Adult Neurogenesis and Olfactory Activity Regulate Olfactory Bulb Volume

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Introduction: Olfactory blub (OB), a central organ that plays important role in processing olfactory information, displays remarkable plasticity that is associated with an on-going neurogenesis from cells that are derieved from the subventricular zone (SVZ).[1] OB volume has been shown to display propensity to change in response to olfactory sensory level. However, SVZ neurogenesis and olfactory activity regulate OB volume and the interaction among them remains unclear. Here, we utilized in situ MRI labeling of neural progenitors with micron-sized iron oxide particles (MPIO)[2] in combination with MRI volumetry to elucidate interplaying of olfactory activity level and SVZ neurogenesis on the OB volume.

Methods: To control olfactory activity, 3-week old Sprague-Dawley rats were subjected to reversible unilateral naris occlusion.[3] MRI images of the OB were acquired weekly following occlusion. In separate set of animals, after 3 weeks of occlusion, 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 animals underwent a reopening of the naris plug, allowing recovery of olfactory activity, two days prior to MPIO injection. MPIO injection allows a portion of migrating NPCs to be labeled near the SVZ and then migrating into OB. Serial MRI was used to monitor the appearance of hypo-intense spots, which were caused by MPIO-containing cells migrate into OB. To manipulate SVZ neurogenesis, we utilized transgenic rat model that express thymidine kinase under the GFAP promoter (GFAP-TK). Production of new neurons in SVZ was ablated under treatment of animals with ganciclovir (GCV) that can selectively kill proliferating GFAP (+) cells.[4] Narisocclusion was performed on both transgenic (TK) and wild-type rats (WT). After 3 week of occlusion, the nose plugs were removed from animals to allow activity to return to normal state. All MRI experiments were performed on 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. OB volumes were caluculated from manually drawn serial VOIs using MIPAV (www.mipav.cit.nih.gov) program.

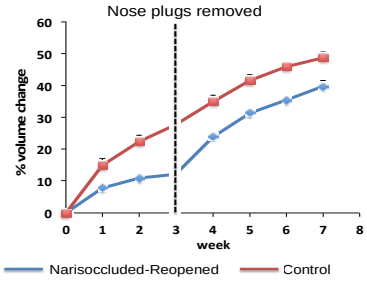

Figure 1. Growth profile of OB during narisocclusion (from week 1 to week 3), and reopened of the occlusion (from week 3 to week 7).

Results: Figure 1 shows changes of OB size changes during and after 3 weeks of unilateral narisocclusion. Narisocclusion causes the bulb to stop its normal growth rate. Upon reopening, OB regains its size with rapid growth rate within 1-2 weeks, before resuming a normal growth rate. Figure 2A-B shows migration of MPIO-containing NPCs into the outer layers of OB. Immohistorchemistry indicated that MPIOs (green) were, indeed, found inside neural precursors (Fig 2C) and interneurons (Fig 2D). Increase of hypointense pixels within the OB were monitored serially by MRI, and areshown in panel 3E as a function of time. The reopening of the narisocclusion led to a transient increase of new cell addition into OB before returning back to baseline. Figure 3 shows the MRI images of OB of WT (3A-B) and TK (3C-D) rats that were treated with GCV drug. Panel A and B represent a time-point after 3 weeks of occlusion and panel B and D represent a time point after 3 weeks of recovery following occlusion. Panel 3E clearly shows that the OB size could only recovery in the WT but not TK animals.

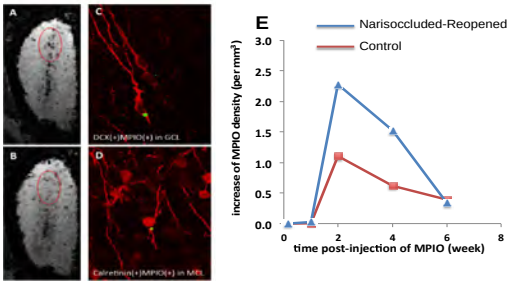

Figure 2 MRI of the same animal showing cells (hypo-intense spot) at different time point (A) in middle layer at 2 weeks post-injection and (B) outer layer of OB at 4 week post-injection. Panels C and D provides representative images of MPIO inside the cells. Panel E shows dynamic increase of cell integration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

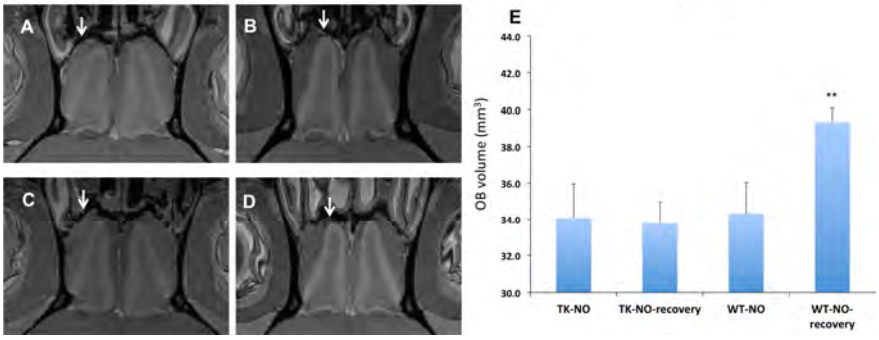

Figure 3 MRI images of OB from wild-type (WT) and TK-GFAP (TK) animals that were treated with ganciclovir to ablate generation of new cells from the SVZ. A) WT animal with narisocclusion for 3 weeks (WT-NO), B) WT animal after 3 week of recovery following 3 weeks of occlusion (WT-NO-recovery), C) TK animal with narisocclusion for 3 weeks (TK-NO), and D) TK after 3 week of recovery following 3 weeks of occlusion (TK-NO-recovery). Panel E shows average OB volumes from animals in groups A-D. (arrows indicate OB sides that were manipulated)

Discussion: Following narisocclusion (NO), the bulbs display slower growth rate, which could be attributed by decrease of new precursor cells migrating into OB due to lack of sensory input. Interestingly, the reopening of occlusion led to ~ 100% increase of new cell integration in OB. The increase of new cells coincides with the regrowth of the bulbs. To ask whether this new cells are necessary for the bulb to regain its volume after returning of activity, we performed narisocclusion in transgenic animals (TK), whose production of new neurons can be abolished. As seen in figure 3E, without SVZ neurogenesis, the OB could not develop and regain its volume even that the afferent olfactory activity has been returned.

Conclusion: We have clearly demonstrated that the growth of OB and its volume is regulated by olfactory activity and that SVZ neurogenesis is important for the regrowth after recovery of activity. In normal rats, OB shows reversible response to olfactory activity level. However, this plasticity was abolished in the animals with lack of SVZ neurogenesis. Our results indicated that SVZ neurogenesis is not only crucial to maintain and restore bulbar circuitry[5], but it is also necessary for maintaining dynamic volume changes of the olfactory bulbs.

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). [4] Snyder J. S. et al., *Nature* 476:458-461 (2011), [5]Cummings D. et al., *J Neurosci*. 34: 13801-13810 (2014).