What's Special about the Ethical Challenges of Studying Disorders with Altered Brain Activity?

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Abstract Where there is no viable alternative, studies of neuronal activity are conducted on animals. The use of animals, particularly for invasive studies of the brain, raises a number of ethical issues. Practical or normative ethics are enforced by legislation, in relation to the dominant welfare guidelines developed in the United Kingdom and elsewhere. Guidelines have typically been devised to cover all areas of biomedical research using animals in general, and thus lack any specific focus on neuroscience studies at the level of the ethics, although details of the specific welfare recommendations are different for invasive studies of the brain. Ethically, there is no necessary distinction between neuroscience and other biomedical research in that the brain is a final common path for suffering, irrespective of whether this involves any direct experience of pain. One exception arises in the case of in vitro studies, which are normally considered as an acceptable replacement for in vivo studies. However, to the extent sentience is possible, maintaining central nervous system tissue outside the body naturally raises ethical questions. Perhaps the most intractable challenge to the ethical use of animals in order to model neuronal disorder is presented by the logical impasse in the argument that the animal is similar enough to justify the validity of the experimental model, but sufficiently different in sentience and capacity for suffering, for the necessary experimental procedures to be permissible.

Keywords Reduction · Refinement · Replacement · Neuroscience · Cost–benefit analysis · Speciesism

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Pre-clinical studies of the brain may be conducted on both animal subjects and human participants. Thus, neuroethics cover human neuroimaging and psychopharmacology, for example, as well as the direct study of human disorders with altered neuronal activity. Here, the focus will be on pre-clinical work of the kind that is argued to necessitate the use of animals.

The ethical challenges of experimentally inducing illness in a subject or experimental species for the benefit or potential benefit of the agent or experimenter species are many. For present purposes, I will focus on practical or normative ethics, as enforced by legislation, in relation to the guiding principles of reduction, refinement and replacement (the 3Rs; Russell and Burch 1959). These are applied to animal work in the United Kingdom, embedded as Article 4 in the new European Directive 210/63/EU (European Commission 2010) and promoted as a key concept in the US Guide for the Care and Use of Laboratory Animals (National Research Council 2011). The importance of evidence-based welfare follows from due consideration of species typical behaviour. Finally, returning to ethics in its broader sense, I will consider the perception that there is an ethical demand to ease human (and animal) suffering through scientific advance, which may only be possible through the use of animals. However, scientific advances may also be used to improve functions that are already in the normal psychological range, or to alleviate arguably self-inflicted conditions such as drug addiction. Contemporary views of the ethics of animal use in the neurosciences may take into account, for example perceptions of need for the treatment, as well as human culpability in relation to the development of mental illness.

1 Ethics and Legislation

The use of cannabis, even for medical reasons, is still illegal in many countries or states. In contrast, the general use of excess alcohol, at doses that result in a range of social and health costs, is legal in most countries. Specific actions with potentially fatal consequences such as driving when drunk are generally illegal, particularly where others may be harmed. In contrast, driving after a sleepless night might

involve an equivalent risk of accident but drivers (and their employers in the case of shift workers) are much less likely to be prosecuted. In other words, appropriate ethical codes are not necessarily enforced by legislation and are subject to contextual factors. A full discussion of the general issue of the rights and wrongs of using animals—as companion animals, in food production, as well as in biomedical research—is beyond the scope of this current topic. Briefly, influential positions include the view that the use of animals amounts to 'speciesism', reflecting a discrimination similar to racism and nepotism (Ryder 1975), and that if animals are considered to have rights (Regan 1984), then actions such as killing animals for any purpose are intrinsically wrong. Alternatively, if science is to progress through the study of living organisms, then perhaps experiments on both humans and animals should be considered on an equivalent basis. The fact that sequences of the human genome have been found in other animals has been argued to lend support to the argument that to sacrifice the 'non-human' for the sake of the 'human' animal cannot be legitimate (Hoeyer and Koch 2006). The utilitarian position takes the consequences of progressing science through the use of animals (or not conducting these experiments) into account (Singer 1975).

With respect to utility, the distinction between pure and applied research will not be addressed. In any case, with increasing emphasis on translation to practical benefit through the consideration of impact, as required by many research funding bodies, much fundamental 'curiosity-driven' research in the life sciences may be viewed as pre-clinical in so far as its implications for future clinical benefits are in sight. Similarly, increased ethical regulation and legislation has an impact on the study of animal behaviour for its own sake, yet in the longer term, further developments will be essential both for animal welfare science and to further inform public debate as to the legitimacy of animal use in general (Dawkins 2006; Barnard 2007; Patterson-Kane et al. 2008).

The ethical codes applied to animal use are practical or normative in that all are enforced by legislation, with current European Union guidelines considered gold standard. The general area of biomedical ethics is of still broader scope, covering also non-neuroscience animal work to which the same considerations apply. Conversely, many of the ethical issues raised by work in the neurosciences are of course generic, applying to any in vivo research, rather than specific to in vivo studies of the effects of altered neural activity. Moreover, as the brain provides a final common path for the perception of suffering, distinctions based on how that suffering has been induced may not be pertinent to the outcome from the animal's point of view. In other words, the perception of suffering will be the same irrespective of how the underlying neural substrates have been activated, though the likely benefits of the research may well vary depending on the field of study. The challenges presented by the legislation applied to enforce appropriate ethical standards are in part technical, for example whether the anaesthetic regime is optimal for the species and procedure in use (Fornari et al. 2012; Ideland 2009). There are also practical challenges given that resources will be limited. For example, continuous out-of-hours monitoring on an individual animal basis might be desirable after some kinds of procedure, but even the best research facilities are

unlikely to have the resources to provide a level of care beyond that routinely provided for sick humans. The ethical guidance provided by the 3Rs (Russell and Burch 1959) and their application to neuroscience research (Blakemore et al. 2012) will be considered in relation to the feasibility of using non-invasive techniques developed for use in human, either by way of replacement of animal work or as a refinement. As it is the ultimate goal of those ethically opposed to animal experimentation, the replacement of such use will be considered first.

1.1 Replacement

Replacement is the most challenging of the 3Rs as applied to neuroscience. Altered neuronal activity can be studied directly in human participants using the non-invasive techniques of the cognitive neurosciences, such as electroencephalography (EEG), which reveals patterns of association between the electrical activity of the brain and behavioural changes, and functional magnetic resonance imaging (fMRI), to measure brain activity in so far as this is reflected in blood flow. These approaches are for the most part correlational in that possible brain substrates, which are identified without any neural intervention, and the data recorded provide only indirect measures of neural activity and with limited spatial and temporal resolution (Logothetis 2008). Invasive experimental studies of the human brain are conducted using techniques that apply stimulation to the scalp rather than surgical intervention. Although the spatial resolution is limited, areas of the brain can be temporarily inactivated in normal participants by means of transcranial magnetic stimulation (TMS) or transcranial direct current stimulation (tDCS). Thus, TMS and tDCS can be used to model altered neuronal activity.

Over the last three decades, an explosion of work conducted in human participants claims to relate recorded neuronal activity to a bewildering variety of psychological processes. This work has even gone so far as to include ethical reasoning: the 'neuroscience of ethics' as distinct from the ethics of neuroscience (Funk and Gazzaniga 2009; Kahane et al. 2011). Beyond the localisation of specific or more likely non-specific psychological processes to specific brain regions or networks, it is not clear what such studies necessarily add to our theoretical understanding of psychology (Sarter et al. 1996; Coltheart 2006). However, the contribution of such methods to the field of neuroscience is more widely accepted. Moreover, in principle, disorders characterised by altered neuronal activity can be studied directly in clinical populations. However, such observations may be confounded by the use of medication and, whatever precautions are in place, in cases of psychological and psychiatric disorder, the ability to give informed consent may be compromised.

In the short term, the continued use of animal models has been argued to be essential to our understanding of the relationships between neuronal activity and behaviour, for example the mechanisms of learning and memory and their disorder (Blakemore et al. 2012). Only in animals and in vivo can we conduct direct

manipulations of a brain system to test its role in psychological processes (in vitro tests cannot substitute for behavioural tests of psychological responses to drugs and lesions). This approach is complementary to those approaches that involve measuring neural changes in human subjects, but the animal work is necessary because the human evidence is largely correlational and therefore inconclusive on its own, for example if we study human subjects who take drugs, we cannot know whether the effects we observe are a consequence of the drug or of psychiatric illness. TMS and tDCS techniques are promising but unsuitable for deep brain structures. Compared to controlled intervention studies in animals—using techniques such as microdialysis and electrophysiology—fMRI has limited temporal and spatial resolution. Computer simulations cannot substitute for experiments until we have sufficient data to successfully model the real nervous system. Thus, for some purposes, it has been argued that the use of animals cannot be replaced.

Related to the principle of replacement, further justification of precisely which animal species has been selected for a programme of work is required. Neuroscientific studies in which the nervous system is directly manipulated typically use rats rather than mice or some other small mammal to make use of the huge body of evidence already collected on the rat (both behavioural and neuroanatomical). There are excellent stereotaxic atlases for rats and a wealth of behavioural studies provides a sound basis for the selection of experimental parameters. Rats are also a hardy species, well able to tolerate the mild food or water deprivation necessary to motivate responding in order to test the behavioural consequences of altered neuronal activity. Some behavioural tests of activity or exploration are unconditioned and require no motivation for their expression but learning can only be demonstrated by testing the effects of a conditioned cue on a motivated response.

Arguably, the mouse has yet to demonstrate the same level of behavioural sophistication as the rat, in part because many mouse strains are hyperactive and aggressive and therefore difficult to work with. For example, being much smaller than the rat, the mouse is less well able to tolerate the deprivation schedules that can be essential to motivate reliable response rates. However, excellent progress is nonetheless being made in adapting benchmark tests of learning for use in the mouse (Schmitt et al. 2003, 2004; Deacon 2006; Bonardi et al. 2010). Mice remain the species of choice for studies of the effects of genetic modifications and cognitive effects have been clearly demonstrated in relation to genotype (Schmitt et al. 2003, 2004). However, for studies that manipulate neural activity directly, the smaller brain of the mouse can make some brain lesions and injections harder to restrict to their intended locations than is the case in the rat. Overall rodent species give quite a good trade-off between complexity of brain (necessary to meet the scientific objectives) and the need to consider phylogenetic position. Although invertebrates may suffer more than is commonly believed (Sherwin 2001; Crook and Walters 2011), animals in 'higher' phylogenetic positions are generally considered to have an increased capacity for suffering. Such judgements in relation to level of species are reflected in the introduction of legal protection (UK Animals [Scientific Procedures] Act 1986; European Directive 2010/63/EU) at the level of more neurologically complex invertebrates such as the octopus, as well as in the special

considerations that apply to mammals of the primate genus. Thus, the use of rodents can be viewed as a replacement for the use of primates.

In addition to the scientific limitations of in vitro studies of nervous function raised above, the demarcation between in vivo and in vitro is dubious in the case of brain tissue. Indeed, one early study reported the use of an isolated whole brain preparation in the rat, which on some criteria was still alive up to 5 h after removal from the rest of the animal: in addition to metabolic activity showing glucose utilisation, there was both spontaneous EEG activity and an EEG response to drug administration as well as to a loud sound (Andjus et al. 1967). More recently, an isolated guinea pig whole brain has been reported viable as a preparation for the study of the auditory system (Babalian et al. 1999) and to provide a useful in vitro model of cerebral ischaemia (Breschi et al. 2010). Again to the extent such an in vitro whole brain preparation shows viable physiological activity, conscious perception cannot be assumed to have been removed by decerebration. Logically, the use of smaller samples of brain tissue may present similar challenges. The olfactory-hippocampal circuit of the guinea pig has similarly been reported to be viable in vitro and over an even longer time frame, at least with respect to its electrophysiological properties (de Curtis et al. 1991). This preparation can be seen as a significant scientific advance on the use of traditional slice preparations to study smaller samples of brain tissue and has clearly had translational impact for our understanding of temporal lobe epilepsy (Paré et al. 1992). However, maintaining parts of a brain, such as emotional or pain centres, or even a collection of nerve cells from such a region in vitro clearly poses ethical challenges that are different from working with, for example, an isolated heart. Thus, in the case of nervous tissue, it should be emphasised that replacement by way of in vitro tests raises particular issues.

The use of immature forms of vertebrates can also be presented as replacement. However, particularly for studies of the nervous system, there is compelling evidence that age matters. Even adolescent organisms respond quite differently from those of adults, and this constrains interpretation of both in vitro tissue studies as well as in vivo studies of juvenile systems (McCutcheon and Marinelli 2009).

Finally, replacement is not a logical objective in areas of animal science, where the animals are the object of study rather than acting as a model for a human condition (Barnard 2007). In this sense, studies of animal behaviour, which may include investigation of its underlying neural substrates, should have special status.

1.2 Reduction

Rigorous peer review of applications for funding, as well as of articles submitted for publication, should ensure that animal studies are well designed and appropriately analysed statistically. However, reduction is not simply a matter of using fewer animals. Rather the objective is to use a sample appropriate to detect the effect size of interest, otherwise statistically small effects that are nonetheless of potential

scientific importance will remain undetected. Potential clinical significance is also a consideration: a small improvement to a serious illness such as Alzheimer's disease, or a delay in the onset of symptoms could represent an important advance. With appropriate statistical advice, reduction within any particular experimental protocol is achievable and generally considered best practice. However, to achieve an overall reduction in the number of animals entering regulated procedures is more challenging because of rapid progress in the development of genetically modified mouse models. These are providing vital information with respect to both normal function such as learning and memory and disorders such as neurodegenerative diseases. A consequence of this success has been an increase in the number of laboratory animals used in neuroscience as well as other forms of biomedical research (Blakemore et al. 2012).

1.3 Refinement

General improvements to laboratory animals' conditions are discussed in Sect. 2 below. The most obvious refinement specific to studies of altered neuronal activity would be to adopt the cognitive neuroscience techniques used in human studies to make all studies of altered neuronal activity, including those conducted in animals, non-invasive. However, as discussed in Sect. 1.1 above, these techniques are insufficiently advanced to allow the replacement of animal experimental subjects with willing human participants. In common with all neuroscientific techniques, the presently available non-invasive methods to study brain function in animals also have technical limitations which restrict their usefulness, in animal studies in particular. One particularly important limiting factor is the level of spatial resolution, which can be achieved. Functional imaging techniques are insufficiently advanced to allow us to address the anatomical subdivisions of interest, for example the distinction between shell and core sub-regions of nucleus accumbens. This is because the resolution is too poor for deep structures, and resolution <1 mm would be required. Anatomically, it is possible to achieve resolution of the order of 1 mm with a standard scanner. However, for functional imaging, which is necessary to address functional questions, it is very difficult to get images with voxels this small. Moreover, the temporal resolution of fMRI is at best around 1 s, which is insufficiently precise to capture neuronal activity in relation to behavioural reaction times, which are of the order of milliseconds. Relatedly, the question as to what the activity measured in functional imaging studies reflects remains controversial because blood flow is an indirect measure of neural activity (Logothetis 2008).

Therefore, although the same non-invasive (EEG and fMRI) or less invasive (TMS and tDCS) techniques can in principle be applied in animals, there would be no particular advantage to this line of work for its own sake and some additional disadvantages. For example, animals typically have smaller brains and do not keep still without the use of anaesthetic or restraint. However, structural imaging in animals will allow for refinement in so far as it can be used to verify experimental

lesion placements prior to assessment of the brain post-mortem. Additionally, pharmacological MRI can be combined with the administration of experimental drugs to animals to delineate their effects without the need for any stressful procedure beyond the administration of the drug itself and the anaesthetic or restraint required for the MRI.

Animal work to study altered brain activity typically involves the use of invasive surgical procedures, which cannot be used experimentally in humans, to allow examination of the effects of experimental manipulation of neuronal activity on behaviour. The adverse effects resulting from these procedures can be broadly categorised into unintended or incidental effects, as distinct from the intended experimental effects intrinsic to the changes in neuronal activity induced. The routine management of these adverse effects is described below.

1.3.1 Incidental Effects

Without proper precautions, rats could experience pain during or after the surgical procedures necessary to access the brain. This is avoided by authorising only trained and competent staff to administer the most suitable anaesthetic for the species in use, under veterinary guidance for current best practice. Analgesics are routinely administered to minimise post-operative discomfort. Long-lasting systemic analgesics administered pre-operatively are ideal, in that pain relief will be in place immediately after the anaesthetic wears off. As an additional precaution to ensure long-term pain relief, local anaesthetic may be applied peri-operatively to the region of the wound. Animals showing subsequent signs of pain or discomfort are given a follow-up treatment systemically and treated topically if the operation wound is scratched.

Post-operative experimental procedures commence only once animals have made a full recovery from surgery. Animals are typically checked at least daily by the experimenters and the technicians and at more frequent intervals when an animal is sick. Malaise is recognised as, for example lethargy, loss of appetite, or poor coat condition. As a last resort, animals showing recognised signs of illness or discomfort that do not respond to treatment may be humanely killed. In particular, any animals showing gross locomotor deficits or serious impairment of the special senses, or that show other symptoms that exceed the severity limit of the agreed programme of work, are put down immediately.

The majority of the invasive techniques used in the neurosciences are classed as moderate under the UK legislation as they require surgery with recovery. However, animals, usually rodents, generally recover rapidly from these surgeries and the established techniques used have no long-term impact on the health and welfare of the animals. The combination of surgery techniques with systemic or localised pharmacological manipulations is unlikely to impose any additional health risks, and in all cases, animals are fully recovered from surgery at the time of any drug administration. Even after an animal has made a full recovery from surgery, it might in consequence of that surgery show altered sensitivity to some other treatment.

For example, it might show a shifted-dose response to a drug treatment and the objective might be to determine whether lesion-induced deficits can be reversed with drug treatments. Interactive effects that result in suffering or malaise for the animal typically occur relatively rarely. Predicting when such interactive effects will occur remains challenging. However, in general, the successful management of unwanted side effect of experimental treatments, together with ongoing improvements to husbandry, is a matter of routine in institutions authorised to conduct experimental work with animals. Refinement is perhaps the most readily achievable principle of the 3Rs and at the same time improves the quality of the science.

1.3.2 Intended Effects

Some aspects of the adverse effects seen post-operatively are an inevitable consequence of the scientific objective, in the case of the current topic, to study altered neuronal activity. Behavioural changes seen post-operatively after brain surgeries can include hyperactivity and increased aggression. These changes are usually relatively innocuous (e.g. hyperactivity) and can be within the species typical range (e.g. slightly increased aggressive behaviours). Such non-specific changes typically subside as the animal recovers, and if not veterinary treatment may be indicated. Additionally, it may be necessary to cage separately any rats which show increased aggression post-operatively.

Hyperactivity or other alterations in typical behaviour can also be seen as a lasting effect of some experimental brain treatments. Some of these effects are functionally related to the psychological changes under experimental investigation, and in this case, the incidence should be high (approaching 100 %) because the changes induced specifically relate to the scientific objectives. These adverse effects present an ethical challenge: to the extent they are integral to the scientific programme (the defined purpose for which the legal authority to conduct the work has been granted), they are of necessity left untreated. Such an experimental programme must be legal, but nonetheless represents a significant challenge ethically. The successful simulation of distressing psychological, psychiatric or neurological disorders, such as anxiety, schizophrenia or Huntington's disease, requires sufficient comparability in the level of suffering induced, in order for the science to be valid.

1.4 Rules and Recommendations: The Need for Flexibility

There is a clear difference between a rule and a recommendation and applying the 3Rs as a routine prescription may not work as intended when a number of considerations need to be taken into account. Viable strategies for replacement are insufficient for reduction to meet this target, and the ethical gap may effectively set reduction against refinement (Olsson et al. 2011). In other words, reuse or continued use in order to achieve reduction results in more harm on fewer animals,

rather than the alternative of less harm on more animals to achieve the same experimental objectives in a more refined way.

More specific challenges arise when one proposed refinement can be seen to work against another. For example, with respect to the outcome to be learned about, there may be grounds to motivate conditioning procedures using aversive (e.g. mild foot shock) rather than appetitive (e.g. food reward) stimuli. At first sight, the selection of an aversively motivated procedure might seem to represent an unnecessary increase in the overall severity of the procedure. However, such aversively motivated procedures typically use mild foot shocks, just sufficient to produce reliable associative learning and within just two conditioning trials (Nelson et al. 2011a, b). This rate of learning is much faster than the equivalent appetitively motivated procedures in which the outcome is food reward (Cassaday et al. 2008; Horsley et al. 2008). Thus, aversive procedures allow the refinement of studies that require the use of microinjection procedures (in order to examine the effect of localised drug administrations) because the number of injections that can be administered without causing local damage at the point of infusion is limited (Nelson et al. 2011a, b).

Similar considerations arise in that proposed refinements can work against reduction if important experimental baselines are shifted. For example, studies investigating the neural substrates of associative learning require that a behavioural response first be established (in order that changes in associative strength can be detected). Food-motivated responses such as lever pressing can provide suitable baseline responses but have the disadvantage that they take some time to establish. Associative learning has also been investigated using licking for water as the motivated response, and these variants have the advantage that the licking response is readily established. In principle, these procedures could be refined to exclude the requirement for water deprivation, by the use of sweetened milk or sucrose solution as a food reward. However, there can be barriers for making such a switch: most importantly, to introduce the use of high incentive rewards would increase the behavioural baseline response. The incentive value of rewards as demonstrated behaviourally is known to be significantly affected by quite minor changes to experimental procedure such as a change in the reinforcer in use (Randall et al. 2012). Behavioural analyses of reinforcement-value measure responding on schedules requiring animals to make progressively more and more responses (such as pressing a lever within a Skinner box) to secure the same level of food reward. This provides a measure of their level of motivation for different reinforcers, in other words, their reinforcing strength relative to other ‘less rewarding’ reinforcers. Systematic comparisons of responding for different reinforcers on progressive ratio schedules, controlling for calorific content, suggest that the level of sucrose determines the reinforcing properties of novel foods that contain a mix of nutrients and flavours (Naleid et al. 2008). Moreover, the neural activity underlying the processing of reinforcers can show differences in relation to the reinforcer in use. For example, antagonists at both dopamine D_1-like and D_2-like receptors reduce the incentive value of sucrose, whereas the incentive value of corn oil is more sensitive to blockade of D_2-like than D_1-like receptors (Olarte-Sánchez et al. 2013).

Thus, there is a particular issue with respect to shifts in the baseline behavioural response in studies, which directly or indirectly manipulate dopaminergic neuronal activity in a manner likely to result in changes in hedonic tone (Wise 2008). When tasks are adapted to run with different reinforcers, direct comparability between task variants is compromised and there may be a substantial body of work completed with the reinforcer originally adopted. Moreover, where the neuronal activity under study modulates incentive salience and this is not the objective of the study, any shift in the behavioural baseline response would be predicted to compromise identification of the associative learning effects of interest. Whilst the above examples were selected from behavioural neuroscience studies, of course similar considerations arise in other areas of biomedical research.

Particularly where recommendations may have an unforeseen impact on the quality of the scientific outcomes, a two-way dialogue is essential. For example, refinements such as 'environmental enrichment' might seem unlikely to affect experimental outcomes. However, depending on the nature of the study, statistical power may be affected (Baumans and Van Loo 2013). Statistical power could be improved to the extent variability is reduced in animals better accustomed to novelty and change but results might be more variable between laboratories if standardisation of more varied environments is harder to achieve. For example, depending on strain and previous housing conditions, increased cage size and other forms of enrichment can significantly increase aggression in some male mice, most likely because of increased territoriality (Barnard 2007). Increased aggression can be a particular problem in studies involving some neural manipulation but could equally adversely affect the outcome of other kinds of biomedical research.

Importantly, institutional ethical review procedures debate such issues. However, it must be acknowledged that the effectiveness of such committee ethics has been questioned on a number of grounds. The general barriers to the debate and implementation of best practice include lack of resources and administrative burden (Illes et al. 2010). Additionally, researchers actively engaged in animal research, and others who may be seen to have a vested interest in animal research, have been suggested to be over-represented on such committees in the USA (Hansen 2013). The proportion of lay members on the equivalent committees in the United Kingdom is comparable, but in Sweden, for example, animal ethics committees have a much higher proportion of laypersons, including animal rights activists (Ideland 2009). However, even with such wider representation, interview methods confirm that such committees remain focused on refinement and optimisation of experimental protocols rather than questioning whether the research should be done in the first place. Thus, the context of the committee meeting may be sufficient to constrain the scope of its effectiveness (Ideland 2009). Moreover, non-specialists are unlikely to have sufficient knowledge to predict the effects of proposed refinements, either on other aspects of refinement or on the experimental outcomes that relate to the objectives of the study. Thus, lack of representation by other neuroscientists with relevant expertise extending to the behavioural techniques in use, could be a particular issue with respect to the evaluation of experimental programmes to study altered neuronal activity.

2 Species Typical Behaviour and Evidence-Based Welfare

Species differences mean that welfare guidelines should be evidence-based rather than rely on anthropomorphism. Moreover, consideration of species typical behaviour is fundamental to the assessment of potential suffering or lasting harm, which may be inflicted in the course of neuroscientific studies of any particular species of laboratory animal.

Laboratory housing conditions are the most important non-specific factor, affecting the well-being of laboratory animals. In the past, caging for laboratory animals was primarily designed on the basis of practical requirements such as construction and maintenance costs, space limitations and convenience of use for the experimenter. These practical considerations are still important and budgets for upgrading facilities are a precious resource. Since animal welfare is a major driver for upgrading laboratory housing, it is vital to be clear about the costs and benefits of proposed innovations from the animals' point of view. For example, modern split-level cages allow greater opportunity for exploration and separate areas provide the opportunity for the animal to retreat to hiding places. Moreover, they are suitable for animals with brain implants such as indwelling cannulae.

Within these improved caged environments, further opportunities can be provided. Standard laboratory feeding regimes deny the animal the opportunity to forage which in a natural habitat would take a high proportion of their time. Additionally, the provision of ad libitum food results in shortened life span due to overfeeding and inactivity. Environmental refinement refers to modifications to the housing of laboratory animals intended to enhance welfare, for example by simulating natural foraging conditions as far as possible or through the provision of other stimuli appropriate to the animals' species-specific needs (Baumans and Van Loo 2013). Other species typical behaviours include nest building and a variety of opportunities for social contact. Nesting and chewing materials can be provided as part of the environmental refinement. The five freedoms, first established by the Brambell Committee as a set of guiding principles to promote the welfare of farm animals, are specifically framed in terms of the 'freedom adequately to react to' a variety of aversive situations including injury and stress, in addition to the freedom to display normal species-specific behavioural patterns. However, breeding is not desirable in standard experimental colonies. Similarly, aggressive encounters may be part of the animal's repertoire but cause problems in the laboratory environment because they inflate the severity banding. Yet adaptive cost is not necessarily tantamount to suffering in that defending a territory is a normal behaviour for many species and one that would ordinarily confer reproductive advantage (Barnard and Hurst 1996; Dawkins 2006; Ohl and Staay 2012).

Knowledge of an animal's natural habitat and behaviour provides an excellent starting point for laboratory animal husbandry. For example, species such as the African mole rat, which lives in dark burrows, should be provided with burrowing and foraging opportunities in the laboratory. Moreover, there is evidence to suggest

that such environmental refinement may be an important determinant of their cognitive performance in experimental studies (du Toit et al. 2012). Conversely, exposure to novel stimulation of the wrong kind, particularly under brightly lit conditions, would most likely result in stress rather than 'enrichment' for such a subterranean species. However, in general, anthropomorphism provides an unreliable basis from which to gauge animal welfare and we lack insight into how the animal in question would normally wish to spend its time. Animals' choices may result in short-term discomfort yet make excellent functional sense in terms of 'adaptive self-expenditure' (Barnard 2007). Since the same refinements will not be appropriate for all species, it is essential that the effectiveness of environmental refinements be evaluated, for example through the use of preference tests and other behavioural and physiological parameters (Chmiel and Noonan 1996; Dawkins 2006; Fitchett et al. 2006; Patterson-Kane et al. 2008; Baumans and Van Loo 2013).

Neuroscience studies do not raise special challenges with respect to general refinements to standard animal husbandry practices within the laboratory environment. However, additional considerations do arise with respect to the deprivation schedules used to motivate some behavioural neuroscience studies of learning and memory. Such studies may, for example, rely on stable baseline response rates in order to assess the degree of learning to a conditioned stimulus. For example, conditioned suppression of drinking provides a reliable measure of conditioned fear: to the extent animals (typically rats or mice) are fearful of the conditioned stimulus, they should be hesitant to drink. The experimental induction of fear and thirst, compounded by the trade-off between emotion and motivation inherent to the use of conditioned suppression of drinking to measure learning and memory, can be seen to raise concerns from an anthropomorphic perspective.

The justification for refinement, however, depends on the evidence that the water deprivation schedule in use results in adverse effects. The weights of rats on water deprivation are closely monitored daily since restricted water access tends to reduce food intake and routine welfare checks include the examination of skin elasticity, to check for any signs of dehydration. Additionally, the evidence base includes a systematic study of the health effects of restricted access to water: schedules of deprivation typical of those used in conditioned suppression studies have been reported to have no adverse physiological effects on rats and, moreover, to be appropriate to the experimental objectives (Rowland 2007; Hughes et al. 1994). In the wild, rat species inhabit a wide range of environments including desert, and the deprivation schedules adopted in laboratories may represent little in the way of deviation from the species typical range of intake patterns. Similarly, there is no evidence that the foot shocks used in such conditioned suppression studies result in lasting trauma in that when tested, the animals do not show total suppression, either to the experimental context or the conditioning cue (Nelson et al. 2011a, b).

3 Ethical Demand to Ease Human and Animal Suffering

The legitimacy of essential medical research is widely accepted amongst the general public and also a dominant theme at ethical review committees (Ideland 2009) and amongst researchers who use animals (Hobson-West 2012). Indeed, the ethical guidelines arising from the 1947 Nuremberg Code require that experiments should be based on the results of animal experiments, to minimise unnecessary human suffering. There was a historic context to this directive and contemporary views on the ethics of animal experimentation take into account (for example) perceptions of need for the treatment, as well as human culpability. For normal individuals, cognitive enhancers may be seen as inessential psychological cosmetics. Individuals who suffer addiction to drugs or who become obese could be argued to be less worthy of research effort necessitating the use of animals (see Sect. 4). Thus, the interpretation and implementation of the objective of the code—to minimise unnecessary human suffering—varies between counties, and for many disorders, there is no universally accepted animal model (Nature Neuroscience Editorial 2010).

Advances in veterinary science that alleviate animal suffering are also dependent on experimental studies of other (laboratory) animals. The animals that principally benefit are companion, farm and laboratory animals; thus, such advances can still be argued to be of benefit to the human owners, compounded by potential commercial gain in the case of farm and laboratory animals. However, curiosity-driven work in animal science is essential to an understanding of the normal behavioural repertoires, which should as far as possible be made available to any captive animal. This provides the evidence base for evolutionarily salient welfare (Barnard 2007; Ohl and Staay 2012).

Many scientists and lay persons would share the view that the capacity for feelings, both positive and negative, is of central concern (Balcombe 2009). That animals should have a comparable level of sentience is essential to the validity of models of psychological and psychiatric disorder. However, it is precisely this comparability, especially in respect of the capacity to suffer pain, which raises the issue as to whether animal experiments should be conducted in the first place. At the same time, points of difference in cognitive and other capacities can be argued to justify the demarcation of ethical responsibility in relation to species. For example, neuronal correlates of almost every imaginable facet of higher order processing are now being extensively studied in human participants, including ethical decision-making itself (Funk and Gazzagina 2009; Kahana et al. 2011). Cognitive processes unique to ethical decision-making are beyond the scope of animal models. However, non-human primates in particular show compelling behavioural evidence of a variety of cognitive capacities that provide rational justification for their continued protection (Mameli and Bortolotti 2006). At the same time, the use of pigs in neuroscience research has increased (Lind et al. 2007). In turn, the scientific advantage of the resemblance of the pig to the human brain raises ethical concerns. The use of pigs may be seen as ethically preferable to the use of primates but their use in neuroscientific studies is likely to remain less

acceptable than the use of rodents. This use of 'sentientism' has been argued to be formally analogous to speciesism (Würbel 2009). Furthermore, the majority of judgements of sentience are clouded by prejudice based on species, for example pigs are widely perceived as intelligent emotional animals. Whilst a high proportion of individuals may empathise with pigs, for many empathy breaks down with 'pest animals' such as rodents (Würbel 2009).

Some of the same considerations apply to other areas of biomedical research, but the issue is particularly sensitive where sentience is the direct object of study as is the case in studies of altered neuronal activity. Moreover, particularly in the case of disorders that might have been avoided, cost–benefit analyses take human culpability into account.

4 Getting a Grip: Human Culpability for Behavioural Disorders

Animal work to test cosmetics for recreational use, as distinct from dermatological products for what might be seen as medical use, receives relatively little public support. Similarly, research to identify cognitive enhancers suitable for general use in normal individuals could be viewed as less ethically defensible than that directed towards identifying treatment for age-related cognitive decline. In extreme form, the former could amount to intellectual vanity. In contrast, the latter can manifest as severe dementia, resulting in significant human suffering and economic cost. However, such a distinction is blurred in that many of the new treatments for neurological diseases are also likely to have uses for people without disease, to the extent they can also improve normal brain function via their effects on cognition or affect (Chatterjee 2004). In practice, controlling the use of drugs (with or without prescription) is difficult. Prozac, whether obtained under prescription or purchased online, is already widely used in cases of mild depression and to some extent in individuals unlikely to meet contemporary diagnostic criteria.

Animal work intended to alleviate the consequences of 'self-inflicted' problems such as those related to alcohol consumption and cigarette smoking is already falling into a similar category: this despite the increasing recognition of addiction as a disease process. Obesity is similarly a disorder with a recognised neuronal component that could to some extent be argued to be self-inflicted, thus raising additional questions as to the acceptability of animal models in obesity research. This widening concern with the use of animals for laboratory research, which aims to alleviate human suffering which could have been avoided through behavioural change, could be further extended to raise questions with respect to a range of stress-related psychological and psychiatric disorders (Lund et al. 2013). Arguably, human individuals should take some responsibility for their exposure and reactions to stressors. Similarly, in addition, to the direct risks associated with drug taking, from overdose to accidents in consequence of impaired judgement, drugs too can

increase the risk of psychological and psychiatric disorders. For example, there is good evidence that cannabis use increases the risk of psychosis (Verdoux et al. 2003; Moore et al. 2007), there is some evidence that the use of MDMA ('Ecstasy') is a risk factor for depression (Parrott 2001) or at least acute mood swings (Baylen and Rosenberg 2006). In short, psychological and psychiatric disorders are commonly seen in relation to substance use and direction of causality can be extremely difficult to establish (Verdoux et al. 2003; Soar et al. 2006; Moore et al. 2007). Head injuries are preventable to the extent that they result from engaging in sport, riding a bicycle without a helmet, driving a car without due care and attention. Thus, a wide range of disorders based on altered brain activity have some lifestyle aspect. Accidents aside, given what we now know about the importance of the epigenetic processes that determine gene expression in relation to environmental exposures, it would be surprising if they did not. However, to dismiss sufferers of conditions to which their own behaviour could be seen to be a contributing factor would raise further questions about individual responsibility in relation to social factors such as economic deprivation and level of education, as well as early environmental effects (such as the pre-pregnancy body weight of the mother), which obviously could not be controlled at the level of the affected individual (Lund et al. 2013). Obesity in companion animals is also relatively commonplace. The same arguments can be seen to apply to the owners of obese companion animals: arguably, they should know better, but their capacity effectively to take responsibility for their animal's diet may again be affected by economic deprivation and level of education.

5 Conclusions

Pre-clinical studies involving animal use face many of the wider challenges of neuroethics: not all neuronally mediated treatments or improvements are necessarily ethical in the wider sense, particularly in cases when there is no underlying disease in need of treatment. Thus, one commonly raised issue is whether we necessarily want to advocate the use of drugs by way of 'cosmetic' cognitive enhancements that might—like any performance-enhancing drug—permit unfair advantage advantages in assessment situations (Farah 2012). Such challenges are compounded to the extent advances can be seen to derive from invasive animal work. Surgical interventions to the brains of animals allow the precise experimental manipulation of neuronal activity in order to establish its effects under controlled experimental conditions. This kind of work presents additional ethical considerations in that it involves direct manipulation of animals' emotional and cognitive systems. Direct experimental manipulation of the brain might seem more ethically dubious than invasive studies of other essential organs such as the heart. Certainly, human patients needing invasive medical procedures may be justified in having a greater fear of brain compared with open-heart surgery: the brain is more identifiable with the human sense of self than is the heart; assuming they survive, the side effects of

brain surgery are more difficult to predict with any certainty. However, peripheral procedures can impact on the brain, for example if altered sensory experience or suffering result from the procedure. Pain and suffering are mediated by a network of brain areas, which thus provide a final common path for suffering arising in consequence of all aspects of animal usage, including neuroscientific studies, invasive biomedical research on other organ systems, as well as non-invasive work which may nonetheless result in suffering or distress. Yet pain is not a direct consequence of tissue damage in the brain in that there are no pain receptors in the brain itself. Therefore, the ethical guidelines to be followed are general rather than specific to the organ system or behaviour, which is the subject of study. The legislation surrounding all such work ensures that animals' experience of pain and suffering is the minimum necessary to achieve the scientific objectives and moreover limited in relation to the likely benefits of the programme of work. One important exception to the applicability of the 3Rs arises in the case of in vitro studies that are normally considered as an acceptable replacement to in vivo studies. However, to the extent sentience is possible, maintaining central nervous system tissue outside the body raises ethical questions.

The debate around the moral justification for the ethical norms in place is another matter. Indeed, recognising the difficulty inherent in identifying moral absolutes applicable under every conceivable circumstance, Aristotle's 'virtue ethics' focused on the character of the moral agent rather than the fundamental ethical principles underlying the available guidance. In particular, virtue ethics point to the extent to which the agent—in this case, the experimenter using animal subjects—can be seen to reflect morally on his or her actions.

Many of the key questions surrounding the ethics of research involving animals were raised in the comprehensive 2005 report published by the Nuffield Council on Bioethics. This document remains an excellent summary. From the researchers' perspective, the fundamental challenge is presented by the logical impasse in the argument that the animal is similar enough to justify the validity of the experimental model, but sufficiently different in sentience and capacity for suffering, for the necessary experimental procedures to be in principle permissible (their implementation being highly regulated). The evidence of continuity provided by functional genomics has been used to support the argument that research has undermined its own legitimising principle (Hoyer and Koch 2006).

Distinctions drawn on the basis of species have of course been central to some of the ethical arguments made *against* animal use, principally that such use amounts to speciesism, similar in connotation to racism (Ryder 1975). However, although the term speciesism was intended to highlight discrimination against animals in a negative way, some researchers do now nonetheless describe themselves as speciesist in Ryder's sense (Hobson-West 2012). Moreover, distinctions drawn on the basis of species can also be an inevitable part of the justification for such animal use, based on criteria that indicate level of sentience. Essentially, cost–benefit analyses seek to quantify the suffering experimentally inflicted on 'lower' animals and offset this against potential benefit for the human species. Thus, the legislation concerning animal experimentation could be described as inherently speciesist in

that special protection is afforded to primates and all but one of the invertebrates are excluded. More generally, the law could be said to be speciesist in that euthanasia is enforced for sick animals likely to be suffering in excess of what is considered acceptable. The regulatory frameworks require the use of a humane endpoint, whereas the very option of euthanasia of terminally ill humans is highly controversial. Indeed, speciesism could be said to be widespread in that, for example, the vast majority of individuals of both our own and other species only attempt to mate with members of their own species. As a species, we do not love other animals in the same way that we love other people. Any matings with a member of another species that do occur are by definition unsuccessful in a biological sense in which any viable offspring will not be fertile. Similarly, the conservation of endangered animal species attracts far more public attention than does the conservation of rare plant species. This wider consideration of what it might mean to be speciesist is not intended to trivialise the discussion: the acknowledgement of the role of speciesism seems essential to the logic of arguments for as well as against the use of animals in neuroscience. By definition, humanism is 'species-centric' to the extent its philosophies and morality are centred on human interests and needs. As an ethical stance, biocentrism that recognises the value of all non-human life in nature may very well be more ethically defensible. However, rightly or wrongly, the vast majority of human activity promotes human interests and needs. This is the context in which the ethics of animal use, for experimental neuroscience as well as for other human purposes, are situated.

Sentience is not a uniquely human attribute and sentientism or using the ability to feel and perceive as a criterion for the level of protection an animal should receive can also amount speciesism. With the exception of those presented by in vitro studies of altered neuronal activity, ethical challenges are not unique to the use of animals in neuroscience studies. Naturally, the ethical challenges of animal work are particularly emotive when sentience is the direct object of study, as is the case in studies of altered brain activity.

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