Non-human Primates in Neuroscience Research: The Case Against its Scientific Necessity

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Summary — Public opposition to non-human primate (NHP) experiments is significant, yet those who defend them cite minimal harm to NHPs and substantial human benefit. Here we review these claims of benefit, specifically in neuroscience, and show that: a) there is a default assumption of their human relevance and benefit, rather than robust evidence; b) their human relevance and essential contribution and necessity are wholly overstated; c) the contribution and capacity of non-animal investigative methods are greatly understated; and d) confounding issues, such as species differences and the effects of stress and anaesthesia, are usually overlooked. This is the case in NHP research generally, but here we specifically focus on the development and interpretation of functional magnetic resonance imaging (fMRI), deep brain stimulation (DBS), the understanding of neural oscillations and memory, and investigation of the neural control of movement and of vision/binocular rivalry. The increasing power of human-specific methods, including advances in fMRI and invasive techniques such as electrocorticography and single-unit recordings, is discussed. These methods serve to render NHP approaches redundant. We conclude that the defence of NHP use is groundless, and that neuroscience would be more relevant and successful for humans, if it were conducted with a direct human focus. We have confidence in opposing NHP neuroscience, both on scientific as well as on ethical grounds.

Key words: *brain, electrophysiology, fMRI, monkey, neurology, neuroscience, primate*.

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Introduction

Experiments on non-human primates (NHPs) are highly controversial. Opinion polls consistently show that members of the public are particularly troubled by the practice (1–4), with many only supporting it with important caveats, or indeed opposing it outright. A recent MORI poll in the UK found that only 16% of respondents supported the use of macaque monkeys for medical research to benefit people (5). This is not surprising, since NHP experiments in neuroscience, for example, involve many invasive and stressful methods, including intracranial electrodes, various restraint and training techniques, and water deprivation (6). Nonetheless, tens of thousands of NHPs continue to be used each year in experiments in the EU and the USA. According to EU statistics from 2011, the majority of NHPs were used in drug testing, but a significant number, i.e. 631 (10% of the total), were used in fundamental biological research, a large proportion of which would encompass basic neurological investigations, such as those discussed in this paper (7). Unfortunately, more-exact figures are not available.

The justification provided by scientists who use NHPs, in neuroscience research as well as in other areas, centres around a harm–benefit analysis (now inherent in the EU Directive on the use of animals in science, *Directive 2010/63/EU*; 8), i.e. that harms to the NHPs used are mitigated by benefits to humans. However, the benefit to human health of NHP-based neuroscience research appears to be considered only superficially in the project applications that we have seen. In addition, public information proffered by those that use NHPs in this way amounts to simple statements about the severity of the human disease in question and the need to use monkeys. The actual extent to which NHP neuroscience research benefits human health has not been considered in any depth or truly independently.

Many publications offer generalisations with regard to the indispensability of NHP research, based on anatomical, physiological, genetic, functional and behavioural similarities, which are often superficial and unreferenced (e.g. 9–11). Claims can be as vague as: "Nonhuman primates have a unique position in biomedical research related to their close phylogenetic proximity to humans. This close proximity often serves as the basis for scientific justification of their use in research" (9); and, with regard to neuroscience, "Neuroscience is an area in which research with non-human primates has played a major part in our understanding of basic neurobiology and the causes and potential treatments for human disor-

ders. This animal model is especially valuable because of the many similarities between human and non-human primates that derive from their common ancestry, such as complex cognitive capabilities, great social complexity, details of reproductive biology, and intricacy of brain organisation… In neuroscience, non-human primates continue to have important roles in basic and translational research, owing to their behavioural and biological similarity to human beings" (12), and the model has a "far-reaching relevance that is irreplaceable for essential insights into cognitive functions, brain disease, and therapy" (13). Many of these general claims are augmented by issues that are not relevant to, and have no place in, the NHP research debate, and which should be dismissed. For example, it is frequently stated that animal research as a whole uses a fraction of the animals that are used and killed by humans, and that, of those used in research, just a fraction of 1% are NHPs (e.g. 13).

This pro-NHP experimentation 'canon' is supported by inquiries into NHP research conducted over the past decade, the findings and conclusions of which have been influenced by such expressions of opinion from NHP researchers. For instance, the 2006 report commonly known as the Weatherall Report (14), and the consequent Bateson Review (15), both concluded, broadly, in favour of the need for NHP experimentation. There are, however, important and serious caveats, in addition to well-founded concerns about the review processes themselves and, accordingly, about their conclusions. These include: their potential lack of objectivity (i.e. they were funded and overseen by organisations and individuals who support NHP research); the lack of genuine harm–benefit analyses and systematic reviews; and questionable processes and criteria for assessing the value of the research. Therefore, it is particularly notable that they advocated a greater focus on the development of alternatives, improved animal welfare and systematic review of NHP research. In addition, they expressed concerns over the following issues: welfare costs; the application and relevance of the research to humans; the overstating of medical benefits by researchers; that benefits were speculative and not commensurate with welfare costs; that, often, little or no evidence of actual medical benefit is available; and that NHP work sometimes repeats previous work and/or 'confirmed' prior human studies (16). Of particular importance in relation to neuroscience, Recommendation 8 of the Bateson Review stated that, "Highly invasive and long-term NHP research often carries a high welfare cost. In such cases, funders should take particular care only to fund projects with a very high likelihood of producing scientific, medical or social benefit. Wherever possible, funders should take steps towards encouraging a preferential or complementary use of less invasive techniques such as neuroimaging and transcranial magnetic stimulation". Section 4.2.5 notes that almost half (46.2%) of the reviewed studies were neuroscientific, and that half of these had "a high welfare impact on the animals", while "In most cases, however, little direct evidence was available of actual medical benefit in the form of changes in clinical practice or new treatments". Additionally, the "effectiveness of knowledge transfer from basic to applied research" — something frequently claimed to be beyond doubt and, indeed, "the only way to successfully cope with devastating disorders and reduce the invasive character of many clinical procedures applied for their diagnosis and treatment" (17) — has been questioned by many (e.g. 18). This is acknowledged by Recommendation 4 of the Bateson Review, which suggests developing a mechanism "…to identify research results with potential to deliver improvements to healthcare or other significant benefits to society, and to assess the extent to which the potential benefits are achieved". Unfortunately, three years later, at the time of writing, the Medical Research Council (MRC) has indicated that this recommendation "is not currently being taken forward, as we have prioritised work on other recommendations in the Bateson Review" (18). Factual information on NHP research results and their medical relevance (as opposed to claims of efficacy and human relevance by NHP researchers) therefore remains unavailable to the public and the wider medical profession. Overall, few, if any, of the recommendations from the above inquiries and reports have been upheld and enforced.

This review examines the important question of the degree (if any) to which NHP neuroscience benefits humans, firstly by reviewing the scope, capacity and potential of 'alternatives' to the use of NHPs, such as human research and neuroimaging, asking whether the resultant benefit to humans from these approaches alone is not sufficient and, therefore, whether NHP experiments are needed at all; and secondly, by critically assessing salient claimed human benefits of specific NHP neuroscience studies, asking if there is substance to such claims, and whether any such benefits absolutely relied on the use of NHPs. Much of the latter is in large part in response to statements made recently by, for example, the Max Planck Institute for Biological Cybernetics (Germany) and Newcastle University (UK), in support of NHP neuroscience.

Overall, we examine, *inter alia*: historical claims of the necessity of NHP use and its contribution to human medical progress, both in general terms and with regard to specific examples frequently proffered by its advocates; claims of future indispensability based on the perceived limitations of alternative approaches; the nature of inquiries into the efficacy of NHP research in recent years, and if

they are fit for purpose; the degree of contribution of other neuroscientific investigative methods to the field, and how they compare to NHP use; and the extent to which confounding factors affect NHP data and their extrapolation to humans. In short, we seek to establish what human neuroscience can do and, as the scientific method demands, we *ask questions* of NHP neuroscience, testing the hypothesis that it is necessary and relevant to humans, rather than seeking to justify it.

The Increasing Power of Human Neuroscientific Investigative Methods

Human-based methods must be the goal, from both a scientific and an ethical perspective. There follows a detailed consideration and description of these, in order to gauge how capable they are, and to confront allegations that these methods are inferior and less able to tackle important research questions that must be answered, in order for progress to be made in the diagnosis and treatment of neurological diseases. These examples relate to particular investigative techniques and to areas of neuroscience that are particularly common, such as vision (categorisation, face and colour recognition and processing), memory function and vision-associated memory function, neuronal control of movement, and analysis of neural oscillations of various frequencies and synchrony. Some of these human-focused methods are also described in the subsequent section of this review, as part of individual cases against specific claims of NHP research necessity.

Transcranial magnetic stimulation (TMS)

TMS remains, in its fourth decade of use, an extremely useful and productive technique. It allows the non-invasive stimulation of discrete brain areas via magnetic pulses through the scalp, activating the cortex over a focused area of around a few square centimetres.

A 2010 review of TMS outlined its historical and current importance in neuroscience research (19). Originally developed to investigate the propagation of neural signals along the corticospinal tract, spinal roots, and peripheral nerves in humans, it owes its development to human studies. Single-pulse, paired-pulse and repetitive TMS "allows routine evaluations of the excitability and conductivity of corticospinal motor pathways" to investigate movement physiology in healthy patients and those with neurological disorders; allows researchers to transiently interfere with behaviour in various domains (i.e. create 'virtual lesions'), to enhance understanding of cortical functions; permits the mapping of motor cortical outputs and the study of motor conduction; provides measures of intracortical facilitation, inhibition and cortico-cortical interactions; allows the study of cortical excitability in neurological diseases, the functional relevance of cortical areas in cognitive task performance, brain–behaviour relations, and the pathophysiology of various neurological and psychiatric disorders.

Much information about the mechanisms of TMS (and indeed transcranial electrical stimulation [TES]) — for example, which neurons are specifically activated by it — has been gleaned via human experimentation over the past 30 years. Studies involving anaesthetised patients, as well as conscious volunteers, have elucidated how waves of neural activity travel down the corticospinal tract, measured by epidural electrodes implanted in the spinal cord (see Di Lazzaro & Ziemann [20]).

fMRI

fMRI is a method of measuring brain activity, in which sophisticated scanning machines and computers detect changes in blood oxygenation and flow that reflect neural activity. Human fMRI investigations are revealing how areas of the brain interact to transform particular sensory information into specific motor outputs, to achieve goal-directed movements (21). Resting state functional connectivity (rs-fc) fMRI has successfully identified altered intrinsic neural networks in many neurological and psychiatric disorders, including Alzheimer's disease and schizophrenia, as well as other disorders, such as tinnitus (22). It has also been of use in the identification of neuroimaging biomarkers of various conditions and disorders. With regard to tinnitus, for example, rs-fc fMRI analysis has implicated several inherent neural networks. In patients, the neural networks affected seem to show consistent modifications, including differential connectivity between limbic areas and cortical networks, and brain regions involved in attention and auditory processing.

fMRI and other neuroimaging technologies are also improving dramatically with time, increasing their power and resolution. It is acknowledged that "Advances in the new-generation of ultra-high-resolution, brain-dedicated PET/MRI systems have begun to provide many interesting insights into the molecular dynamics of the brain" (23). Such ultra-high field MRI is, at present, around five times more powerful than some systems that are used routinely. When combined with brain-dedicated high-resolution research tomograph (HRRT) positron emission tomography (PET), which is an order of magnitude more sensitive than a whole-body system, the visualisation of many of the fine structures of the human brain is possible, includ-

ing the hippocampus, thalamus and the brainstem. Investigation of the neural and functional activity of these regions, such as during memory tasks, is also possible (see Cho *et al.* [23]).

Electrocorticography (ECoG)/intracranial electroencephalography (EEG) and magnetoencephalography (MEG)

ECoG is an invasive technique involving arrays of electrodes implanted subdurally on the brain's surface. Clinical monitoring to identify epileptic foci involves craniotomy, placement of electrode strips on the surface of the cortex (often beneath the dura), as determined by prior EEG and MRI, then stimulation and recording over several days, followed by explantation and therapeutic resection. This procedure often provides an opportunity for research allied to therapeutic use (24).

ECoG permits the acquisition of brain signals that have "an exceptionally high signal-to-noise ratio, less susceptibility to artefacts than EEG, and a high spatial and temporal resolution (i.e. < 1cm/< 1 millisecond, respectively)" (24). This increased resolution makes it superior to non-invasive techniques, in that it helps to explore in detail the very short-lived dynamics of brain processes. ECoG "carries substantial information about task-related activity, such as motor execution and planning, auditory processing and visual-spatial attention"; "can reveal functional connectivity and resolve finer task-related spatial-temporal dynamics, thereby advancing our understanding of large-scale cortical processes"; "has especially proven useful for advancing brain–computer interfacing (BCI) technology for decoding a user's intentions to enhance or improve communication and control"; and "yields information that clinicians can subsequently use to guide the process of functional mapping by electrical stimulation"; see, for example (24).

Human-based invasive research into visual perception and selectivity is commonplace, including the area of facial recognition. Intracranial local field potentials (LFPs) were recorded in 14 epilepsy patients by using ECoG, while they were presented with images of faces and other objects. ECoG is regarded as showing "superior selectivity (and hence spatial resolution) compared with fMRI" (25), and, in this study, it permitted recording with up to 187 electrodes for up to 10 days. This revealed exemplar selectivity in the face-related cortex — findings that were related to similar, previous studies with single-unit electrodes in NHPs as well as fMRI in humans.

ECoG has revealed information about functional selectivity in the human cortex, in response to audiovisual stimuli. Twelve neurosurgical patients implanted with subdural electrodes on the cortical surface (590 in total) showed different and unique electrode responses, with selectivity to stimuli. In other words, the human sensory cortex is arranged "as a mosaic of functionally unique sub-regions in which each site manifests its own special response profile" (26).

Subdural arrays of electrodes were used to investigate the neural processing of partial visual information in the recognition of objects (27). This built on prior human research involving fMRI, EEG and invasive recordings. LFPs were recorded in 18 epileptic patients, each with an average of 94 implanted electrodes, when they were presented with visual stimuli. Analysis of neuronal activity along the visual stream revealed that the human visual cortex remains selectively active, even when presented with as little as 9% of an object. These signals were delayed, however, indicating that additional neural processing, involving spatial integration and extrapolation from prior knowledge, was occurring, to enable the reliable recognition of partial objects.

The human cerebellum has scarcely been explored in neuroscience, mainly due to the major focus of neuroscience on the cortex, but also due to its relative inaccessibility by non-invasive methods such as EEG and MEG. Most human information has come from lesion studies, as well as fMRI and TMS, though some EEG and MEG reports exist. For instance, correlation between resting-state fMRI activity and beta-band activity in MEG has been shown. In addition, there are some historical reports of intracranial electrode-based (including electrocorticographic) investigations of the human cerebellum, which elucidated neuronal oscillations and perturbations during task performance (28). Thus, invasive investigation of the human cerebellum is not only possible, but has been achieved, and has produced very useful data to augment non-invasive studies.

Such is the power and importance of ECoG, it has been argued that "ECoG/icEEG [intracranial EEG] informs unresolved questions in the study of human memory and is yielding insights necessary for the development of novel interventions to facilitate memory function in the damaged brain" (29). While imaging such as fMRI reveals the *where* of memory function, and non-invasive (scalp) EEG/MEG the *when* of memory and, in some instances and circumstances, also the *how* of memory, ECoG reveals the "*how* of human memory across an extended scope of the neurophysiology of memory in humans" (29). One example is the spatio-temporal functioning of human 'subsequent memory'.

The importance of EEG and MEG to brain research and functional mapping is exemplified by its central role in multi-national collaborative projects delineating neural networks, known as the 'brainnetome', such as the Human Connectome Project in the USA and the CONNECT Project in

Europe (30). This is due to the "outstanding temporal resolution" of these methods, and because "they are the primary clinical techniques used to capture the dynamics of neuronal connections". The most advanced EEG systems now have sampling rates faster than 1kHz, a level of precision that means they are able to record "subtle and swift changes in neuronal activity", such as epileptiform spike waves with durations of less than 50ms. The power of EEG and MEG has been increased substantially by the development of multi-channel systems, which are able to record 256 channels simultaneously, permitting the analysis of the functional integration of the brain across multiple regions. MEG, which records magnetic fields produced by electrical activity in the brain, has a comparable resolution to that of EEG, but has higher localisation accuracy. Together, by relating the results of these approaches to fMRI data and psychiatric/behavioural/clinical performance, it is believed that neuroscience and clinical research will benefit (30).

Single unit/microelectrode recordings

Similarly to ECoG above, this often takes place in patients undergoing neurosurgery for a variety of reasons, including epilepsy, autism, movement disorders, with the patients' consent. Single microelectrodes can be inserted into various areas of the brain, for short-term or even longer term periods, to permit readings to be taken of single-cell electrical activity during a variety of tasks, including vision, memory and navigation (see Fried *et al.* [31]).

Single-unit spiking activity was recorded in 14 epilepsy patients undergoing surgery, to investigate grid-like neuronal activity in human spatial navigation, in both the entorhinal and cingulated cortex (32). These cells had been identified previously in human fMRI investigations, as well as in rats, bats and monkeys. Notably, the human grid-like cells seemed to have "noisier firing maps than some grid cells reported in rodents".

Single-unit recordings are augmenting extensive fMRI investigations of human episodic memory. Research, directed to the hippocampus and associated brain regions via these human fMRI investigations, coupled with clinical findings in patients with amnesia, confirmed the hypothesis that so-called place cells are involved in the encoding and retrieval of episodic memory (33).

Ten epilepsy patients were implanted with chronic depth electrodes for up to 10 days, to investigate the perception and recognition of faces. This involved measuring the activity of single neurons in the medial temporal lobe (MTL), and the authors concluded that the firing of MTL neurons in humans (including various regions, such as the hippocampus, amygdala, and the entorhinal and parahippocampal cortices) depends on perceptual decisions (i.e. recognising faces), rather than on the actual visual features of the stimuli (34). Single-neuron and LFP activity in the MTL have been concurrently measured in humans during visual recognition tasks, to determine the relationship between those measurements (35). Building on previous similar investigations in humans, as well as related human fMRI, and scalp and intracranial EEG experiments, this research revealed that single neuron and LFP responses in the gamma and theta ranges indicate the conscious processing and recognition of perceived visual stimuli, which were phase-locked. Crucially, in humans, there existed a post-stimulus latency in neuron firing of around 300ms, which is much longer than similar latency in monkeys of just 100–200ms. This greater latency in humans is thought to reflect the greater processing of stimuli for memory functions.

Prior human-based research into autism implicated the amygdala in autism-associated abnormal processing of faces. Autistic patients undergoing neurosurgery facilitated the investigation of single neuron firing in the amygdala, which revealed normal neural electrophysiology, but underlay abnormal neural responses to facial features, compared to control epileptic patients undergoing neurosurgery (however, the nature of the control group, being epileptic, may be an important caveat; 36). Recordings were made via 56 electrodes implanted in the two autistic patients, compared to 88 neurons in the eight controls.

Combinations of invasive single-unit and cell-assembly recordings

A range of applications and successes of invasive human neuroscience were reviewed by Engel *et al.* (37). This 2005 review illustrates the breadth of human invasive neuroscience ten years ago, and, of course, the power and ability of investigative techniques have greatly improved in the intervening decade. This shows that human neuroscience has indeed been diverse and flourishing, and not limited to non-invasive imaging techniques, for some time. Examples of invasive human neuroscience cited by the Engel *et al.* review include:

— identification and characterisation of the relationship between single-neuron activity in various structures of the brain and movement of body parts and/or sensory stimulation, such as the somatotropic organisation of the subthalamic nucleus (STN);

— discovery of the cellular correlates of tremor;

— elucidation of the function of the basal ganglia, including details of oscillations/synchrony/coherence, and the effects of dopamine agonists;

— investigation of pathophysiological changes in epilepsy;

— study of neural coding and representation, particularly with regard to language;

— examination of learning and many types of memory (e.g. declarative, episodic, implicit, recognition, verbal);

— general cortical function and associated neural processes;

— confirmation of results obtained non-invasively; and

— analysis of movement-related synchronisation in different frequency ranges.

Many, if not all, of the above have been investigated in humans at varying resolutions, ranging from single-unit and multi-unit activity to local or more distributed cell assemblies. This not only informs neural coding, specificity and tuning, but also neural topography and maps, and the formation, function and spatiotemporal interactions of neural cell assemblies. Such investigations are not limited to brief experimental protocols just because they are in humans; many investigations take place over days or even weeks.

Electrical brain stimulation

Cortico-Cortical Evoked Potentials (CCEP) constitute an investigational approach that permits the *in vivo* human-specific 'mapping of brain networks', i.e. the study of anatomical and functional connectivity between motor regions of the cortex. This has previously involved NHP-based invasive electrophysiology, though non-invasive human-based methods — such as diffusion tensor imaging (DTI), TMS, transcranial direct-current stimulation (tDCS), PET/fMRI, EEG, MEG and *post-mortem* human brain dissection — are increasingly employed (38, 39). These human-based methods arguably make the NHP work redundant, particularly when one considers the associated advantages of species specificity.

Connectivity is tracked by applying electrical impulses to chronically implanted subdural electrodes, for example in epilepsy patients undergoing pre-surgical evaluation, and then recording evoked potentials elicited at distant cortical sites. While these non-invasive techniques are valuable and continue to be informative for mapping the human brain, CCEP augments them and provides more detail, by aiding the resolution of functionality and directionality of anatomical links — in other words, while non-invasive imaging establishes connections, CCEP can establish whether these connections are actually used and in which direction. Further, CCEP helps to reveal the actual neural basis of imaging signals specific to humans, and facilitates the study of perturbations in brain network function during cognitive processing. To date, many human brain regions have been examined and mapped, including the fronto-parietal network, hippocampus and language networks, and observations have been made on human spatial memory, perseverance, motor braking and visual perception, among others (see Keller *et al.* [39]).

A review of direct electrical stimulation (DES) of the brain comprehensively summarises how this method of neuroscience research has contributed to our understanding of human brain function (40). It has informed the organisation of human brain networks associated with movement, language and cognition, as well as basic neuroscience concepts such as neural transmission, localisation of brain functions and arrangement of many sensorimotor areas. Specific examples include impulses to act, face recognition, detection of motion, and production of language.

Specific Claims of NHP Researchers

Advocates of NHP neuroscience have, over time, cited various specific areas of research in which they regard NHP use as crucial. We critically examine some of the recent, commonplace and most vociferous here.

Single-neuron studies

One of the most common refrains from NHP researchers is that experiments on the activity of single neurons in the brain — as opposed, for example, to studying the inputs and outputs of groups of neurons, studying neuronal connections, structure, function and so on — are fundamental to neuroscientific investigation, and that they must be conducted in NHPs because they cannot easily be done in humans, and that NHPs are most likely to provide data of human relevance (e.g. 14, 15, 41, and many more publications, declarations in various forums, and personal communications). It is informative to examine these and related claims:

'Single-neuron studies of the human brain are difficult, and therefore rare'

Superficially, single-neuron studies in humans are ostensibly difficult to conduct due to their highly invasive nature, and it seems plausible that NHP-based experiments should be easier to sanction and perform. However, scrutiny of the neuroscientific literature reveals that purportedly rare human single-neuron studies are not rare at all. Since the first human single-neuron recording in

1955 (42), through experiments "during cognitive measures" that began in the 1970s (see Ojemann [43]), a search of scientific literature databases suggests that hundreds of such human experiments have been performed. This is borne out by the recent publication of a 376-page book on the subject (31), describing in detail many human single-neuron studies across many fields of investigation over this period of 60 years. These studies continue to be performed, for example, in epilepsy patients undergoing neurosurgical procedures for the condition (e.g. 44, 45).

That said, human single-neuron studies could be, and should be, more routine than they are. They "provide unique insights into the neural mechanisms of human cognition", and this insight could be achieved with little effort. However, there are many missed opportunities — for example, during surgery for dyskinesias, in which electrodes are used to locate specific brain areas. The additional studies might not be undertaken, despite the available opportunities, because there could be pressure on neurosurgeons not to do so — though it could be considered "a tragedy for human knowledge when an effort is not made to utilise the available opportunities" (43).

Encouragingly, any possible historical impediments to human investigations, such as limited clinical scenarios, relatively poor electrodes and primitive hardware and software, have been overcome. For instance, there are now flexible microwire bundles and tetrode arrays, permitting chronic implantation for long-term analysis. Such advances have led to a "mini-explosion in the field of human microelectrode recording", the power of which has been augmented by pre-amplifiers, head stages, noise reduction circuitry, better spike-sorting algorithms, etc. (46).

'Epileptic brains (normally studied in human research) are different from non-epileptic brains'

It is claimed that the study of human single neurons is confounded by the fact that, usually, the brains being studied are those of epileptic patients. However, it has been argued that any differences between epileptic and non-epileptic brains may be gross in nature, and therefore that there is no evidence that single neuron activity is affected (43), and also that any potential differences are mitigated by the recording of activity in tissue away from the epileptogenic focus (i.e. with no epileptiform activity). Further, investigation is not restricted to areas of the brain around sampling sites, and the use of other sites (such as the sensorimotor cortex, for example) is generally accepted if there is informed consent by the patient, as well as Institutional Review Board approval (43). Indeed, in any surgery, the epileptogenic focus is not known *a priori*, and as such requires electrode implantation into several sites to determine its location, many of which will be unrelated to the seizure network/focus (47). In addition, data are evaluated in the context of data from other studies, such as fMRI of non-epileptic brains, and there are no confounding effects from general anaesthesia, as there tend to be in NHP studies (see below; 47).

'The scope of human invasive research is limited'

Human single-neuron studies have, in fact, been greatly informative and powerful in many and varied ways, and their scope continues to increase. Examples include:

— *Declarative memory*: This type of memory enables the rapid transformation of experiences ('episodes') into long-term memories that are subsequently accessed or 'declared' by free recall or familiarity. The MTL has been an important area of research (hippocampus and surrounding structures) in this regard, which is central to a type of declarative memory known as episodic memory (memories of the details of one's own personal experiences), as well as spatial memory (memories of locations, spatial relationships, navigation, etc.). Notably, the former is thought to be almost exclusive to humans, though even if it were not, its study in non-humans would be close to impossible, as it chiefly relies on verbal reporting. This is why it has been asserted that single-neuron recordings in humans are "uniquely positioned to contribute toward our understanding of the neural mechanisms of MTL-dependent memories above and beyond what can be learned from animal models" (48). Spatial memory is being investigated in humans by using combined techniques, such as single-neuron, LFP and fMRI approaches. The impracticality of making humans under study navigate mazes (used in the past as a reason to use animals) has been overcome by simply asking human subjects to navigate a computer program. Human studies also involve the use of wireless electrodes to provide a more complete picture of the neural basis of spatial memory and navigation, by assessing vestibular and proprioceptive inputs (49).

— *Sleep*: Human studies of slow waves, their synchrony and their underlying activity, are considered invaluable for elucidating the link between sleep and cognition, as the simultaneous recording of activity from multiple brain areas bilaterally, and sampling of activity across cortical and sub-cortical structures, is rarely achieved in animal studies (50).

— *Visual cognition*: Interest in human-focused research is increasing, due to improved tech-

nologies, such as diffusion tensor imaging, building on historical human contributions to the field from lesion studies — in, for example, epilepsy patients who have undergone resective surgery. Much focus has been on regions of the MTL such as the parahippocampal gyrus, but also the inferior temporal cortex (ITC) (51).

— *Thoughts and deliberations*: Much mental activity is outside the realm of standard 'stimulus-response' that is frequently central to neuroscientific investigation. Thought processes not related to direct external input, such as imagery, free recall, deliberations and so on, are difficult, if not impossible, to study in animals, as they involve verbal reporting and/or responses to instructions. In these cases, human study is not merely desirable, it is necessary. While EEG, PET, MEG and fMRI have all aided the inference of thoughts from patterns of neural activity, greater clarity has been provided by single-neuron studies. Studies involving the use of intracranial depth electrodes implanted in the MTL have determined correlates of internal visualisation, and that neurons selective for objects, animals, etc., are selective for both visualisation and actual vision. These studies have also involved the investigation of recollection (not possible in animals), and have shown that the firing rate of specific neurons can indicate the type of object being visualised/observed (52). The latter has implications for brain–machine interfaces (BMIs; control of external devices, e.g. robotic arms) via patterns of neural activity detected in real time.

— *Reward processing; investigation of deep brain structures*: Human single-neuron studies have not been limited to superficial parts of the brain. Studies of deep structures (such as the nucleus accumbens, caudal anterior cingulate, anterior cingulate, dorsal anterior cingulate and substantia nigra) have also been conducted, enabling human-specific research into the processes in which these structures are involved. These include reward processing, often investigated with NHPs (53).

— *Facial processing and recognition*: Single-neuron studies of the human amygdala have investigated the perception of faces, moods and emotions, revealing how human single neurons respond to different parts of the face selectively; this is often studied with NHPs (54).

— *Language, memory and learning*: The output of just one laboratory over a period of 24 years illustrates what can be achieved with human single-neuron studies. Perception, object naming, verbal memory and association, among others (with many protocols not possible in animals), were elucidated by using almost 200 patients, more than 250 recording sites, and almost 500 single neurons (55).

— *Reach and grasp, motor prostheses*: Single-neuron human studies in patients undergoing deep brain stimulation (DBS) surgery have aided the discovery and understanding of neurons encoding directional movement and intent, and the modulation of gripping force. Work in amyotrophic lateral sclerosis (ALS) patients implanted with neurotrophic electrodes has enabled patients to control cursors, speech synthesisers and robotic fists (56). Recent advances in BMI technology allow the exclusive study of human motor cortical control and neurophysiology, with any (arguable) past contribution of NHP experiments having no bearing on what could, and should, be done now and in the future.

— *Seizure generation*: Invasive and non-invasive human research, including electrode-based measurements of LFPs and action potentials of individual neurons, have been conducted since the 1950s, in patients with movement disorders and pharmaco-resistant focal epilepsy. It has been suggested that there is "plenty of room to address interesting new questions by using the recording approaches presently available", which include subdural arrays of penetrating electrodes. It is notable that the techniques have evolved from using a few electrodes, recording over a few minutes under general anaesthesia, to arrays of microwires, implanted at depth, recording multiple activity in multiple brain areas semi-chronically. Such investigations have yielded important insights into human epilepsy (57).

fMRI development and basis/interpretation of the BOLD (Blood Oxygen Level Dependent) signal

It is claimed that invasive NHP experiments were critical to the development of our understanding of fMRI imaging, by way of elucidating the nature of the neural activity underlying the BOLD fMRI response (17, 58, 59), based on what some regard as a seminal paper published in 2001 by Max Planck Institute researchers: the Logothetis *et al.* (63) paper (hereafter referred to as 'the Logothetis paper', not to be confused with subsequent reviews by Logothetis, also referred to here [58, 60]). Indeed, it has been claimed that fMRI images "only became interpretable by doctors at nerve-cell level thanks to the work of Max Planck researchers", that work with monkeys was the first to show "that BOLD fMRI actually does measure changes in the activity of nerve cells" (61), and that it allowed us to "comprehend the neural processes underlying

such metabolic changes in order to be able to correctly interpret the functional scans used to assess the condition of patients with various neurological or psychiatric diseases" (17). This is of major consequence, as fMRI has become one of the core techniques of neuroscience, and has revolutionised it. These are very bold and significant claims, so what follows here is a detailed and critical assessment of them — as is warranted by the associated harms to the monkeys involved. It is not intended to be, and should not be inferred as, an overt criticism of the individual(s) who made the claims and who conducted the research. Nonetheless, our examination leads us to contend that these claims are incorrect, given the history of fMRI development and understanding described in the literature.

Given the salient consideration of the direction in which neuroscience research should be heading, and what techniques current research should be employing, these claims are redundant in any case. What went before, even if such claims were true, is of no consequence. It is of academic interest to analyse these claims, to help gauge the veracity and validity of statements asserting the worth of NHP neuroscience. We show here that there already existed substantial weight-of-evidence from human studies to support the hypothesis, and that, despite the argument put forward by Logothetis, and in view of related human evidence, the Logothetis paper, and the monkey research it entailed, simply cannot be considered seminal.

Human fMRI was first reported in 1991 (a decade prior to publication of the Logothetis paper in 2001), followed by the first BOLD-based human brain activation results and other human imaging experiments in 1992–93. These experiments built upon developments in PET imaging in the 1970s and 1980s (reviewed in Bandettini [62]). The crux of the debate is whether NHP experiments were indispensable to this progress, and in the deciphering of the nature of the fMRI signals. Would fMRI have been developed at all, or over a similar time scale, without them? Would we really be less able to understand the basis of BOLD fMRI without monkey experiments?

What Logothetis *et al.* did was to perform simultaneous intracortical recordings (LFPs, and single-unit and multi-unit spikes) of neural signals and fMRI responses, in the monkey visual cortex, in order to investigate and establish the link between neural activity, i.e. what we want to measure (and what is indirectly measured) in fMRI, and the haemodynamic response (changes in blood flow/oxygenation, etc.) in response to neural activity, i.e. what fMRI directly measures. This showed that increased BOLD contrast did reflect an increase in neural activity, and, because the greatest correlation was between LFP (synaptic activity) and BOLD signals, that BOLD reflects neural input to a given area, rather than its 'spiking' output (63). The main claims are that: a) this was the first simultaneous study of intracranial recordings with fMRI; b) this simultaneous approach was essential to fully address the nature of the BOLD response; c) prior simultaneous recordings of fMRI and EEG or optical imaging suffered from poor spatial resolution (EEG) compared to intracortical recordings; d) this research enabled the aforementioned conclusions to be drawn; and e) establishing the detailed relationship between BOLD signal and neuronal activity will continue to depend on NHP experiments. We argue, however, that this represents an exaggeration of the importance of these monkey experiments, and overlooks both the contributions of, and potential of, human-based investigations at that time. For example:

— *Prior weight-of-evidence suggested the same conclusion:* While the Logothetis paper may have been the first to directly investigate neurovascular coupling in the manner it did, prior weight-of-evidence strongly suggested the same conclusion. The paper itself cited previous human research examining concurrent EEG signals and fMRI images, though the importance of this was downplayed by the authors, based on the 'poor spatial resolution' of EEG. One such cited human study, published five years previously and also involving the same area of the visual cortex ('V1' or the 'striate cortex'), set out "to understand how the fMRI response relates to neural activity" and reports the results of three tests supporting the hypothesis that fMRI responses are directly proportional to local average neural activity over a period of time, with which Logothetis *et al.*'s data were "consistent" (64). Another review cites human work going back to 1995 that productively investigated neurovascular coupling: examples include fMRI of patients performing motor tasks, coupled with electrophysiological mapping of the sensory-motor regions of the cortex via cortical stimulation of the motor cortex to elicit hand movement and evoked-response recording in the somatosensory cortex during tactile stimulation of the hand; and comparison of gamma-band LFP signals in the premotor cortex with fMRI activation in patients performing tasks (see Mukamel & Fried [65]). Meanwhile, a 2002 review (66) acknowledges that Logothetis *et al.* "pioneered the simultaneous acquisition of electrical and fMRI data in primates", but also noted that, regarding their conclusion, "This is in agreement with recent data that show a significant correlation between fMRI BOLD responses and evoked potentials in humans, and the literature regarding evoked field potentials and cerebral blood flow in animals". All in all, many examples are provided of human studies that eluci-

date the degree of neurovascular coupling, i.e. the relevance of fMRI imaging to actual neural activity, in various areas of the human brain and at different power bands.

— *Many questions remain regarding neurovascular coupling in humans, including the validity of the findings of Logothetis et al.:* Arguments over linearity of haemodynamic response, and indeed between BOLD signals and underlying neural activity, continue to this day: they are "still a matter of debate, even after decades of research" (67), and "…a detailed understanding of the neurovascular coupling process remains elusive" (68). Thus, some 15 years after the studies reported in the Logothetis paper in 2001, they still cannot be considered definitive. Some reports suggest that the response is linear under certain conditions (e.g. 64), while others show non-linear haemodynamic responses (e.g. 69); some reports show BOLD fMRI correlates with underlying LFP rather than spiking activity (as in the Logothetis paper, and also shown in experiments involving cats [70]); others show, in contrast, that fMRI does correlate with spiking, while other studies suggest it correlates with both (human-based studies in 2005 [71]). These findings have been summarised by Kim *et al.* (67). Ekstrom also discusses this conundrum, accepting that "…the relation between the blood oxygen-level dependent signal and underlying neural activity remains an open and actively researched question", with "much for us to understand" (72), and also asserts that the model supported by Logothetis *et al.*'s 2001 results (BOLD reflecting perisynaptic activity, i.e. LFP rather than neuron spiking [63]) is challenged by situations in which BOLD, LFP and spiking dissociate. This uncertainty is further acknowledged and discussed at length in another review published in 2012 (73).

In view of the above, the Logothetis paper — and, therefore, the issue of the basis of the BOLD signal, as reported by it and the other papers cited here — is not an 'open-and-shut case', and important questions remain. The explanation of neurovascular coupling, it offers may be only partly correct, and/or may be only correct in certain circumstances. Or it might actually be incorrect overall.

— *Human studies were possible instead, and had to be conducted anyway:* The authors of a 2013 review of neurovascular coupling, citing the Logothetis paper, acknowledge that the extrapolation of those studies to the human brain was unclear, and that human studies were necessary (74). They cite multiple human studies that have, since, suggested a similar correlation between the BOLD signal and ECoG/LFP signals in the gamma range (30–130Hz), and also between BOLD and single-neuron firing when there is also firing of nearby neurons. Such human studies have been conducted and published since the late 1990s.

A review contemporary to the Logothetis paper (75) illustrated many examples of simultaneous electrophysiological and haemodynamic studies in humans, broadly of the type performed by Logothetis *et al.* in monkeys. While none used intracranial deep electrodes, as did Logothetis *et al.*, they at least show that such studies are possible in humans, and include a variety of combinations of PET, EEG, fMRI, MEG and invasive recordings. A 2006 review, citing many human studies, described how combined fMRI and functional near-infrared spectroscopy (fNIRS) studies have illuminated the relationship between neural activity and the BOLD signal (76). It cites the first 'combined study' that took place, in humans, in 1996, as well as more than a dozen other human studies. Further, it concludes that, while most studies focus on changes in haemoglobin (Hb) concentrations and correlation with BOLD signal, various other contributory factors have often been overlooked, such as blood flow dynamics, blood volume and changes in oxygenation. The authors remark that "…the details of the translation between an ensemble of neurons firing and the ensuing increase in focal cerebral blood flow remain controversial. The lack of a detailed understanding of the underlying physiology did not hinder an overwhelming success of fMRI; on the other hand, the more complex the paradigms investigated the more mandatory is a thorough understanding of the imaging signal". The point is that, undoubtedly, fMRI has been an overwhelming success without the detailed understanding of the underlying processes that NHP researchers (who use monkeys to investigate it) claim they are providing with their research; and, while a deeper understanding may or may not be desirable, this could be achieved solely via human investigation.

Other salient examples include a human study by Arthurs *et al.* in 2000 — a year prior to the Logothetis paper — that measured somatosensory evoked potentials (at the scalp) in five healthy, unanaesthetised volunteers, alongside fMRI BOLD changes (77). While these two approaches were not simultaneous, that appears to be a minor detail, given the convincing nature of the results and conclusion. It concluded that "…the BOLD response correlates with synchronized synaptic activity, which is the major energy consuming process of the cortex". This is the main conclusion of the 2001 Logothetis paper. Further human work by the same (i.e. Arthurs) group was published in 2003 (78), which cemented their findings "as had recently been shown in primates (Logothetis *et al.*, 2001)". In another study, combined EEG and fMRI

revealed how different components of EEG signals are related to positive and negative BOLD signals, aiding their interpretation, and helping to "further isolate the neural mechanism underlying both EEG and fMRI responses" (79). A 2013 review of fMRI stated that 'current' techniques (which will have improved further since publication) provided a resolution of $1mm^3$ spatially, and 1 second temporally (80).

While advocates of invasive NHP experiments frequently use the spatial resolution provided by invasive electrodes as a defence of this approach, this resolution is sufficient for it to be central to the Human Connectome Project, which is mapping the connectivity of the human brain. Furthermore, this review mentions fMRI machines with even greater resolutions: 9.4T systems, for example, and 7T systems that have detected BOLD responses with a $0.7mm^3$ resolution. As previously stated, this is improved when fMRI is used in combination with other techniques such as EEG. Finally, a 2012 review summarised the contribution of simultaneous human EEG/fMRI studies to the understanding of BOLD signals/neural activity, which elucidated the relationship between BOLD signal and alpha rhythms (81). This review also cited a 1998 study, in which the relationship between activity-dependent increases in cerebral blood flow and single-unit activity and LFPs was examined in the rat cortex (82). This study showed that there was a strong correlation between activity-dependent cerebral blood flow and LFPs. This conclusion is similar to that of the Logothetis paper, in that it links the basis of fMRI signal to LFP/synaptic activity, rather than spiking. In other words: while this study did not use fMRI as Logothetis *et al*. did, it provided evidential weight to the hypothesis that fMRI is reporting synaptic activity/LFPs (in rats, rather than monkeys, and three years before the Logothetis paper). If these human studies were required in any case, due to questions over the extrapolation of NHP data, and were conducted/could have been conducted instead, then the fact that Logothetis *et al*.'s 'seminal' experiments were performed in NHPs is superfluous: their major conclusion was not dependent on NHP use.

— *Human studies alone are more than capable of addressing the ongoing issue now and in the future, and are being used to do so:* The 2012 Singh review (73) goes on to suggest that neurovascular coupling can be (and is being) investigated and resolved by means of non-invasive human EEG/fMRI experiments, as it has been since the mid-1990s (prior to Logothetis' invasive NHP work) as well as "electrode recordings in implanted human epilepsy patients with BOLD fMRI in healthy human participants". Regarding the former, non-invasive human studies, "At the invasive microscopic level, these oscillatory signals can be found in LFP recordings, where they reflect the integrated post-synaptic potentials of neurons within a millimetre of the recording electrode. However, such signals can also be measured macroscopically at the cortical surface by using either [ECoG, EEG or MEG] — these signals then represent the synchronous activity of many square millimetres or centimetres of cortex". In fact, such studies have revealed which components of the electrophysiological signals positively and negatively correlate with the BOLD response: even simple tasks have shown (via MEG) reductions in alpha power, increases in gamma power, and evoked sustained DC changes all closely localised with BOLD response (73). The authors conclude that a combination of MEG, fMRI and MRS can address this complexity in human studies. Other recent examples include: a) simultaneous icEEG and fMRI in epilepsy patients, providing good quality results as well as more events reported than with scalp EEG (83); b) investigation of the link between BOLD response and neuronal activity by using fMRI and high-density ECoG grids in humans (84, 85); and c) investigation of the association between fMRI activation and electrical activity, as well as brain connectivity, with simultaneous intracranial electrodes and fMRI in humans (86, 87).

— *A detailed understanding of BOLD fMRI may be of academic interest only, and not necessary:* fMRI results generally 'make sense', meaning that knowledge of the mechanistic basis may not actually be needed for their successful application. One analogy that has been proffered is that of a new telescope, offering astronomers unprecedented clarity in their view of the heavens. Would it matter, if nobody fully understood how it worked, particularly if it had been calibrated with well-known objects and previous observations (68)?

In conclusion, the use of the Logothetis paper as evidence of the necessity of NHP neuroscience must be considered specious.

Deep brain stimulation (DBS)

DBS has been, and remains, an effective therapy for the tremor-related symptoms of PD for tens of thousands of patients. It involves the insertion of stimulating electrodes into deep brain structures called the basal ganglia, which, among other functions, control movement and posture, and whose normal function is disrupted in Parkinson's disease (PD). Advocates of NHP research claim that macaque experiments (subsequent to the development of the so-called 'MPTP macaque model' of PD in 1983)

have been indispensable to its development, and specifically to the development of DBS of the subthalamic nucleus (STN). Indeed, this argument, in addition to the others described in this review, is repeatedly used as a 'flagship' to showcase NHP neuroscience as a means of convincing the public, as well as regulators and legislators, that NHP neuroscience is vital to progress in neurological medicine (e.g. 17, 41, 88–92). However, in common with BOLD fMRI above, this is of historic interest only, and has little or nothing to add to the critical consideration of the current and future value of NHP neuroscience. For the same reasons as apply to BOLD fMRI, it is worth addressing here, in order to rebut those claims, as well as to illuminate the true nature of such 'arguments of necessity.'

First, it must be appreciated that such claims have not been made via a robust, systematic review of all the available literature. Anecdotal evidence, citing NHP experiments in which STN-DBS has been investigated, is not sufficient; the mere use of NHPs in DBS research/investigation of the STN is no measure of their crucial nature or of their contribution to the field. Indeed, a critical and comprehensive review of the literature that includes all methods of investigation, alongside other summaries of evidence, provides a compelling case against the necessity of NHP research in the development of STN-DBS, and in support of human observation and neurosurgical investigation alone as the foundation of DBS treatment of PD (93, 94), and these accounts should be consulted for an in-depth argument against it. These arguments are summarised in a recent 'Letter to the Editor' published in *ATLA*, dedicated to the issue (95). Briefly, they demonstrate:

— the major role of human studies historically in uncovering the functional anatomy of the brain, as well as confounding species differences from parallel animal studies, including of the basal ganglia (and the STN);

— that the STN had been linked to movement disorders in humans, as long ago as the 1920s, as a result of both clinical and *post mortem* studies, before similar observations were made in NHPs (e.g. 96); and

— that basal ganglia generally were being operated on in the 1940s, to alleviate movement disorders, belying claims that this was down to NHP experiments (see examples in various articles [97–100]).

Further, they describe:

— the use of electrostimulation in humans since the 1960s, initially to establish the correct placement of needles for making thalamic lesions during surgery (see various articles [101–104]);

— how, during these procedures, it was noted that the stimulation of particular brain structures could suppress the symptoms of movement disorders, including PD (see various articles [105–108]);

— how DBS could therefore be a probable alternative to therapeutic lesioning (108, 109); and

— the use of human DBS, in the late 1970s and in the 1980s, in various parts of the brain, including the basal ganglia, to control tremor, among other things (110–113).

All these points illustrate that human studies predated, by decades, the first report of the MPTP monkey in 1983, as well as its subsequent use to investigate and characterise the basal ganglia and STN in PD. Clearly, claims such as "...the treatment of PD by delivering DBS to the STN owes everything to the research in non-human primates..." (114) cannot be correct. Regarding the application of DBS to the STN specifically, in addition to the association of the STN with movement disorders almost a century ago (as stated above), and as part of myriad human investigations of the basal ganglia since then (see 115), it is also obvious that the STN would have become more and more implicated and investigated in humans as a matter of course, without the need for investigations in the 'new and interesting' MPTP monkey.

Though the question of *deliberate* targeting of the STN in humans prior to work with MPTP monkeys is perhaps debatable, it is clear that the STN was, in any case, flagged as a potential therapeutic target in human investigations, prior to the availability of MPTP monkey data. The type of brain lesions performed in these human surgeries inherently and unavoidably affected an area within several millimetres (4–6mm, typically) of the tip of the electrode used in those procedures (116, 117). Given that the STN is within a similar several millimetres of various structures that *were* targeted, it follows that there will, undoubtedly, have been associated STN lesions. In other words, the consequences of STN lesioning *were* studied, however unwittingly. In fact, this was overtly discussed by researchers in the 1960s, such as Andy *et al.* (116) and Hassler *et al.* (118).

Over and above human-based investigation underpinning the development of DBS, it is also clear they are central to its further development and refinement, as well as to the understanding of PD pathology, with no need for recourse to NHP experiments. Illustrative examples include:

— studying the effects of DBS-mediated stimulation of the internal globus pallidus (GPi) on local neurons in unanaesthetised PD patients undergoing surgery for stimulator implantation, revealing details of changes in firing rate and patterns of neurons in the vicinity of the GPi (119);

— extracellular single-unit recordings in the basal ganglia revealing the presence of bursting neurons and their firing rate; determination of how STN activity is modified by L-dopa therapy, and how the pallidal complex functions in terms of bursting patterns and oscillations (120); and
— neuroimaging studies linking abnormal activation patterns in the insula to PD-related cognitive decline, behavioural abnormalities and somatosensory disturbances (121).

Neural oscillations and memory

It has been claimed that researching how visual stimuli are translated into memories, which involves examining how different areas of the brain collate information, "can only be examined in apes" (122). This type of research often involves analysing neural oscillations, and determining how their phase and amplitude correlate with spiking, how this underpins 'phase coding' (whereby variable neuronal spiking at particular phases of oscillations permits the coding of information such as spatial location), and how gamma-phase oscillations correlate with BOLD signals from fMRI. These are central to memory formation and function, and an important area of research, as they "exhibit specific spatiotemporal patterns that show active brain regions, indicate the types of neuronal computations that occur, and reveal how information flows through the brain" (123). We contend that the claim that this is only possible in apes is false. Historically, there are reports of intracranial electrode-based investigations of the human cerebellum, which elucidated neuronal oscillations and perturbations during task performance (28). There are many more recent instances of the human-specific investigation of neural oscillations at various frequencies, including the theta band, during processing of (often visual) stimuli, alongside associated memory formation and retrieval (e.g. 24, 124–127). Indeed, such human research, even involving intracranial electrodes, has become quite routine: this permits greater spatial and temporal resolution, all in a human-specific environment, and because these electrodes can be implanted for days or even weeks, complex human cognitive and task-related processes can be investigated in conscious individuals (123).

The use of both surface and depth electrodes expands the scope of investigation from the cortex to deep brain structures, and often microelectrodes are also implanted to record concurrent action potentials of single neurons. This has elucidated the neural basis of four complex cognitive domains: working memory, episodic memory, language, and spatial cognition (123). A 2005 review of invasive recordings from the human brain cites more studies, including the elucidation of the function of the basal ganglia, including details of oscillations/synchrony/coherence and the effects of dopamine agonists (37).

A 2014 review augments the above, and underlines the capabilities of human-based research into neural oscillatory mechanisms (128). This review cites numerous human studies that have shown how these oscillations complement neural firing in the neural representation of sensory perceptions and memory, and how they contribute to the encoding and retrieval of memory. For example, transcranial alternating current stimulation (TACS) in humans has implicated oscillatory phase in sensory processing; human studies have revealed content-specific LFPs, showing both category-specific and stimulus-specific neuronal firing and LFP responses at different frequencies in the temporal lobe; and human EEG studies have helped to reveal the information contained in oscillatory power, frequency and phase in facial representations and in auditory stimuli (128).

Neural control of movement

It is claimed that NHP neuroscience is vital for a greater understanding of the neural control of movement, i.e. how the brain, spinal cord and associated sensory and motor neurons interact to generate synchronised, controlled, movements. Much of this involves investigating different 'tracts' of the central nervous system (CNS), such as the corticospinal tract (the major tract of nerves descending from the brain, particularly motor areas of the brain, through the spinal cord), the reticulospinal tract (the tract of nerves descending from the reticular formation of the brainstem to the trunk and limbs, involved in motor functions such as posture and locomotion), and the dorsal root ganglia of the spine, which relay sensory stimuli to the spinal cord. Both NHP and human investigations are conducted, with the major difference between them being the methods used to stimulate and record neural activity.

In common with other areas of research, much of the justification for NHP use rests on the claim that measuring the activity of individual neurons provides greater information and resolution than is possible via non-invasive methods, and that this is difficult in humans. Indeed, it appears that many invasive NHP experiments are replicative of previous human or NHP investigations involving non-invasive approaches, and/or invasive research with other species (such as cats or rodents), performed to obtain greater detail and/or species-specific data. For example, the recent research of a group at Newcastle University that focuses on this field, has involved the extensive use of NHPs, many of which have undergone craniotomies and

laminectomies to facilitate the insertion of stimulatory and recording electrodes into the brain and spinal cord. Much of this work had already been conducted in cats (129–131) or, in some cases, was very similar in nature to work already completed in the same NHP species and/or in humans, but added very little (or nothing) to existing knowledge (130, 132, 133). Further, some papers reported important species differences between monkeys and humans that confounded their findings (131, 132, 134, 135), while others, by referring to prior human studies of a very similar nature, suggested (at least indirectly) that further studies could be done non-invasively in humans (131, 133–136). For example, non-invasive, surface-recording, high frequency EEGs were shown to accurately reflect the timing of spikes in single neurons in one of the studies described below (136), thus validating this approach as a non-invasive method, which also happens to be frequently used in human studies. For instance, non-invasive monitoring of spike activity in the human somatosensory cortex is possible, and effective, when scalp electrodes are used in combination with improved techniques to minimise the 'noise' that confounded previous studies (137). In addition, tactile — as opposed to electrical — stimulation has been shown to be effective, permitting non-invasive investigation of the somatosensory system in children (138). Combined with imaging techniques, such as MRI, fMRI and PET, we argue that these techniques render this type of invasive single-neuron experimentation with monkeys redundant.

Vision/binocular rivalry

The Max Planck Institute uses monkeys to investigate the neurological basis of binocular rivalry, a phenomenon where, when two different images are presented to the two eyes simultaneously, the viewer is only conscious of one of the two images at a time (139). However, this is also being investigated in humans, such as a study of the modulation of responses of visually selective neurons in the human MTL with alternating percept, with findings consistent with those in NHP experiments (140). Indeed, reviews of the phenomenon talk about both monkey and human studies interchangeably (51).

Confounding Factors Adversely Affecting NHP Neuroscience

Those who use NHPs in their research often argue that the results are conclusive or provide crucial evidence, such as in the fMRI case above. These claims are also commonly stated in submissions by chimpanzee researchers to the US Institute of Medicine's (IOM) chimpanzee research inquiry (see below; 141), and many others. What they often ignore are the numerous confounding factors that mean that the results of NHP research must be viewed with much more scepticism.

Species differences: Genetic

Claiming that similarities in brain structure and function are sufficient evidence to support the use of NHPs in neuroscience is superficial and inadequate. This is axiomatic, because in a complex living system such as an individual NHP, ostensibly minor differences can cause significant disparities in biological processes and their outcomes. It is clear from some of these biological and phenotypic differences that studies of NHP brains can only provide definitive information about the species studied, and may be misleading, if used as analogues for, or to predict responses in, human brains. This limitation is rarely acknowledged by NHP researchers, and it should be much more fully appreciated and considered. Proponents of NHP neuroscience must be able to demonstrate a comprehensive correlation with, and predictive nature for, human brain function that has resulted in translation to clinical practice, as a result of data that could not have been obtained in any other way.

The dearth, up to now, of comparative biology relating to the suitability of NHPs as a model for human neuroscience can be considered surprising, given the extent of their use and the associated costs and harms. Encouragingly, however, recent years have seen increased effort to investigate and understand these species differences, though their application to the critical questioning of how they affect inter-species extrapolation of experimental results remains poor. Some of these fundamental genetic and biological differences, as well as observable physiological and functional disparities, are summarised here.

First, it is useful to appreciate the degree of significant differences between humans and even our 'least different' relative, the chimpanzee, which was used in biomedical research (at least in the USA) until recently, when it was deemed to be unnecessary following an in-depth review by the US IOM (141). A comprehensive review of these differences highlighted disparities in all aspects of gene expression and protein function, from chromosome and chromatin structure to post-translational modifications (142). The collective effects of these differences are extensive and widespread, and they revealed the superficial similarity between human and chimpanzee genetic sequences to be of little consequence for biomedical research. These differences included some that were particular to the brain, and thus are pertinent to this report:

— A study examining the expression of around 12,000 genes in the prefrontal cortex of the brain found that almost 1,000 were expressed in the human, but not in the chimpanzee, while the reverse was the case for 344 genes. In addition, of the genes that were expressed in both species, 20% showed a different expression profile (for example, 19 genes linked to Alzheimer's disease, PD and Huntington's disease in humans were expressed differently in chimpanzees; 143). In the cerebral cortex, at least 169 genes are expressed differently (many of which are involved in neuroprotection and synaptic transport), and 916 genes are expressed at least two-fold differently in the cerebellum (144). Furthermore, many genes involved in oxidative metabolism and mitochondrial function are expressed to a higher degree in the human brain than in the chimpanzee brain.

— Of approximately 10,500 genes studied in various human and chimpanzee organs, 34% showed differential expression in the brain (145).

— The expression levels of 90 transcription factor genes were significantly different in human and chimpanzee brains. These gene networks are enriched for primate-specific *KRAB-ZNF* genes, which are central to human and chimpanzee brains and are associated with genes involved in the development and maintenance of this organ (146).

— In humans, but not in chimpanzees, 61 genes are up-regulated and another 55 are down-regulated by the FOXP2 protein. The genes involved are important for brain development and function (for example, those involved in craniofacial formation and in establishing the neural circuitry and physical structures needed for spoken language via cerebellar motor function), and in the formation of cartilage and connective tissue (147).

— Many splicing factors are differentially expressed in humans and chimpanzees, including 20 in the brain. This will result in many protein variants, which may have distinct functions in the brains of humans and chimpanzees (148).

It follows that these profound differences in gene complement and expression between human and chimpanzee brains will be even more significant between humans and monkeys, and therefore, are likely to adversely affect the translation of data to humans to a greater degree. A more recent review (from 2014) illustrates this in detail (149), with many of the cited differences affecting the brain:

— Parallel duplications and losses of the *RHOXF2* gene in humans and 16 NHP species, alongside different patterns of expression, are thought to have important inter-species biological implications due to the role of the gene as a transcription factor (modulating the expression of genes under its control) and in developmental processes. Notably, *RHOXF2* is expressed differently in the brains of human newborns and embryos, and it regulates the expression of at least three other genes involved in the function of the CNS. It is therefore thought to be involved in CNS function and brain development, with significant implications for inter-species differences (150).

— Humans have a rate of gene turnover 2.5-times that of all other mammals, which includes several gene families, notably genes preferentially expressed in the brain (151).

— One study reported that over 7% (corresponding to 893) and 6% (corresponding to 789) of 12,473 genes in the cerebellum showed increased and decreased expression, respectively, in humans compared to rhesus macaques (152), while another study noted that 91 genes were differentially expressed in human brains relative to those of rhesus macaques, as well as chimpanzees (144).

— An investigation of micro-RNA (miRNA) expression and regulation in the brain, specifically in the prefrontal cortex and the cerebellum of humans, chimpanzees and rhesus macaques, noted that up to 31% of the 325 miRNAs examined "diverged significantly" between humans and rhesus macaques, and that human-specific miRNAs were associated with neurons and with target genes involved in neural functions, supporting the theory that miRNAs have contributed to the evolution of human cognitive functions. Of the 413 miRNAs expressed in the human brain, 11% were not detected in rhesus macaque brains, and almost one third (31%) of miRNAs common to the human and rhesus macaque prefrontal cortex were differentially expressed in those two species. Of those differentially expressed prefrontal cortex genes, 77% were also differentially expressed in the human and the rhesus macaque cerebellum (153).

— Such is the degree of change of miRNA expression and the repertoire of their target genes across NHP species, developmentally throughout NHP lifespan, and developmentally throughout lifespan across NHP species, that miRNAs are thought to be the basis and major driving force of the evolution of the human brain. This was evidenced by a study of the prefrontal cortex and cerebellar cortex transcriptomes of humans, chimpanzees and rhesus macaques of different ages, which revealed significant variation between these types, in addition to sequence divergence in *cis*-regulatory regions (154).

— One human-specific miRNA, miR-941, is highly expressed in the brain and has been implicated

in neurotransmitter signalling via the roles of some of its target genes. Of note, the host gene of miR-941 (mi941 is an intronic miRNA), *DNAJC5,* encodes cysteine-string protein-α (CSPα), which has been linked to neurodegenerative diseases including Huntington's and Parkinson's diseases, and adult neuronal neroid-lipofuscinosis; and miR-941 may be associated with hedgehog and insulin signalling pathways, with associated roles in human longevity and in some cancers (155).

— The adenosine-inosine editing rate, and therefore the resultant changes in gene function and expression, is higher in humans than in NHPs, including rhesus macaques, due to primate-specific *Alu* sequences. This appears to particularly affect the human brain, via genes associated with neuronal functions and neurological diseases including bipolar disorder, motor neuron disease, Alzheimer's and Parkinson's diseases, schizophrenia, multiple sclerosis, and amyotrophic lateral sclerosis (156).

— A recent connectome study reported major species differences in the architecture of the inferior parietal cortex, and polar and medial prefrontal cortices (157). These findings augmented previous studies demonstrating a greatly expanded, lightly myelinated region of prefrontal cortex in humans when compared with that in rhesus macaques and chimpanzees (158), and a more gyrified prefrontal cortex in humans compared to other primates, even allowing for differences in brain size (159). Functional consequences of these differences may involve sensory perception, visceral functions, higher order cognitive functions, and emotional and reward-related behaviours (see 157).

— Comparative studies of human, chimpanzee and rhesus macaque genomes identified, for example, different numbers of long inverted repeats (LIRs) associated with orthologous genes in these species. There were 546 of these in humans, of which 421 (77%) were human-specific, but there were only 130 in the rhesus macaque, of which 107 (82%) were rhesus macaque-specific. Genes associated with the human-specific LIRs were involved in neural development and function, and in cell communication (160).

— The study of the *MCPH1* gene (one of at least seven key genes known to be involved in the regulation of brain size during development) illustrates how specific mutations can result in functional changes, leading to altered regulatory effects in downstream genes, and ultimately to significant species-specific phenotypes and evolution (161). The regulatory effects of human and rhesus macaque *MCPH1* were different in three out of eight downstream genes tested, and the human-specific mutations altered the regulatory effects on the downstream genes.

— The profound effects of genetic differences can be illustrated by the *SRGAP2A* gene, which produces a truncated protein in humans, but not in great apes. This appears to underlie differential morphology and density of dendrites, linked to different behaviour and cognition.

— Several human-accelerated enhancers have been discovered. These are non-coding DNA sequences involved in enhancing the expression of one or more genes, which have evolved in humans to be significantly different to their corresponding sequences in other species. Recently, one has been directly linked to differential brain development. This enhancer (HARE5) of the *Fzd8* gene has features unique to humans in terms of sequence, temporal and spatial expression, and transcript abundance, notably between humans and macaques, which affect "…the cell-cycle dynamics of a critical population of stem cells during corticogenesis and may underlie some distinctive anatomical features of the human brain", including brain development and size (162).

Genetic differences such as these clearly have biological sequelae. Indeed, it has been postulated that "…the entire topology of a complex brain network can be reprogrammed by subtle adjustments of many genes that act additively to produce a given phenotype" (163). Some work has uncovered the functional consequences of differential gene complement and expression in the brain, while the genetic basis of other, empirical, biological differences has yet to be established.

Species differences: Physiological and functional

Primate brains are known to "differ in aspects of structural detail, as well as in overall size" (164). For example, the human neocortex differs from that of other great apes in several ways, including having an altered cell cycle, prolonged corticogenesis, and increased size (see Boyd *et al.* [162]). It is now accepted that various primate brains are far from simply scaled-up or scaled-down versions of each other. Over and above this general observation, detailed knowledge of important genetic, structural and functional differences is beginning to emerge as the question of inter-species brain differences is addressed. While some features of cortical organisation are common to various mammalian species, it is clear that "phyletic variation in cortical organization is far more extensive than has generally been appreciated or acknowledged" (165). Examples include differences in cortical neuron genetics and biochemistry, as well as their connectivity, organ-

isation and function in rats, and visual system differences in monkeys (165). Varied techniques such as fMRI, PET imaging and diffusion-weighted imaging (DWI) have revealed human brain specialisations (compared to other primates) with regard to development, cortical organisation, connectivity, ageing, and visual and auditory pathways (166). The insular cortex in humans is involved in various somatosensory and visceral sensorimotor functions, emotions, music, language, and other aspects of awareness and perception, and it shows extreme morphological variability between species. These differences include not only gross morphology, but also "laminar organization, cellular specialization, and structural association" (167). Due to this, and to its connectivity with important and well-researched brain areas, such as the anterior cingulate cortex, the frontal pole and the dorsolateral prefrontal cortex, the parietal and temporal lobes, the entorhinal cortex, and the amygdala (167), associated functional differences between species will confound translational research.

A review in 2013, outlining the biological basis of cortical evolution, outlined many human-specific and species differences in the cerebral cortex (168). For example:

— "The human cerebral cortex has expanded significantly relative to other hominids, including introduction of new regions in the frontal and parieto-temporal lobes in humans."

— "…although the basic principles of brain development in all mammals may be conserved, the modifications of developmental events during evolution produce not only quantitative but qualitative changes as well."

— Differences in brain size (such as between humans and monkeys) reflect not only differences in cell number, but also in the arrangements and connectivity of those cells.

— Much of cerebral expansion and evolution is due to the action of genes involved in the control of cell division/the cell cycle, in addition to the very different durations of cortical neurogenesis (humans 100 days; macaques 60 days; mice six days).

— Cerebral evolution/expansion is governed and affected by many genes, and in turn by small modifications in those genes and their regulatory elements. This is evidenced by mutations in these genes causing intellectual disability in humans.

— Human-specific gene networks (mainly involved in neuronal morphology and synaptic function) have been linked to differences in the cerebral cortex, most specifically the frontal lobe, for example.

— Human neuropil (in effect, grey matter) is significantly expanded compared to other primates, especially the prefrontal cortex, and processes such as dendritic and synaptic maturation and synaptic elimination are prolonged in humans compared to other primates.

A comparative analysis of the macro-scale connectivity of the human and macaque brains has been conducted by Goulas *et al.* (169). While it was concluded that, "on the whole" they are "similarly wired", there were also instances of "diverging wiring patterns" and "novel evolutionary aspects" in particular areas, leading to concerns that the suitability of macaques for human neuroscience may be challenged by unique human features, including connectivity reconfigurations. It was discovered that just over half (45/82 regions, 55%) of the brain can be considered as significantly similar — meaning, of course, that almost half (45%) cannot. Overall, the authors concluded there were differences in macro-scale connectivity in the prefrontal, parietal and cingulated regions of the cortex: all regions extensively studied in macaques. Such differences are thought to underlie cognitive processes unique to humans. Further, there are "pronounced changes" in the arcuate and inferior fronto-occipital fasciculi; there are differences in the functional and connectional architecture of some regions of the parietal cortex, notably in the medial region; the different connectivity in, and divergent functional roles of, the anterior cingulate cortex may cause differences in decision-making, cognitive, motivational and motor processes, while that of the posterior cingulated may differentially influence social cognition. Notably, these differences are underpinned by both genetic, epigenetic and environmental factors, and further, the functions of various brain regions depend on other factors in addition to connectivity, such as the laminar patterns of meso-scale connections (169). Consequently, it is acknowledged that macro-scale connective similarities do not guarantee functional similarity; functional divergence is known in conserved networks. It is therefore not enough for advocates of macaque neuroscience to use any degree of connective similarity between macaques and humans to support their claims of the validity and human relevance of their NHP model.

The impact of genetic, epigenetic and environmental factors on the composition and function of the cortex has been thoroughly reviewed (170). As expected, intrinsic cortex genes are directly involved in the development and specification of cortical fields, which are also affected by extrinsic gene products that are associated with sensory receptor type, location and function, as well as epigenetic factors that depend on environment and stimuli. Examples include numerous genes that affect the expression of transcription factors and other regulatory genes, which regulate patterning in the developing cortex and cortical field size and

location. In summary, "…it is clear that genes act in a sequential and combinatorial fashion, and that an alteration in the spatial and temporal pattern of expression at any stage could result in dramatic changes in the resulting cortex" (170).

A recent study, also acknowledging the paucity of work comparing the connectomes of primate brains, compared inter-regional brain connections across humans, chimpanzees and rhesus macaques (157). In common with the Goulas *et al*. study described above, this revealed a largely conserved structural architecture in the three species, but also revealed "major differences" in the inferior parietal, polar and medial prefrontal cortices, including hubs present in these areas of the NHP brains that were absent in humans. Due to the functional roles of these areas, these differences may affect high-order cognition, emotional and reward-related behaviours, visceral functions, and sensory processing. Notably, the human prefrontal cortex is one of the most enlarged brain regions compared to that in NHPs. It is more gyrified, shows major differences in functional organisation, perhaps especially in visual pathways, and structural differences are supported by fMRI data. The "pronounced changes" in the arcuate fasciculus mentioned by Goulas *et al*. were investigated by Rilling *et al*. (171), revealing its substantial expansion in the human brain compared to the brains of macaques and chimpanzees, compatible with its role in language. Other temporal-frontal pathways have expanded, too. Indeed, the temporal cortex seems to have "undergone a substantial reorganisation since the last common human–macaque ancestor some 29 million years ago", and the functional connectivity between higher-order auditory areas and the medial and lateral frontal cortex differs between humans and macaques. There is a parietal-frontal network in humans that "cannot be matched to any macaque network" (172).

The impact of genetics on CNS function has been elegantly illustrated by studies of neuroplasticity, which is defined as "a multifaceted and dynamic process involving gene–environment interactions that result in both short- and long-term changes in gene expression, cellular function, circuit formation, neuronal morphology, and behaviour" (173). Both genetic and epigenetic changes mediate "various aspects of experience-dependent plasticity, such as learning and memory, stress responsivity, and cognition", and regulate normal brain function, including memory (173). The intricate relationship between genetic/epigenetic factors and brain function is illustrated by the link between mutations in genes that encode chromatin binding/modifying enzymes and many different neurological disorders; as well as the link between environmentally-induced chromatin alterations in the absence of mutations that have been shown to be critical for neuronal functions including synaptic activity and cognition (173). Histone modifications may influence gene expression so heavily that they have been linked to various neurodegenerative diseases of the CNS (Friedreich's ataxia, and Huntington's and Alzheimer's diseases; 174).

It has been concluded that primate brains are qualitatively, as well as quantitatively, different, which explains species differences in cognitive abilities (164). Some differences in the neocortex — particularly the prefrontal and temporal areas — have already been discussed above. Notably, the frontal lobes of humans, in absolute terms, 'dwarf' those of NHPs, which is thought to be of great relevance in explaining inter-species cognitive differences. However, the frontal lobes are, at the same time, much smaller than would be expected for a primate of our brain size. The primary and premotor cortices occupy a much smaller proportion of the cortex in humans than in NHPs, and the branching complexity of layer 3 pyramidal-cell basal dendrites is markedly higher in the human prefrontal cortex than in those of macaques or marmosets, reflecting increased cortical connectivity. Major differences have also been noted in the cerebellum, which is extensively connected to the cerebral cortex, and which is involved in movement as well as cognition. It has areas that are unique to humans and apes, and is larger in humans, even accounting for body weight — although, relative to cortex size, the human cerebellum is smaller than that of NHPs.

The impact of such differences as those summarised here is acknowledged in some papers. For example, Boynton (71) discusses the growing discrepancy between monkey electrophysiological data and human fMRI results, for which one of the possible explanations is species differences. The author suggests that, due to such differences, the monkey model will undoubtedly "break down" at some point, as science pushes toward "higher-level processes such as consciousness, learning and decision making", based on, for example, observations that the firing rate of neurons in the primary visual cortex of macaques (V1) is less affected than that in the human V1 by attention and by saccadic and binocular suppression, as evidenced by strong modulation of the BOLD signal.

Other issues with NHP experimentation, affecting its human relevance

Experimental

Various significant and confounding species differences have been described:

— In vision research: Discrepancies between humans and NHPs have been noted in experiments concerning the neurological basis of the

control of eye movements and gaze. Specifically, "with regard to the role of SMC [supplementary motor complex] in response cancellation, non-human primate and human studies have yielded somewhat different conclusions, potentially due to differences in species, response effector, and/or methodology" (175). It remains unclear what the human homologue of the monkey ITC actually is, and while data from LFP investigations in humans are "coarsely comparable" to those from NHPs, it is cautioned that "functional similarities should not be interpreted to imply direct homology at the anatomical, cellular, or connectivity levels between human and monkey structures, species whose common ancestor lived about 30 million years ago." One "intriguing aspect of MTL responses" is that latency times differ greatly between species — human responses generally seem longer than those of monkeys by two-fold or three-fold. In the ITC, human latency times are also "considerably longer" than those in the macaque. It is not known why, though it has been postulated that major inter-species structural differences mean the human ITC does not project directly to the MTL, and/or includes more synapses (51).

— In spatial navigation, it is acknowledged, "Whether results from rodent studies can be directly mapped onto humans is unclear, since subtle anatomical differences in MTL circuitry between species do exist" (49).

— There are notable (and important) differences between results from the study of oscillations in humans and non-humans (see Jacobs & Kahana [123] for specific citations). In working memory, cortical oscillatory-phase synchrony occurs during memory retention in the beta range in humans, in contrast to the gamma range in non-humans. Hippocampal activity related to episodic memory differs in humans and non-humans. Human memory formation is associated with decreased hippocampal activity at many frequencies, while in non-humans hippocampal theta oscillations increase in amplitude during memory encoding. Finally, "the timing of neural responses and oscillations differs between humans and monkeys in general" (176).

— In sleep research: Human microelectrode studies of sleep 'slow waves' revealed "exciting findings" that were not expected based on non-invasive studies and the animal literature. Slow oscillations were "remarkably synchronous" in animals under anaesthesia, but human studies of natural sleep concerning multiple brain areas revealed that slow waves, and underlying active and inactive neuronal states, occur locally, i.e. some regions can be active, while others are silent. Also, wake-like and sleep-like activities have different durations in different cortical areas in different species (50).

— The confounding issue of species difference has also been acknowledged in BOLD fMRI: "because the BOLD signal is dependent on many physiological and biophysical parameters, which could vary between different species, these relationships [between BOLD and neural activity] can be considered as semi-quantitative" (confounding results regarding the correlation of LFPs and spikes with BOLD), and "the haemodynamic response can vary widely across cortical areas and between species. Different aspects of the haemodynamic response might change on different timescales, and might have different neural determinants and different consequences for the BOLD signal" (66).

— In seizure generation: While animal research concomitant to human studies has been on a large scale, confounding species differences exist. For example, it appears that there is "a more distributed epileptogenesis in human epileptogenic cortex compared to a more focal and concentrated epileptogenic neuronal aggregate in experimental [animal] models" (57).

— Finally, and more generally, it is acknowledged that, relative to other animals used in experiments, NHPs "…possess substantial outbred genetic variation, reducing statistical power and potentially confounding interpretation of results in research studies" (177).

Anaesthesia

One major, and often overlooked, inherent problem with animal models in neuroscience is the use of anaesthesia. This is used to mitigate suffering in the animals used, such as that associated with the placement and use of electrodes, and also to prevent movement during data collection (178). Though some protocols on animals do not involve anaesthesia (though crucially these tend to be limited to research into cortical structures only), many do. This is a significant concern, because anaesthesia adversely affects the translation of findings from animals to humans, over and above species differences. For instance, anaesthetic agents disrupt neurovascular coupling in several ways, such as altering baseline haemodynamic parameters, and different agents differentially affect the systemic effects of experimental stimuli, with consequent differential BOLD fMRI responses (178). Anaesthetic agents appear to delay haemodynamic response functions, and, further, they disrupt neurovascular coupling in different ways, posing particular problems for pharmacological and neuroimaging studies (for a review see 179).

Welfare issues and stress

While not as commonplace as it once was, the capture and confinement of wild monkeys continue for the purposes of breeding and supply to laboratories worldwide. For example, almost 4,200 monkeys were exported from Mauritius to the USA in 2014, including hundreds which were caught in the wild (180). This unavoidably causes severe stress and distress, with lifelong consequences. However, once laboratory-housed (whether wild-caught or purpose-bred), the handling, routine laboratory procedures, experimental protocols and so on, are all part of life for the monkeys, and all of these factors cause unavoidable stress.

Neurological and vision experiments often cause significant suffering, classed as 'severe'. For example, electrophysiology experiments often require head restraint, with experiments typically involving such procedures as: the removal of an area of skull to expose the brain; posts being cemented onto the skull for restraining the monkey by the head during recording and stimulating sessions; and, in some cases, scleral search coils being implanted in the eye to monitor eye movements. While it must be noted that some refinements have been reported in this area, such as the use of minimally invasive 'halos', face masks and head caps in place of surgically implanted posts (181), and infra-red eye tracking systems in place of implanted scleral eye coils (182), our investigations show that the former, more invasive approaches still seem to be widely used. Either way, the associated restraint causes significant welfare problems.

In addition, animals are sometimes deprived of food or water for many hours prior to the experiments, to motivate them to perform visual tasks. During recording or stimulating sessions, which can last for several hours each day, NHPs are usually conscious and restrained in chairs by the metal fixtures cemented to the skull. To avoid other NHPs tampering with the implants, in some laboratories the animals are singly housed for the duration of experiments, which may last for months or even years. Due to the investment made in 'preparing' these animals, such NHPs are often used and re-used in similar experiments for very long periods of time, and so inevitably suffer long-term stress when subjected to neuroscientific investigations. All of this has attendant consequences for their welfare, as well as for the scientific data derived from such experiments. Water deprivation — often core to some behavioural research involving task training — is accepted as a stressor, as the UK refinement working group acknowledged: "…restricting access to food or fluid can elicit behavioural and physiological responses that compromise animal health and welfare and may affect the scientific data being collected" (183). This also applies to restraint, the associated stress of which "must be carefully taken into account as this is likely to have a range of general physiological and neurophysiological effects" (178).

Stress-related elevations of heart rate, blood pressure and a variety of hormone levels (including cortisol) are well known to affect scientific data obtained from animals in laboratories (184–187), particularly those involving the nervous system. Indeed, warnings have been issued about the consequences of disregarding the effects of stress due to laboratory routines (185–187), yet this remains under-reported in scientific studies, or not reported at all (188). Thus, stress associated with neuroscience experiments could, *inter alia*:

— lead to alterations of blood pressure and flow that could impact on fMRI research, which relies on blood flow to the brain for its measurement;

— increase the time taken to train macaques for certain procedures, such as eye tracking tasks, as stressed animals take longer to train;

— affect the length of sessions that macaques will tolerate, causing research to take longer than necessary; and

— affect vision in macaques, with increased likelihood of eyestrain impacting on the length of sessions or the quality of data through issues such as poor accommodation (focusing on images) and increased likelihood of involuntary eye movements.

Human-specific attributes

One other consideration is that animal models — even if the above species differences and confounding factors were overcome to any significant degree — can never inform human cognitive processes that simply cannot be studied well, or at all, in animals, such as "language, imagery, episodic memory, volition, and even consciousness" (65), as well as dreams, imagined future scenarios, etc. (189). Further, it is contended that non-humans may not even be sharing the same perceptions as humans when presented with visual stimuli, and therefore it is desirable to perform relevant electrophysiology experiments in awake humans who can report their perceptions (see 190). The repertoire and depth of emotions that can be studied in animals are also limited, rendering human research essential (189).

Concluding Remarks

NHP experiments — in neuroscience, as well as in many other areas of biomedical research — are conceived, funded and conducted on the basis of a

general assumption of human relevance and eventual benefit, rather than firm scientific evidence of their value. This default position is maintained superficially, on the basis of opinions of those who practise NHP research, and anecdotal claims of worth that fail to withstand scrutiny, or which, at the very least, are controversial. Instead, any rationale for NHP use should be based firmly on systematic, robust, critical and independent evaluation.

In defending their practices and in condemning any criticism (or even questioning) of them, many NHP researchers overstate the human relevance of neuroscience research involving NHPs, its contribution to human neuroscience in the past, its current necessity, and its likely future contribution, with little or no substantiation. At the same time, there is a gross understatement of the contribution of human-based research to neuroscience, the significance of what this has achieved, the powerful and ever improving performance of non-invasive methods, the scope of what can be done in humans (both non-invasively and invasively), and the significance of species differences between monkeys and humans.

This defence of NHP neuroscience, based on an inflated portrayal of its importance alongside undervalued and denigrated alternatives to it, is consequently poor and misleading. This review aims to reset the balance of the argument, by showing that: humane human-relevant neurocognitive (and associated) research is much more capable, widespread, important and powerful than those who use NHPs accept; claims by NHP researchers of the exclusive capabilities of NHP neuroscience are incorrect; and NHP experiments are unjustifiable, due to both the lack of scientific necessity, and the existence of significant interspecies differences that confound any results derived from them.

We have shown in this review that this applies both to NHP neuroscience generally, as well as to a number of salient, specific, claims, such as the use of NHPs in the development of BOLD fMRI, DBS, etc. Overall, the value of current NHP research cannot be supported, either by anecdotal evidence or by any claimed historical successes. Any successes, even if they could be proven, must be weighed against failures. If there are a few 'success stories' in historical NHP neuroscience where NHP experiments have contributed significantly with data that could not have been derived by another means, the many thousands of research programmes that did not translate to human benefit must be taken into account. A few successes do not validate a model, if there are also orders of magnitude more failures. Secondly, in any case, this has little bearing on the need for NHP neuroscience now and in the future. New and improving non-NHP technologies, not available to science in the past, provide ethical, novel and unprecedented means of human-specific investigation.

Overall, the case that neuroscience would be much improved, and more relevant to and ultimately more successful for humans, should it be conducted with a solely human focus, is supported by comprehensive and robust evidence. Given the great ethical and financial cost of NHP neuroscience, the inherent suffering and cruelty involved (as revealed by several recent investigations [see *crueltyfreeinternational.org*]), as well as the human ethical aspect in terms of the urgent need for greater understanding of human neurology and neurological disorders, the onus must be on those who use NHPs in neuroscience to make an evidence-based case for what they insist they must do. Given the content of this review, we contend that such a case cannot be made, and have confidence in our position in opposing NHP neuroscience scientifically and ethically.

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