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Supplemental Information

Delineating the Macroscale Areal Organization of the Macaque Cortex *In Vivo*

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Supplemental Data

Dataset 1, NKI-Macaque
The Macaque dataset 1 consisted of two rhesus monkeys (Macaca mulatta, one male, age 6 years, 6.4 kg, marked as NKI-R; one female, age 7 years, 4.5 kg, marked as NKI-W), which was collected from the Nathan Kline Institute for Psychiatric Research. All methods and procedures were approved by the NKI Institutional Animal Care and Use Committee (IACUC) protocol. The monkeys were previously implanted with an MRI-compatible custom built acrylic head post.

Data Acquisition
Structural MRI data were obtained with a 3.0 Tesla Siemens Tim Trio scanner with an 8-channel surface coil adapted for monkey head scanning. T1-weighted anatomical images (TE=3.87 ms, TR=2500 ms, TI=1200 ms, flip angle=8 degrees, 0.5 mm isotropic voxel) were collected for each macaque. Each macaque was sedated with an initial dose of atropine (0.05 mg/kg IM), dexdomitor (0.02 mg/kg IM) and ketamine (8 mg/kg IM) intubated, and maintained at 0.75% isoflurane anesthesia for the duration of structural MRI procedures. Functional MRI scans were obtained using a gradient echo EPI sequence (TR=2000 ms, TE=16.6 ms, flip angle=45 degree, 1.5x1.5x2mm voxels, 32 slices, FOV=96 x 96 mm). Seven sessions were acquired for NKI-R (294 min in total) and eleven sessions (668 min) for NKI-W. Each session included 4-7 scans, 8-10 minutes per scan. We collected data under anesthesia and while the monkeys were awake. Monocrystalline iron oxide ferumoxytol (MION) solution was injected at iron doses of 10 mg/kg IV prior to the MRI scanning for all MION sessions. See Table S1 for details of the parameters of the data analyzed. Additionally, we collected somatosensory task (2 sessions for NKI-R and 6 sessions for NKI-W) while the monkeys were awake without MION contrast agent. The air puff was used to stimulate the finger tips. The pseudo-randomized left and right stimulation blocks (20 sec) were separated by 20 sec periods of rest. During the awake scanning, the head motion was controlled using head holder, which was implanted stereotaxically on the cranium of NKI-R and NKI-W under anesthesia before the awake studies.

Dataset 2, OHSU-Macaque
A second macaque dataset consisted of two male rhesus macaques (Macaca mulatta, one male, age 5 years, 8.6 kg, marked as OHSU-1; one male, age 5 years, 7.6 kg, marked as OHSU-2), which were collected at the Oregon Health and Science University. Animal procedures were in accordance with the National Institutes of Health guidelines on the ethical use of animals and were approved by the Oregon National Primate Research Center (ONPRC) Institutional Animal Care and Use Committee.

Data Acquisition
Structural MRI data were obtained with a 3.0 Tesla Siemen Tim Trio scanner with a 15-channel knee coil adapted for monkey head scanning. T1-weighted anatomical images (TE=3.33 ms, TR=2600 ms, TI=900 ms, flip angle=8 degrees, 0.5 mm isotropic voxel) were collected for each macaque. Functional MRI scan were obtained using a gradient echo EPI sequence (TR=2070 ms, TE=25 ms, flip angle=90 degrees, 1.5x1.5x1.5 mm voxels, 32 slices, FOV=96 x 96 mm). Each macaque was sedated with an initial dose of ketamine (10 mg/kg) for intubation, and thereafter maintained on <1% isoflurane anesthesia during each scan session. Eight sessions were acquired for each monkey; For each session, 30 min BOLD scan without MION and 30 min scan with MION were acquired at the same day; The BOLD scan started at 45 min after the monkey was anesthetized, followed by the MION scan. The MION solution was injected at iron doses of 8 mg/kg IV. We collected all data at this site under anesthesia. See Table S1 for details of the parameters of the data analyzed.

Supplemental Experimental Procedures

Image Preprocessing
Structural image processing included the following steps: 1) spatial noise removal by a non-local mean filtering operation (NKI-dataset) (Zuo and Xing, 2011, 2014), 2) constructing an average structural volume from multiple T1-weight images, 3) brain extraction and tissue segmentation into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF); this was performed by FSL, FreeSurfer and ANTs, followed by manual editing to fix the mis-segmentations, 4) reconstructing the native white matter and pial surfaces using FreeSurfer, and 5) registering the native surface to a hybrid left-right template surface (Yerkes19 macaque atlas; (Donahue et al., 2016). The Yerkes19 template was created from 19 adult macaques T1 images acquired at the Yerkes Primate Research Center

and processed with customized Human Connectome Project (HCP) pipeline (Donahue et al., 2016; Glasser et al., 2013). The left and right hemisphere surfaces were registered with each other and down-sampled from 164k_fs_LR surface to a 10k_fs_LR (10,242 vertices) surface.

Functional image preprocessing included the following steps: 1) discarding of the first five volumes of the time series and compressing temporal spikes (AFNI 3dDespike), 2) slice timing correction, motion correction, and bias field correction (OHSU-dataset), 3) normalizing the 4D global mean intensity, 3) regressing out nuisance signals including the mean time series from WM and CSF masks, the Friston-24 motion parameters (Yan et al., 2013), as well as linear and quadratic trends, 4) band-passed filtering ($0.01 < f < 0.1$Hz) of the residuals to extract the low-frequency fluctuations, 5) registering the functional image to the anatomical space and projecting it onto the native middle surface, 6) spatial smoothing with a 4 mm full width at half maximum kernel along the native surface and down-sampling to the 10k surface.

Quality Control Procedure

Frame-wise displacement (FD) and mean FD were calculated to quantify the head micro-movements. The mean FD of the scans included in our analysis were less than 0.25 mm during awake scanning and 0.05 mm under anesthesia. The average mean-FD was 0.11 (SD=0.05) across all the awake scans of two macaques and 0.023 (SD=0.006) across all the anesthetized scans of all four of the macaques.

Surface-based FC Calculation and Gradient-based Parcellation

The cortical surface was reconstructed to provide an accurate representation of morphology and topology of the brain for each macaque. The volumetric fMRI data was aligned to the anatomical space and then projected to the native middle cortical surface. Then the time series were smoothed along the high-resolution (about 164k vertices for each hemisphere) native middle surface (FWHM = 4 mm) and down resampled to a coarser (10,242 vertices for each hemisphere) template surface. Based on the prior study in human work (Xu et al., 2016), the smoothing kernel is chosen about twice that of the surface (10k surface) that the gradients were calculated on.

The procedures of surface-base FC calculation and gradient-based parcellation were similar as previous studies described in Laumann et al. (2015) and Xu et al. (2016). In brief, the functional connectivity (FC) map in full cortex of the gray matter tissue was first computed using the time course for each vertex, resulting in a (10,242 vertices x 20,484 vertices) matrix. The distributions of the resulting correlation values were standardized to the normal distribution using Fisher's r-to-z transform. Then a FC similarity profile was calculated for each vertex on the surface from the spatial correlation between the vertex's FC map and the FC map of every other vertex, resulting in a 10,242 vertices x 10,242 vertices symmetric matrix. Each column (or row) of this matrix represents the FC similarity map for each surface vertex. The spatial gradient (i.e., the first spatial derivative) of each FC similarity map was computed on the native middle surface to measure the degree of the transition in FC profile at each vertex, resulting in 10k gradient maps for each hemisphere. A 'watershed by flooding' algorithm (Gordon et al., 2016) was then applied to each of the gradient maps - resulting in 10k binarized edge maps. Finally, the 10k gradient and edge maps were averaged to generate the final gradient and an edge density map. By applying the same watershed algorithm to the edge density map to yield the final parcel map.

Parcellation Validation

For each Macaque, in order to compare the homogeneity across different conditions (awake vs. anesthesia, MION vs. no MION), the parcel homogeneity was evaluated using Kendall' coefficient at all vertices in the parcel for each condition (Xu et al., 2016). Consistent with previous human work, the overall homogeneity of the parcellation was compared to a null model created from 1000 random rotations of the parcellations on the cortical surface (Gordon et al., 2016).

Network Assignment

To characterize a large-scale system of parcellations created in the current study, we assigned a network identity to parcels using the previously established network definition from Ghahremani et al. (2016) The matching procedure was similar with Gordon et al. (2016). Specifically, we extracted the time series and averaged it within each parcel. The averaged time series were then correlated against all other times series across the cortical surface to obtain a connectivity map. Connections were excluded if the geodesic distance between parcel centers was less than 10 mm to remove the purely local connectivities (Power et al., 2011) from spatial smoothing. After that, we thresholded and binarized the connectivity map at the top 5% of connectivity strengths. This resulted in a binarized map of regions

with high connectivity between parcels. Then we examined the overlap of this binarized map to the binarized group ICA Z-map (Z>2.33, p<0.001) from Ghahremani et al. (2016) and defined the best match using the Dice coefficient. Then neighboring parcels assigned to the same network were merged to create a final network-patch (see Figure 7).

Evaluating the Stability of FC, Gradients, and Edge Density

A key challenge for imaging efforts focused on brain parcellation is the determination of minimal data requirements to reliably and consistently capture individual parcellations. While the human imaging literature is actively working to establish data requirements, functional MRI imaging in non-human primates faces additional challenges. In particular, decreased signal to noise characteristics relative to humans due to smaller voxels size required, and the usage of anesthetics. The iron-based contrast agent, MION, is increasingly being used to overcome these challenges, particularly when scanning at 3.0T (Gautama et al., 2003; Grayson et al., 2016; Leite et al., 2002); however, the necessity of MION, particularly in the awake macaque, is not clear. In the present study, for each macaque, we randomly split the data from all sessions into halves within each condition, setting aside one half of the data as a reference (subset 2). We then randomly selected samples from subset 1 of 8, 12, 16, 20, 24, 28, 32, 40, 48, 56, 72, and 88 min (up to the half of the sample for each macaque in each condition) to examine convergent estimates of FC, gradient and edges. Of note, previous findings suggested that shorter sampling of contiguous data over more sessions can facilitate the convergence of stability. Hence the subsamples of 8 to 88 min data were generated from multiple 2 min contiguous segments from the subset 1. Parcel-based FC was calculated to evaluate the areal-level cortical network and permuted 1000 times at each time point. Gradient and edge density maps were also calculated to examine the convergence of the parcellation. Of note, to decrease the computational costs of gradient and edge density calculation for the random samples at each time point, we only used 100 random samples and based our calculation on a reduced (i.e., downsampled) set of vertices (i.e., 400) (prior testing found the results obtained to be comparable with those obtained for the higher resolution representations).

Parcellation Homogeneity and the Parcel Size

As expected, we noted a close relationship between homogeneity and parcel size in Figure 2C (Lowess fit line). Specifically, we found a negative correlation (r=-0.24, p<0.001) for functional-defined parcellations across 4 monkeys (both awake and anesthetized states), as well as five atlases (Figure S5). We tested the significance of differences in homogeneity among parcels as a function of size, by: 1) divided parcels (across all parcellations) into five subgroups based on the parcel size (parcel size: below 15 mm^2, 15-30 mm^2, 30-45 mm^2, 45-60 mm^2, above 60 mm^2), and then 2) conducting a two-way ANOVA to compare the homogeneity among parcellations (factors: parcel size group, parcellation). The pair-wise post hoc test demonstrated that the homogeneities of four functional-defined parcellations were significantly greater than those of the atlases (all p<0.001), including the PHT00 atlas which had the highest number of parcels.

Data Requirements for Mapping the Reliable FC, Gradient and Edges

During the awake state (Figure 5), the averaged similarities (spatial correlation) from subset 1 and subset 2 (reference) increased more slowly up to 88 minutes for NKI-W, where they were 0.92 (SD=0.007) for FC, and r=0.74 (SD=0.009) for gradients; the Dice coefficient was 0.72 (SD=0.009) for parcellations with 88 min of data. Similar results were found in subject NKI-R, though the overall similarities were lower than subject NKI-W as less data was collected. During the anesthetized state, at the 8 min point, the average similarities (spatial correlation) across four monkey from subset 1 with subset 2 (reference) were only 0.77 (SD=0.388) for FC and 0.61 (SD=0.310) for gradient; mean Dice coefficient was 0.69 (SD=0.020) for parcellations, while increased to 0.92 (SD=0.037) for FC, 0.83 (SD=0.343) for gradient, and 0.73 (SD=0.365) for parcellations at 40 min. The similarity is increased to 0.91 (SD=0.026) for FC, 0.87 (SD=0.033) for gradient, and 0.75 (SD=0.014) for parcellation at 110 min in the OHSU dataset.

Effect of MION

As can be seen in Figure S6 (right panel), compared to the MION data within the same macaque, the FC, gradient and edge density maps obtained without MION have a relatively modest correspondence with those obtained using MION in anesthesia data (dark green dots). Similar but relatively lower correspondences were observed in the awake data from NKI-dataset (light green dots). These findings suggest that the signal obtained with MION is notably improved relatively to standard BOLD fMRI.

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Table S1. Related to Experimental Procedures. Parameters of the datasets included in this study.

Subject ID	Site	Awake (movie) MION	Awake (rest) MION	Awake (movie) BOLD (without MION)	Anesthesia MION	Anesthesia BOLD (without MION)
NKI-R	NKI	2 sessions (88 min) mean FD = 0.127 (0.046)	NA	3 sessions (96 min) mean FD = 0.075 (0.023)	2 sessions (110 min) mean FD = 0.017 (0.002)	NA
		subset 1: s1 (32min) subset 2: s2 (56 min)		subset 1: s2 (32min) subset 2: s1, s3 (64 min)	subset 1: s1 (50min) subset 2: s2 (60 min)	
NKI-W	NKI	4 sessions (240 min) mean FD = 0.119 (0.042)	1 sessions (80 min) mean FD = 0.164 (0.044)	3 sessions (180 min) mean FD = 0.106 (0.037)	3 sessions (188 min) mean FD = 0.021 (0.002)	NA
		subset 1: s1, s3 (120 min) subset 2: s2, s4 (120 min)		subset 1: s1 (60 min) subset 2: s1, s3 (120 min)	subset 1: s2 (48 min) subset 2: s1, s3 (140 min)	
OHSU-1	OHSU	NA	NA	NA	8 sessions (240 min) mean FD = 0.024 (0.001)	8 sessions (240 min) mean FD = 0.023 (0.003)
					subset 1: s2, 4, 5, 8 (120min) subset 2: s1, 3, 6, 7 (120 min)	subset 1: s2, 4, 5, 8 (120min) subset 2: s1, 3, 6, 7 (120 min)
OHSU-2	OHSU	NA	NA	NA	8 sessions (240 min) mean FD = 0.033 (0.007)	8 sessions (240 min) mean FD = 0.029 (0.006)
					subset 1: s2, 4, 5, 8 (120min) subset 2: s1, 3, 6, 7 (120 min)	subset 1: s2, 4, 5, 8 (120min) subset 2: s1, 3, 6, 7 (120 min)

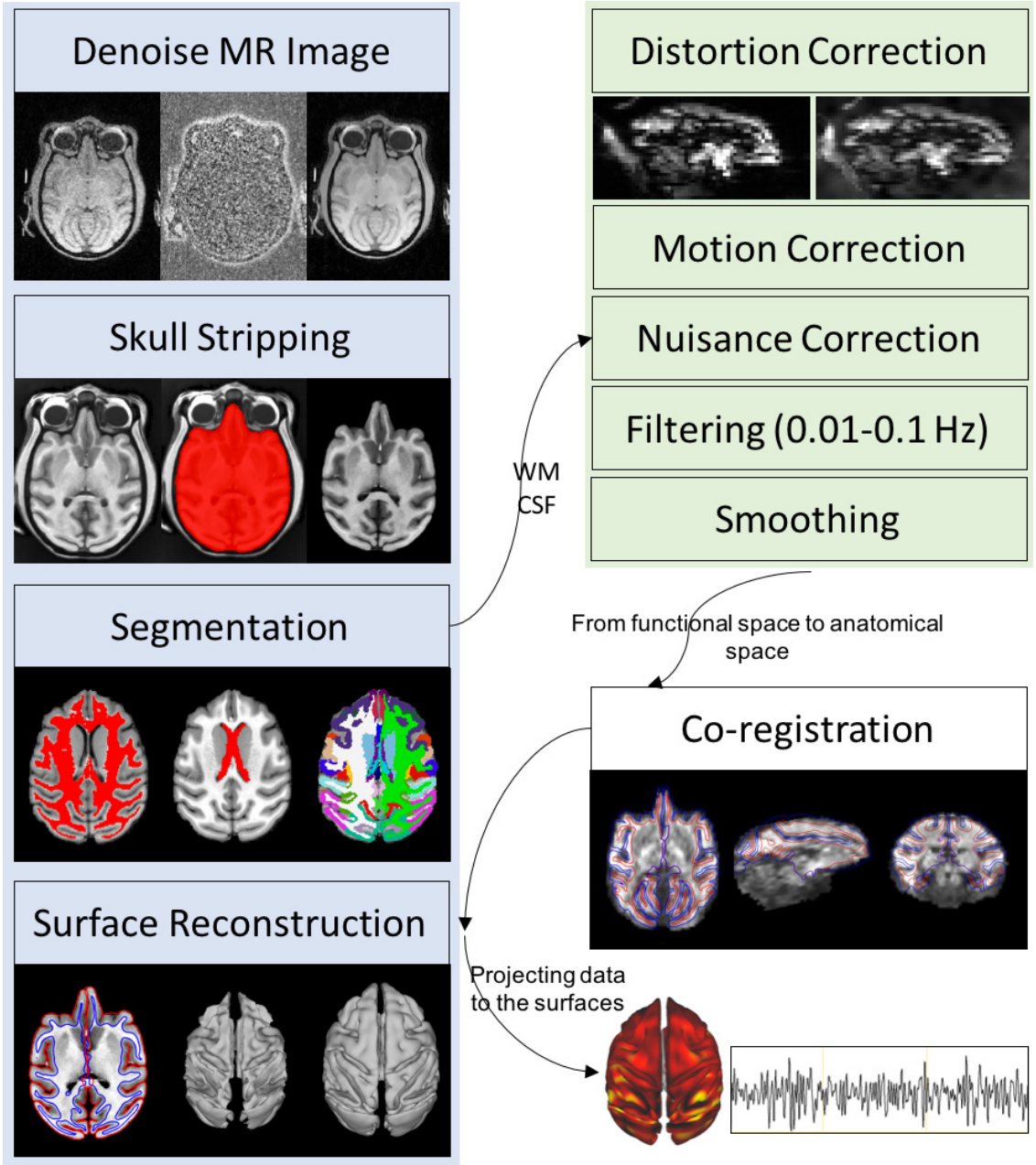

Figure S1. Related to Experimental Procedures. The workflow of the data-preprocessing.

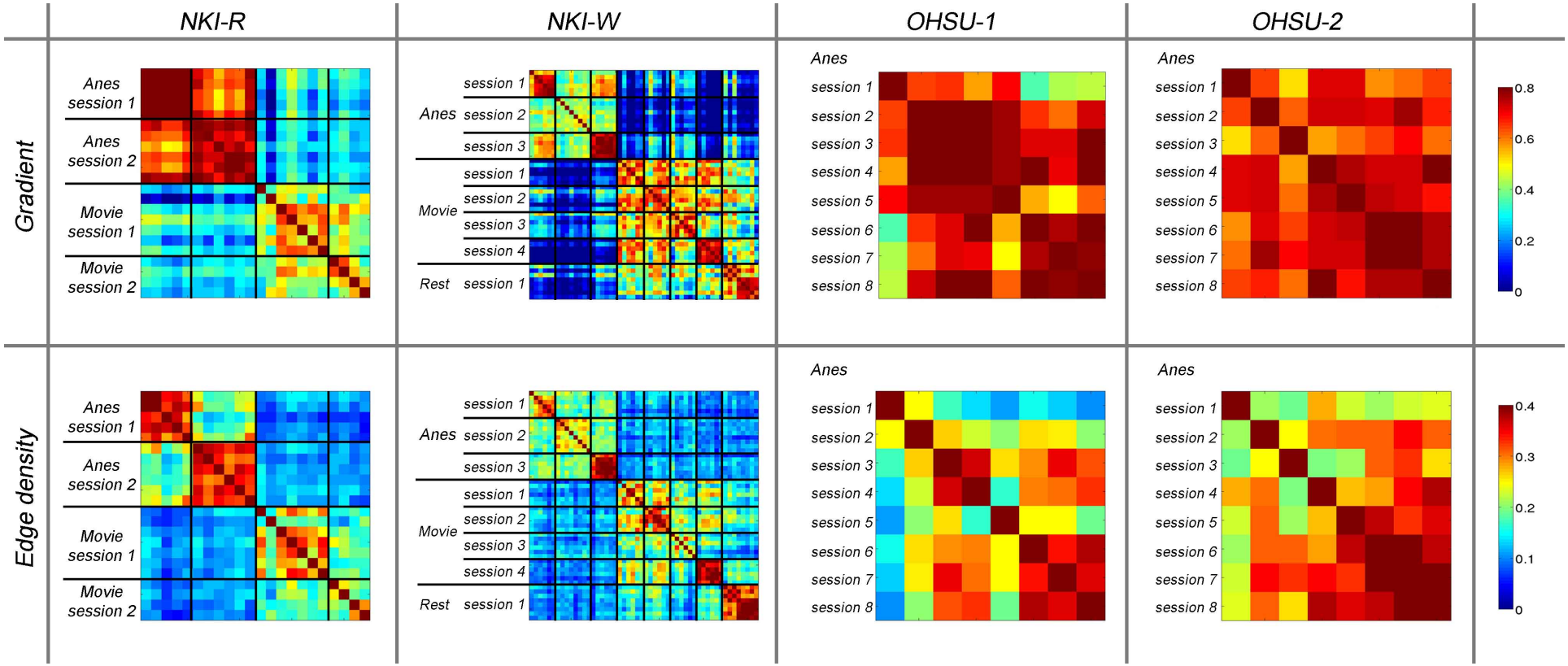

Figure S2. Related to Experimental Procedures and Figure 1. The spatial correlation matrixes of gradient and edge density maps between scans across four monkeys and sessions. NKI-dataset acquired 8-10 min per scan and 5-7 scans per sessions while OHSU-dataset acquired only one 30 min scan per session.

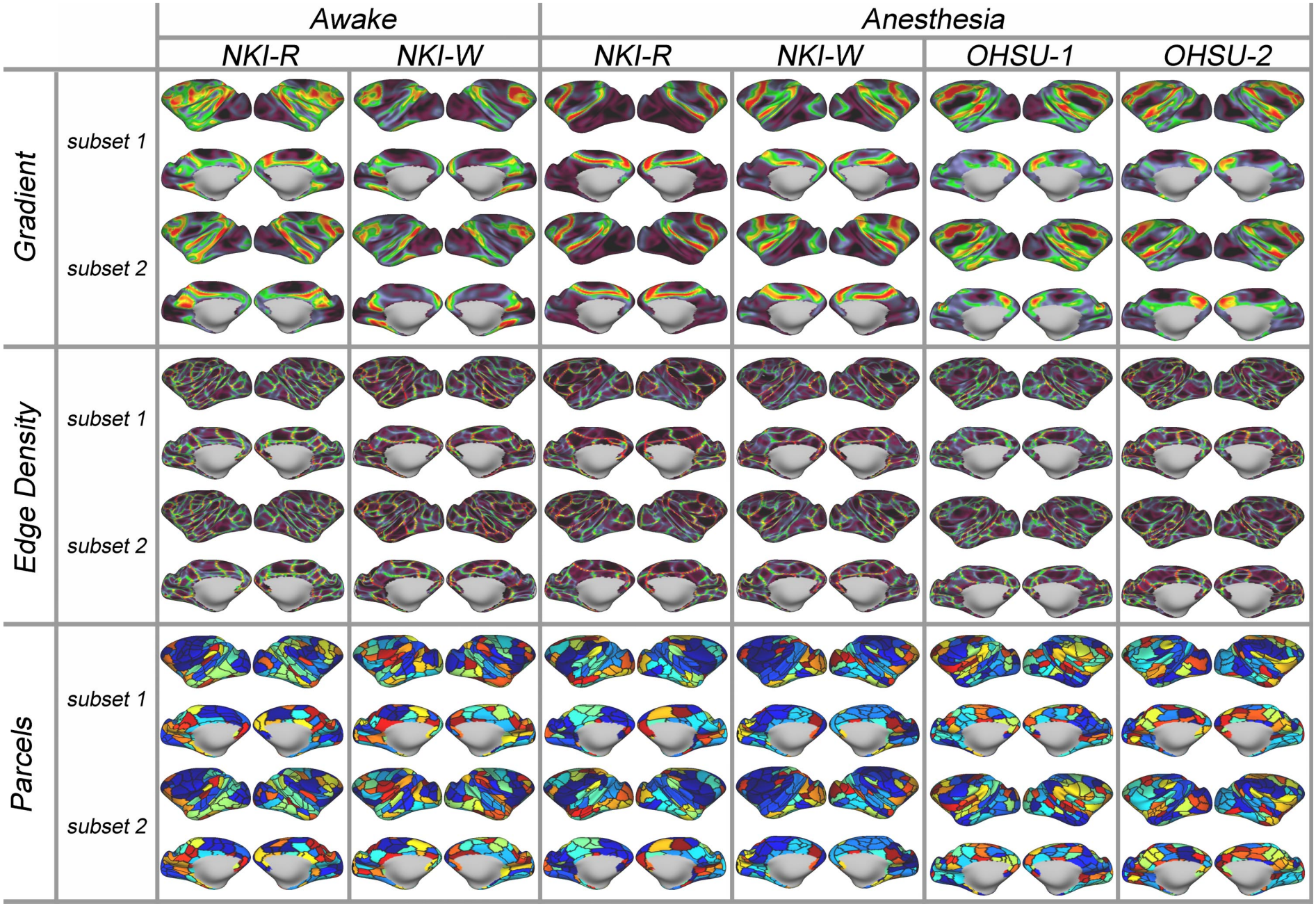

Figure S3. Related to Figure 1. Subject-specific areal organization (gradient, edge density and parcellation) from two independent subsets under awake and anesthesia in four macaques.

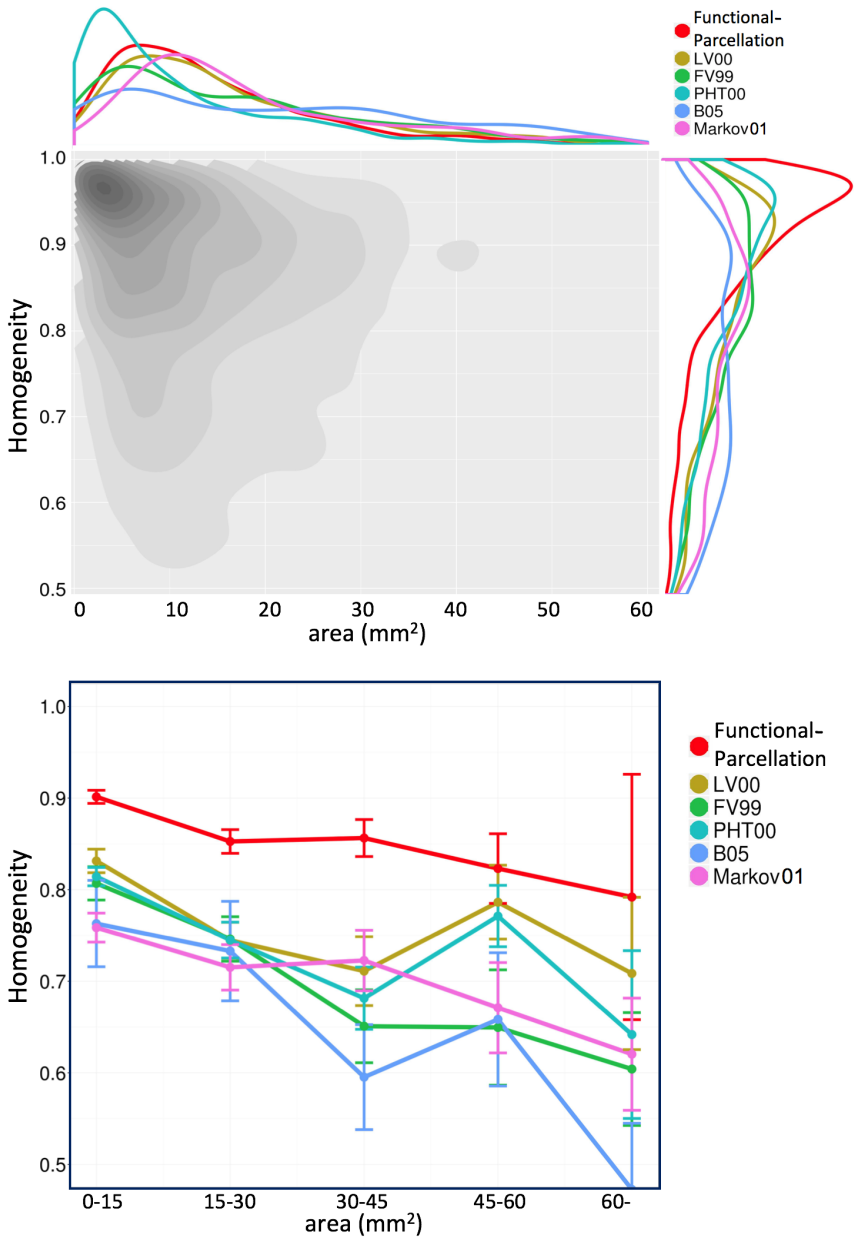

Figure S4. Related to Figure 2D. The homogeneities of different parcellations by parcel size. Top: the distribution of homogeneity and parcel size of functional-defined individual parcellation and five atlases across 4 monkeys including both awake and anesthetized states. Bottom: mean homogeneities (with confidence intervals) of parcels at each parcel-size group.

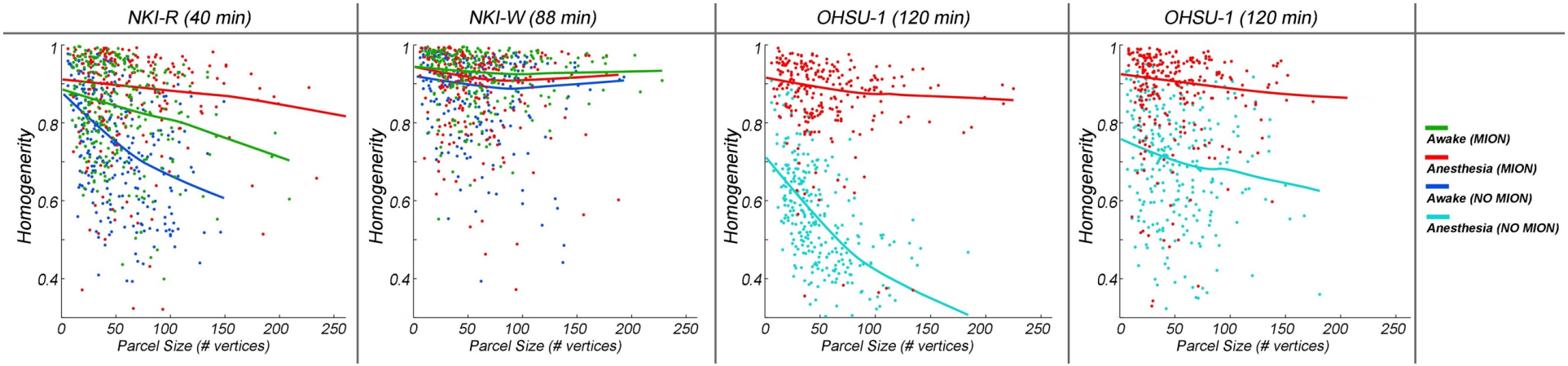

Figure S5. Related to Figure 2A. Homogeneity of subject-specific parcels by parcel size in different conditions (awake and anesthesia, MION vs. BOLD).

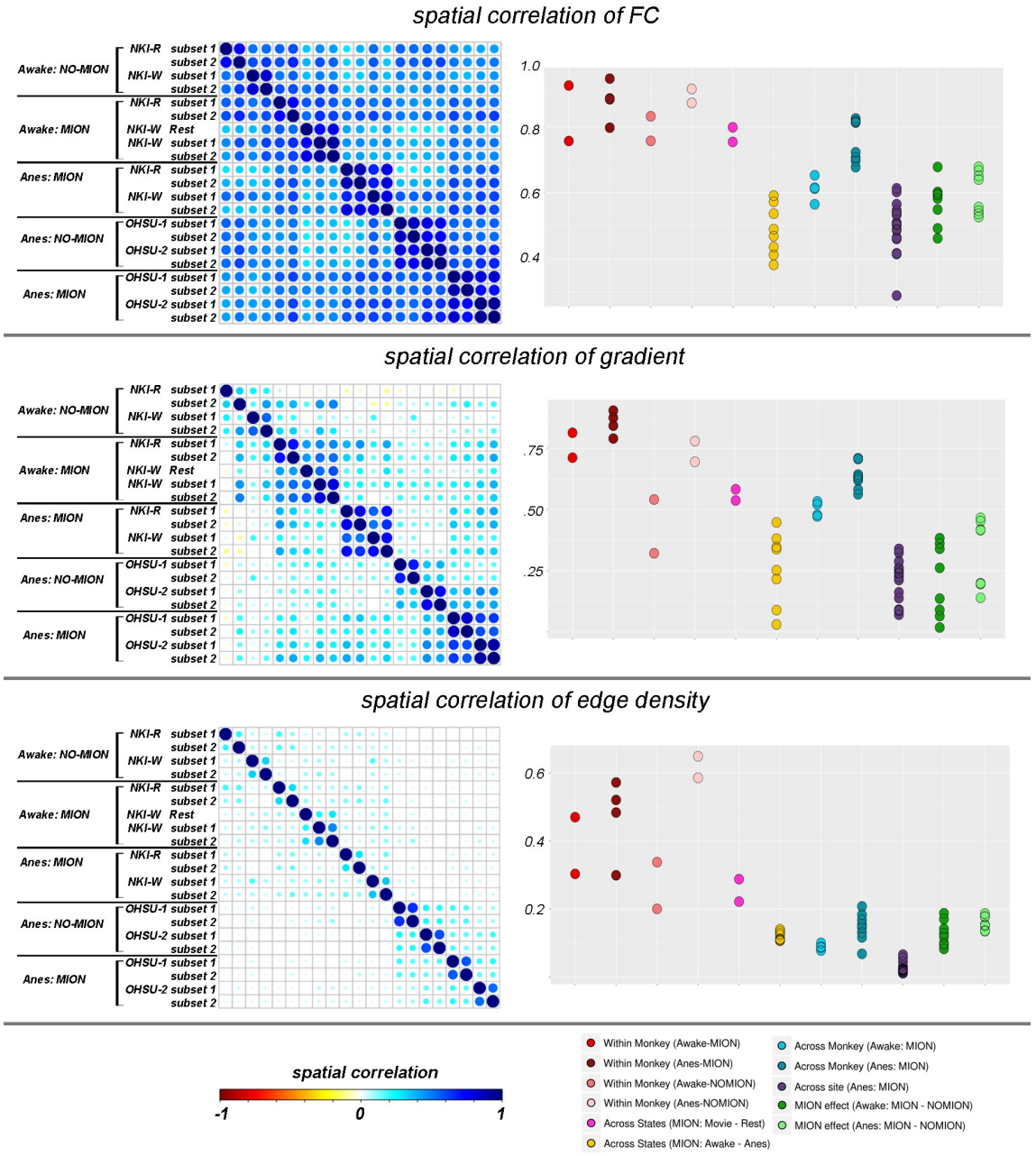

Figure S6. Related to Figure 7. The spatial correlations of FC, gradient and edge density maps across four macaques and subsets under different states from two sites (left panel). The distribution of correlations within- and between-monkey, states, and sites (right panel).