and response elicited by flashes were generally weaker [1]. It has also been found histologically that there are less vessels in the VC of DR rats than normal rats [3]. In the subcortical visual system, previous histological studies showed mixed results on the impact of dark rearing on the morphological development in rats. Some found a significant reduction in the number of neurons and synapses in SC/LGN of the DR rats [7, 8], while others observed no changes in either densities of neurons and synapses in SC at P30 [9] or optic nerve fiber counts [10]. Our study did not find significant differences in BOLD signal changes between the DR and normal groups in SC and LGN. This may due to insufficient dark-rearing time since some previous study claimed to find a significant reduction of numerical density of neurons in SC at P65[9]. Further improvement can be made by prolonging the dark-rearing time.

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