The role of MRI in applying the 3Rs to non-human primate neuroscience

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ABSTRACT

Magnetic resonance imaging is playing a significant role in applying the 3Rs to neuroscience studies using non-human primates. MRI scans are contributing to refinement by enhancing the selection and assignment of animals, guiding the manufacture of custom-fitted recording and head fixation devices, and assisting with the diagnosis of health issues and their treatment. MRI is also being used to better understand the impact of neuroscience procedures on the welfare of NHPs. MRI has helped to optimise NHP use and make greater scientific progress than would otherwise be made using larger numbers of animals. Whilst human fMRI studies have replaced some NHP studies, their potential to directly replace NHP electrophysiology is limited at present. Given the considerable advantages of MRI for electrophysiology experiments, including improved welfare of NHPs, consideration should be given to focusing NHP electrophysiology laboratories around MRI facilities. Greater sharing of MRI data sets, and improvements in MRI contrast and resolution, are expected to further advance the 3Rs in the future.

1. Introduction

Non-human primates (NHPs), particularly macaques (*Macaca* spp.), are important models for exploring the neural basis of cognition (Roelfsema and Treue, 2014). Non-invasive imaging techniques, including magnetic resonance imaging (MRI), are increasingly being applied in neuroscience research using NHPs to localise cognitive processes and to compare the workings of the human and macaque brain (Passingham, 2009). MRI allows for precise in vivo analysis of the organisation of brain structures in relation to each other and, using functional MRI (fMRI), indirect measurement of neuronal activation by detecting changes associated with blood flow. Advances in scanner technology, image acquisition protocols, experimental design and analysis methods are improving the quality of data collection and enabling previously unobtainable insights into brain anatomy and function (Mars et al., 2014; Herrmann et al., 2015). Initiatives such as the PRIMatE Data Exchange (PRIME-DE) aim to accelerate the pace of advancement and scientific discovery through greater collaboration and sharing of NHP imaging datasets (Milham et al., 2018, 2020).

Whilst a great deal can be learnt about brain function using fMRI in human volunteers and patients, much of the information gained is correlative. To establish causal links between brain function and behavioural performance it is necessary to directly alter brain function in some way, either prior to or during fMRI, using surgical, chemical or physical means (Vanduffel and Farivar, 2014; Bell and Bultitude, 2018). Because many of the procedures used to manipulate and monitor brain elements are invasive, only rarely is it considered ethical for them to be carried out in humans (Vermeire et al., 2017). Such techniques are permitted in NHPs, with appropriate justification, enabling causal inferences about the contribution of brain areas to behaviour and correct interpretation of the results of non-invasive human studies (Lemon, 2012; Rilling, 2014). In addition, the ability to manipulate the physical and social environment of NHPs allows for the investigation of environmental impacts on brain structure and function.

Wherever NHPs are used in research with the potential to cause pain and distress, there is a requirement for researchers to implement the 3Rs principles of replacement, reduction and refinement (Russell and Burch, 1959; www.nc3rs.org.uk/3Rs). These principles are widely embedded in national and international legislation and regulations on the use of animals in scientific procedures, as well as in the policies of organisations that fund or conduct animal research (European Union, 2010; UK Government, 2014; NC3Rs/BBSRC/Defra/MRC/NERC/Royal Society/Wellcome Trust, 2019; United States Department of Agriculture, 2020; MacArthur Clark and Sun, 2020). Fully applying the 3Rs to research involving NHPs is not only an ethical imperative but can also improve the quality of the science and its translation, as well as public support (Prescott et al., 2017). It is therefore important that, wherever relevant and practical, new technologies are used actively to deliver 3Rs improvements in the use of NHPs (Bateson et al., 2011). MRI is playing a significant role in applying the 3Rs to neuroscience studies using NHPs, with potential to further advance the 3Rs in the future.

2. Refinement

Refinement is about minimising harm caused to animals in experiments, and about exploiting the latest in vivo technologies to improve understanding of the impact of animal welfare on scientific outcomes. Over the last decade, MRI has enabled substantial improvements in the welfare of NHPs used in neuroscience studies. It is now common place for structural MRI (sMRI) images to be used to create custom-fitted recording chambers and head fixation devices that are computer numerical control (CNC)-machined or 3D-printed to precisely fit the surface topography of an individual macaque's skull. Custom-fitted devices improve bone integration and stability, reducing the likelihood of infection, bone necrosis and loosening of the device (Mulliken et al., 2015; Chen et al., 2017; Ortiz-Rios et al., 2018). Surgery time can be shorter, and the hygiene of implant margins is significantly improved compared to traditional acrylic implants (McAndrew et al., 2012; Ortiz-Rios et al., 2018). There are scientific advantages too, in that fewer complications means data collection is less likely to be interrupted to allow animals to recover. While custom-fitted approaches were initially limited to electrophysiology dedicated implants, they now have also been applied to titanium-free implants compatible with fMRI. Using a custom-fitted implant manufactured from PEEK (polyetheretherketone) coated with hydroxyapatite, Ortiz-Rios and colleagues found the quality of blood oxygen level dependant (BOLD) fMRI signal to be improved, compared to traditional approaches using acrylic for head stabilisation. However, a potential limitation of this refined implant is its relatively large footprint that might interfere with the placement of recording chambers and/or the use of non-invasive stimulation. Implant customisation relies on precise reconstruction of the animal skull, which is usually done using computerised tomography (CT), due to the poor quality of bone imaging using traditional MRI sequences (Basso et al., this issue). CT is also used to monitor bone regrowth around implants and problems with device integration and infection (Guerriero et al., 2019). To avoid the ionising radiation of CT, techniques borrowed from human craniofacial surgery such as "black bone" MRI (Eley et al., 2012; 2017), which provide a good contrast between bone and soft tissues, are currently being tested. A variety of non-invasive approaches for head restraint of NHPs in fMRI studies have been reported in recent years (Shrihasam et al. 2010; Hadj-Bouziane et al. 2014; Slater et al., 2016). Whilst these represent significant refinements, and are applicable in some circumstances, data quality is usually highest with surgically-implanted headposts.

MRI has improved the quality of electrophysiological data by allowing more accurate, submillimetre targeting of recordings, stimulation and microinjections, helping to optimise animal use and scientific outcomes (Subramanian et al., 2005; t'Hart et al., 2006). Subramanian and colleagues found MRI-guided stereotaxic targeting of cells into basal ganglia nuclei of parkinsonian monkeys to be more than twice as accurate as atlas-guided stereotaxis. In optogenetics and ultrasound stimulation experiments, sMRI of each individual is being used to target virus-injection sites or stimulate brain regions more precisely that could be achieved using atlases (Gerits et al., 2012; Yang et al., 2018; Yazdan-Shahmorad et al., 2018; Folloni et al., 2019). Non-invasive, MRI-guided approaches for temporary inactivation can be considered a refinement over more traditional methods that require craniotomy, with an associated risk of infection and/or long-lasting impairments in the animals. sMRI is likewise being used to guide the placement of recording chambers before surgery, helping to reduce the risks associated with cranial implants and generally minimise the need for multiple invasive surgeries owing to poor chamber placement (Johnston et al., 2016). Initial MRI scans can also enhance the selection and assignment of animals to experiments, thereby avoiding potentially compromised data and/or wastage of animals where there are underlying problems, such as spontaneous brain abnormalities. Several cases of brain anomaly have been reported in which the MRI diagnostic led to refinement of the experimental plan (Sadoun et al., 2015; Bridge et al., 2019). Prior to the development of MRI tools, brain pathologies would only be detected at the post mortem examination, so scientific benefit from such discoveries could only be achieved *a posteriori* (Schmidlin et al., 2009). Given these advantages, greater use should be made of MRI scans to detect potential issues before enroling animals into experimental plans. MRI is also used for diagnosis of underlying CNS damage linked to invasive brain experiments (e.g. oedema; bleeding vs abscess), enabling better treatment plans and assessment of treatment success in-study (Winkelmann et al., 2012; Doane et al., 2018; Li et al., 2018). In such instances, MRI can help researchers and veterinarians establish a quicker diagnostic (Basso et al., this issue) and avoid extensive treatment (Yoshizawa et al., 2010). Where prognosis is poor, informed decisions can be made about early euthanasia to limit suffering (Johnston et al., 2016).

MRI has the potential to be used to assess the long-lasting affective states of NHPs. A recent analysis of the mammalian hippocampal plasticity literature suggests that the variation over time in the local amount of hippocampal grey matter measured by sMRI can track the cumulative affective experience of individuals during their whole life (Poirier et al., 2019). This approach offers the opportunity to assess the welfare impact of repeated procedures and to test scientifically the efficacy of putative refinements.

Whilst there are numerous benefits of using MRI to refine NHP experiments, it does come with its own potential risks for NHP welfare. In many NHP imaging studies, the monkeys are anaesthetised to facilitate their positioning in the scanner and to prevent motion during image acquisition (Winkelmann et al., 2012). While each single anaesthetic episode is considered to be safe, whether repeated anaesthesia is associated with a long-lasting welfare cost is currently unknown. Various anaesthesia protocols are currently used by different research groups. The welfare impact of repeated anaesthesia might therefore be different depending on the choice of anaesthetic drugs and the frequency of anaesthesia.

Capability for fMRI in awake, behaving macaques has increased considerably since the original work by Logothetis and others (Logothetis et al., 1999; Goense et al., 2010), enabling laboratories to map higher cognitive functions. Several research groups have also succeeded in collecting fMRI data from conscious marmosets, *Callithrix jacchus* (e.g. Hung et al., 2015). Multiple scan sessions are often required for monkey fMRI experiments (Janssens et al., 2012). However, the need for invasive techniques (e.g. implanted head posts), combined with restraint of the animal, extensive training and behavioural monitoring etc., make fMRI studies with awake NHPs challenging to perform (Goense et al., 2010; Srihasam et al., 2010). There is a risk of inducing stress if the animals are not acclimated progressively, using positive reinforcement, to the restraint chair, scanning environment, and radio frequency noise (Stefanacci et al., 1998; Chen et al., 2012; McMillan et al., 2017). In addition, as with electrophysiology, awake MRI still relies on fluid control/restriction, which can have welfare implications depending on the protocols used and how individual animals respond to them (Prescott et al., 2010, 2012). Guidance on refining fluid control is available (Prescott et al., 2010; Association of Primate Veterinarians, 2014).

The macaque brain is much smaller than the human brain, so there is a need for higher resolution than in human fMRI studies (Dubowitz et al., 2001). One approach is to do macaque imaging at higher applied magnetic fields (>3T). However, many laboratories do not have access to high field scanners due to prohibitive costs (Milham et al., 2020). One possibility to overcome this limitation is to use exogenous, iron-based contrast agents. At 3T, contrast agents improve the signal-to-noise ratio and spatial specificity of fMRI (Vanduffel et al., 2001; Leite et al., 2002; Janssens et al., 2012). However, the MRI signal obtained with this approach tracks changes in cerebral blood volume rather than the BOLD signal classically measured in human fMRI studies, complicating comparisons with human data. Contrast agents need to be injected intravenously. When the injection is performed on conscious animals (e.g. for awake fMRI experiments) it can potentially induce stress if the animal has not been previously trained to voluntarily present its leg or arm for injection. While no obvious short-

term behavioural effects have been observed (Vanduffel et al., 2001), the long-term impact of repeated injections still needs to be investigated. In addition, repeated injections of iron-based contrast agents induce an increase of iron plasma level that can be dangerous for animal health (Vanduffel et al., 2001). To prevent this problem, researchers treat animals with intra-muscular injection of chelating agents. Besides increasing the number of injections, allergic reactions to chelators have been reported in macaques (pers. comm to CP and MP). Sharing information between laboratories about the frequency of this adverse effect and how to best treat it offers the opportunity to refine this practice. In fact, there is huge potential for refinement from greater sharing of MRI protocols and metadata relevant to animal welfare, for example via PRIME-DE, on topics such as animal training, scanning and anaesthesia, enabling others to appreciate and implement ways to achieve their science with reduced impact on animals.

3. Reduction

Reduction is about minimising the number of animals used per experiment or research programme consistent with the scientific objectives, but also about using appropriately designed and analysed experiments that are robust, reproducible and truly add to the knowledge base. Achieving more science from each animal is also a form of reduction, if it avoids the need for additional animal use.

Along with advances in electrophysiology (e.g. new probes and better recording systems) (Thiele et al., 2006), MRI has helped to optimise NHP use and make greater scientific progress than would otherwise be made using larger numbers of animals; for example, by guiding and improving neuronal recordings (Freiwald et al., 2009; Vanduffel et al., 2014). Vanduffel et al. write that, by enabling quick examination of many neurons of similar selectivity in a hierarchical network of interconnected, category-selective areas, 10 years of MRI-guided single neuron studies have yielded greater progress in understanding face processing in inferior temporal (IT) cortex than did the previous 30 years of unguided single cell work. Comparative anatomy studies, for example to understand evolution of the primate brain, have benefited tremendously from sMRI, enabling data to be rapidly collected from living, healthy, non-elderly adult subjects without sacrificing animals or waiting for them to die. This has, in turn, facilitated larger within-species sample sizes, permitting formal statistical tests of between-species differences (Rilling, 2014). MRI has also facilitated post mortem 3-D analyses of primate brains and allowed extending the number of species available for comparative analyses, while preserving the brains intact for future research and educational purposes (Heuer et al., 2019; Barett et al., 2020; Roumazeille et al., 2020).

Greater sharing of imaging data sets has the potential to significantly increase the amount of scientific knowledge derived from each animal. Greater statistical power resulting from increased sample size allows researchers to tackle questions that could not be previously answered. In addition, it helps to alleviate concerns about the potential lack of generalisation of results coming from experiments with only two or three animals and allows investigation of individual variation in brain structure and function, an important aspect to understand brain states in health and disease. Comparing similar datasets from different laboratories offers the ability to test for replicability of findings. The opportunity to interrogate existing animal data sets also tends to attract researchers who do not have access to NHPs or MRI scanners. Widening the pool of researchers to people with different skills and/or different interests (e.g. computational scientists) can result in new ways to analyse the data and new questions being asked. Under the auspices of PRIME-DE, international NHP research centres have compiled 27 neuroimaging data collections into an openly available, shared database, accessible at http://fcon_1000.projects.nitrc.org/indi/indiPRIME.html. Currently, MRI data is available for over 200 animals in the standardised Brain Imaging Data Structure (BIDS) format. Such data sharing initiatives have already led to publication of new scientific findings, without further NHP use (Amiez et al., 2019; Xu et al., 2020).

4. Replacement

Replacement methods are those which avoid or replace the use of animals where they would otherwise have been used. The possibility to use fMRI studies in humans to replace electrophysiology experiments in NHPs has been repeatedly proposed. Whilst human fMRI has avoided some NHP use (Bateson et al., 2011), the potential for direct replacement of NHP electrophysiology is limited at present. Functional mapping techniques such as fMRI record a haemodynamic signal rather than neuronal activity itself, which makes it impossible, for example, to make inferences about the relative timing of events at a fine temporal scale. Alternative techniques such as magnetoencephalography (MEG) can offer greater insights into neuronal timing and do directly reflect the electrical activity of a region, but the MEG signal reflects synchronised activity across populations of cells, rather than the single cell level information that is available from electrophysiological studies in animals (Bateson et al., 2011; Lopes da Silva, 2013). Were the temporal and spatial resolution of fMRI (or other neuroimaging methods) to approach that of electrophysiology in the future, then a proportion of NHP studies may be able to be replaced. There would still be a need to establish causality of neural interactions on behaviour, but non-invasive approaches for reversible inactivation may be applicable (Lee et al., 2016; Bell and Bultitude, 2018). These are becoming ever more precise, with transcranial focused ultrasound (tFUS)/focused ultrasound neuromodulation (FUN) offering spatial resolution on the millimetre scale as well as the capability to target sub-cortical structures safely (Di Biase et al., 2019). However, in many cases, there would be a continued need for NHPs because of, for example, the requirement for disease modelling, systematic manipulation of the physical or social environment, or interspecies comparisons to study evolution. Nonetheless, significantly improving the spatial and temporal resolution of non-invasive imaging technologies should be a high priority given the potential, after appropriate validation, to advance the 3Rs in NHP research (Bateson et al., 2011; Vermeire et al., 2017).

5. Conclusion

Advances in imaging technologies over the last decade have created new opportunities to derive information from the study of NHPs, but also opportunities to apply the 3Rs, especially refinement. Given the considerable advantages of MRI for electrophysiology experiments, including improved welfare of NHPs, consideration should be given to focusing NHP electrophysiology laboratories around MRI facilities. Economies of scale could then allow greater investment in other welfare-related improvements, such as larger and better-quality housing, or access to specialist expertise in training animals using positive reinforcement techniques. Many of the 3Rs advantages of MRI for NHP use in neuroscience would apply equally to the use of other species such as rodents. The ever-increasing ability to obtain better contrast and higher resolution in MRI scans means that quality of the study data can be expected to continue to improve well into the future, which is expected to bring additional 3Rs benefits. Opportunities to further apply the 3Rs should be implemented as soon as they have been validated.

Declaration of Competing Interest

The authors declare no competing interests.

Credit authorship contribution statement

Mark J. Prescott: Conceptualization, Writing - original draft, Writing - review & editing. **Colline Poirier:** Writing - original draft, Writing - review & editing.

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