Modulating Activity in the Olfactory Bulb Leads to Reversible Changes in Size and Alter Migration of New Neurons

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Purpose: It is known that olfactory blub (OB) plasticity is extended throughout life and is closely associated with on-going neurogenesis. One of main players in this process is the production of neural progenitor cells (NPCs) in the subventricular zone (SVZ) niche.[1] These NPCs migrate from the SVZ through the rostral migratory stream (RMS) to the olfactory bulb (OB), where they differentiate into various types of interneurons. The migrating cells play an important role in maintaining neuronal homeostasis in the OB. Various techniques based on optical and electron microscopy have been used to study cell migration and neuronal turnover in OB. However, none of these techniques enable longitudinal study of the whole brain within the living subject. The purpose of this work is to apply MRI as a complementary tool to study plasticity and new neuronal turnover. We utilized in situ MRI labeling of neural progenitors with micron-sized iron oxide particles (MPIO)[2] in combination with MRI volumetry to address the question of whether change in olfactory sensory level alter migration speed and pattern of NPC integration in OB in relation to changes in OB size in response to activity deprivation and recovery.

Methods: To deprive olfactory activity, 3-week old Sprague-Dawley rats were subjected to reversible unilateral naris occlusion.[3] After 3 weeks, the animals were stereotactically injected with 20 µL MPIOs (average diameter of 1.63 µm, Bangs Laboratories, IN Fishers) into lateral ventricle near SVZ ipsilateral to the side of the occluded naris. Another group of animal underwent a reopening of naris plug, allowing recovery of olfactory activity, 7 days prior to MPIO injection. Serial MRI data was acquired on an 11.7 T animal MRI system (30 cm 11.7 T horizontal magnet, Magnex Scientific, Oxford, England, MRI Electronics, Bruker Biospin, Billerica, MA, and 12 cm integrated gradient shim system (Resonance Research Inc, Billerica, MA) using a custom built volume transmit coil and a custom built, 2.5 cm diameter, receive-only surface-coil. Flash 3D gradient echo sequences were used for all MRI acquisitions with the following parameters: FOV 1.92 cm^2, matrix size 256^3 (75 µm isotropic resolution), 12.5 kHz bandwidth, TE 8 ms, and TR 25 ms. MRI was performed at 4-6 and 22-24 hour post-injection. The migration rate was calculated by measuring the distance that cells travel in a given period between the first and second MRI session. OB volumes were caluculated from manually drawn serial VOIs using MIPAV(www.mipav.cit.nih.gov) program.

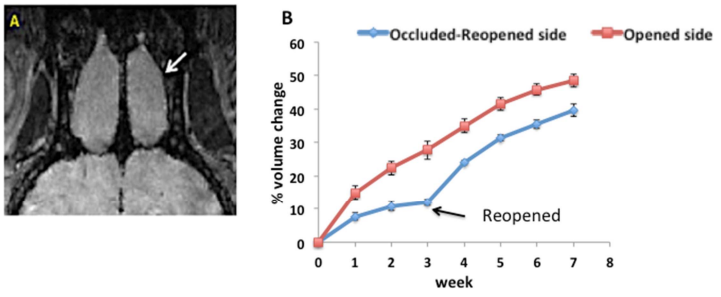

Figure 1. (A) Coronal view of MR image of the OB after 3 weeks of unilateral naris occlusion. Arrow indicate smaller right OB in comparison to the left OB. (B) plot of growth rate of the OB during naris occlusion and after reopening of the naris plugs (n =5). Arrow indicates when the plugs were removed.

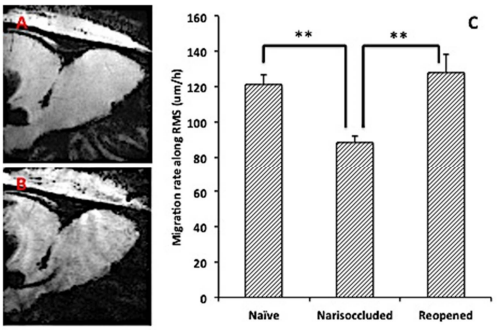

Figure 2. MRI of OB with NPCs seen migrating from SVZ into OB at different time point (A) 4 h and (B) 22 h after MPIO injection. Panel D show migration rates of NPCs groups of animals (n=5)

Results: Figure 1 shows 25% reduction of OB volume after 3 weeks of unilateral naris occlusion as expected. Upon reopening, OB regains its size with exponential growth rate within 1-2 weeks after reopening before resuming normal growth rate. As seen in figure 2, following the injection of MPIO, labeled cells are detectable as dark spot due to signal loss in T_2*-weighted images. Within 4 hours post injection (Fig 2A), MPIOs could be detected with MRI in the early RMS and then migrated to the OB within 20-24 h (Fig 2B). Figure 3A-B show radially migration into outer layers of OB. Immohistorchemistry indicated that MPIOs (green) were found inside neural precursors (Fig 3C) and interneurons (Fig 3D). Increase of hypointense pixels within the OB were monitored serially by MRI, and shown in panel 3E as function of time. The reopening of the naris occlusion led to significant increase of new cell addition into OB.

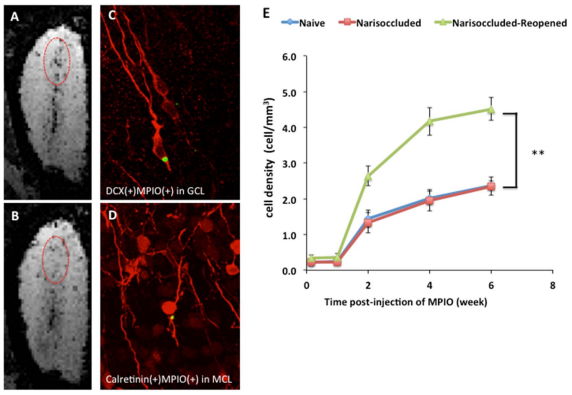

Figure 3 MRI of the same animal showing cells (hypointese spot) at different time point (A) in middle layer at 2 weeks post-injection and (B) outer layer of OB at 4 week post-injection. Panels C-D provides representative images of MPIO inside the cells. Panel E show dynamic increase of cell integration into outer layer of OB

Discussion: It has been shown naris occlusion leads to significant loss of interneurons and survival of precursor cells in OB due to lack of sensory input.[3] Here, using in situ MRI labeling, we have shown that there was a 30 % decrease of the speed of cell migrating into the OB in naris-occluded rats. This decrease in speed could be recovered after reopening the naris. It is possible that reduction of activity could suppress cellular turnover to such extent that results in slower rate of precursor cell migration. Using high resolution MRI, we observed that there was no difference in cell integration among the control and narisoccluded animals suggesting the controlled balance of OB cellular homeostasis and plasticity. Interestingly, the reopening of occlusion led to ~ 100% increase of new cell integration in OB. This could be partially attributed to increase of survival of NPCs due to a sudden recovery of sensory input.

Conclusion: We have demonstrated that the OB is highly plastic and show reversible response to olfactory activity level. Furthermore, we have determined the rates of NPCs migration under influences of different circumstances in correlation with activity level that the OB received. High resolution MRI also provides a powerful tool to longitudinally investigate the fate of NPCs integration within the same living animal subjects.

References: [1] Lledo P. M. et al., Trends Neurosci 31:392-400 (2008) [2] Sumner J. P. et al., *Neuroimage* 44: 671-678 (2009) [3] Cummings D. et al., J Neurosci. 19: 7433-7440 (1997).