Delineating the macroscale areal organization of the macaque cortex in vivo

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Abstract

Complementing longstanding traditions centered around histology, functional magnetic resonance imaging approaches are rapidly maturing in their ability to delineate brain areal organization at the macroscale. In particular, automated approaches focused on the detection of gradient-based boundaries in functional connectivity (FC) properties between cortical areas have demonstrated the ability to characterize human brain organization at the individual level and recapitulate previously established cytoarchitectonic brain areas. The use of non-human primates (NHP) provides the opportunity to overcome critical barriers in the advancement of translational research. Here, we establish the data and scanning condition requirements for achieving reproducible, stable and internally valid areal parcellations at the individual levels, which have good correspondences with previously established postmortem areas; the inclusion of data from two independent imaging sites ensures the reproducibility of our findings. We demonstrate that highly reproducible areal organizations for fingerprinting can be achieved whether subjects were scanned under anesthesia or awake (rest, naturalistic viewing), though differences between awake and anesthetized states precluded the detection of individual differences across states. Individual differences were notably more stable across differing awake states. Comparison of awake and anesthetized states suggested a more nuanced picture of changes in connectivity for higher order association areas, as well as visual and motor cortex. These results establish feasibility and data requirements for the generation of reproducible individual-specific parcellations in NHP, as well as provide insights into the impact of scan state on findings.

Introduction

The non-human primate brain model remains one of the most promising vehicles for advancing translational neuroscience[1–7]. This is particularly true for investigations requiring the definition or manipulation of cortical areas and their associated circuitry. However, lines of inquiry tend to focus on the delineation of cortical area or circuit, in part due to the reliance on histological techniques that inherently necessitate a more targeted window of examination. Beyond their labor intensiveness, such methodologies require sacrifice of an animal to achieve the intended results, which inherently limits the longitudinal examinations needed to study neuroplasticity, neuromodulation and brain development. The availability of histological atlases for various non-human primate species provides an alternative means of guiding intracranial measurements and interventions. However, such atlases are inherently limited in their ability to account for differences in brain areal organization among animals, which can negatively impact experimentation when not properly considered[8–11].

Recent fMRI studies in the humans have demonstrated the feasibility of using resting state fMRI (R-fMRI) methodologies to delineate the areal organization of the cortex at the macroscale based on intrinsic brain function[12–16]. Inspired by the postmortem histological parcellation approaches used to differentiate spatially contiguous areas into functional units based on differences in their cytoarchitecturel and connectivity properties, Cohen et al.[17] proposed a novel approach to performing areal parcellation in the human brain that measures gradients in intrinsic functional connectivity among neighboring vertices, which can in turn be used to identify transitions across distinct functional units. This approach has been extended to the full neocortex, with applications revealing functional discrete areas at the group-average level that are homogenous and reproducible across studies [8,18,19]. Several of the borders have been highlighted for their correspondence to task activations and cytoarchitectonically defined areas[8,13,20]. Most recently, this gradient-based boundary mapping approach has been applied at the individual-level, successfully mapping full-brain gradient and transition

patterns that despite commonalities appear to be individual-specific[8,9,21,22] and have moderate to high test-retest reliability using as little as 20 minutes of data.

Work by several groups has suggested the possibility of parcellating the macaque brain in-vivo as well, using methods such as functional MRI, diffusion tractography and invasive neural recordings. However, to date, these studies[6,23–25] have primarily focused on more limited sections of cortex, e.g visual cortex, including occipitotemporal, parietal and frontal cortex. The logical extension of this work – fMRI-based parcellation of the neocortex of the macaque in its entirety – would be extremely valuable in evaluating the strengths and limitations of the macaque as a translational model[26–31].

In the present work, we pursue this aim using gradient-based fMRI parcellation methods to arrive at a putative map of the areal organization of the macaque cortex in its entirety. Although promising in human literature, there are several challenges that need to be addressed to achieve this goal, namely, 1) the feasibility of the boundary mapping parcellation in an individual macaque; 2) the validity of the parcellation; 3) the reliability of the gradient-based boundary mapping approach across scans in an individual macaque; and 4) the stability of the parcellation and the quantity of individual data required for an accurate estimate of full-brain functional areal organization. To address these challenges, we made use of four adult rhesus macaque datasets from two independent research institutions: Nathan Kline Institute (NKI-Dataset; n=2) and Oregon Health and Science University (OHSU-Dataset; n=2). Each macaque dataset includes between 294 and 688 min of awake and/or anesthetized fMRI scans. Using these data, we first identified the individual-specific functional boundaries (i.e. edge density) and areal parcellation for each dataset and compared it against the available anatomical borders from previous studies. Then, we assessed the functional homogeneity of the gradient-based areal parcellation and demonstrated the uniqueness of the result obtained for each specific monkey. We also evaluated the stability of functional connectivity (FC) profiles, gradient and boundary maps to investigate the total quantity of data required for estimating full-brain functional areal organization. In addition, we examined the impact of a monocrystalline iron oxide ferumoxytol (MION) contrast

agent, on the quality of data needs for parcellation. as prior work has demonstrated its utility in increasing the ability to detect functional connectivity patterns for non-human primates, when scanning at 3.0T[32]. Similarly, we examined the potential influences of anesthesia, which is known to decrease measures of FC somewhat[33]. Together, these analyses establish feasibility and data requirements for the generation of reproducible individual-specific parcellations in the non-human primate, as well provide insights into the impact of varying factors (e.g., scan state) on findings.

Results

High Reproducibility of Gradient-based Parcellations

The gradient, edge density and corresponding parcellations were first calculated using awake fMRI data with MION contrast (see Table S1 for the details of experiments), collected from the same macaques at the NKI site. The gradient evaluates the spatial transitions in the similarity of neighboring FC profiles across the native cortical surface. Corresponding edges and parcels across animals were identified from the gradient maps using the 'watershed' algorithm[34]. The details of these methods were described in previous studies[21,22]. Of note, the awake fMRI data were acquired with naturalistic viewing paradigm – movies were playing during the awake scans for the NKI dataset (monkey ID: NKI-R, NKI-W). To investigate the impact of awake states (movie and rest), we additionally collected one pure rest session for one animal (NKI-W). In the following section, 'awake' states without any specifications refer to naturalistic viewing states.

In the awake states with MION contrast, within individual animal, the gradients and edge density detected by this method demonstrated high reproducibility across individual scans (see Supplement Figure S1). Consistent with prior work in humans, gradient maps showed a higher degree of reproducibility than edge maps when equivalent amount of data were used. In order to obtain a better assessment of the upper bound for reproducibility, we randomly divided the scans for each macaque into two subsets (i.e., split halves) and regenerated gradient and edge maps for the data available in each subset. Markedly higher reproducibility was shown for each of the measures, as well as the resultant parcellation maps (Figure 1). The spatial correlations between two

gradient maps for macaque NKI-R and NKI-W while awake were 0.79 and 0.92; for edge density, the correlations were 0.42 and 0.71. The Dice coefficient between two parcellations were 0.69 and 0.76.

INSERT FIGURE 1. HERE

Next we looked at data obtained during the anesthetized state using MION in subjects from each site, NKI and OHSU. Similar with the awake results, we observed a high degree of reproducibility in gradients, edge density and corresponding parcellations (Figure S1). The spatial correlations in gradients between subsets were 0.96 (NKI-R), 0.87 (NKI-W), 0.84 (OHSU-1), and 0.90 (OHSU-2); for the edge density were 0.60, 0.48, 0.52 and 0.57. The Dice coefficient between parcellations were 0.69, 0.74, 0.70, and 0.72, respectively.

In addition, we examined the reproducibility of gradient, edge density and parcellations using data obtained without MION (NO-MION). Compared with the data using MION, the findings were similar but less reproducible. Specifically, in the awake states from NKI dataset, the spatial correlations between two subsets were 0.48 and 0.76 in gradients, 0.24 and 0.53 in edge density, 0.67 and 0.72 in parcellations. In the anesthetized data from OHSU, the spatial correlations were 0.69 and 0.78 in gradients, and 0.65 and 0.59 in edge density. The Dice coefficient were 0.70 and 0.67 for the final parcellations.

Comparison of Parcellation with Known Topographic Areas

To further assess the validity of the parcellation maps using an external reference, the putative boundaries delineated by our functional gradient-based parcellation was overlaid with the areas 3, 4, and 17 borders previously established using post-mortem histology [35,36]. As depicted in Figure 2, the parcels derived by gradient-based mapping were approximates overlapped with areas 3, 4, and 17 borders. This suggests that gradient-based boundaries represent differences in function within a topographically organized area that are captured by histologic and topographic mapping. It is important

to note that while there were clear correspondences between our findings and those of the postmortem map obtained from prior work, some notable differences exist. While such differences may suggest greater detectability, or the detection of a unique aspect of the functional architecture in the macaque, the possibility of artifactual findings related to the imaging also need to be considered. For example, in early visual cortices (e.g., the fractionates in area 17), we found additional edges not apparent in the postmortem map; examination of the raw data for these divisions suggests they may reflect an artifact arising from incomplete coverage, though alternatively, they may reflect the presence of further subdivisions. Future work with optimized imaging will be required to help differentiate between these possibilities.

INSERT FIGURE 2. HERE

Parcellation Homogeneity

Consistent with prior work in humans, we assessed the internal validity of the parcellation maps obtained for each macaque. Specifically, we calculated the homogeneity of FC similarity in the parcels generated from subset 2 (reference subset) using the data from subset 1 (test subset). The mean homogeneity across all parcels and two macaques in awake with MION condition was 0.88 (SD=0.044), while under anesthesia across four macaques was 0.89 (SD=0.015) (Figure 3, red line). Then, we assessed the degree to which this parcellation was more homogenous than a null distribution of mean homogeneities generated from 1000 'random' parcellation maps for the same subject; each of these maps were generated by randomly rotating the parcellation units around the cortical surface for each subject[34]. The mean homogeneity of null model parcellations was no more than 0.70 (mean=0.68, SD=0.013) for four macaques (these findings are comparable to those previously reported in humans[21,22]; in each macaque, the real parcellations were significantly greater than that obtained in null model parcellations (awake: Z score = 8.62, 9.66, $p<0.001$; anesthesia: 9.85, 6.38, 8.95, 7.25, $p<0.001$, Figure 3).

INSERT FIGURE 3. HERE

Additionally, we compared the homogeneity across different states (awake vs. anesthesia) and contrast types (MION vs. NO-MION). To facilitate a relatively fair comparison between states within each subject, we subsampled the reference and test subset to the same amount of time. The MION data exhibited higher homogeneity than NO-MION condition regardless of awake (Figure 4, blue line) or anesthetized states (Figure 4, turquoise line); the z scores between MION and NO-MION data in awake state are 7.55 (NKI-R), 7.61 (NKI-W), both $p<0.001$, while under anesthesia are 22.16 (OHSU-1) and 14.12 (OHSU-2), both $p<0.001$. These differences between awake and anesthesia are examined and discussed in the later sections.

INSERT FIGURE 4. HERE

Data Requirements for Mapping the Reliable FC, Gradient and Edges

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[32,37,38]; however, the necessity of MION, particularly in the awake macaque, is not clear.

Here, we first examined the stability of FC, gradient and edge maps derived from data obtained during the awake state using MION in the NKI-dataset. More specifically, for each of the two macaques imaged in the awake state, we randomly split the data obtained with MION into two independent subsets (120 minutes for each subject NKI-W; 44 minutes each for NKI-R) and used subset 2 to derive reference FC, gradients and edge density. Next, we randomly sampled subset 1, started at 8-minute amount, adding in six 4-min increments, followed by three 8-minute increments of data, and then (for subject NKI-W only) two 16 minute increments (i.e. 8, 12, 16, 20, 24, 28, 32, 40, 48, 56,

72, 88 min); the maps generated from each increment were compared to the reference maps in the same macaque. The results convergent estimation for FC are averaged from 1,000 random samplings of data at each time point. Of note, to facilitate the computational capacity for gradient and edge density calculation, the results of gradient and edge density were calculated on the down-resampled 400 regions of interest vertices for 100 times at each time point. The similarity coefficient (overlap of adjacent matrix from the test to the reference parcellation) were used to measure the similarity of the parcellations.

INSERT FIGURE 5. HERE

Consistent with prior human work, the average FC, gradients and parcellation from awake state (Figure 5, green line) were progressively increased by the amount of data incorporated into the analysis. Focus on the subject NKI-W who has more data, the average similarity (i.e. correlation, similarity coefficient) of 8 min from subset 1 with subset 2 (reference) was 0.87 (SD=0.021) for FC, 0.76 (SD=0.054) for gradient and 0.72 (SD=0.018) for edge density. The correlations were rapidly increased to 0.95 (SD=0.008) for FC, 0.86 (SD=0.032) for gradients with 28 min of data, and 0.78 (SD=0.013) for edge density with 40 min of data. The averaged similarities were then slowly increase to 0.97 (SD=0.003) for FC, r=0.90 (SD=0.009) for gradients and 0.80 (SD=0.011) for edge density with 88 min of data. Similar results were found in subject NKI-R while the overall similarities were lower than subject NKI-W as less data was collected.

Next we looked at data obtained during the anesthetized state using MION in subjects from each site, NKI and OHSU. Again, we compared findings obtained using two distinct subsets of data in each subject. We found that a stability profile was highly similar to that observed in the awake state (Figure 5, red line). For each of the monkeys, the parcel-based FC, gradient and corresponding parcellations uninterruptedly increased from 8 min to 28 min and began to plateau after 40~56 min. At the 8 min point, the average similarities across four monkey from subset 1 with subset 2

(reference) were only 0.81 (SD=0.055) for FC, 0.65 (SD=0.105) for gradient, and 0.69 (SD=0.020) for parcellations, while increased to 0.92 (SD=0.037) for FC, 0.83 (SD=0.044) for gradient, and 0.73 (SD=0.028) 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 parcellatino at 110 min in the OHSU dataset.

In order to establish the impact of MION, for each of the four macaques, we also examined the stability of FC, gradient, edge density generated using the data obtained without MION (Figure 5, awake: blue line, anesthesia: turquoise line). When the data obtained for each monkey without the use of MION was divided into two subsets, the correspondence remains notably lower than obtained with MION - regardless of whether anesthesia was used (OHSU-1, OHSU-2) or not (NKI-W, NK-R).

Identification of Unique Individual Areal Boundaries (Fingerprinting)

Next, we worked to confirm that despite apparent commonalities, the parcellations obtained were unique to each individual. To accomplish this, we assessed the similarity of the gradients and edge density generated across the four macaques and subsets (i.e. subset 1 and subset 2 for each macaque); Figure S2 depicts the spatial correlations between 21 gradient maps (2 macaque x 2 subsets x 3 conditions from NKI and 2 macaques x 2 subsets x 2 conditions from OHSU), as well as between 21 edge density maps (Figure S2). To facilitate the visualization, we highlighted the spatial correlations from MION data in Figure 6. The correlations between subsets within a given subject in the same state (awake or anesthetized, red and dark red dots), are notably greater than those between different subjects (blue and turquoise dots). When looking within the same state, an individual can be successfully identified from others using half of dataset for each macaque. Of note, the correlations in gradient and edge density across the monkey while under anesthesia (cadet blue dots) is higher than while awake (turquoise dots) - possibly suggesting that functional boundaries are more prone to variation while awake. Across states, measures obtained from data in the same macaque under two different awake states (i.e., naturalistic viewing, rest exhibited a relatively high degree of similarity (fuchsia dots). However, the correlations in findings between awake and

anesthetized (yellow dots) states in the same macaque were much less than those obtained in the same state. In addition, we also observed site effects, with correlations between monkeys across two sites (purple dots) being lower than within the same site while under same circumstance (Anesthesia with MION).

INSERT FIGURE 6. HERE

Effect of MION

As can be seen in Figure S2 (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.

Effect of State During Awake Imaging

Here, we examined the impact of differences among awake states by comparing functional connectivity, gradient and edge maps produced during the viewing of movies with those obtained during rest. Human imaging studies have drawn attention to the overall stability of functional connectivity patterns across differing awake conditions, despite more fine-grained modifications in connectivity associated with manipulations of state (e.g., rest, movies, task performance). Consistent with the findings from humans, we found a high degree of similarity between functional connectivity patterns (r=0.79) associated with rest and those observed with movie viewing. At the network level, a similar connection profile was found within and between networks except lateral visual cortex, which showed greater within network connectivity in movie viewing than rest. Regarding the gradient and edge density, we also found a relatively high similarity for each, the gradient maps and the parcellation maps generated using the two awaken states (movie and rest). The spatial correlation of gradient was 0.59 and Dice coefficient of parcellations was 0.68. As the similarity of FC, gradient and edge density between two awake states (movie and rest) within each monkey (see Figure 6 fuchsia dots) is

higher than across monkeys in the awake movie state, this suggests that naturalistic viewing, which is more favorably tolerated by non-human primates, can be used for the fingerprint.

Effect of Anesthesia

As reported in the prior section, the FC, gradient and edge density generated from differing subsets of data obtained with anesthesia showed a high degree of correspondence, similar to what was observed when subsets of data collected while awake were compared. However, the correspondence of areal organization measures was substantially lower when compared across the awake and anesthetized states in the same subjects (NKI-R, NKI-W). Specifically, compared to the awake condition, the spatial correlations in FC between awake and anesthesia was 0.52 (SD=0.08) (Figure 6 top panel, yellow dots). The gradient and edge density showed even less similarity between awake and anesthetized, with the spatial correlations across states with half subsets of data in NKI-dataset being 0.25 (SD=0.14) and 0.12 (SD=0.02) respectively (Figure 6, lower panel, yellow dots).

The lower correspondence of findings between states (awake, anesthetized) relative to within a state was not necessarily surprising. Previous studies have suggested that the spatial and temporal properties of functional connectivity observed in non-human primates can be impacted by anesthesia, with the profundity of the efforts observed depending on the depth of anesthesia (Hutchison et al., 2012; 2013; Vincent 2007). Visual examination of the FC matrices for the awake states (rest, movies) and anesthesia revealed notable regional connectivity differences that appeared to be specific to anesthesia (Figure 7B). To facilitate detection of those connections with the greatest change, for each monkey, we: 1) calculated the difference score (FC[awake] - FC[anesthesia]) for each connection, and then 2) transformed them to Z-scores based upon the mean and standard deviation for difference scores throughout the brain (Figure 7C). To make network-specific statistical inferences, for each network, we compared FC across each set of connections within the network using a paired t-test; to provide conservative estimates that avoid inflation based on the number of connections,

significance was estimated by randomization (10,000 times) for a given pairing of networks. The same procedure was carried out to look at connections between pairings of networks. Bonferroni correction was used to correct for multiple comparisons (Figure 7D).

INSERT FIGURE 7. HERE

Consistent with prior work, we found evidence of anesthesia-related decreases within and between most networks, with specific findings differing somewhat between the two monkeys. Decreases in within-network FC for the dorsal salience, dorsal motor, lateral visual, and medial visual networks were consistent across the two subjects. Of note, although more limited, there was also evidence of anesthesia-related increases in FC - most notably in the posterior cingulate cortex default mode network (PCC-DMN) for the two subjects. Complementing these within-network findings were anesthesia-related increases in between network FC for the visual networks; these increases were most consistent for FC with default network and fronto-parietal components. Of note, while comparison of awake and anesthetized states was not possible for the datasets collected at OHSU, the sparsity of the data collected there was more similar to that obtained under anesthesia at NKI, suggesting some level of consistency.

Discussion

The present work assessed the ability of a gradient-based FC boundary mapping approach to map the areal organization of the non-human primate brain at the individual level and yield reproducible results. In particular, the present work demonstrated the ability to move beyond network- and region-specific parcellations, to provide full cortical parcellations. Importantly, we found that: 1) areal boundaries are highly reproducible in individual macaques, though substantial amounts of data are required, 2) resultant parcellations appear to exhibit construct validity per homogeneity analyses and comparison to parcellations obtained from histologic and topographic mapping, 3) functional connectivity patterns, and their resultant gradients and edge maps appear to be stable across waken states, though markedly different across awake and

anesthetized states, 4) enhancement of non-human primate imaging using MION appeared to be essential for obtaining reasonable signal stability in a reasonable time period, 5) similar to humans, despite gross similarities, individual-specific functional connectivity and parcellation patterns were readily detectable per fingerprinting analyses, 6) alignment of nonhuman and human parcellation results into a common space gives rise to the potential for enhancing trans-species studies.

Gradient-based Areal Boundaries Are Highly Reproducible in Individual Macaques with Sufficient Data

Our findings regarding reproducibility and data requirements are not surprising given recent work carried out in humans[8,21,22]. Similar to the findings from humans, more data is generally better when addressing the reproducible FC[21], though with the largest gains being observed as the data used increases up to ~32 min - a finding that echoes the findings from humans, where the most substantial gains were seen up to around 27-30 minutes. It should be noted that the data needs increase with each additional step of processing (i.e., from FC to gradient and edge calculation). Improvements in reliability for gradients and parcellation slowly but continuously increased as data increased to 40 min, while the largest reproducibility values (spatial correlation and Dice coefficient) were still relatively lower than what can be achieved for FC. These findings suggest that extended data are required to achieve a similar level of reproducibility of parcellations. Additionally, our findings extend recent works emphasizing the importance of contrast agents when scanning non-human primates at 3.0T by demonstrating that the use of MION is a prerequisite to achieving maximal reproducibility for FC, gradients, and functional boundaries maps[32,37,38]; this is true regardless of whether anesthesia was used or not.

The Gradient-based Parcellation Show Internal Validity

Directly relevant to the present study, prior studies in humans suggest that the boundary map-based parcellation had highly homogeneous FC patterns at the group and individual level[8,21,22]. In the current study, we tested the homogeneity of boundary map-derived parcellations using 50 percent subsets of the data (subset 1), such that the

parcels were generated completely independently from the other half of the dataset (subset 2) for each status within each macaque. On average, the parcel homogeneity was above 0.9 with 120 min of awake data. This high degree of homogeneity indicates that most parcels represented regions of similar functional connectivity pattern. It is notable that the homogeneity varied across parcels. As discussed in a previous study[34], the homogeneity of parcels is associated with parcel size, where small parcels are more likely to have higher homogeneity than larger parcels. A few parcels had low homogeneity and some of them, e.g. in the frontal pole, anterior inferior temporal lobe, may be caused by the low-SNR or distortion signal in those areas. Accordingly, it is necessary to use a null model to truly evaluate the homogeneity. By creating the null model from 1000 randomly rotating of the identical parcellations around the cortical surface, we found that the homogeneities of parcels were significantly higher than the null model in both awake and anesthetized states. Consistent with the finding in the human literature[34,39], this suggested that the overall gradient-based parcellations can successfully delineate the functional homogeneous cortical areas in individual macaque.

A key challenge for any effort focused on cortical parcellation, whether in the human or non-human primate, is determination of the 'correct' number of parcels. Regardless of which modality or parcellation method is used, a number of differing criteria can be used to make such determinations. In the current study, the watershed flood algorithm yielded between 110 and 140 parcels per hemisphere, for each macaque. An advantage of this methodology, is the lack of need for a priori specification of the number of parcels to be produced. However, the results can be impacted by properties of the data, such as spatial resolution and smoothness; in this regard, a priori specification of parameters (e.g., number of parcellations) can have advantages. Our estimate for number of parcels converge with current estimates generated from histology-based studies (i.e. cytoarchitecture, myeloarchitecture, and chemoarchitecture), which have increased from 78-88[40–43] to 130-160 in each hemisphere[40,44]. Future work will benefit from integrative examinations across assessment modalities (e.g., functional MRI, diffusion, histology) in the same animals.

Rest and Naturalistic Viewing during Awake Imaging Show Similar Functional Properties

Our findings regarding the relative stability of functional connectivity patterns across awake and sensitivities to level of arousal (i.e., sleep, anesthesia) help to synthesize previous findings from the human and non-human primate literatures. Over the past decade, numerous studies have demonstrated the ability to extract highly similar intrinsic functional connectivity networks, regardless of whether imaging is carried out during an active task state or rest[45–47]. Recent work has increasingly highlighted the potential value of using non-rest states, particularly naturalistic viewing, for assessing functional connectivity, as head motion appears to be lower and tolerability higher. While systematic differences in connectivity patterns are undoubtedly present across states, as revealed by within subject comparisons, differences in connectivity patterns across individuals remain largely intact - at least for static functional connectivity patterns[48–51]. Given the various behavioral demands of awake imaging for non-human primate, we have found that naturalistic viewing conditions can be particularly valuable - at least for full-brain parcellations. For those interested in more fine-grained parcellations or temporal dynamics, it is important to further investigate the temporal stability vs. state-dependence of more subtle distinctions within cortical areas (e.g., polar angle and eccentricity mapping in early visual cortex).

Individual Areal Organization Is Unique While the Awake and Anesthetized States Show Distinct Profiles

As suggested by prior work in humans, we found that individual areal organization in the monkey can be used for fingerprinting. Within the same condition (awake-awake, anesthesia-anesthesia), the within-individual spatial correlations of gradient and edge density maps are explicitly higher than between-individual correlations. Consistent with prior work[46,48,52], the ability to fingerprint could be achieved across different awake conditions (i.e., movie, rest), though some decrements in similarities for FC, gradients and edge maps were noted. Importantly, the ability to fingerprint using data collected with anesthesia was notably decreased when attempting to match the same subject across the awake and anesthetized states. This is not surprising, as the similarities for

full-brain FC, gradient maps and edge maps were dramatically reduced when looking across the awake and anesthetized states (e.g., ~40% in whole-brain FC similarity).

While the presence of differences in FC properties between awake and anesthetized states was not surprising based on prior work[33,53,54], some of the specific findings suggest a more nuanced picture than previously appreciated. Consistent with prior studies varying levels of anesthesia, we found evidence of anesthesia-related compromises in within- and between-network connectivity for higher-order associative cortices; the specific networks affected differed somewhat across the two monkeys, with the most consistent findings being observed in the salience and dorsal attention networks. However, unlike prior studies, which largely relied on variations of depth of anesthesia, our comparison of awake states and anesthesia also suggested that the visual networks and their connectivity with default and frontoparietal networks actually showed anesthesia-related increases in connectivity. Overall, these findings echo those findings of recent studies of the impact of anesthetics on brain differences in humans, which suggested a loss of complexity in the functional architecture of the brain[55–59].

In sum, the present work suggests that each, the awake and the anesthetized states, as well as their differences, are highly stable. Future work would benefit from more detailed examinations of anesthesia effects that include awake imaging, as well as possibly natural sleep.

Effects of Sites

Finally, it is worth noting a unique aspect of the present work - the examination of monkey datasets across two institutions. A significant advantage of this strategy was the ability to confirm the reproducibility of findings across two independently collected datasets. Not surprisingly, while we were successful in demonstrating the generality of our findings, we did find evidence of site-related differences. Specifically, we found that the correspondence of gradient and edge maps among subjects was higher within a site than between. Possible factors that might explain site-related variation include differences in: 1) anesthesia protocols (e.g. the knockout agent and delay duration after

the time of anesthetics administration); 2) head coils; the OHSU dataset was acquired by a knee coil while NKI dataset was with a surface coil; prior work has demonstrated differences in signal properties (including distortion) of the data obtained from monkeys using these coils[32]; 3) rearing histories. Future work would benefit from more coordinated efforts focused on multi-site imaging in the non-human primate as a means of maximizing the reproducibility of findings between laboratories.

Limitations

It is important to note that despite the various successes of the present work, a number of limitations and potential areas for future optimization exist. As previously discussed, a key limitation of any parcellation methodology is the lack of a gold standard benchmark to help establish validity. The present work made use of comparisons to previously established histologic parcellations to provide insights into validity, as well as the examination of parcel properties (i.e., homogeneity). Future work would benefit from examination of multiple methods of parcellation in the same subjects (e.g., fMRI and diffusion imaging, fMRI and myelin maps, fMRI and histology). Another consideration for future work is examination of the potential impact of preprocessing strategy decisions on the resultant parcels and their stability. Optimization of preprocessing may be particularly useful if focused on bringing down data needs for achieving the same parcellation results. Finally, the present work relied on the FC similarity measure used in prior studies for gradient-based parcellation. Prior work demonstrated this measure to have favorable properties relative to several other possible measures. However, an exhaustive examination of measures to be used for gradient-based parcellation has yet to be carried out; this would be of benefit both - to determine if some measures may be able to lead to more fine-grained parcellations, and to find a less computationally costly measure if possible. On a related note, a range of alternatives exist for the final parcellation step in the gradient-based strategy - future work determining optimality is merited.

Conclusions and Future Directions

In summary, the present work demonstrates the ability to achieve highly reproducible, individual-specific cortical parcellations in the non-human primate. Our findings also highlight the many factors that can contribute to stability, in particular the need for sufficient data and contrast agents. Our findings also emphasized the need for consideration of state (i.e., awake vs. anesthetized) when interpreting findings and their reproducibility across studies, as well as during the study design process. Transformation of our non-human primate findings into a common space demonstrated a future direction that can be further optimized to facilitate comparative and translational studies.

METHODS

Dataset 1, NKI-Macaque

The Macaque dataset 1 consisted of 2 rhesus monkeys (Macaca mulatta, 1 male, age 6 years, 6.4 kg, marked as NKI-R; 1 female, age 7 years, 4.5 kg, marked as NKI-W), which were 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 Siemen 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. The macaques were 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 with 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 11 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. The 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.

Dataset 2, OHSU-Macaque

The Macaque dataset 2 consisted of 2 male rhesus macaques (Macaca mulatta, 1 male, age 5 years, 8.6 kg, marked as OHSU-1; 1 male, age 5 years, 7.6 kg, marked as OHSU-2), which were collected from the Oregon Health and Science University. The animal procedures were in accordance with 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). The 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 knocked out, 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.

Image Preprocessing

Structural image processing included the following steps: 1) spatial noise removal by a non-local mean filtering operation (NKI-dataset)[60,61], 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 incorrect volume, 4) reconstructing the native white matter and pial surfaces by FreeSurfer, 5) registering the native surface to a hybrid left-right template surface (Yerkes19 macaque atlas)[62]. The Yerkes19 template was created by 19 adult macaques acquired from Yerkes Primate Research Center and processed by Human Connectome Project (HCP) pipeline[62,63]. The registered left and right hemisphere surfaces were brought into register 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 the first five volumes of the time series and compressing temporal spikes (AFNI 3dDespike), 2) slice timing correction, motion correction, and field bias correction (OHSU-dataset), 3) normalizing the 4D global mean intensity, 3) regressing out nuisance signals including the mean time series for WM and CSF masks, the Friston-24 motion parameters[64], as well as linear and quadratic trends, 4) band-passed ($0.01 < f < 0.1$Hz) filtering the residuals to extract the low-frequency fluctuations, 5) registering the image to the anatomical space and projecting onto the native middle surface, 6) spatial smoothing with 4 mm full width at half maximum along the native surface and down-sampled to 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 while awake, 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

A cortical surface was reconstructed to provide an accurate representation of morphology and topology of brain for each macaque. The volumetric fMRI data was aligned to anatomical space and then projected to 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.

The procedures of surface-base FC calculation and gradient-based parcellation were similar as previous studies described in Laumann et al. and Xu et al.[39,65]. In brief, the functional connectivity (FC) map in full cortex of 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 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[15] was then used for each of 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 parcel maps.

Parcellation Validation

For each Macaque, to compare the homogeneity across different condition (awake vs. anesthesia, MION vs. NO-MION), the parcel homogeneity was evaluated by Kendall' coefficient from all vertices in the parcel for each condition[65]. Consistent with previous human work, the overall homogeneity of the parcellation was compared to a null model created by 1000 randomly rotating the parcellation on the cortical surface[15].

Evaluating the Stability of FC, Gradients, and Edge Density

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 have suggested that shorter sampling of contiguous data over more sessions can facilitate the convergence. 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 were also calculated to examine the convergence of the parcellation. To facilitate the computational capacity for gradient and edge density calculations, the results of gradient and edge density were performed on the down-resampled 400 regions of interest vertices for 100 times at each time point.

Network Assignment

To characterize large-scale system for parcellations created in the current study, we assigned network identity to parcels using the previously established network definition from Ghahremani et al.[66] The matching procedure was similar with Gordon et al[67]. Specifically, we extracted the mean time series within each parcel, which was 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 were less than 10 mm to remove the purely local connectivity[68] from spatial smoothing. After that, we thresholded and binarized the connectivity map at the top 5% of connectivity strengths. The resulted in a binarized map of regions with high connectivity to that 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. and defined the best match by the Dice coefficient. Then the neighboring parcels assigned in the same network were merged to create a final network-patch.

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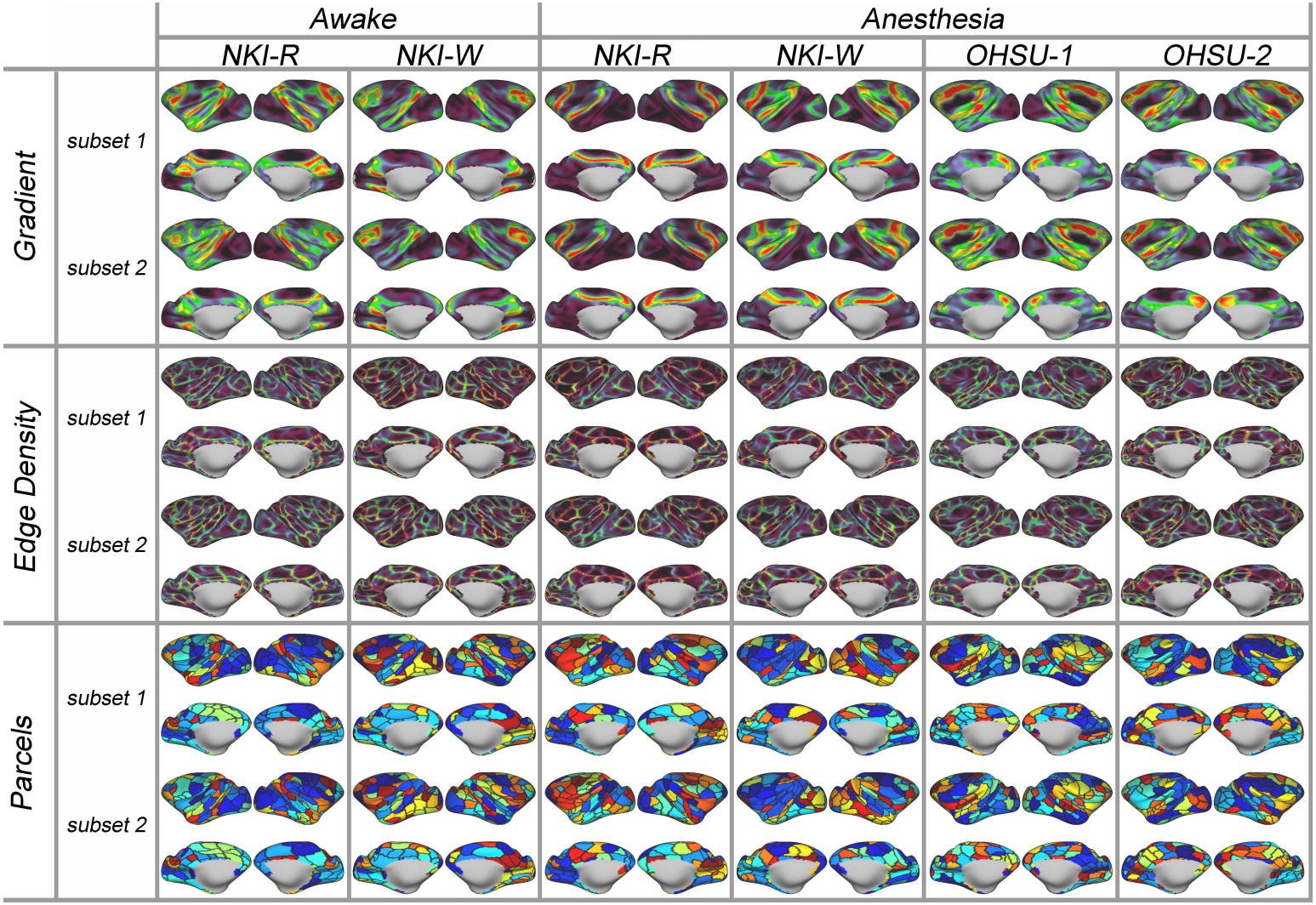

Figure 1. Subject-specific areal organization is highly reproducible across two independent subsets of scans under awake and anesthesia. For awake state, the gradient, edge density and parcellation maps were computed from two 44-min subsets for monkey NKI-R and two 120-min subsets for NKI-W; while under anesthesia, the total amounts of data for each subset are 55-min (NKI-R), 94-min (NKI-W), 120-min (OHSU-1), and 120-min (OHSU-2).

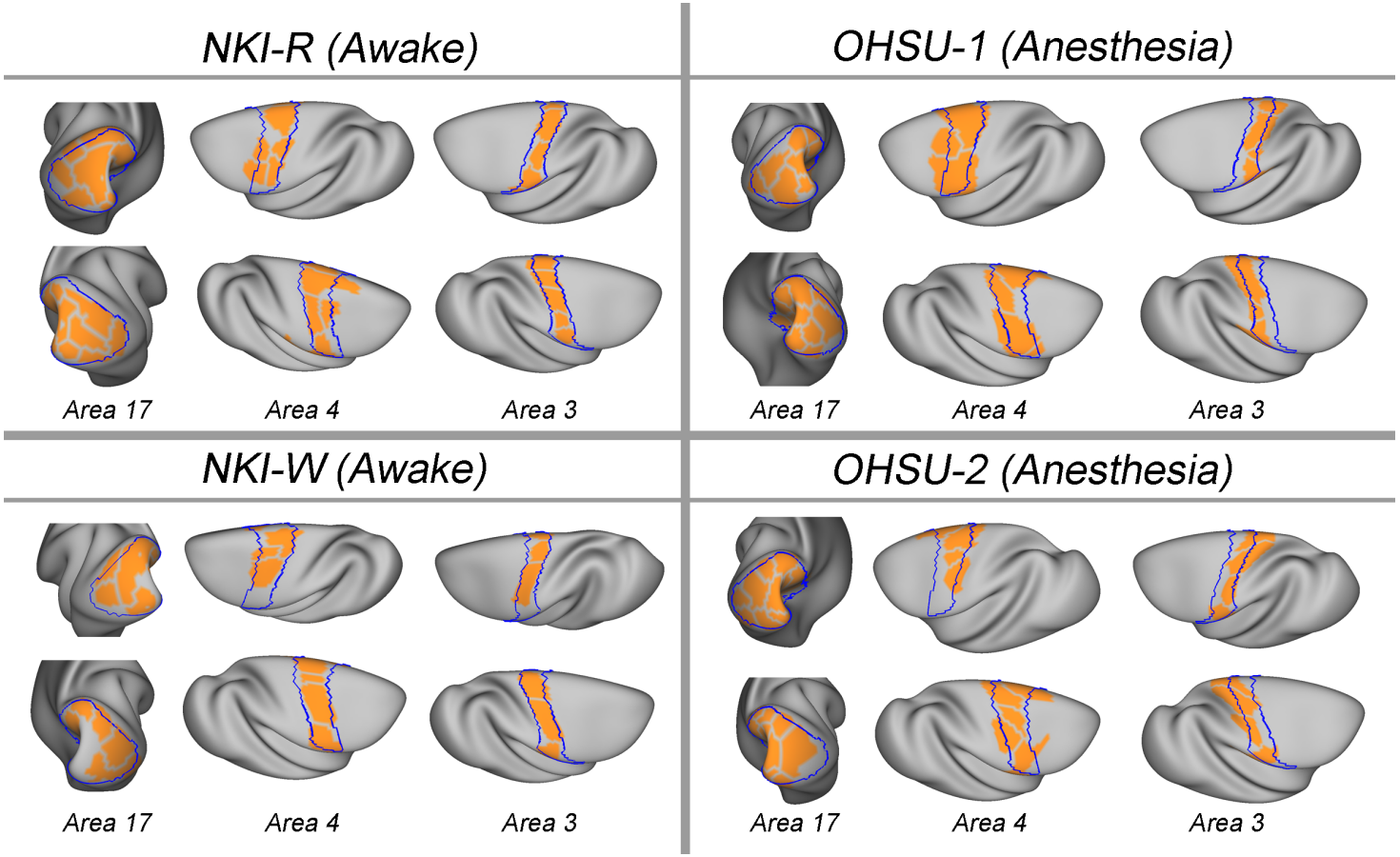

Figure 2. Subject-specific parcels (orange) overlaid on boundaries (blue) of post-mortem histologically defined areas (area 17, area 3, and area 4) from Brodmann [35].

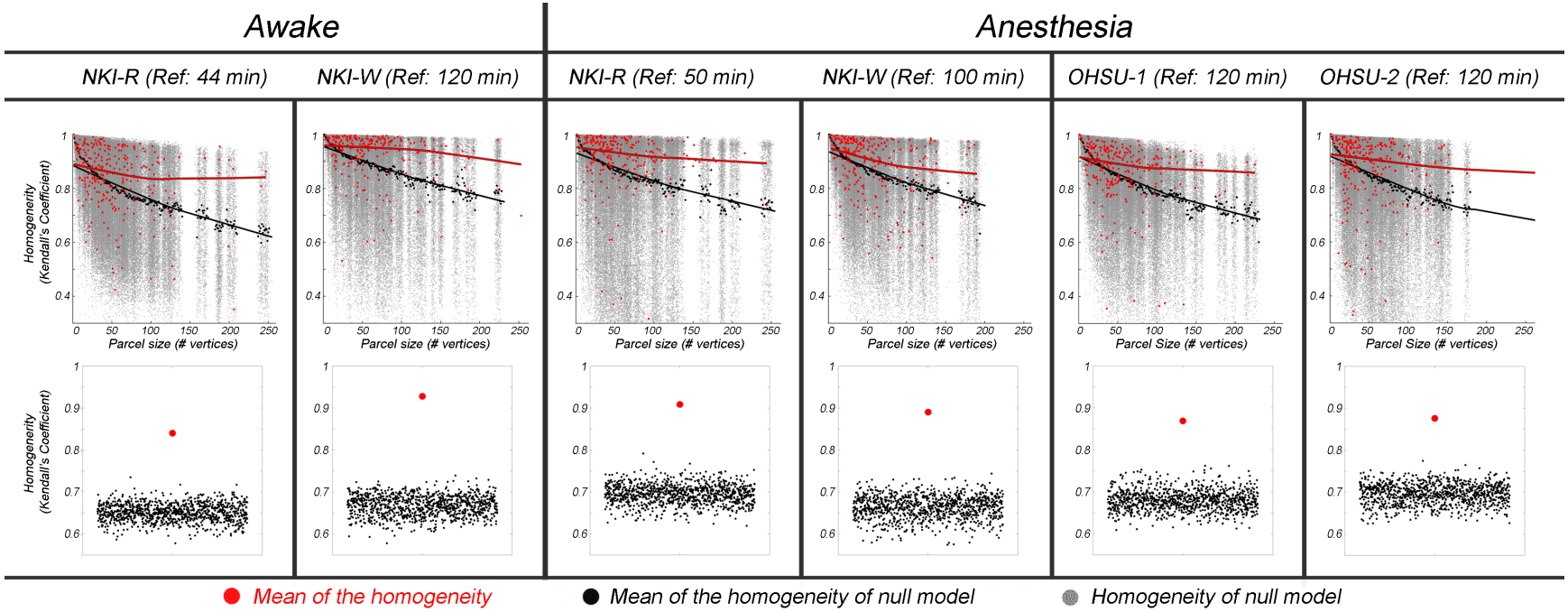

Figure 3. Upper panel: Homogeneity of real parcels (red dots) by parcel size compared to homogeneity of null model parcels (gray dots) under awake and anesthesia states. The real homogeneities were calculated in the parcels generated form subset 2 (reference subset) using the data from subset 1. Black dots were the mean homogeneity across iterations for each null model parcel. The lines are the LOWESS (locally weighted scatterplot smoothing) fit represent the effect of parcel size on homogeneity of the subject parcels (red line) and the null model parcels (black line). Lower panel: The mean homogeneity across real parcels (red dot) for each monkey under awake and anesthesia states are significantly higher than the mean homogeneity from the null model parcellations (black dots).

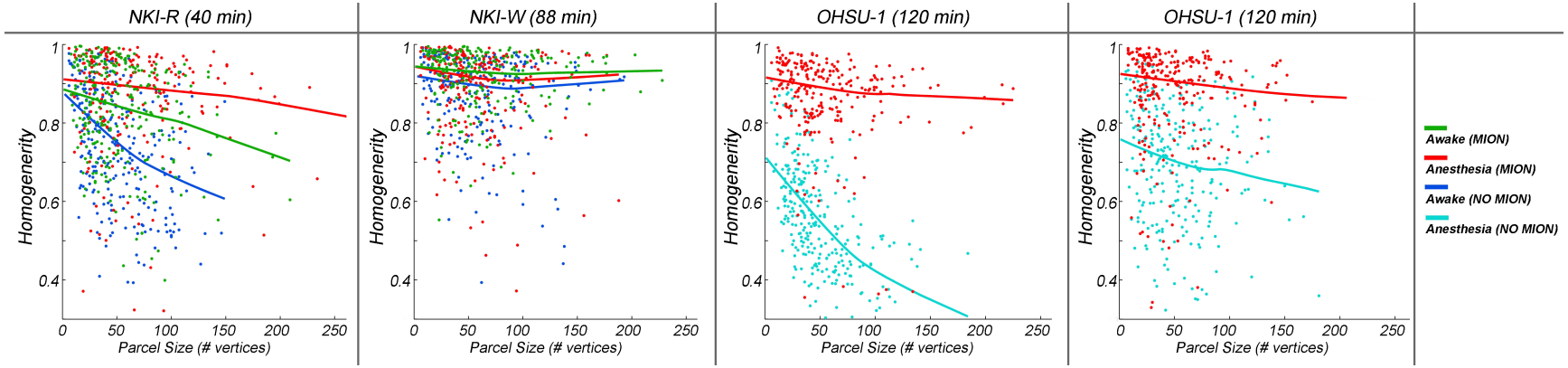

Figure 4. Homogeneity of subject-specific parcels by parcel size based on different conditions (awake vs. anesthesia, with MION vs. without MION).

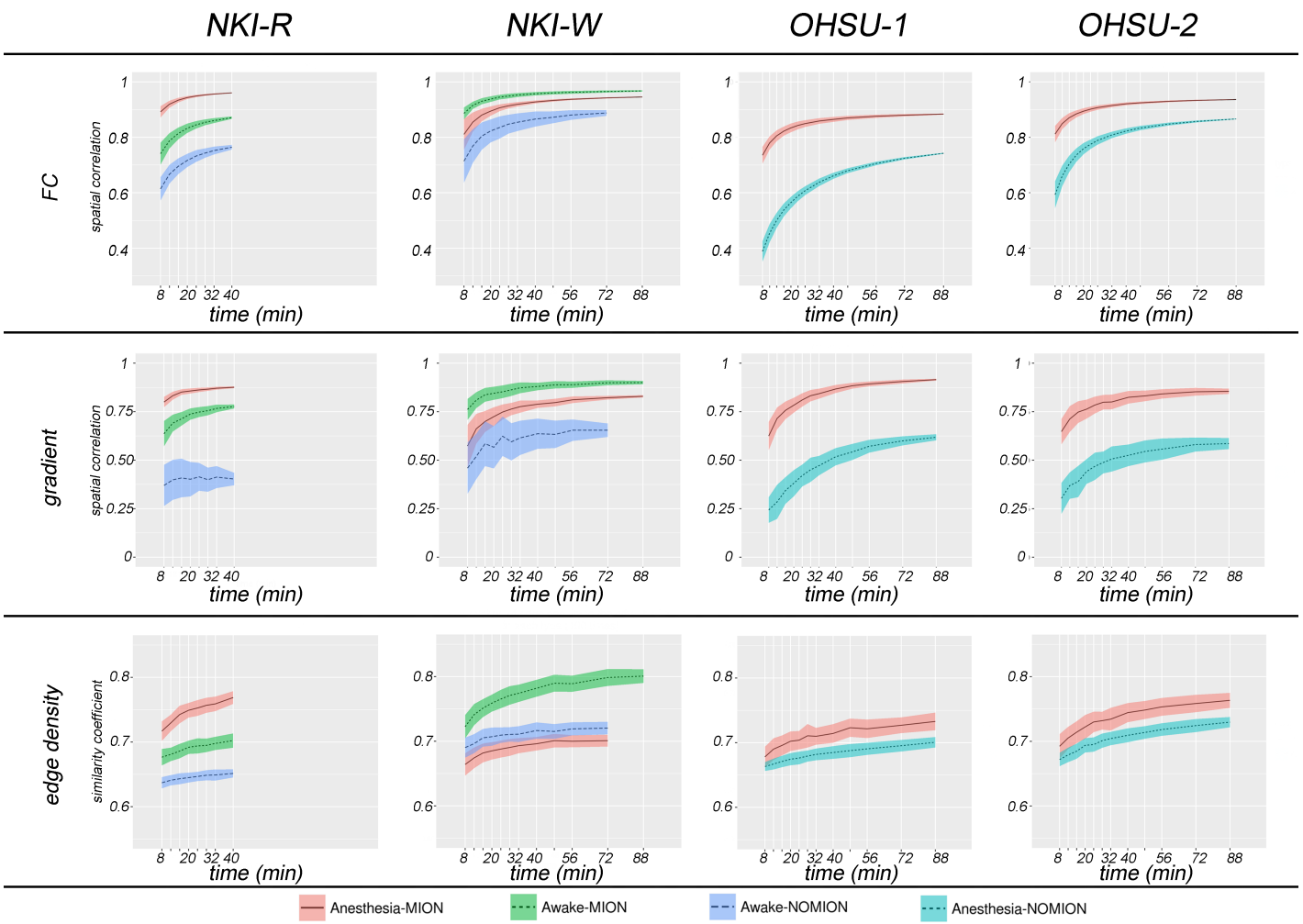

Figure 5. The stability of subject-specific functional connectivity, gradient, and edge density as a function of the amount of data used within different conditions (awake vs. anesthesia, with MION vs. without MION). Each line represents the mean (black line) and SD (colored patch) of similarity within each condition for each monkey; the similarity between maps generated from each incremental amount of data (from subset 1) and those from the other half of data (reference: subset 2).

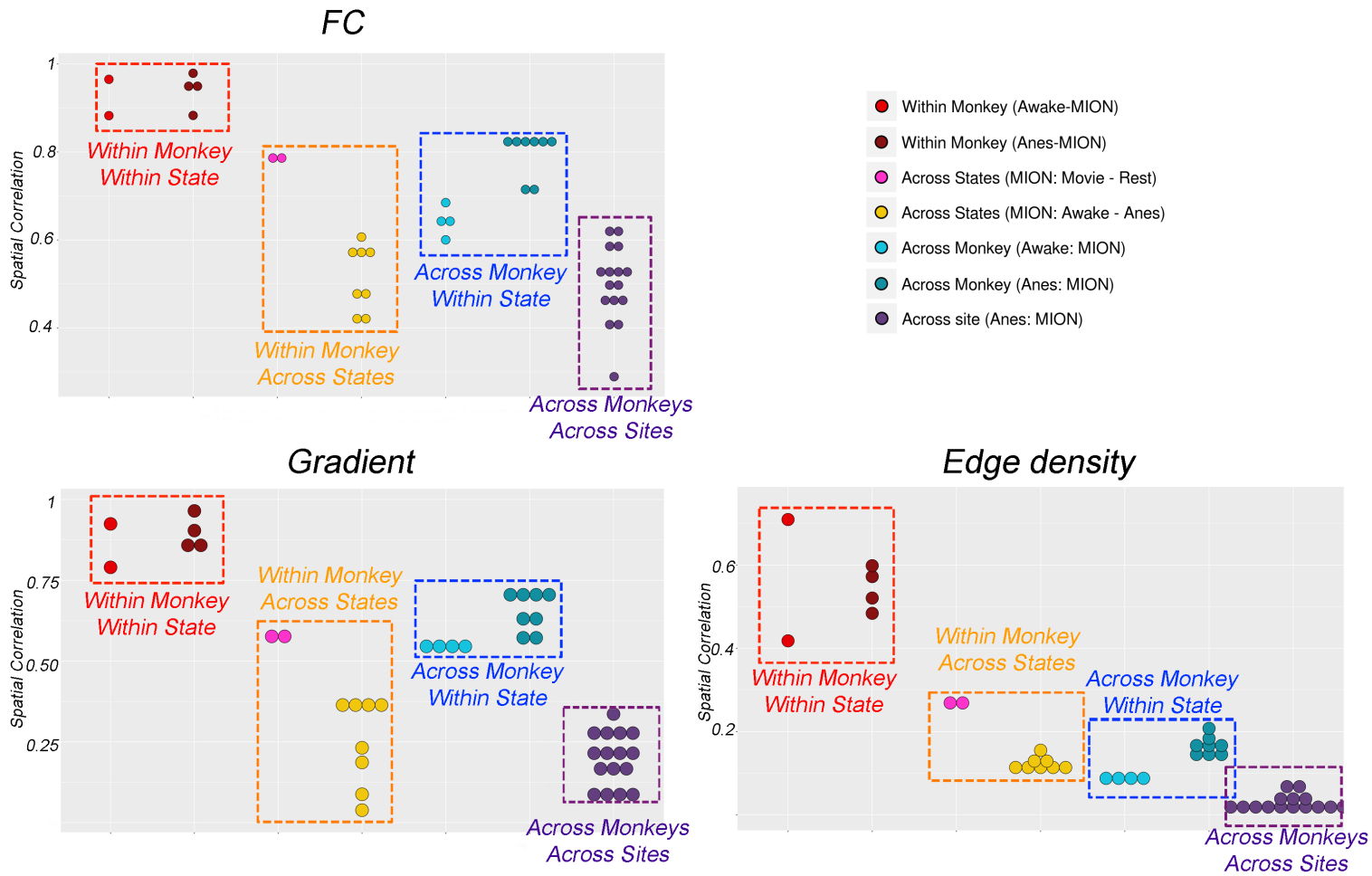

Figure 6. Dots plots of the spatial correlations in FC, gradient and edge density from two different subsets (subset 1 and subset 2), three conditions (awake movie, awake rest, anesthesia), and four monkeys from two sites. To facilitate comparison, we mark off dots to highlight the effects of subset, state, individual monkey and scan site in colored boxes.

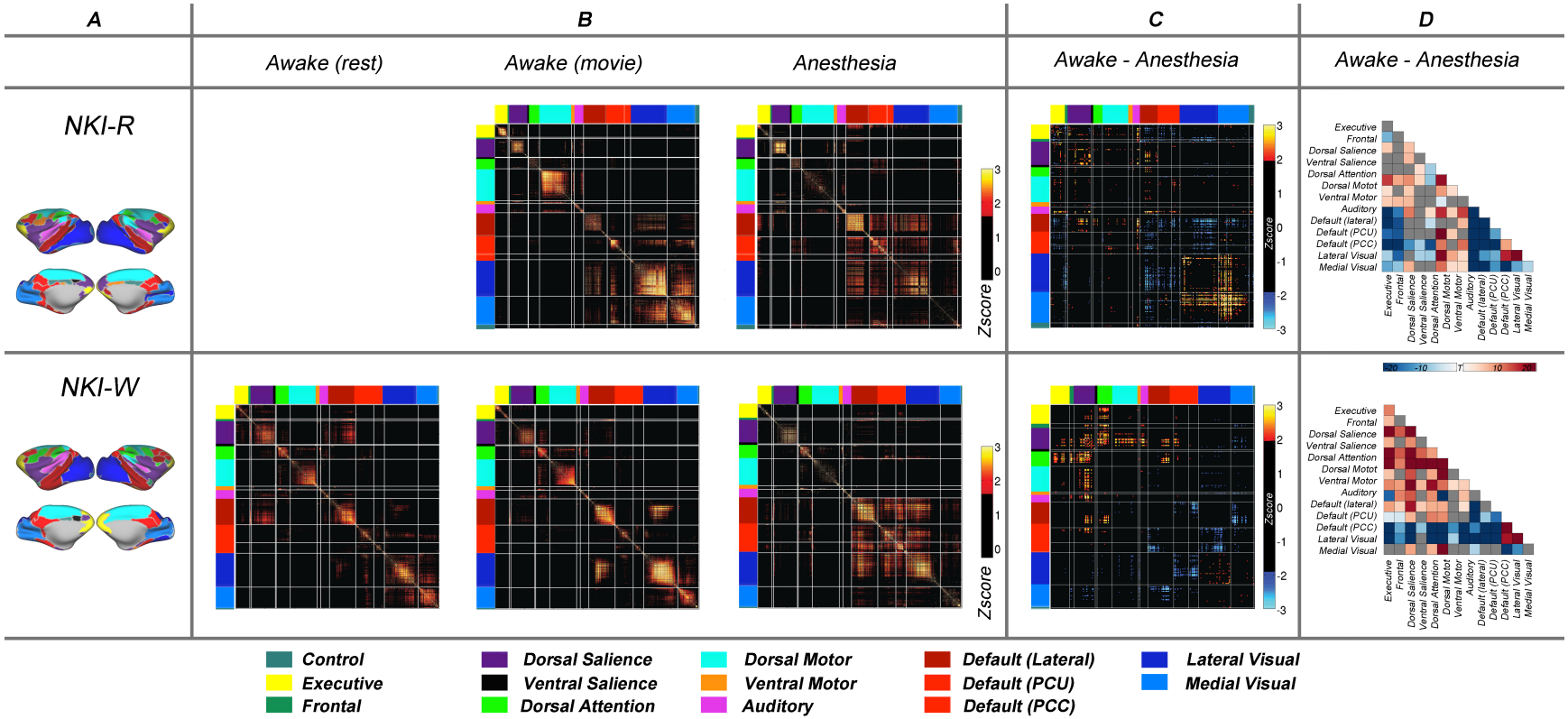

Figure 7. A: The network level of parcellations created by assigning the parcels to the networks defined by group ICA (Ghahremani et al., 2016). B: Functional correlation matrices for the awake states (movie, rest) and anesthesia. To facilitate the visualization, we transformed correlations to z-scores (subtract the mean and divide by standard deviation) and threshold at z-score > 1.645 ($p<0.05$, one-tail). C: The differences of correlation matrices (r[movie] – r[anesthesia]) that were transformed to z-scores and threshold at z-score > 1.960 ($p<0.05$, two-tails). D: The significant differences of FC between awake (movie) and anesthesia at network level. The significance was defined by Bonferroni corrected p-score from randomization < 0.05.

Table S1. Parameters of the datasets included in this study

Subject ID	Site	Awake (movie) MION	Awake (rest) MION	Awake (movie) without MION	Anesthesia MION	Anesthesia 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
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
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)
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)

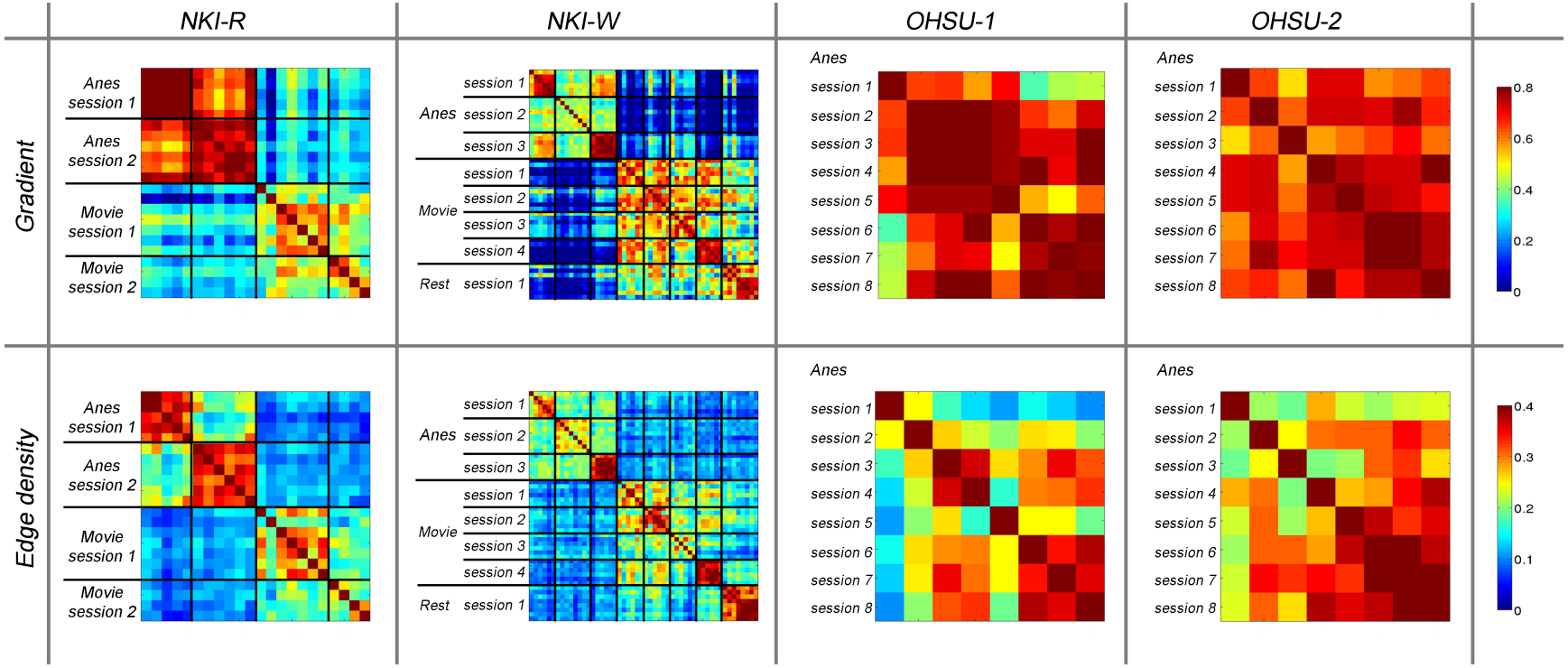

Figure S1. The spatial correlation matrixes of gradient and edge density maps between scans across four monkeys and sessions. NKI-dataset required 8-10 min per scan and 5-7 scans per sessions while OHSU-dataset required 30 min per scan and one scan per session (Anes: anesthesia).

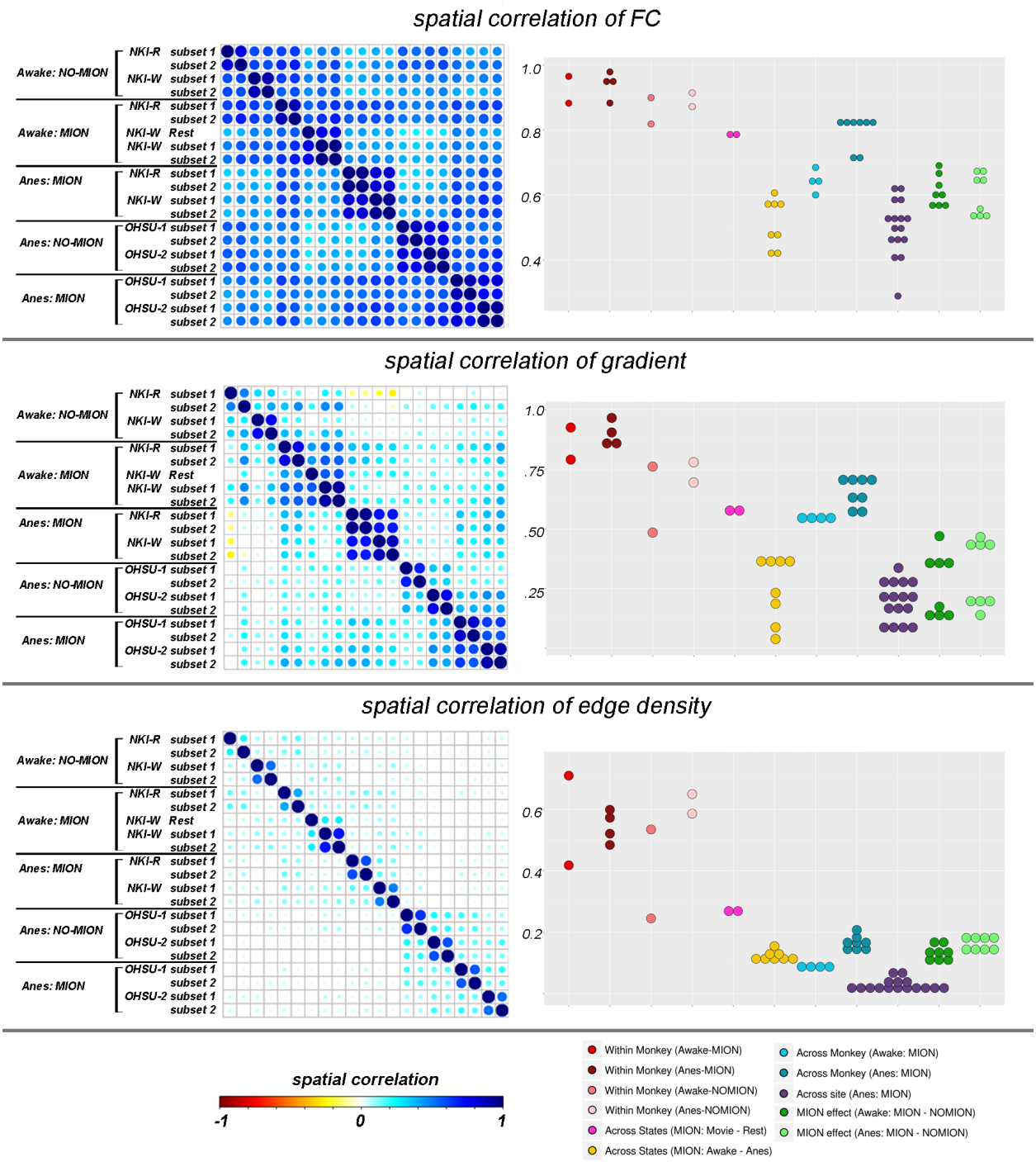

Figure S2. The spatial correlations of FC, gradient and edge density maps across four macaques and subsets under different states from two sites (left panel). The distribution of correlations within- and between-monkey, states, and sites (right panel).