

Accelerating the Evolution of Nonhuman Primate Neuroimaging

The PRIMatE Data Exchange (PRIME-DE) Global Collaboration Workshop and Consortium^{*,*}

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Nonhuman primate neuroimaging is on the cusp of a transformation, much in the same way its human counterpart was in 2010, when the Human Connectome Project was launched to accelerate progress. Inspired by an open data-sharing initiative, the global community recently met and, in this article, breaks through obstacles to define its ambitions.

Nonhuman primate (NHP) neuroimaging carries tremendous translational promise for biomedicine (Phillips et al., 2014; Roelfsema and Treue, 2014). However, progress has been slow, as researchers face not only the many challenges that human neuroimaging has overcome but also unique obstacles that require consensus solutions. To date, the approach has remained largely piecemeal and single-lab driven, causing most NHP researchers to struggle to amass datasets consisting of even 10 to 20 subjects, whereas their human-imaging counterparts now aim for thousands.

The PRIMatE Data Exchange (PRIME-DE) was recently established to accelerate the pace of advancement (Milham et al., 2018) by promoting a culture of collaboration and open science in the NHP neuroimaging community. PRIME-DE established a repository of openly shared data in 2018, followed by a Global Collaboration Workshop (GCW) on September 5–6, 2019 at the Wellcome Trust in London. Through these efforts, the community has made substantial progress toward a global vision and here outlines its ambitious albeit eminently achievable goals. Four key domains of activity in NHP neuroimaging are considered that can dramatically accelerate progress.

Standardizing Data Collection Harmonizing Data Collection Is Key for Reproducibility and Shared Data Value

Minimal Data Acquisition Specifications. There was agreement that a universal data acquisition protocol is not yet practical, but minimal specifications can be defined toward standardization. A shared

lesson from the Human Connectome Project (Van Essen et al., 2013) is that the cortical sheet should be resolvable with isotropic voxels no larger than half the minimum cortical thickness (e.g., 0.5–0.6 mm voxels for macaque cortex and 0.4 mm for marmosets). Acquiring 3D T1- and T2-weighted scans is important for brain segmentation, and T1/T2 ratios can generate “myelin maps” to assist surface mapping and rapid quality checking.

For functional MRI, attainable target spatial resolutions are 1.0-mm isotropic voxels for large NHPs and 0.5 mm for smaller ones. However, these are beyond the 1.2–1.5 mm range currently employed on common 3 Tesla scanners, and manufacturers are phasing out gradient inserts previously used to boost signal-to-noise. A way forward is the adoption of more sophisticated coil systems with higher signal to noise (SNR), enhanced with acceleration methods (multiband imaging) for higher functional and temporal resolution with less acquisition time. These coils are commercially available (24-channel macaque, 16-channel marmoset) though still require customization to accommodate head posts and/or chambers.

Anesthetized Imaging. Although awake imaging is clearly the long-term aspiration for NHP imaging, it is technically challenging and requires training the subject. Thus, anesthetized imaging remains important for resting-state, diffusion, and structural imaging and benefits from minimal head motion. A key factor for establishing common practice is standardizing the anesthetic agents. Many GCW laboratories already use highly similar protocols,

entailing isoflurane anesthesia for structural imaging and IV administration of dexmedetomidine (0.015–0.02mg per kg bolus or 4.5–5.0ug per kg per h infusion) to allow reduction of isoflurane concentrations to between 0.6%–1.0% to improve the functional MRI signal. Other agents are being successfully employed and might be required by researchers for scientific reasons (Flecknell, 2015). Beyond the specific agents employed, opportunities exist to advance the monitoring and control of anesthesia depth throughout scanning by logging temperature, end tidal CO₂, O₂ saturation, respiration rate, heart rate, and blood pressure synchronized to data acquisition.

Awake Imaging. Four identifiable challenges confront awake NHP imaging. First is the challenge of behavioral training for the scanner environment. Second, the placement of head immobilization hardware determines which brain areas are accessible with head coils. This precludes universal acceptance of a single head coil and necessitates customization or generating a range of standardized options. Third, noninvasive eye tracking provides a key control measure in awake NHPs. Finally, head and jaw movements, as well as the apparent head movement and brain distortions produced by changes in susceptibility from body and limb motion, remain a problem for awake imaging, particularly at high magnetic fields. Behavioral training and external monitoring methods, such as magnetic resonance (MR)-compatible video tracking and jaw and/or body motion sensors, can be invaluable for correcting motion. Post-acquisition

methods (e.g., ICA-AROMA, ICA-FIX) will help; and film viewing, when appropriate, can decrease head motion (as reported in human neuroimaging).

Opportunities for Improving Data Quality. Although using higher field scanners is an obvious way to improve data quality, current costs (~1 million USD per Tesla) and operational nuances make them relatively inaccessible to most groups. Recent findings suggest that iron-based contrast agents such as monocrystalline iron oxide nanoparticle (MION) can increase contrast-to-noise ratio (CNR) and spatial specificity at 3 T. However, this has limitations, as the agents tend to be costly, and frequent usage necessitates the introduction of chelating agents to minimize impact on animal welfare by long-term accumulation of iron. Additionally, contrast agents measure cerebral blood volume (CBV) rather than the blood-oxygenation-level-dependent (BOLD) response, complicating comparison to human BOLD fMRI. Unlike human MRI, NHP MRI suffers from dramatic signal variations from coils or other sources. Thus, appropriate quality control strategies should be implemented both for custom and standard coils. An approach to improve fMRI data quality is to increase the number and duration of acquisition sessions (Xu et al., 2018). Prospective motion correction approaches deployed in human research (Maclaren et al., 2013) may also improve structural imaging. Currently, the main way to avoid motion artifacts in awake imaging is to limit head movements (e.g., training or head immobilizing).

Finally, investigators identified the need for creating and sharing NHP “phantoms,” which would allow data-collection sites to check and benchmark their data-collection protocols using a common reference as is done in human imaging. Such phantoms would be created and made freely available as a 3D-printed model of a given species’ brain filled with a contrast agent with known relaxation times to standardize signal-to-noise assessment across sites. Phantoms could be created for any of the primate species (apes, marmosets, baboons, macaques). Importantly, working on good quality data acquisition beats any

post-acquisition cleaning algorithm available and is crucial if we are to create standard pipelines for NHP MRI data analysis.

Animal Welfare, Regulations, and Intellectual Property
NHP Imaging Stakeholders Are Seeking Policy-Making Guidance from and Working with Funding Agencies, Professional Societies, and the Larger Community to Ensure Maximum Benefit and Transparency

Animal Welfare and Regulations. NHP neuroscience is a heavily scrutinized and extremely sensitive area of research with extensive ethical approval processes and oversight. However, NHP research is not governed by a common set of international regulations or ethical statements (e.g., Declaration of Helsinki for human research). National differences in NHP research and NHP welfare regulations are particularly problematic for efforts to collaborate internationally. The community agreed that addressing this challenge going forward will benefit from additional transparency when sharing their datasets, including identification of the relevant regulatory body and reference to their published standards. Additionally, it will be important to increase the collection and sharing alongside MRI data of objective and evidence-based measures of animal health status as metadata, which can also provide scientific insights (e.g., home-cage behavioral data, eye-tracking data, genomic information, rearing and maintenance information, sourcing of animals, anesthesia maintenance values, as relevant). National primate centers and breeding sites can help with collection of this metadata.

Engaging the Public. Candid and transparent communication with the public on the importance of nonhuman animal research is vital for maintaining and increasing governmental and public support. It is not uncommon for institutions and scientists to find themselves in a reactive rather than proactive position, focusing solely on the defense of their work. Recent experience is showing that a proactive stance raises public awareness and support for animal research as a key element of modern science and medicine, balancing the discussion of

concerns raised by activist groups. Politicians are often unaware of the impact of the animal research occurring in their own constituencies, which can lead to legislation being put forward that fails to capture the importance of scientific advances. Institutional and funding-body press offices could better link translational developments directly to the foundational research performed on laboratory animals because the reporting of the fundamental animal research bases is often unmentioned. Researchers and their institutions can find support and public-engagement training from groups such as Speaking of Research (US), Basel Declaration on Animal Research (EU), Pro-Test Deutschland (GER), Pro-Test Italia (ITL), [Understanding Animal Research \(UK\)](#), and Gircor (FRA).

Alongside the importance of the work, the public can learn about the balance between benefits and harms, including evidence-based safeguards for animal welfare. Several institutions have now signed the UK Concordat on Openness in Animal Research (<http://concordatopenness.org.uk>). This now five-year-old agreement, currently signed by 122 institutions, encourages openness and better information sharing about animal research. Rather than being a generic statement on openness that will find nominal support by most institutions, the Concordat annually assesses, supports, and rewards institutional public engagement efforts. Communication efforts emerging from signatories of this agreement have been impressive (more useful information on institutional websites, patient-led activities, virtual tours of animal facilities, and better-balanced social media discussions). Lastly, the community noted the need for increased leadership from the national and international research organizations in efforts to explain the continued importance of NHP research, supporting researchers and engaging the public.

Crediting and Intellectual Property. In NHP research, where substantial costs and efforts are required for training or maintenance of a single individual, investigators hold real concerns about not being appropriately credited or being “scooped” analytically with one’s own data. Recent years have witnessed an increasing acceptance of “data descriptors” or

“data papers” on resource-sharing infrastructures (Neuroimaging Informatics Tools and Resources Clearinghouse [NITRC], Zenodo) as a publication-based means of crediting data generators and encouraging sharing. Digital object identifiers (DOI) assigned to datasets can further assure the rapid identification, crediting, and tracking of datasets. However, such efforts need to be recognized by the institutions and used in promotion reviews (e.g., Declaration on Research Assessment, <https://sfdora.org>). This situation is problematic for the advancement of open science and must be addressed by a coordinated effort involving both institutions and funders recognizing the importance of data generation and sharing. These realities often drive investigators to hold back their newest data from sharing initiatives, instead sharing only those datasets that have already yielded publications.

GCW participants converged on a solution that moving forward, in addition to fully open sharing options, a “collaboration seeking” sharing option will be added with the following terms: (1) early sharing encouraged, but an investigator can accept or reject access requests to these data; (2) the investigative team may receive co-authorship credit on the publication (to be negotiated by the dataset holders and proposed collaborator); and (3) upon publication of the first manuscript, the data status will switch to open sharing. Additionally, GCW participants felt that the generation of a registry of ongoing studies would be immensely important for the NHP research community to avoid duplicating efforts and to foster collaboration. Finally, the issue of using shared data for commercial purposes remains unresolved. In human studies, the data generators can consent to commercial use or not, but for the NHP community, it is less clear if ownership lies with the data generator, institution, or funder.

Data Standards, Quality Assessment, and Analytic Softwares

The Adoption of Data Standards and Open Analytic Solutions Are Readily Attainable

Data Standards. There is a clear need for metadata standards in NHP data acquisition. The Brain Imaging Data

Structure (BIDS) framework (Gorgolewski et al., 2016), used in the initial PRIME-DE data release, is recommended given its rapid maturation and widespread adoption in human neuroimaging, including EEG and MEG. However, the BIDS format will require revision to capture the range of metadata unique to NHPs. Minimally, species and scanning position (upright, sphinx) require specification. Metadata could also include details regarding anesthesia protocol, contrast agents, coil type (surface versus volume), head-fixation information, subspecies, age, sex, universal specimen identifiers, body weight, available genomic information, and animal origin. The NIFTI (Neuroimaging Informatics Technology Initiative) and GIFTI (geometry format under NIFTI) formats, for volumetric and surface datasets respectively, provide a standard for porting data between software packages. The CIFTI (Connectivity Informatics Technology Initiative) format appears to be well positioned as a framework for connectivity analyses that span surface-based representations and subcortical regions.

Quality Assessment. NHP imagers have yet to reach a consensus on quality assessment or assurance. Some datasets might be of higher quality, even if these are from fewer animals. There are also concerns that implementing high QC standards at this initial stage will stall data sharing, and analytic methods may be developed to rescue lower quality data. In the human literature, steps toward universal approaches to quantify data quality are being made (e.g., MRIQC) and could be adapted for NHP imaging. However, most existing tools are optimized for human heads, which have very different tissue profiles and are imaged at lower resolution. Investigators are leveraging technical advances (e.g., multichannel segmentation, deep learning, improved templates) to break through this barrier and avoid manual correction. However, at present, visual inspection and ratings remain key steps for quality assessment and analytical validation. Given these realities, the PRIME-DE consortium has recommended sharing all data regardless of data quality and to share QC ratings for the datasets. Finally, real-time quality assessments have been recently automated in

the human literature (e.g., the Framework Integrated Real-time MRI Monitoring [FIRMM]) and could be adapted for NHP imaging for motion monitoring, feedback, and to assess when sufficient data have been collected.

Pipelines. There is a scarcity of end-to-end NHP image preprocessing pipeline solutions, including surface-based analyses. Investigators identified a range of open-source tools and pipelines that are available or progressing in their development, making it just a matter of time until the reliance on in-house code decreases. This process can be accelerated through establishing mechanisms for rapid communication of developments via wikis, mailing lists, technical notes, code repositories, notebooking sites, and Brainhack events. Such communication is especially important in assisting investigators from outside of NHP imaging to engage with this community’s data. Publication of methods papers is encouraged and their value should be considered in assessing a researcher’s productivity. Lastly, it is worth noting that scientists are making progress in tackling the challenges of within and interspecies alignment. These efforts are crucial not only in advancing our understanding of the NHP brain but also in creating a common terminology between researchers from human imaging and the NHP community who quite often still use different vocabularies. A critical ongoing effort by some groups attending the GCW is the alignment of imaging and digitization of the wealth of histological and tract-tracing data in NHPs. With sufficient investment, such important data could be curated, helping to bridge analytical scales.

Coordinated Paradigm Design Common Ground in Functional Imaging Creates Opportunities for Globally Coordinated Activity

Functional localizers are commonly used in human and NHP imaging, spanning retinotopy, tonotopy, object perception, somatotopy, eye movements, social cognition, and more. To date, labs have tended to use customized approaches by creating and using their own localizer stimuli, typically in a relatively limited number of subjects. Commonalities in focus areas across laboratories create

opportunities for coordinated paradigm design and data sharing. First, the simple sharing of final statistical maps (e.g., via NeuroVault, Open Science Framework, or the Brain Analysis Library of Spatial Maps and Atlases [BALSA]) would generously allow applying meta-analytic techniques and aggregating across site information. Equally important, the sharing of functional localizer stimuli would allow harmonizing efforts and, as a result, improve the likelihood of reproducible findings, dramatically enhancing the value of shared datasets. Complementing lower-level functional localizers are naturalistic stimuli (e.g., films), which can be used to probe a range of systems, including higher-order association areas. Unfortunately, there is great variation in naturalistic stimuli across laboratories and in custom analyses that are needed to extract meaningful information from these localizers. As a first step, the community agreed that small groups will work together on obtaining coordinated localizer data for different modalities in 30 individuals as a basis for creating template-based probabilistic maps. These data will be invaluable to the broader community that often requires information on where functional fiducials reside in specific individuals. Long term, the community wants to work toward generating a collection of natural films and analytical approaches for a rapid (10–15 min) multifaceted “primate global localizer” that could be used by many laboratories. Its usefulness will need to be validated and established alongside information from accepted localizers.

Ambitions for the Next Five Years

Over the course of the next five to ten years, the PRIME-DE GCW attendees agreed that it will be possible to collect and share structural scans from 1,000 NHPs in various species with further grassroots sharing efforts of higher quality and more extensive datasets in 200 animals. With financial support, the coordi-

nation of activities centered around localizers could yield data from 30 animals for a given localizer, as the community works toward a multi-faceted primate functional localizer. More substantial investment would allow the generation of a large-scale, multimodal resource for NHPs similar to the Human Connectome Project, possibly including developmental samples (pediatric, fetal) and metadata (genotyping and phenotyping information, etc.). The integration of digitized neuronal tract tracing data, neurophysiology (high density recordings, laminar, etc.), histology, and neuromodulation approaches (optogenetics, electrical microstimulation, pharmacological inactivation, ultrasound, etc.) would bring unprecedented value to the resource.

Conclusion

We have synthesized a perspective put forward by the GCW meeting on the challenges and opportunities for NHP imaging and the ambitions of the community. Given the grassroots nature of the effort, the community recognized the need to meet regularly to strengthen communication and facilitate progress. Following the lead of its human counterpart, NHP imaging is unquestionably evolving toward reproducible and scalable science. To accelerate the pace of its evolution through increased collaboration, sharing, and investment, large-scale global neuroscience ventures (e.g., the BRAIN Initiative, Human Brain Project) and other funding schemes will need to support the community objectives for the next five to ten years of data generation and sharing. If the PRIME-DE GCW serves as a litmus test, exciting advances, and discoveries will become evident by global collaboration and support.

SUPPLEMENTAL INFORMATION

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Supplemental Information

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