Published in final edited form as:

Neuroimage. 2018 May 15; 172: 586–596. doi:10.1016/j.neuroimage.2018.01.051.

Spontaneous activity forms a foundation for odor-evoked activation maps in the rat olfactory bulb

Garth J. Thompson^{a,b}, Basavaraju G. Sanganahalli^{a,b,c}, Keeley L. Baker^{d,e}, Peter Herman^{a,b,c}, Gordon M. Shepherd^d, Justus V. Verhagen^{d,e}, and Fahmeed Hyder^{a,b,c,f,*}

^aMagnetic Resonance Research Center (MRRRC), Yale University, New Haven, CT, USA

^bDepartment of Radiology and Biomedical Imaging, Yale University, New Haven, CT, USA

^cQuantitative Neuroscience with Magnetic Resonance (QNMR) Core Center, Yale University, New Haven, CT, USA

^dDepartment of Neuroscience, Yale University, New Haven, CT, USA

^fDepartment of Biomedical Engineering, Yale University, New Haven, CT, USA

^eThe John B. Pierce Laboratory, New Haven, CT USA

Abstract

Fluctuations in spontaneous activity have been observed by many neuroimaging techniques, but because these resting-state changes are not evoked by stimuli, it is difficult to determine how they relate to task-evoked activations. We conducted multi-modal neuroimaging scans of the rat olfactory bulb, both with and without odor, to examine interaction between spontaneous and evoked activities. Independent component analysis of spontaneous fluctuations revealed resting-state networks, and odor-evoked changes revealed activation maps. We constructed simulated activation maps using resting-state networks that were highly correlated to evoked activation maps. Simulated activation maps derived by intrinsic optical signal (IOS), which covers the dorsal portion of the glomerular sheet, significantly differentiated one odor's evoked activation map from the other two. To test the hypothesis that spontaneous activity of the entire glomerular sheet is relevant for representing odor-evoked activations, we used functional magnetic resonance imaging (fMRI) to map the entire glomerular sheet. In contrast to the IOS results, the fMRI-derived simulated activation maps significantly differentiated all three odors' evoked activation maps. Importantly, no evoked activation maps could be significantly differentiated using simulated activation maps produced using phase-randomized resting-state networks. Given that some highly organized resting-state networks did not correlate with any odors' evoked activation maps, we posit that these resting-state networks may characterize evoked activation maps associated with odors not studied. These results emphasize that fluctuations in spontaneous activity are relevant for active processing, signifying the relevance of resting-state mapping to functional neuroimaging.

*Address correspondence to: D. S. Fahmeed Hyder, Address: Yale University, N143 TAC (MRRRC), 300 Cedar Street, New Haven, CT 06520, USA, fahmeed.hyder@yale.edu, Phone: +1 203-785-6205, Fax: +1 203-785-6643.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Since the early days of neuroscience, synchronized activity has been observed across the entire brain, often emerging spontaneously at rest. For example, the alpha wave, measured by the electroencephalogram (discovered by Hans Berger in the 1920s (Karbowski, 2002)), emerges spontaneously at rest with closed eyes, and its spatial scale suggests it must involve the synchronized coordination of many neurons. In 1995, using functional magnetic resonance imaging (fMRI) of the blood-oxygenation level dependent signal (BOLD), Biswal and colleagues found that the fMRI signals from distant parts of the brain, at rest, were highly correlated with each other. In addition, they observed that the spontaneously correlated brain regions, or “networks,” closely matched brain regions co-activated during tasks (Biswal et al., 1995). Their findings from “resting-state fMRI” indicated that the spontaneous correlation of brain activity at rest is likely relevant to stimulus-induced activation patterns.

Much evidence exists that spontaneous activity fluctuations in the brain impact subsequent task or stimulus induced activations (Arieli et al., 1996; Hyder and Rothman, 2011; Northoff et al., 2010), but much work remains to link stimulus or task activation to the networks observed in resting state fMRI. Many studies have observed that, over brain regions spanning a few millimeters, spontaneous activities and stimulus-induced activations follow similar spatiotemporal patterns (Kenet et al., 2003; Tsodyks et al., 1999; Vasireddi et al., 2016). On a larger spatial scale, discrete, bilateral patterns are observed in the same regions as stimulus-induced activations (Mohajerani et al., 2010).

Smith and colleagues compared elements of brain networks from resting-state fMRI to elements of task-induced fMRI activation patterns and discovered the two sets were very similar (Smith et al., 2009). Because their study used large fMRI databases and data across different subjects, they did not compare individual activations to resting state networks. By focusing on a single sensory system, and by using multi-modal measurements, hypothetically individual activation patterns can be directly connected to networks. The olfactory bulb (OB) provides a good model for this type of query because it is been hypothesized that within it, the spontaneous fluctuations reverberate and create plasticity which is then used during sensing (Galan et al., 2006; Kollndorfer et al., 2014). Also, a bilateral, resting-state network has already been observed in the rodent OB (Nasirivanaki et al., 2014).

To investigate the relationship between sensory responses and networks at rest, we recorded from the OBs of freely breathing, anesthetized rats. We recorded both the intrinsic optical signal (IOS), which provides high spatial and temporal resolution, and fMRI, which allows imaging of the entire glomerular sheet (the second outer-most layer of the OB, rich in axon terminals carrying the output of olfactory sensory neurons onto olfactory bulb neurons (Lancet et al., 1982)). Data were recorded at rest and also during olfactory stimulation with three different ester molecules. Resting-state networks were used to create simulated activation maps which were compared to actual activation maps to test odor discrimination.

Using IOS, which can image the dorsal OB, one of three odors could be distinguished using our method to combine networks (despite all three odors producing very similar activation maps). Using fMRI, which can image the entire glomerular sheet, all three odors could be distinguished using our method to combine networks. Our results support the hypothesis that odor-induced responses may be formed by recruiting from networks that are present at rest across the entire glomerular sheet.

2. Methods

2.1 IOS Methods

2.1.1 IOS Recording methods

2.1.1.1 Animal preparation (IOS): 6 male Sprague-Dawley rats (300–400 g) were anesthetized with isoflurane (4% for induction, 1.5–3.0% for maintenance). The level of anesthesia was checked regularly using pedal withdrawal reflex. Analgesia (Carprofen 5mg/kg) was administered two hours prior to surgery and atropine (0.03 mg/kg) was administered at the start of surgery. Animals were placed in a stereotaxic frame and the bone overlying the dorsal surface of the OB was exposed, thinned and coated with cyanoacrylate glue to make the bone transparent (Bozza et al., 2004). Throughout the surgery and optical recordings the rat's core body temperature was maintained at ~37 °C with a thermostatically controlled heating pad (Omega Engineering Inc, Stamford, CT).

2.1.1.2 Optical recoding (IOS): For optical recordings, 4–6 days after window surgery, rats were anesthetized with urethane (1.5 g/kg i.p.) and administered atropine (0.03 mg/kg) every two hours. Eye lubricant was used throughout (Lubrifresh P.M. lubricant eye ointment). The rat's skull was glued to a head-bar used to stabilize the rat's head during imaging in a stereotaxic frame.

Intrinsic optical signals from the dorsal OB were recorded using a CCD camera (Redshirt Imaging LLC, Decatur, GA, USA) with 256×256 pixel resolution, and at a frame rate of 7 Hz. The epifluorescence microscope used was a custom-made tandem-lens type (Ratzlaff and Grinvald, 1991) with either 1 x magnification (FOV: 6.67×6.67 mm) or 1.7 x magnification (FOV: 4.3×4.3mm). Imaging lenses were prime Nikon F-mount (ccd lens: 135mm f/3.5, used at f/8; object lens: 135mm f/3.5, used at f/3.5 or 80mm f/5.6, f/5.6). Depth-of-field was empirically established to be $0.26 \times (f/M)$ (f =f-stop of ccd lens, M =magnification of the imaging system), being 0.9–1.5mm, using the corners of the spread function of a Ronchi ruler (14 cy/mm at 45 degrees). A high power LED of 617nm (Thorlabs, Newark, NJ) fluorescence was aimed via optical fiber at the OB, driven by a T-Cube LED Driver (LEDD1B, Thorlabs, Newark, NJ). The fluorescence filter set used was FF01-475/50 (excitation filter, bypassed), FF506-DiO2 (dichroic), and LP515 (emission filter; Semrock, Lake Forest, IL, USA).

At 617nm, the deoxyhemoglobin absorption coefficient is ~10x greater than the oxyhemoglobin absorption coefficient (Kocsis et al., 2006; Steinke and Shepherd, 1992). The sensitivity to deoxyhemoglobin of this IOS signal is thus comparable to BOLD-fMRI, in which deoxyhemoglobin provides the intrinsic contrast (Vasireddi et al., 2016).

Each imaging session consisted of manually-triggered trials with inter-trial intervals of >3 min. In each trial the same concentration (5%) of an odor was presented in one 4 second pulse, using a custom-built multichannel auto-switching flow dilution olfactometer (Lam et al., 2000) with dedicated lines for each odor to avoid cross-contamination. Three single-molecule odorants were presented orthonasally, all fruit-like esters: isoamyl acetate (IAA, a banana-like smell), ethyl butyrate (EB, an apple-like smell), and methyl valerate (MV, a pineapple-like smell). These odors are commonly used in olfactory IOS studies as they produce a strong dorsal response (they primarily activate a medial region and a dorsolateral region) (Johnson et al., 2007; Johnson and Leon, 2007). IAA, MV and EB's odor responses are very similar, but not identical, making them a good set to use for odor discrimination. Odor concentrations are indicated as percentage saturated vapor (% s.v.). Medical-grade air was used to dilute the nitrogen-carried vapor in the headspace of odor reservoirs to generate the desired concentration at a flow rate of 500 ml/min. The olfactometer output entered orthogonally into a Teflon odor tube positioned 5 mm rostral to the nose. Breathing was measured as the movement of the thorax by a piezoelectric strap around the animal's chest as described previously (Gautam and Verhagen, 2012). During each respiration cycle, one sharp upward deflection in the piezoelectric signal occurred during thorax expansion (inspiration). For clean air imaging of the resting state, compressed air at 500 mL/min was presented directly in front on the animal's nose for 384 s (freely breathing, not intubated).

Three resting state trials from rats 2-6 were used. 3 trials of each odor from all rats were used, except for rat 2 from which 4 trials of IAA and 3 trials of the other odors were used.

2.1.2 IOS Data analysis—Except as indicated, all data analysis was done using custom-written *MATLAB* (The Mathworks Inc., 2015, www.mathworks.com/products/matlab.html) code.

2.1.2.1 Data co-registration (IOS): Errors were detected within the first three frames of all resting state data (frame 3 of the total of 2688 was of ~50x lower intensity for unknown reasons), and the 83rd and 84th frames (out of a total 84) for MV stimulation for one rat (frames with reduced intensity due to the LED turning off too early). The resting state frames were removed (reducing all resting state scans by ~0.4s), and the flawed stimulation frames were replaced by the average of prior and succeeding frames (to maintain consistency).

Data were co-registered between rats as follows: All frames from all scans were averaged to create an “anatomical image” for each rat. A translation and a rotation were determined from each rat's anatomical image to the first rat's (larger FOV) anatomical image using visual inspection of overlay comparison and image blending in *Inkscape* (Inkscape Project, 2016, inkscape.org). Data from rats 2-6 were zero-padded to match rat 1's larger FOV, and translation and rotation parameters from *Inkscape* were applied as a rigid body transformation to co-register all rats to the first rat's space. To reduce data size and thus processing time, all functional data were resampled using a resampling filter to 7Hz (if different), reduced in scale by a factor of 3 by averaging 3×3 grids of voxels to one voxel (new resolution: 78×78 μm at 1x magnification). To facilitate inter-rat comparisons, each 2D

image was blurred with a Gaussian kernel ($\sigma=3$ pixels (0.234 mm), size=12 pixels (0.938mm))

2.1.2.2 Resting state functional network detection (IOS): Functional networks have been observed using optical imaging of the rodent (Li et al., 2014b; Vazquez et al., 2014), though most prior work has found functional networks in the brain using fMRI. A common technique used to find networks in brain fMRI data is independent component analysis (ICA) (Calhoun et al., 2001). Here, we applied ICA to IOS data to calculate resting-state networks within the OB. The Group ICA of Functional Connectivity (GIFT) Toolbox (Vincent Calhoun and Srinivas Rachakonda, 2015, mialab.mrn.org), a toolbox optimized to find functional networks in resting state fMRI data, was used. Thus, several modifications were made to the resting state data prior to ICA analysis which did not alter covariance (in regions and frequency bands of interest) but allowed GIFT to operate: 1. Set to zero mean and resampled to 0.5Hz. 2. High-pass filtered above 0.01Hz (to remove drift). 3. Masked to the OB. 4. Reversed in sign (as activation deflects the 617nm IOS signal downward, but deflects the BOLD-fMRI signal upward). 5. The contrast range was adjusted, limited to between 172 and 1,666 (to match the contrast range from BOLD fMRI measured in section 2.2.2.2). Following these adjustments, GIFT was used to estimate the number of independent components using the MDL (minimum description length) criteria, which estimated 16.3 ± 2.9 (mean ± 1 s.d.). Auto-masking was disabled and the mask set to the OB mask used previously. As each run was relatively short (~383s), data were entered as group data with three trials each for five experiments. Other than described above, all default parameters were used to run GIFT and generate 16 independent components. The *T*-maps for all three trials were averaged together to produce 16 “resting state networks.” Networks were sorted into left OB, right OB, and bilateral OB networks.

Random networks were generated as described above for actual networks, except as follows: Prior to blurring each run, the Fourier transformation was taken (for each voxel, over time). The phases, but not magnitudes, were randomly shuffled between all frequencies. The inverse Fourier transformation was then calculated and the real part kept to produce data that preserved the magnitude relationship between the data but randomized phase relationships. 16 random networks were created to match the 16 actual networks.

2.1.2.3 Odor evoked map detection (IOS): Activation maps were created as follows: For each odor-stimulation trial, on a per-pixel basis, a *T*-test was done for 3.1s-7s (odor stimulation) > 0s-3s (pre-stimulus baseline) (two-sample, one-tailed, equal variance). The spatial extent of *T* values was saved as a per-trial *T*-map.

2.1.2.4 Network versus evoked map comparison (IOS): Resting state networks and activation maps were compared as follows: Correlations between each odor-stimulation trial's activation map and each of the sixteen networks were calculated, using the respective rat's anatomical image as a covariate of no interest (partial correlation using Pearson coefficient). Correlation coefficients were converted to *Z* values with an $N(0,1)$ distribution using a normalizing Fisher's transformation (Thompson et al., 2013b). For each odor and resting state network combination, the distribution of *Z* across trials was tested for significance with a one-sample *T*-test. These steps were repeated for both actual networks

and random networks. (Note that, as this is spatial similarity, some random networks are expected to be significantly correlated with odor activation, as some should randomly be in the correct spatial positions.)

Simulated activation maps were constructed from resting state networks as follows: For each odor-stimulation trial a multiple linear regression was done using the 16 networks' *T*-maps, the anatomical image, and a constant term as inputs, and that trial's odor stimulation activation map as an output. This produced one slope value for each trial and network combination. The mean slope was then taken across all trials for each odor and network combination. To avoid over-fitting, and to focus on the hypothesis that networks may be selectively recruited by activation (Galan et al., 2006; Smith et al., 2009), only the mean slopes from networks that had significant correlation with the respective odor's activation (see previous paragraph) were used for reconstruction. The simulated odor activation maps, one per odor, were thus constructed by multiplying the mean slopes by the *T*-maps for each network, then summing together only the significant networks for that odor. This was done for both actual networks and random networks. An example of this process is shown in Fig. S1.

Simulated activation maps were compared to actual activation maps in order to determine odor differentiation as follows: For each odor-stimulation trial, spatial Pearson correlation across all pixels was calculated between that trial's *T*-map and the simulated activation map created from networks for both that odor, and also the other two odors. Correlation coefficients were converted to *Z* values with an $N(0,1)$ distribution using a normalizing Fisher's transformation (Thompson et al., 2013b). To measure odor differentiation, *T*-tests (two-sample, one-tailed, equal variance) were performed between the set of *Z* values for the correct odor pairing, and the set of *Z* values resulting from all incorrect odor pairings. This was done for actual networks and random networks.

2.1.3 IOS Ethical statement—All procedures were performed in accordance with protocols approved by the Pierce Animal Care and Use Committee (PACUC). These procedures are in agreement with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2 fMRI Methods

2.2.1 Advantages and disadvantages of fMRI versus IOS—Different recording and stimulation parameters were used for fMRI versus IOS, as parameters for optimal odor-specific stimulation response under urethane anesthesia differ between modalities. Notably, fMRI requires a longer stimulation time (30s vs. 4s) and higher odor concentration (20% vs. 5%). Conversely, higher odor concentrations can cause increasingly diffuse spatial responses using IOS (e.g. at 630nm, Wachowiak and Cohen (Wachowiak and Cohen, 2003)), whereas they are necessary for fMRI. The 5% used for IOS was hence a compromise between reducing response map diffuseness and deviating from the 20% used for fMRI. For a direct comparison between IOS and fMRI see Sanganahalli and colleagues (Sanganahalli et al., 2016).

Versus IOS, fMRI has the disadvantage of reduced spatial ($250 \times 250 \times 500 \mu\text{m}$ vs. $43 \times 43 \mu\text{m}$ at 1.7 x magnification) and temporal (1Hz activation, 0.5Hz resting state vs. 7Hz) resolution, but has the advantages of covering a greater spatial volume (the entire olfactory bulb (OB) can be imaged vs. only the dorsal surface) and multiple, longer resting state scans can be recorded ($4\text{--}5 \times 1000$ s scans vs. 3×384 s scans).

2.2.2 fMRI Recording methods

2.2.2.1 Animal preparation (fMRI): Sprague-Dawley rats ($n=6$) (different rats than for IOS) of weight ~ 300 g were used. Rats were anesthetized with urethane (1.5 g/kg intraperitoneal), administered atropine to prevent nasal blockage (0.03 mg/kg, subcutaneous) and administered eye lubricant to prevent desiccation (Puralube Vet Ointment, ophthalmic ointment). Rats were not intubated and were allowed to breathe freely throughout the experiment. Blood pressure and rectal temperature were continuously monitored to ensure stable physiology. Temperature was maintained at $\sim 37^\circ\text{C}$ using a water flow heating pad. Blood gas pH, pCO_2 and pO_2 were recorded approximately once per hour. A nose cone was placed over rats' noses to administer both clean air and odors. Rats' head positions were fixed with ear bars and a bite bar in a cradle and placed under the surface coil.

Through the nose-cone, compressed air was continuously given at 250 mL/min, during resting state, during baseline, and during anatomical scans. Odors were given through this air using a custom-built olfactometer (Sanganahalli et al., 2009). Concentration was 20% s.v.

2.2.2.2 MRI recording (fMRI): Data were recorded with a 9.4T Bruker horizontal-bore spectrometer using a home-built 1H surface coil radiofrequency probe (1.4 cm diameter) and a Varian console. Shimming was performed using the B0DETOX software (C. Juchem, 2017, http://innovation.columbia.edu/technologies/cu17326_b0detox) (Juchem et al., 2014; Juchem et al., 2015). EPI images were recorded for odor stimulation and resting state (TR 1000 ms, TE 15 ms, 64×32 matrix, 16×8 mm FOV, 3x initial references, 16 dummy scans, 0.5 mm coronal slices, 2 rats had 8 slices, 4 rats had 10 slices). Stimulation was 150 TRs for 150 s at 1 Hz (0-30 s clean medical grade air, 30-98s odor, 90-150 s clean medical grade air). The same three odors as described in section 2.1.1.2 (IAA, MV, EB) were used. Resting state was 500 TRs using the Varian "full triple" option (a reference image for every volume to prevent signal drift) for a total imaging time of 1,000 s at 0.5Hz (all clean air). Number of trials varied by rat, see Table 1. T2 anatomical images were also recorded for registration purposes (TR 4000 ms, TE 40 ms, 128×64 matrix, 16×8 mm FOV, 24 dummy scans, 0.5 mm coronal slices, same number of slices as EPI).

2.2.3 fMRI Data analysis—Except as indicated, all data analysis was done using custom-written *MATLAB* (The MathWorks Inc., 2015, www.mathworks.com/products/matlab.html) code.

2.2.3.1 Data co-registration (fMRI): Anatomical images were co-registered between rats as follows: Using BioImage Suite (Yale School of Medicine, 2015, bioimagesuite.yale.edu), rats 2-6's anatomical images were registered to rat 1's anatomical image using a nonlinear registration (50 iterations, normalized mutual information, otherwise default). Then, the

average of all six registered images (where data were available) was taken to create a “template” OB image. All rats’ anatomical images were then registered to the template using a nonlinear registration (50 iterations, normalized mutual information, and otherwise default).

Functional images were co-registered between rats as follows: slice-timing correction followed by motion-correction (to the middle volume rather than the first volume) was performed in SPM8 (The FIL Methods group, 2015, www.fil.ion.ucl.ac.uk/spm/software/spm8). Following this, the corresponding rat’s nonlinear transformation to the template was applied using BioImage Suite (see previous paragraph). To facilitate inter-rat comparison, data were blurred, per-slice, with a Gaussian filter ($\sigma=2$ voxels/0.250mm, size=8 voxels/1mm).

2.2.3.2 Resting state functional network detection (fMRI): ICA using the GIFT toolbox (see section 2.1.2.2) was used to calculate resting-state networks. GIFT was used to estimate the number of independent components using the MDL (minimum description length) criteria, which estimated 21.8 ± 6.9 (mean $\pm$ 1 s.d.). As each run was relatively long (1000 s) and the number of runs varied per rat (Table 1), each run was entered as a separate experiment. Other than described above, all default parameters (including automatic masking) were used to run GIFT and generate 22 independent components. The resultant *T*-maps were used as 22 “resting state networks.”

Random networks were generated as described in section 2.1.2.2, except the procedure used for generating fMRI networks in the previous paragraph was used to create them from phase-randomized fMRI data.

2.2.3.3 Odor evoked map detection (fMRI): Activation maps were created on a per-trial basis largely as described in section 2.1.2.3, except as follows: To increase sensitivity (lower in fMRI than IOS), the baseline following stimulation was also used as a control time series, thus the *T*-test for each odor stimulation run was for 30s-90s (odor stimulation) $>$ 1s-29s and 31s-150s (pre- and post-stimulus baseline).

2.2.3.4 Network versus evoked map comparison (fMRI): To focus on the glomerular sheet, a mask of the glomerular layer in each slice in the template OB was drawn by a researcher (BG Sanganahalli). All further analysis described in this section was done only using the glomerular sheet. This mask also facilitated display of images as flatmaps (see section 2.2.4).

Following the methods described IOS in section 2.1.2.4, resting state networks and activation maps were compared; simulated activation maps were constructed from resting state networks and these simulated activation maps were compared to actual activation maps in order to determine odor differentiation .

2.2.4 fMRI Visualization of the glomerular sheet—To convert the glomerular sheet from a mask within 3D MRI data to a 2D image, a custom function was written to extract

“flatmaps,” images of flattened layers in the OB. This function was inspired by OdorMapBuilder (Liu et al., 2004), but is highly simplified.

This algorithm is run on 3D data of the OB of any type with a corresponding mask of one layer on one side.

Within each slice of the MRI data, the layer mask forms a ring shape, or an inverted-U shape. Thus, each row of the image has either one or two sections of the layer in it. If there are two sections, these are the left and right sides of the layer. If there is only one section this is either the top or the bottom (for ring shape) and can be divided evenly into left and right sides. Each side of the layer on each row can be averaged together to produce one voxel, per-side, per-row. Arranging these voxels from left-bottom to left-top, concatenated with right-top to right-bottom, converts the layer into a one-voxel row in the flatmap.

As one row corresponds to one slice in the original MRI, combining all of the rows in slice order creates a flatmap. This flatmap will have a rostral-caudal dimension, assuming rows from coronal slices were concatenated. The other dimension will go ventral-medial-dorsal-lateral-ventral based on how it was unwrapped. Thus, a 2D image is produced of the layer (Fig. 1.)

After use of the flatmap transformation, the following steps were applied to improve the flatmap appearance: 1. The aspect ratio was corrected to isotropic voxels so that the flatmaps would reflect the actual size of the glomerular sheet. 2. A blur was applied with a 3x3 voxel square to make gross spatiotemporal structure more visible.

The creation of flatmaps was for visualization purposes only; all data analysis was done in the original space, within the same mask of the glomerular sheet used to create flatmaps (section 2.2.3). The *MATLAB* function used to create flatmaps (*slice_out_layer.m*) is provided as supplemental data.

2.2.5 fMRI Ethical statement—All procedures were performed in accordance with protocols approved by the ethical committee of Yale University School of Medicine Institutional Animal Care and Use Committee (IACUC). These procedures are in agreement with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

3. Results

3.1 IOS Results

3.1.1 IOS Resting state networks—To determine the organization of spontaneous activity in the olfactory bulb (OB), 16 networks were generated from resting state IOS data (Fig. 2A). Networks appeared concentrated in specific locations and were primarily unilateral with the exception of networks 15 and 16. Many primarily unilateral networks also appeared to have a weaker bilateral mirror, e.g. networks 1-3, 5, 8, 9 and 11. Random networks (Fig. S2A) were also concentrated in specific locations, but were either smaller point-like locations or rows across one side of the OB, potentially reflecting regions of similar signal-to-noise ratio (SNR). No random networks appeared bilateral.

To compare resting state networks to odor-induced activation, *T*-maps from activation due to three ester odors (IAA, MV and EB) were correlated with the 16 resting state networks (Fig. 2B, C, D). Which networks had significantly high correlation with which odors' activation varied from odor to odor, e.g. IAA activation correlated with network 13 whereas EB and MV activation did not, however they correlated with network 3 whereas IAA activation did not. Results for random networks were sufficiently similar to continue our actual vs. random comparative analysis (Figs. S2B-D). This is because some random networks were randomly in the correct spatial positions to correlate with activation.

Some networks, while appearing to have similar structure to the others, did not correlate with any odor's activation map, e.g. networks 1, 4 and 11.

3.1.2 IOS Actual vs simulated activation patterns—To determine if resting state networks form a foundation that may be useful for odor discrimination, simulations of odor activation maps for each odor were created from them (slopes and coefficients are shown in Table S1A). The simulations for each odor from resting state networks (Figs. 3A, C, E) appear similar to their respective actual odor activation patterns (Figs. 3B, D, F). Results from random networks appeared noisier, but were similar enough to continue comparative analysis (Figs. S3 A, C, E).

To test whether the odors used in this study can be discriminated using resting state networks, spatial correlation was calculated between simulated and actual networks, both for one correct and two incorrect odor pairings. If the simulated and actual networks for the correct pairing correlated higher than the incorrect pairings, this suggests that the odor may be discriminable. One odor, IAA, was found to be significantly discriminable from the other odors ($p=0.03$, Fig. 3G). The other odors, MV and EB, were not significantly discriminable ($p=0.51$ MV, $p=0.58$ EB, Fig. 3G). Conversely, no odors were significantly discriminable with random networks ($p=0.084$ IAA, $p=0.18$ MV, $p=0.56$ EB, Fig. 3H), suggesting that the structure of the actual networks is important to discrimination of IAA.

When using only the dorsal surface of the OB, it is difficult to distinguish between these odors. For example, IAA and EB (Blauvelt et al., 2013), and MV and EB (Sanganahalli et al., 2016) activation maps are known to be very similar. Hypothesizing that networks would be present across the entire OB, in the next section we used fMRI to image the entire glomerular sheet.

3.2 fMRI Results

3.2.1 fMRI Resting state networks—For visualization purposes, all fMRI data is shown in terms of a flatmap of the entire glomerular sheet. This process was described in section 2.2.4, and an example of the creation of a flatmap is shown in Fig. 1.

To determine the organization of spontaneous activity in the olfactory bulb (OB), 22 networks were generated from resting state fMRI data (Fig. 4A). Like IOS, fMRI networks appeared concentrated in specific locations, however unlike IOS they were mostly (12/22) bilateral. Random networks (Fig. S4A) were also mostly (14/22) bilateral but smaller, only a

few voxels in size. The exception was random network 16, which appeared similar to an inverse map of the magnetic field strength (likely due to SNR differences).

To compare resting state networks to odor-induced activation, *T*-maps from activation due to three ester odors (IAA, MV and EB) were correlated with the 22 resting state networks (Figs. 4B-D). As with IOS, which networks had significantly high correlation with which odors' activation pattern varied from odor to odor. Results for random networks were sufficiently similar to continue our actual/random comparative analysis (Figs. S4B-D), which however differed from actual networks as there was the largest correlation with random network 16 and much smaller correlation for the other random networks.

As with IOS, some networks appeared similarly structured, but did not correlate with any odor's activation map. This included many more networks for fMRI than for IOS, e.g. networks 4-5, 7-10, 12, 14, 17, 19, and 22.

3.2.2 fMRI Actual vs simulated activation patterns—To determine if resting state networks form a foundation that may be useful for odor discrimination, simulations of odor activation maps for each odor were created from them (slopes and coefficients are shown in Table S1B). The simulations for each odor from resting state networks (Figs. 5A, C, E) appeared similar to their respective actual odor activation patterns (Figs. 5B, D, F). Results from random networks appeared noisier, but were similar enough to continue comparative analysis (Figs. S5 A, C, E).

To test whether the odors used in this study can be discriminated using resting state networks, spatial correlation was calculated between simulated and actual networks, both for one correct and two incorrect odor pairings. If the simulated and actual networks for the correct pairing were correlated higher than the incorrect pairings, this suggests that odor may be discriminable. All three odors were discriminable using actual networks ($p=0.021$ IAA, $p=0.044$ MV, $p=0.044$ EB, Fig. 5G). Conversely, no odors were significantly discriminable with random networks ($p=0.13$ IAA, $p=0.44$ MV, $p=0.38$ EB, Fig. 5H), suggesting that the structure of the actual networks is important to discrimination of all three odors.

Thus, we speculate that using the entire glomerular sheet allowed more odors to be discriminated versus using only the dorsal surface (see section 3.1.2).

4. Discussion

4.1 Stimulus-response may recruit from organization which also exists at rest

Spontaneous activity at rest in the brain (Biswal et al., 1995) and OB (Li et al., 2010; Nasirivanaki et al., 2014) is highly synchronized across large spatial regions. Spontaneous activity in the OB has a high energy demand (Nawroth et al., 2007), is affected by disease (Liu et al., 2013), and is necessary for maintaining and developing the organization of glomeruli (Yu et al., 2004). Higher brain regions connected to the OB also show functional connectivity (Wilson et al., 2011; Wilson and Yan, 2010). These observations indicate that spontaneous activity in the OB reflects an underlying, biologically important function.

Studies of the visual system provide evidence that spontaneous activity is strongly linked to evoked activity. In the visual system, on a spatial scale of a few millimeters, spontaneous activity impacts subsequent stimulus-induced activation (Arieli et al., 1996), and the spatial patterns of spontaneous activity closely match stimulus-induced activation patterns (Tsodyks et al., 1999). Spontaneous activity moves through several discrete patterns, many of which match specific responses to visual stimuli at different orientations (Kenet et al., 2003; Vasireddi et al., 2016).

Across larger spatial scales, in the somatosensory system in mice (Mohajerani et al., 2010) and humans across many tasks (Liu et al., 2011; Smith et al., 2009), high correspondence between components of task-induced activation and resting state networks is also observed.

Galan et al. observed the glomeruli of honeybees under odor stimulation, and hypothesized that odor-evoked maps could form a basis for the neural code of olfaction. They proposed a network equivalent to the “perceptron” concept in machine learning, where weights on inputs are learned, and these combine to form a binary output, here indicating either perceiving or not perceiving an odor (Galan et al., 2004). The structure of axonal connections in the bulb or the brain hypothetically constrains the spontaneous patterns that can be displayed, forming a basis set to act as inputs (Galan, 2008). Supporting this concept, in honeybees, spontaneous fluctuations in glomeruli following a stimulus were sufficient to reconstruct activation (Galan et al., 2006). Similarly, in monkeys, varying task parameters alters “internal models” (observed using neural electrophysiology), and the internal models impact future errors in task performance (Stavisky et al., 2017). Results such as these indicate that spontaneous fluctuations reinforce correlated spatial patterns that may be recruited as needed for the current task or stimulus.

Our work provides direct evidence in support of such a basis set present at rest, as the olfactory bulb is a system ideally suited for testing this. The glomerular responses for different odors are distributed over the whole glomerular sheet, and these activation patterns provide unique responses for different odors. Therefore within one modality (smelling) we created different patterns to study the different recruitments of resting state networks. Using data from IOS and fMRI, networks within the actual spontaneous fluctuations of the resting-state reconstructed odor activation patterns, and these were able to distinguish one or more of the odors tested. However, random networks were not able to distinguish any odor using any modality. This indicates that the actual resting-state networks form a foundation, which describes odor activation patterns better than random networks.

fMRI was able to distinguish all three odors, versus only one odor for IOS. Given that these odors’ activations also include a medial portion of the OB (Johnson et al., 2007; Johnson and Leon, 2007), the dorsal activity maps imaged with IOS are likely to provide less information about odor circuits (i.e. report a smaller extent thereof) than the entire glomerular sheet imaged with fMRI. We speculate that this may explain why the odors are more separable based on the BOLD signal across the entire sheet than dorsal IOS. (Other possibilities for discrepancy exist; for example, unlike fMRI, the overlying large vessels could not be avoided and could potentially optically obscure IOS networks, or the odorant concentration differences between modalities could cause different patterns, see last paragraph of section

4.3.) If it is true that the better discrimination was due to increased OB coverage, potential new methods could image the lateral or ventral OB with IOS simultaneously with the dorsal OB, providing both high resolution and improved classification.

As several networks were highly structured but uncorrelated with activation patterns for the odors used in the present study, the spontaneous fluctuations observed may be related to odors beyond those we studied.

Thus, we hypothesize that the synchronized activity patterns, observed across the glomerular sheet for over 40 years (Sharp et al., 1975), may be reinforced at rest through spontaneous fluctuations and mechanisms such as Hebbian plasticity (Galan et al., 2006) across inherent structural connections (Galan, 2008). Thus, presentation of an odor may selectively activate networks to differing degrees (Fig. S1). This hypothesis presents a likely function of spontaneous fluctuations and a potential mechanism for stereotyped, synchronized activation.

4.2 Differences in neuroimaging modalities

While the purpose of this study was not to compare optical and MRI methods (see (Sanganahalli et al., 2016) for such a comparison), a few differences emerged which are worth noting.

ICA on phase-randomized fMRI data produced an apparent field strength map (#16, Fig. S4A), whereas there was no equivalent for phase-randomized IOS data (Fig. S2A). This is because IOS is detecting a flat-like dorsal surface, whereas in fMRI there is greater spatial variation in SNR due to placement of an RF surface coil on top of the head. Even with inclusion of the anatomical image as a covariate, the field strength map correlated highly with every actual activation map. This is likely because field strength directly impacts SNR, to which activation maps are sensitive. Thus we observed much higher correlation for simulated (from random networks) versus actual activation maps for fMRI (Fig. 5H) vs. IOS (Fig. 3H).

fMRI networks were much more likely to be strongly bilateral than IOS networks (55% vs. 13% for actual, 64% vs. 0% for random). While this may reflect the better spatial coverage available with fMRI, we must be cautious as fMRI is more affected by partial volume in adjacent voxels than IOS. In particular, the numerous small bilateral networks observed using fMRI with random phase (Fig. S4A) indicate that bilaterality itself cannot be used to claim a network reflects neuronal fluctuations. However, as several actual IOS networks showed weak bilaterality (Fig. 2A) whereas random IOS networks did not (Fig. S2A), larger regions of bilaterality are still likely due to neuronal fluctuations (considering reports on the neural basis of functional connectivity (Magnuson et al., 2014)).

4.3 Other considerations and limitations

As activation within the IOS can be seen through veins (Chaigneau et al., 2003; Lecoq et al., 2011) and as specific vein positions vary by rat, large veins were not removed for IOS data analysis. However, regions where many rats have veins in approximately the same location showed decreased *T* values, e.g. network 5 caudal left bulb (Fig. 2A) and odor activation

center right bulb (Fig. 3B, D, F). This vascular artifact was avoided in fMRI by performing data analysis only on the glomerular sheet.

It is likely that some of the networks, even if they represent neural activity, do not relate to an activation response. It should also be considered that spontaneous fluctuations may interfere with functional activation, rather than facilitate it (Schulz et al., 2012; Weissman et al., 2006). In particular, sniffing with or without odor invokes a dorsal-rostral activation response (Carey et al., 2009), similar to IOS network 15 (Fig. 2A) and fMRI network 11 (Fig. 4A). Interestingly, IOS network 15 and fMRI network 11 were both significant and at a similar positive correlation value for every odor, indicating no odor specificity. While olfactory sensory neurons can also respond to mechanical stimuli (Grosmaître et al., 2007), measurement of networks in the brain using combined resting state fMRI and simultaneous neural electrical recordings (Magri et al., 2012; Pan et al., 2011; Pan et al., 2013; Shmuel and Leopold, 2008) strongly suggests a neural origin for networks. In addition, our initial analysis of IOS recordings from rats following tracheotomy has thus far indicated similar networks, even without air flow through the nose and mouth. However, those data present many methodological differences from the present data, so are the subject of a separate ongoing investigation.

To obtain reliable fMRI/IOS responses to external stimulation, rodent experiments must be performed in anesthetized animals. However, anesthesia directly affects the characteristics of the hemodynamic response, i.e. the shape of the hemodynamic response function (HRF), and influences the spatial specificity of the BOLD signal (Schlegel et al., 2015). Furthermore, brain activity levels can be manipulated readily by depth of anesthesia and choice of anesthetic. In the current study we used urethane anesthesia for both fMRI and IOS studies. Urethane-anesthetized animals are thought to have similar physiological and pharmacological behaviors as unanesthetized animals (Neville and Haberly, 2003). However, some previous studies reported that urethane anesthetized animals show steady and periodic alteration between slow and fast-wave states (Murakami et al., 2005).

Here, we focused on comparing the spatial maps during rest and activation. However, activation may also be characterized by temporal dynamics (Martin et al., 2007; Poplawsky et al., 2015) and temporal dynamics of resting state fMRI are an area of much recent interest (Thompson, 2017). Adding IOS to such fMRI studies will be useful to attain a higher temporal resolution which may result in better discrimination of faster network dynamics (Kiviniemi et al., 2016). For example, even though a rodent may be able to discriminate odors based on a small section of the OB (e.g. after partial bulbectomy), static ICA methods may lack the sensitivity of downstream neural decoding mechanisms that also incorporate temporal properties which are known to allow discrimination of the bulbar responses (Li et al., 2014a; Rebello et al., 2014; Smear et al., 2011). In addition to temporal dynamics, more complex models such as increasing the number of networks, or non-linear comparisons (Magri et al., 2012) could be considered for future work.

It is important to consider that the computational model used in our study does not detect all possible ground-truth resting-state networks and all possible activation-network interactions. For example, the number of independent components (used to identify resting-state

networks) could be increased or decreased, and these components' correlations with activation maps are limited by SNR as well the correlation method used. In addition, our measurements are also limited as both fMRI and 617nm IOS report fluctuations in deoxyhemoglobin, not raw neural activity (e.g. spikes or synaptic potentials), and each method has its limitations in sensitivity, spatial resolution and spatial coverage. Further work including electrophysiology and data analysis is thus needed to determine both the ground-truth behind resting-state networks and activation maps in the olfactory bulb, and also why fMRI could significantly differentiate all three odors but IOS could only make the larger differentiation of IAA vs. MV and EB. While it is likely that some alternative analysis or recording method could distinguish all three odors from the smaller area on which IOS was imaged (or potentially even more odors across even smaller OB areas), the significant differentiation of IAA from MV and EB using IOS (which was not significant using randomized networks) indicates that the networks detected are linked to the underlying "neuronal" networks, and their spatial pattern is linked to odor discrimination.

4.4 Future work: From correlations to plasticity

Using human fMRI data, Smith et al. (Smith et al., 2009) decomposed task activation maps and resting-state time courses into spatial independent components, and demonstrated a high degree of correspondence between task and resting components. While their work extended clinical application of resting-state fMRI data (e.g. pre-surgical brain mapping (Sair et al., 2016)), the question remains as to why there is correspondence between maps of task and resting state. By focusing on a single sensory system, and within individual olfactory stimulation trials, our work demonstrates that the spatial patterns observed during rest likely form a foundation which, when networks are combined, can re-create individual activation patterns. Since we demonstrate that such re-creations can distinguish different olfactory stimuli better than re-creations from random networks, we provide evidence of a function for the spatial patterns of networks present at rest. In other words, these resting patterns are related to reconstructing stimulus-evoked patterns.

Our work suggests that observations by Smith et al. (Smith et al., 2009) are due to the importance of resting-state networks in sensing. But why, if they are measured when no active sensing is occurring, are these networks important? It has been proposed that resting-state fluctuations reinforce models of Hebbian plasticity (Galan et al., 2006; Hebb, 1949; Stavisky et al., 2017). Since "neurons wire together if they fire together," (p. 211 (Lowel and Singer, 1992)), a coordinated resting-state creates plasticity within a pattern which can be easily recruited for activation.

For example, consider studies that have examined the resting-state network activity prior to tasks where a sensory stimulus is detected. Poor performance has been associated with activation, prior to performance, of regions that interfere with the task performed (Eichele et al., 2008; Weissman et al., 2006). Good performance has been associated with activation, prior to task performance, of regions similar to the evoked activation patterns of either the stimulus itself or vigilance in general (Boly et al., 2007; Sadaghiani et al., 2009; Wohlschläger et al., 2016), or stronger establishment of canonical network structure (Thompson et al., 2013a). Such results suggest that well-established active internal networks

present during the resting state are a critical component of good performance on sensory tasks.

If this is true, how could plasticity in relation to resting-state networks be studied? Studies of rest-stimulus interaction (Hyder and Rothman, 2011; Northoff et al., 2010) suggest that varying global metabolic level can be used as a controlled variable by the experimentalist. Using anesthesia to modulate the brain's metabolic state, and hence neural firing rate in animal models, it can be observed that whether at a lower or higher metabolic "baseline" state, a similar peak response can be reached (Hyder et al., 2002; Pasley et al., 2007; Smith et al., 2002; Zhu et al., 2009). This indicates that the differential change in activity is smaller from a globally higher metabolic state, indicating that such a higher metabolic state presents less opportunity for plasticity, and in support of this hypothesis, the higher metabolic state would present a less specific spatial activation pattern (Maandag et al., 2007).

Similar to the anesthesia modulation, using eyes open/eyes closed as high/low global metabolic states in human subjects, the higher state presented globally higher functional connectivity (derived from resting state fMRI) with little difference in local variance (Thompson et al., 2016). The global fMRI signal was later found to account for 65% or more of the variance in resting state fMRI networks (Vos de Wael et al., 2017). This indicates that the shifting baseline metabolic state moves by either shifting all networks upward or downward rather than selecting specific networks for specific states, an idea supported by quantitative positron emission tomography results of cerebral metabolism (Hyder et al., 2016).

Thus, a strong possibility for future work is to use the methods herein, but also to modulate the baseline metabolism itself (e.g. through anesthesia in animal models, or by behavioral alterations in humans) to globally vary the specificity of functional connectivity, and thus vary Hebbian plasticity. Specific networks can be located and tested in terms of effects on activation with both high temporal resolution (using IOS) to study temporal dynamics and good spatial coverage (using fMRI) to locate more networks. Hypothetically lower metabolic states are hypothesized to result in greater changes, less global functional connectivity, and thus greater plasticity. Much future work is possible including both short-term changes over the course of a single imaging session, and also long-term reinforcement at different metabolic levels. Such future work carries the promise of allowing researchers to better understand the function of the brain's resting state, specifically in relation to task-based networks that still dominate studies of cognitive neuroscience.

4.5 Conclusion

Of the three odors we tested, one had an activation pattern which could be significantly differentiated using resting-state networks found within the dorsal surface of the OB. Furthermore, all three odors we tested could be significantly differentiated using resting-state networks found within the entire glomerular sheet. We also observed networks that were structured, but unrelated to the odors we presented, suggesting these networks may characterize odors beyond those we studied. We thus hypothesize that odor-induced responses may recruit from a repertoire of networks found within the field of spontaneous fluctuations of the entire glomerular sheet, and these networks are also present at rest. Our

work suggests a function for spontaneous fluctuations at rest and provides a hypothetical foundation for future studies of the OB's spontaneous activity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Authors thank Bei Wang for her help in animal preparation for fMRI. G. J. Thompson is now working at the iHuman Institute at ShanghaiTech University; however, this work was done at his former affiliation, shown above. Funding for this research included NIH grants (R01 DC-011286, R01 MH-067528, P30 NS-052519), and an NIH fellowship (T32 DA-022975) supporting GJT.

References

- Arieli A, Sterkin A, Grinvald A, Aertsen A. Dynamics of ongoing activity: explanation of the large variability in evoked cortical responses. *Science*. 1996; 273:1868–1871. [PubMed: 8791593]
- Biswal B, Yetkin FZ, Haughton VM, Hyde JS. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med*. 1995; 34:537–541. [PubMed: 8524021]
- Blauvelt DG, Sato TF, Wienisch M, Knopfel T, Murthy VN. Distinct spatiotemporal activity in principal neurons of the mouse olfactory bulb in anesthetized and awake states. *Front Neural Circuits*. 2013; 7:46. [PubMed: 23543674]
- Boly M, Balteau E, Schnakers C, Degueldre C, Moonen G, Luxen A, Phillips C, Peigneux P, Maquet P, Laureys S. Baseline brain activity fluctuations predict somatosensory perception in humans. *Proc Natl Acad Sci U S A*. 2007; 104:12187–12192. [PubMed: 17616583]
- Bozza T, McGann JP, Mombaerts P, Wachowiak M. In vivo imaging of neuronal activity by targeted expression of a genetically encoded probe in the mouse. *Neuron*. 2004; 42:9–21. [PubMed: 15066261]
- Calhoun VD, Adali T, Pearlson GD, Pekar JJ. A method for making group inferences from functional MRI data using independent component analysis. *Hum Brain Mapp*. 2001; 14:140–151. [PubMed: 11559959]
- Carey RM, Verhagen JV, Wesson DW, Pirez N, Wachowiak M. Temporal structure of receptor neuron input to the olfactory bulb imaged in behaving rats. *J Neurophysiol*. 2009; 101:1073–1088. [PubMed: 19091924]
- Chaigneau E, Oheim M, Audinat E, Charpak S. Two-photon imaging of capillary blood flow in olfactory bulb glomeruli. *Proc Natl Acad Sci U S A*. 2003; 100:13081–13086. [PubMed: 14569029]
- Eichele T, Debener S, Calhoun VD, Specht K, Engel AK, Hugdahl K, von Cramon DY, Ullsperger M. Prediction of human errors by maladaptive changes in event-related brain networks. *Proc Natl Acad Sci U S A*. 2008; 105:6173–6178. [PubMed: 18427123]
- Galan RF. On how network architecture determines the dominant patterns of spontaneous neural activity. *PLoS One*. 2008; 3:e2148. [PubMed: 18478091]
- Galan RF, Sachse S, Galizia CG, Herz AV. Odor-driven attractor dynamics in the antennal lobe allow for simple and rapid olfactory pattern classification. *Neural Comput*. 2004; 16:999–1012. [PubMed: 15070507]
- Galan RF, Weidert M, Menzel R, Herz AV, Galizia CG. Sensory memory for odors is encoded in spontaneous correlated activity between olfactory glomeruli. *Neural Comput*. 2006; 18:10–25. [PubMed: 16354378]
- Gautam SH, Verhagen JV. Retronasal odor representations in the dorsal olfactory bulb of rats. *J Neurosci*. 2012; 32:7949–7959. [PubMed: 22674270]
- Grosmaître X, Santarelli LC, Tan J, Luo M, Ma M. Dual functions of mammalian olfactory sensory neurons as odor detectors and mechanical sensors. *Nat Neurosci*. 2007; 10:348–354. [PubMed: 17310245]

- Hebb, DO. The organization of behavior; a neuropsychological theory. Wiley; New York: 1949.
- Hyder F, Herman P, Bailey CJ, Moller A, Globinsky R, Fulbright RK, Rothman DL, Gjedde A. Uniform distributions of glucose oxidation and oxygen extraction in gray matter of normal human brain: No evidence of regional differences of aerobic glycolysis. *J Cereb Blood Flow Metab.* 2016; 36:903–916. [PubMed: 26755443]
- Hyder F, Rothman DL. Evidence for the importance of measuring total brain activity in neuroimaging. *Proc Natl Acad Sci U S A.* 2011; 108:5475–5476. [PubMed: 21441108]
- Hyder F, Rothman DL, Shulman RG. Total neuroenergetics support localized brain activity: implications for the interpretation of fMRI. *Proc Natl Acad Sci U S A.* 2002; 99:10771–10776. [PubMed: 12134057]
- Johnson BA, Arguello S, Leon M. Odorants with multiple oxygen-containing functional groups and other odorants with high water solubility preferentially activate posterior olfactory bulb glomeruli. *J Comp Neurol.* 2007; 502:468–482. [PubMed: 17366613]
- Johnson BA, Leon M. Chemotopic odorant coding in a mammalian olfactory system. *J Comp Neurol.* 2007; 503:1–34. [PubMed: 17480025]
- Juchem C, Herman P, Sanganahalli BG, Brown PB, McIntyre S, Nixon TW, Green D, Hyder F, de Graaf RA. DYNAMIC Multi-coil TEchnique (DYNAMITE) shimming of the rat brain at 11.7 T. *NMR Biomed.* 2014; 27:897–906. [PubMed: 24839167]
- Juchem C, Umesh Rudrapatna S, Nixon TW, de Graaf RA. Dynamic multi-coil technique (DYNAMITE) shimming for echo-planar imaging of the human brain at 7 Tesla. *Neuroimage.* 2015; 105:462–472. [PubMed: 25462795]
- Karbowski K. Hans Berger (1873–1941). *J Neurol.* 2002; 249:1130–1131. [PubMed: 12420722]
- Kenet T, Bibitchkov D, Tsodyks M, Grinvald A, Arieli A. Spontaneously emerging cortical representations of visual attributes. *Nature.* 2003; 425:954–956. [PubMed: 14586468]
- Kiviniemi V, Wang X, Korhonen V, Keinänen T, Tuovinen T, Autio J, LeVan P, Keilholz S, Zang YF, Hennig J, Nedergaard M. Ultra-fast magnetic resonance encephalography of physiological brain activity - Glymphatic pulsation mechanisms? *J Cereb Blood Flow Metab.* 2016; 36:1033–1045. [PubMed: 26690495]
- Kocsis L, Herman P, Eke A. The modified Beer-Lambert law revisited. *Phys Med Biol.* 2006; 51:N91–98. [PubMed: 16481677]
- Kollndorfer K, Kowalczyk K, Hoche E, Mueller CA, Pollak M, Trattng S, Schopf V. Recovery of olfactory function induces neuroplasticity effects in patients with smell loss. *Neural Plast.* 2014; 2014:140419. [PubMed: 25544900]
- Lam YW, Cohen LB, Wachowiak M, Zochowski MR. Odors elicit three different oscillations in the turtle olfactory bulb. *J Neurosci.* 2000; 20:749–762. [PubMed: 10632604]
- Lancet D, Greer CA, Kauer JS, Shepherd GM. Mapping of odor-related neuronal activity in the olfactory bulb by high-resolution 2-deoxyglucose autoradiography. *Proc Natl Acad Sci U S A.* 1982; 79:670–674. [PubMed: 6952218]
- Lecoq J, Parpaleix A, Roussakis E, Ducros M, Goulam Houssen Y, Vinogradov SA, Charpak S. Simultaneous two-photon imaging of oxygen and blood flow in deep cerebral vessels. *Nat Med.* 2011; 17:893–898. [PubMed: 21642977]
- Li A, Gire DH, Bozza T, Restrepo D. Precise detection of direct glomerular input duration by the olfactory bulb. *J Neurosci.* 2014a; 34:16058–16064. [PubMed: 25429146]
- Li AA, Gong L, Liu Q, Li X, Xu F. State-dependent coherences between the olfactory bulbs for delta and theta oscillations. *Neurosci Lett.* 2010; 480:44–48. [PubMed: 20594948]
- Li B, Liu R, Huang Q, Lu J, Luo Q, Li P. Coherent slow cortical potentials reveal a superior localization of resting-state functional connectivity using voltage-sensitive dye imaging. *Neuroimage.* 2014b; 91:162–168. [PubMed: 24434676]
- Liu N, Xu FQ, Marengo L, Hyder F, Miller P, Shepherd GM. Informatics approaches to functional MRI odor mapping of the rodent olfactory bulb - OdorMapBuilder and OdorMapDB. *Neuroinformatics.* 2004; 2:3–18. [PubMed: 15067166]
- Liu Q, Li A, Gong L, Zhang L, Wu N, Xu F. Decreased coherence between the two olfactory bulbs in Alzheimer's disease model mice. *Neurosci Lett.* 2013; 545:81–85. [PubMed: 23628669]

- Liu X, Zhu XH, Chen W. Baseline BOLD correlation predicts individuals' stimulus-evoked BOLD responses. *Neuroimage*. 2011; 54:2278–2286. [PubMed: 20934521]
- Lowel S, Singer W. Selection of intrinsic horizontal connections in the visual cortex by correlated neuronal activity. *Science*. 1992; 255:209–212. [PubMed: 1372754]
- Maandag NJ, Coman D, Sanganahalli BG, Herman P, Smith AJ, Blumenfeld H, Shulman RG, Hyder F. Energetics of neuronal signaling and fMRI activity. *Proc Natl Acad Sci U S A*. 2007; 104:20546–20551. [PubMed: 18079290]
- Magnuson ME, Thompson GJ, Pan WJ, Keilholz SD. Effects of severing the corpus callosum on electrical and BOLD functional connectivity and spontaneous dynamic activity in the rat brain. *Brain Connect*. 2014; 4:15–29. [PubMed: 24117343]
- Magri C, Schridde U, Murayama Y, Panzeri S, Logothetis NK. The amplitude and timing of the BOLD signal reflects the relationship between local field potential power at different frequencies. *J Neurosci*. 2012; 32:1395–1407. [PubMed: 22279224]
- Martin C, Grenier D, Thevenet M, Vigouroux M, Bertrand B, Janier M, Ravel N, Litaudon P. fMRI visualization of transient activations in the rat olfactory bulb using short odor stimulations. *Neuroimage*. 2007; 36:1288–1293. [PubMed: 17512755]
- Mohajerani MH, McVea DA, Fingas M, Murphy TH. Mirrored bilateral slow-wave cortical activity within local circuits revealed by fast bihemispheric voltage-sensitive dye imaging in anesthetized and awake mice. *J Neurosci*. 2010; 30:3745–3751. [PubMed: 20220008]
- Murakami M, Kashiwadani H, Kirino Y, Mori K. State-dependent sensory gating in olfactory cortex. *Neuron*. 2005; 46:285–296. [PubMed: 15848806]
- Nasirivanaki M, Xia J, Wan H, Bauer AQ, Culver JP, Wang LV. High-resolution photoacoustic tomography of resting-state functional connectivity in the mouse brain. *Proc Natl Acad Sci U S A*. 2014; 111:21–26. [PubMed: 24367107]
- Nawroth JC, Greer CA, Chen WR, Laughlin SB, Shepherd GM. An energy budget for the olfactory glomerulus. *J Neurosci*. 2007; 27:9790–9800. [PubMed: 17804639]
- Neville KR, Haberly LB. Beta and gamma oscillations in the olfactory system of the urethane-anesthetized rat. *J Neurophysiol*. 2003; 90:3921–3930. [PubMed: 12917385]
- Northoff G, Qin P, Nakao T. Rest-stimulus interaction in the brain: a review. *Trends Neurosci*. 2010; 33:277–284. [PubMed: 20226543]
- Pan WJ, Thompson G, Magnuson M, Majeed W, Jaeger D, Keilholz S. Broadband local field potentials correlate with spontaneous fluctuations in functional magnetic resonance imaging signals in the rat somatosensory cortex under isoflurane anesthesia. *Brain Connect*. 2011; 1:119–131. [PubMed: 22433008]
- Pan WJ, Thompson GJ, Magnuson ME, Jaeger D, Keilholz S. Infralow LFP correlates to resting-state fMRI BOLD signals. *Neuroimage*. 2013; 74C:288–297.
- Pasley BN, Inglis BA, Freeman RD. Analysis of oxygen metabolism implies a neural origin for the negative BOLD response in human visual cortex. *Neuroimage*. 2007; 36:269–276. [PubMed: 17113313]
- Poplawsky AJ, Fukuda M, Murphy M, Kim SG. Layer-Specific fMRI Responses to Excitatory and Inhibitory Neuronal Activities in the Olfactory Bulb. *J Neurosci*. 2015; 35:15263–15275. [PubMed: 26586815]
- Ratzlaff EH, Grinvald A. A tandem-lens epifluorescence microscope: hundred-fold brightness advantage for wide-field imaging. *J Neurosci Methods*. 1991; 36:127–137. [PubMed: 1905769]
- Rebello MR, McTavish TS, Willhite DC, Short SM, Shepherd GM, Verhagen JV. Perception of odors linked to precise timing in the olfactory system. *PLoS Biol*. 2014; 12:e1002021. [PubMed: 25514030]
- Sadaghiani S, Hesselmann G, Kleinschmidt A. Distributed and antagonistic contributions of ongoing activity fluctuations to auditory stimulus detection. *J Neurosci*. 2009; 29:13410–13417. [PubMed: 19846728]
- Sair HI, Yahyavi-Firouz-Abadi N, Calhoun VD, Airan RD, Agarwal S, Intrapromkul J, Choe AS, Gujar SK, Caffo B, Lindquist MA, Pillai JJ. Presurgical brain mapping of the language network in patients with brain tumors using resting-state fMRI: Comparison with task fMRI. *Hum Brain Mapp*. 2016; 37:913–923. [PubMed: 26663615]

- Sanganahalli BG, Bailey CJ, Herman P, Hyder F. Tactile and non-tactile sensory paradigms for fMRI and neurophysiologic studies in rodents. *Methods Mol Biol.* 2009; 489:213–242. [PubMed: 18839094]
- Sanganahalli BG, Rebello MR, Herman P, Papademetris X, Shepherd GM, Verhagen JV, Hyder F. Comparison of glomerular activity patterns by fMRI and wide-field calcium imaging: Implications for principles underlying odor mapping. *Neuroimage.* 2016; 126:208–218. [PubMed: 26631819]
- Schlegel F, Schroeter A, Rudin M. The hemodynamic response to somatosensory stimulation in mice depends on the anesthetic used: Implications on analysis of mouse fMRI data. *Neuroimage.* 2015; 116:40–49. [PubMed: 25979665]
- Schulz K, Sydekum E, Krueppel R, Engelbrecht CJ, Schlegel F, Schroter A, Rudin M, Helmchen F. Simultaneous BOLD fMRI and fiber-optic calcium recording in rat neocortex. *Nat Methods.* 2012; 9:597–602. [PubMed: 22561989]
- Sharp FR, Kauer JS, Shepherd GM. Local sites of activity-related glucose metabolism in rat olfactory bulb during olfactory stimulation. *Brain Res.* 1975; 98:596–600. [PubMed: 1182541]
- Shmuel A, Leopold DA. Neuronal correlates of spontaneous fluctuations in fMRI signals in monkey visual cortex: Implications for functional connectivity at rest. *Hum Brain Mapp.* 2008; 29:751–761. [PubMed: 18465799]
- Smear M, Shusterman R, O'Connor R, Bozza T, Rinberg D. Perception of sniff phase in mouse olfaction. *Nature.* 2011; 479:397–400. [PubMed: 21993623]
- Smith AJ, Blumenfeld H, Behar KL, Rothman DL, Shulman RG, Hyder F. Cerebral energetics and spiking frequency: the neurophysiological basis of fMRI. *Proc Natl Acad Sci U S A.* 2002; 99:10765–10770. [PubMed: 12134056]
- Smith SM, Fox PT, Miller KL, Glahn DC, Fox PM, Mackay CE, Filippini N, Watkins KE, Toro R, Laird AR, Beckmann CF. Correspondence of the brain's functional architecture during activation and rest. *Proc Natl Acad Sci U S A.* 2009; 106:13040–13045. [PubMed: 19620724]
- Stavisky SD, Kao JC, Ryu SI, Shenoy KV. Trial-by-Trial Motor Cortical Correlates of a Rapidly Adapting Visuomotor Internal Model. *J Neurosci.* 2017; 37:1721–1732. [PubMed: 28087767]
- Steinke JM, Shepherd AP. Effects of temperature on optical absorbance spectra of oxy-, carboxy-, and deoxyhemoglobin. *Clin Chem.* 1992; 38:1360–1364. [PubMed: 1623605]
- Thompson GJ. Neural and metabolic basis of dynamic resting state fMRI. *Neuroimage.* 2017
- Thompson GJ, Magnuson ME, Merritt MD, Schwarb H, Pan WJ, McKinley A, Tripp LD, Schumacher EH, Keilholz SD. Short-time windows of correlation between large-scale functional brain networks predict vigilance intraindividually and interindividually. *Hum Brain Mapp.* 2013a; 34:3280–3298. [PubMed: 22736565]
- Thompson GJ, Merritt MD, Pan WJ, Magnuson ME, Grooms JK, Jaeger D, Keilholz SD. Neural correlates of time-varying functional connectivity in the rat. *Neuroimage.* 2013b; 83C:826–836.
- Thompson GJ, Riedl V, Grimmer T, Drzezga A, Herman P, Hyder F. The whole-brain “global” signal from resting state fMRI as a potential biomarker of quantitative state changes in glucose metabolism. *Brain Connect.* 2016; 6:435–447. [PubMed: 27029438]
- Tsodyks M, Kenet T, Grinvald A, Arieli A. Linking spontaneous activity of single cortical neurons and the underlying functional architecture. *Science.* 1999; 286:1943–1946. [PubMed: 10583955]
- Vasireddi AK, Vazquez AL, Whitney DE, Fukuda M, Kim SG. Functional Connectivity of Resting Hemodynamic Signals in Submillimeter Orientation Columns of the Visual Cortex. *Brain Connect.* 2016
- Vazquez AL, Murphy MC, Kim SG. Neuronal and physiological correlation to hemodynamic resting-state fluctuations in health and disease. *Brain Connect.* 2014; 4:727–740. [PubMed: 25300278]
- VosdeWael R, Hyder F, Thompson GJ. Effects of tissue-specific fMRI signal regression on resting-state functional connectivity. *Brain Connect.* 2017; 7:482–490. [PubMed: 28825320]
- Wachowiak M, Cohen LB. Correspondence between odorant-evoked patterns of receptor neuron input and intrinsic optical signals in the mouse olfactory bulb. *J Neurophysiol.* 2003; 89:1623–1639. [PubMed: 12612023]
- Weissman DH, Roberts KC, Visscher KM, Woldorff MG. The neural bases of momentary lapses in attention. *Nat Neurosci.* 2006; 9:971–978. [PubMed: 16767087]

- Wilson DA, Hoptman MJ, Gerum SV, Guilfoyle DN. State-dependent functional connectivity of rat olfactory system assessed by fMRI. *Neurosci Lett*. 2011; 497:69–73. [PubMed: 21530613]
- Wilson DA, Yan X. Sleep-like states modulate functional connectivity in the rat olfactory system. *J Neurophysiol*. 2010; 104:3231–3239. [PubMed: 20861440]
- Wohlschlagel AM, Glim S, Shao J, Draheim J, Kohler L, Lourenco S, Riedl V, Sorg C. Ongoing Slow Fluctuations in V1 Impact on Visual Perception. *Front Hum Neurosci*. 2016; 10:411. [PubMed: 27601986]
- Yu CR, Power J, Barnea G, O'Donnell S, Brown HE, Osborne J, Axel R, Gogos JA. Spontaneous neural activity is required for the establishment and maintenance of the olfactory sensory map. *Neuron*. 2004; 42:553–566. [PubMed: 15157418]
- Zhu XH, Zhang N, Zhang Y, Ugurbil K, Chen W. New insights into central roles of cerebral oxygen metabolism in the resting and stimulus-evoked brain. *J Cereb Blood Flow Metab*. 2009; 29:10–18. [PubMed: 18781163]

Fig. 1.

Creating flatmaps of the entire glomerular sheet using fMRI data. **(A)** An outline is drawn of the glomerular layer on each slice. The outline is cut at the bottom, flattened and combined with other slices to produce a flatmap. The orientation is shown on the right (V=ventral, L=lateral, D=dorsal, M=medial, R=rostral, C=caudal). **(B)** The blue outline represents the dorsal surface of the OB, as measured with IOS, and is shown above flatmaps of the glomerular sheet, as measured with fMRI. The blue outline is scaled (in the R-C direction) to match in size. (In B, the flatmaps have had both sides rotated by 90 degrees and the left side has been flipped horizontally.) The dorsal surface measured by IOS is comparable to 15-20% of the entire glomerular sheet measured by fMRI.

Fig. 2.

IOS-derived resting state networks in the rat olfactory bulb and relationship to activation. Images are masked to show only dorsal OB, nose upward. (Resting state N=5 rats/15 total trials, stimulation N=6 rats/18-19 total trials per odor) **(A)** *T*-maps of 16 networks. The networks are structured and mostly unilateral, though some are weakly bilateral. (Random networks are shown in Fig. S2A.) **(B)** Correlation (*Z* values on y-axis) between 16 networks (x-axis) and odor activation maps from individual trials (mean $\pm$ 1 S.E.) for IAA. Asterisks indicate significantly high correlation. **(C)** As B except MV odor. **(D)** As B, except EB odor. Specific numbers are shown in Table S1A.

Fig. 3.

Simulated activation maps from IOS-derived resting state data versus actual activation maps. Images are masked to show only dorsal OB, nose upward. (Resting state N=5 rats/15 total trials, stimulation N=6 rats/18-19 total trials per odor. Random networks are shown in Fig. S3.) **(A)** Simulated IAA odor activation map. (This map was created by combining resting state networks shown in Fig. 2A.) **(B)** Mean of actual IAA odor activation maps (taken across all trials, all rats). **(C)** Simulated MV odor activation map. **(D)** Mean of actual MV odor activation maps. **(E)** Simulated EB odor activation map. (How it was created is shown in Fig. S1.) **(F)** Mean of actual EB odor activation maps. **(G)** Spatial correlation coefficients (Z values, y-axis) between actual activation maps from individual trials (x-axis, mean $\pm$ 1 S.E.) and simulated networks (color) from resting state data. Asterisk indicates significant difference between correct and incorrect map pairings. **(H)** As G, except using random networks (see section 2.1.2.2). Specific numbers are shown in Table S1A.

Fig. 4. fMRI-derived resting state networks in the rat olfactory bulb and relationship to activation. See Fig. 1A for flatmap orientation. (Resting state N=6 rats/29 total trials, stimulation N=6 rats/12-16 total trials per odor) (A) *T*-maps of 22 networks created. The networks are structured and half are strongly bilateral. (Random networks are shown in Fig. S4A.) (B) Correlation (*Z* values on y-axis) between 22 networks (x-axis) and odor activation maps from individual trials (mean $\pm$ 1 S.E.) for IAA. Asterisks indicate significantly high correlation. (C) As B except MV odor. (D) As B, except EB odor. Specific numbers are shown in Table S1B.

Fig. 5.

Simulated activation maps from fMRI-derived resting state data versus actual activation maps. See Fig. 1A for flatmap orientation. (Resting state N=6 rats/29 total trials, stimulation N=6 rats/12-16 total trials per odor. Random networks are shown in Fig. S5.) (A) Simulated IAA odor activation map. (This map was created by combining resting state networks shown in Fig. 4A.) (B) Mean of actual IAA odor activation maps (taken across all trials, all rats). (C) Simulated MV odor activation map. (D) Mean of actual MV odor activation maps. (E) Simulated EB odor activation map. (F) Mean of actual EB odor activation maps. (G) Spatial correlation coefficients (Z values, y-axis) between actual activation maps from individual trials (x-axis, mean $\pm$ 1 S.E.) and simulated networks (color) from resting state data. Asterisks indicate significant difference between correct and incorrect map pairings. (H) As G, except using random networks (see section 2.1.2.2). Specific numbers are shown in Table S1B.

Table 1

Number of trials per rat for resting state and for each type of odor stimulation for fMRI.

Rat number	Total Rest	Total IAA	Total MV	Total EB
1	4	2	0	0
2	5	4	4	0
3	5	3	3	3
4	5	3	3	3
5	5	3	3	3
6	5	0	3	3
Total	29	15	16	12