A Report On The Use Of

(Non-Human) Primates

In Brain Research

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Prepared by The Dr Hadwen Trust for Humane Research

84A Tilehouse Street, Hitchin, Herts. SG5 2DY, UK

Registered Charity No. 261096

www.drhadwentrust.org.uk

Introduction

This document has been prepared by the Dr Hadwen Trust, a registered medical research charity with 30 years' experience of developing alternatives to replace animal experiments. The Trust is opposed to animal experiments on both scientific and ethical grounds, however this document intends to focus on the scientific arguments against using (non-human) primates in brain research.

Within the Animal Kingdom primates are biologically our closest relatives and thus share many attributes with us. They are intelligent with highly developed brains, complicated patterns of behaviour and intricate social relationships.

There are compelling ethical arguments against using primates in any type of experiment. Their special and complex needs makes it virtually impossible to house and maintain them adequately in laboratories. Most importantly, the capacity of primates to experience pain, suffering and distress is similar to our own. It is their very similarity to humans that both makes them the subjects of research, whilst at the same time strengthens the ethical argument against confining them in laboratories and exploiting them in experiments.

Primates used in laboratories in the United Kingdom are mainly species of monkeys, such as marmosets, macaques, and baboons. Experiments on great apes (ie. chimpanzees, gorillas, and orang-utans) are not permitted in the UK, for ethical reasons. The same is true in New Zealand and the Netherlands. The latest Home Office statistics record 3,690 experiments on primates in the UK during 2000 (1).

Building a new neuroscience research centre at Cambridge will almost certainly increase the use of primates in the UK. The Dr Hadwen Trust believes there are humane alternative methods of research that can allow brain research to continue, without the use of primates, or other animal species.

This report is divided into four sections. The first section explains the general scientific arguments against the use of primates in brain research. Following this are three sections that deal in more detail with the specific research fields of Alzheimer's disease, Parkinson's disease, and fundamental brain research.

Limitations of animal research

The wrong species

In medical research the species of interest is the human species. Thus the 'gold standard' medical research technique is clinical studies of volunteers and patients. Any 'model' of the human situation is one step removed from the ideal.

Despite the fact that we share 98% of our genes with chimpanzees and 92% with macaque monkeys, at the molecular level there are many small differences between us. The molecular level is the very basis of how things work in the body. Any minor variations there can be amplified, in a cascade effect, at the cellular, organ and whole animal levels, creating troublesome species differences.

- For example, a mutation of a single gene has been identified in humans that causes subtle changes to the chemistry of cell surfaces throughout the body, and has implications ranging from susceptibility to disease-causing organisms to brain development. This form of the gene does not occur even in the closely related great apes (2).

- Differences in the quantity and activity of liver enzymes produced by humans and monkeys, mean that they metabolise drugs differently, and can respond differently to them (3).

Because of species differences, results from animal experiments are unreliable at predicting human responses. Findings from animals may, or may not, apply to humans, and the only way of finding out is to study the human situation. This is a major limitation of all animal research - it produces results of unknowable relevance to humans.

- Monkeys are susceptible to poliovirus through the nose, but humans become infected via the mouth and intestine. This species difference led scientists astray in the early part of the 20th century, when experiments on monkeys eclipsed research with patients, and led to the development of useless nasal sprays to prevent infection (4).

- HIV can infect both humans and other great apes, but only humans develop AIDS. Monkeys are not susceptible to HIV infection and do not develop the illness, and no one knows why. Primate research to find a therapeutic AIDS vaccine has essentially been a failure according to a scientific review published in 2000 (5).

Artificial models

In medical research animals are often used to 'model' a human illness. The symptoms of the human illness are artificially induced in animals in an attempt to mimic the disease, even though the species used may not naturally succumb to that illness. Anything revealed by these 'models' is of dubious relevance to humans, and can even be seriously misleading or delay progress.

For example, in humans a stroke occurs when the blood supply to the brain is disrupted or blocked by a blood clot. Stroke is modelled in animals, including primates, dogs, cats and rodents, by clamping or blocking a blood vessel to the brain. This surgical onslaught mimics the gross effect of a stroke, but does not duplicate the development of the full human condition that is often accompanied by atherosclerosis or high blood pressure. A scientific review of stroke drugs found that of 25 different compounds of proven efficacy for treating stroke in animal models over 10 years, none had been successful in stroke patients (6).

Parkinson's Disease is a slow degenerative disease in humans of unknown cause. Some of the symptoms of Parkinson's, tremors and abnormal movements, are induced in monkeys by injecting a toxic chemical that damages the brain. Despite its devastating effects, MPTP poisoning is not the same as Parkinson's disease, and there are numerous differences. The animals can gradually recover once the poisoning stops, but the human condition is irreversible.

The cost of animal experiments

The cost of animal experiments is both human and animal. Animals suffer directly from being used in experiments. All too often the findings of animal experiments confuse and delay our understanding of the real human problem, and thus humans suffer as a result.

Despite strict legislation in the UK to regulate animal experiments, animal suffering is inherent in much animal research, most especially when it involves primates. Animal experiments covered by UK legislation [The Animals (Scientific Procedures) Act 1986] are by definition procedures carried out on living animals that are "liable to cause pain, suffering, distress or lasting harm". Inflicting brain damage on animals or inducing the symptoms of devastating disorders, such as Alzheimer's, Parkinson's disease, stroke or depression, are certainly liable to cause substantial suffering to the animals involved.

Animal experiments are only one method of scientific research, and they are not the sole means of pursuing medical progress. Although animal experiments represent just a small proportion of medical research, they still equate to millions of animal lives. At the same time, the over weighted emphasis put on them means that researchers can be misled about the nature of human illnesses. Research that focuses on animal experiments is wasting time, effort, money and lives.

Alternatives to animal experiments

At the dawn of the 21st century there are more advanced methods of conducting medical research than archaic animal experiments. An increasing range of safe and non-invasive methods of studying patients and volunteers are available. There are improved techniques for keeping and studying human tissue samples and cells alive and functioning in the laboratory. Computers are being used to model the complexities of human body systems, such as the immune system. Sophisticated analytical and 'test-tube' techniques are enabling unparalleled advances in molecular and genetic analysis. These cutting-edge tools are expanding the horizons of today's researchers and paving the way for future medical progress.

The proposed Centre for Behavioural Neuroscience at Cambridge intends to conduct research, including animal experiments, into how the brain works and disorders of the brain. The next section of this document will consider some of the more specific scientific limitations of using animals in some areas of brain research, and will outline how humane approaches could be used instead to expand our knowledge and further medical progress.

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ALZHEIMER'S DISEASE

Alzheimer's disease (AD) causes confusion, memory loss and dementia in millions of elderly people. The cause remains unknown and there is no effective treatment. In the brains of AD patients an abnormal build-up of proteins occurs, in the form of plaques and tangles. No one knows if these protein deposits are a cause or a result of the disease. Eventually the brain cells start to die, the brain shrinks and there is a loss of mental abilities with increasing age.

Animal research

No animals suffer from AD in the way humans do, yet many animals have been used in an attempt to model AD. Much current research focuses on the use of transgenic mouse models of AD. These are mice genetically modified to possess human genes associated with AD Ñ genes identified from studies of patients. Various mice have been created and each display some of the characteristics of AD, but none of them accurately reflect the full spectrum of symptoms seen in humans (1).

For example, various transgenic mice have been created that overproduce the proteins that accumulate in the brain in human AD. However in these mice the protein plaques are not distributed at the same levels or in the same pattern as seen in human AD, the brain cells do not die, and the mice do not suffer from the severe behavioural changes seen in humans (2). So it seems you can tinker with a mouse and insert human genes, but basically it's still a mouse.

Macaque monkeys also develop protein deposits in the brain as they age, but they do not suffer from the severe form of disease seen in humans. Because monkeys live beyond 30 years, it is generally too expensive to keep them in laboratories for so long before using them in an Alzheimer's research programme. Consequently, mice are the species of choice in AD research, for economic rather than scientific reasons.

Studies of the brains of ageing great apes have shown that like humans they develop protein deposits in their brains. However, they do not suffer the brain damage and brain cell death seen in

humans. Genetic studies have identified genes associated with AD in humans, but confusingly, chimpanzees who also possess these genes do not develop the disease (3).

Overproduction of a brain chemical, galanin, may be associated with Alzheimer's disease. Galanin is found within a distinct population of brain cells in humans, but is much more widely distributed in the monkey forebrain (4).

The distressing behavioural symptoms of human dementia, such as confusion, memory loss, delusions, hallucinations, depression, decline in reasoning and lack of speech, are simply not seen in animals or are not measurable.

Alternatives - the humane way forward

Many of the most significant findings about AD have been learnt from humane research techniques and not from animal experiments. Alois Alzheimer first described the main features of the illness in 1907 when studying post-mortem brains of AD patients. Since then, research on post-mortem brain tissue has provided much of what we now know about the pathology of the human disease, and continues to reveal new insights, such as the potential involvement of toxins and viruses (5).

There is a wealth of humane research techniques that could be used to continue to study AD without resorting to animal experiments. Brain cell cultures have been used to study cellular processes involved in the accumulation of protein deposits, and identify potential treatments (6,7). New molecular techniques are revealing important genetic components to the disease, and providing new insights that may lead to better treatments (8,9). Brain scanners, such as CAT, MEG and MRI are being used to non-invasively "look inside" at the functioning brains of living AD patients. PET scans can be used to diagnose AD, and may even be able to detect changes in the brain several years before the symptoms appear, enabling a vital chance for intervention (10). Population studies have identified risk factors associated with AD, and revealed that drugs such as aspirin and ibuprofen may reduce the risk of developing AD (11).

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PARKINSON'S DISEASE

Parkinson's disease is another common disease of the elderly, affecting 1% of the population over 65. Patients suffer from tremors, stiffness, rigidity, slow shuffling movements, and hunched posture. These symptoms are the result of irreversible brain decay. The condition is slowly debilitating and there is no cure. Some treatments are available, but they are of limited effectiveness and have serious side-effects.

Animal research

Parkinson's disease (PD) is widely modelled in monkeys by injecting them with a toxic chemical, called MPTP. The animals suffer brain damage and succumb to symptoms superficially like those seen in PD patients. These brain damaged monkeys are used in laboratories to study the disease and investigate potential treatments. Rodents and cats are also susceptible to MPTP poisoning.

There are however major differences between the induced condition in animals and the human disease:

- In spontaneously occurring human PD the symptoms develop slowly, and grow progressively worse over time. In experiments, healthy monkeys are subjected to a toxic assault, the symptoms appear rapidly and then gradually lessen. Unlike PD patients, the poisoned monkeys can actually recover if the MPTP poisoning stops.

- The brain damage caused to monkeys by MPTP poisoning is very selective affecting only specific parts of the brain, whilst in PD patients brain damage is much more widespread.

- There are significant changes in the levels of brain chemicals in PD patients, that are not seen in MPTP poisoned monkeys.

- A hallmark of human disease is the presence of Lewy Bodies, protein bodies that accumulate in the brains of AD patients. Lewy bodies are either not present in monkeys' brains, or they occur at reduced levels in some monkey species.

Despite these significant differences monkeys continue to be widely used in Parkinson's research. The monkeys injected with MPTP suffer substantially. They display tremors, rigidity, abnormal posture, loss of balance, drooling, incontinence, compulsive behaviour, constipation, and may be incapable of feeding themselves. At the end of experiments, sometimes after many months, the animals are killed and their brains examined.

Although the MPTP monkey model of Parkinson's was devised in the 1980s, the animal research has done little to improve the treatment of PD patients. The standard treatment for PD remains L-DOPA, which has been in use since the 1960s. Treatment with L-DOPA alleviates symptoms, but its effectiveness wanes after several years, and side-effects develop that are similar to PD itself.

One advance in the treatment of PD in the last 30 years has been "deep brain stimulation". This involves surgery to implant electrodes into the brain to stimulate a part of the brain called the subthalamic nucleus, which can help to control tremors in some PD patients. This therapy was not discovered by animal experiments, but resulted from the observations of French scientists in the 1980s, who noticed that in patients undergoing brain surgery, stimulation of certain parts of brain appeared to stop tremors (1). Although animals have subsequently been used to investigate the technique, trials in PD patients have shown it to be an effective in some cases and have helped to refine the technique (2).

Studies of families have revealed genetic elements to some forms of PD. This knowledge has immediately been used by some scientists to create genetically engineered mice with genetic defects, although they have so far failed to produce a mouse model displaying all the characteristic symptoms of human PD.

Much recent research has focused on the idea of treating PD by transplanting embryonic cells into the brain, in attempts to replace damaged brain tissue with new healthy brain cells. After partial successes in animal experiments, brain cell implants were attempted in humans in the 1980s, but initial results were disappointing. Researchers have continued to pursue this form of therapy, and subsequent improvements in the technique yielded some promising results in a few limited human trials (3). However, such hopes were recently dealt a severe blow when the first full

trial of the technique showed no improvement in PD patients over 60, and tragically left some patients with irreversible side effects (4). Five patients in the trial suffered devastating effects, with uncontrollable movements worse than the disease, although in animal models of PD brain cell implants had the very opposite effect (5). There are also ethical concerns over using human embryonic tissue as a source for implants, yet some researchers continue to hope that this approach might one day provide a cure for PD, and other brain disorders, such as Huntington's and Alzheimer's disease.

Alternatives - the humane way forward

Studies of human populations (epidemiology) have suggested interesting connections between an increased risk of PD and exposure to environmental toxins, such as pesticides, herbicides, or metals (6, 7). There are also indications that diet and smoking may play role in susceptibility to PD (8, 9). Further investigation into confirming these links could help to prevent future cases of PD.

Non-invasive brain scanners such as PET and MRI are already being used to study PD patients and will continue to increase our understanding of the effects of PD on the living human brain. Future improvements in these methodologies are likely to shed more light on the illness; help with diagnosis and monitoring of PD; provide a means of assessing new therapies and improve current ones, such as deep brain stimulation.

Post-mortem research on the human body after death is invaluable to medical science, particularly in following-up the progression of a disease, and the effects of treatments and interventions. A few brain banks already exist, to which people can donate their brains after death, although more could be done to encourage the public to donate their organs to research via a properly regulated system. Studies of human tissues and cells are providing insights into the cellular mechanisms that bring about the death of brain cells in PD, and indications of possible treatments and interventions.

Follow-up post-mortem studies of the brains of PD patients who have received brain lesioning treatment, could provide vital information to optimise the lesion procedure that would be of direct benefit to future patients. Such studies would be a direct alternative to current experiments in which monkeys have their

brains purposely damaged to induce Parkinson's symptoms and to mimic the lesion treatment.

Computer simulations of the interactions between brain cells are being used to shed light on what causes the symptoms of PD, and how therapies work (10).

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FUNDAMENTAL BRAIN RESEARCH

Animal research

Some of the most distressing brain research on primates is "fundamental research" which aims to find out which parts of the brain do what. Monkeys are trained to carry out tasks and then have their brains damaged to investigate the functions and locations of different regions of the brain.

This type of research often involves implanting brain electrodes for recording or electrically stimulating different parts of the brain. These are fitted to the animal by drilling into the skull under anaesthesia. Animals prepared like this will be used when conscious in experiments, recordings being made from their brains, whilst they carry out tasks that they have been previously trained to do, usually for rewards of food, or water. Later their brain may be damaged to see how this affects the way they carry out their tasks. These experiments can involve the animals being strapped into restraining chairs, sometimes for several hours at a time. Their heads may be fixed still by means of a peg cemented into their skull. Experiments may continue for many months.

For example, in vision research monkeys may be taught to recognise the size or position of a shape on a computer screen. To do this, they are held captive in restraint chairs in darkened rooms for several hours a day for up to nine months whilst they learn the visual task. Recording from implanted electrodes may continue for up to six months at a time. Some animals will have parts of their brains damaged under anaesthesia, and subsequently undergo recordings whilst conscious, to see the effects of the brain damage. At the end of experiments animals are usually killed.

Animals fitted with electrode chambers may be kept alone in separate cages, to prevent cage mates from tampering with or damaging the equipment, even though single housing of primates is not recommended in official guidelines as it is known to be stressful and cause psychological suffering.

Scientific limitations

A limitation of the animal experiments is that they record the activity of single brain cells. Single cell recordings from the human brain are not possible, so there is simply no way to compare findings to see if they are relevant to humans. Recording the activity of just a few brain cells, out of several billion in a monkey's brain, might be of academic interest, but such precise recording is not of practical or medical relevance.

Despite some broad similarities between animal brains, there are also clear differences in the brain anatomy of even closely related species, such as humans and monkeys. Science currently knows a great deal about monkey brains, but relatively little about the human brain. However, as new technology allows the investigation of the human brain there are increasing differences being found between the human and monkey brain.

For example:

- The primary visual area of the human brain is twice the size of, and in a different location to, that of macaque monkeys.

- Research with volunteers has demonstrated that the region of the brain associated with eye movements is in a different part of the brain in humans compared to monkeys (1).

- A visual area known as 4A has a unique arrangement in humans, different even to that in chimpanzees (2).

- There are differences between humans and great apes in both the organisation and relative size of an area of the brain known to be involved in taking initiatives and planning future actions (3).

- Differences have been found between chimps, monkeys and humans in the organisation and arrangement of cells in the cerebral cortex, an important part of the brain whose functions include control of movement, co-ordination and posture (4).

- Brain tissue from humans, marmosets, vervet monkeys, guinea pigs, rats and mice have shown significant species differences in the levels and distribution of 'receptors' in the brain that detect chemicals thought to control behaviour and mood (5).

- A study of blood from chimps and humans found significant differences in thyroid hormone metabolism, which is known to play a major role in the development and metabolism of many organs, including the brain (6).

- An area identified in the monkey brain as important in aspects of working memory, is located in a different position in humans (7).

Alternatives - the humane way forward

Non-invasive imaging techniques, such a fMRI, PET, MEG have revolutionised brain research. Scientists can now safely probe the intact human brain to produce 3D images and to watch the brain in action. These technological advances have made studying animals brains less appropriate, as human brains are now accessible. Brain imaging techniques are still relatively novel, and continued improvements in accuracy and sophistication are likely to expand their potential even further in the future.

At present brain imaging techniques may not provide exactly the same precise data as single cell recordings from implanted electrodes, but studying humans has innumerable advantages over experiments on monkeys, such as:

- Studying the species of interest and producing results that are directly relevant to medical progress.

- Lack of species differences.

- Humans are quicker to train than monkeys.

- Humans are far easier to communicate with and can provide direct feedback.

- Restrained monkeys are likely to be highly stressed, which can confuse the results.

- Patients suffering from spontaneously-occurring illness or brain damage can be studied, rather than artificially induced brain damage in monkeys.

- Imaging techniques can provide an overview of the human brain, revealing the interaction of different parts of the brain and the timing of events, aspects which cannot be studied with single brain cell recordings.

A highly innovative technique called transcranial magnetic stimulation (TMS) uses a magnetic pulse to temporarily and reversibly disrupt areas of the brain in human volunteers. This method allows researchers to safely and momentarily mimic the effect of brain damage in human volunteers, instead of inflicting permanent brain damage of monkeys (8). TMS is being utilised for both fundamental research, and in studies of brain disorders such as migraine.

Analysis of human post-mortem brain tissue can contribute greatly to our understanding of the structure and function of the brain. Analysis of patients' brains after death can help validate some of the new imaging technologies, and fresh post-mortem human brain tissue can be used to track connections between individual brain cells and different parts of the brain (9)

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