AN INVESTIGATION

BY THE BUAV

INTO PRIMATE RESEARCH

AT CAMBRIDGE UNIVERSITY

REVISED VERSION

AUGUST 2002

Contents

A. INTRODUCTION

1. This report sets out the principal findings of the undercover investigation conducted by the BUAV into brain research on marmosets carried out at Cambridge University. A BUAV investigator worked as an animal technician at the research unit between March 2001 and January 2002. The research – carried out under three project licences issued under the Animals (Scientific Procedures) Act 1986 ('the 1986 Act')[1] – aimed to improve understanding of the human brain, with the eventual aim of developing treatment for stroke, Parkinson's disease and Huntingdon's disease. The research is a continuation of primate research which has been going on at Cambridge University for a number of years

2. The BUAV fully accepts the importance of research into these debilitating diseases. However, in our view it is morally unacceptable deliberately to cause brain damage to animals such as marmosets which are just as capable of suffering as are people. In addition, we believe that the Cambridge University research, as well as being deeply inhumane, is in many respects scientifically flawed.

3. Like most animal experiments in the UK, the research at Cambridge University is carried out under conditions of great secrecy[2]. Research institutions and the Government join forces to ensure that the public is not told what really goes on. Where details of research are published in scientific journals – as some of the Cambridge University research has been over the years – the suffering experienced by the animals is downplayed if it is mentioned at all.

4. It is only through undercover investigations that the full picture can be revealed. The BUAV believes that it is overwhelmingly in the public interest that our findings are disseminated widely. This is for a number of reasons, which we summarise here and develop in the remainder of the report:

- very large (and increasing) numbers of people are concerned about experiments on animals in general and experiments on primates in particular, as shown by successive recent opinion polls[3]. Self-evidently, they need to be told the facts about primate research before they can engage in informed debate

- as an university, Cambridge receives substantial public funding. In addition, the Medical Research Council ('MRC'), which also receives public funding, part-funds the research in question. Taxpayers have a legitimate interest in knowing how their money is spent

[1] The 1986 Act implements EC Directive 86/609

[2] section 24 of the 1986 Act makes it an offence if 'otherwise than for the purpose of discharging his functions under this Act [a person] discloses any information which has been obtained by him in the exercise of those functions and which he knows or has reasonable grounds for believing to have been given in confidence'. Historically, the Home Office has accepted virtually all information from animals researchers as having been given in confidence. Home Office ministers regularly cite the section as the reason why they cannot reveal information about particular animal experiments even to Parliament.

[3] see, for example, polls in the *New Scientist* on 22 May 1999, the *Times* on 8 September 1999 and the *Guardian* on 23 January 2001

- Cambridge University has made a planning application for a larger primate unit for its neuroscience work. The application has proved highly controversial. Lord Sainsbury, the science minister, took the highly unusual step of supporting the application, even though he has no ministerial responsibility for planning matters. He specifically told the university that he was 'content for you to release this letter to [the planning authorities], and for them to make it available to all parties with an interest in any planning application for the proposed Centre'[4].The application was rejected but Cambridge University are appealing the decision. The Government has reportedly said it will meet any increased security costs necessitated by protests if the site is developed.

  The Prime Minister, in a speech to the Royal Society on 23 May 2002, made it clear that the Government wants the new centre to be built. The appeal will, directly or indirectly, be determined by John Prescott MP, Secretary of State for Transport, Local Government and the Regions. Given the Government's clearly expressed wish for planning permission to be granted, it is difficult to see how Mr Prescott can possibly reach an impartial view. The need for public scrutiny into the primate research work which Cambridge University wish to expand is therefore all the more pressing

- Cambridge University claimed, and the Home Secretary accepted (on the advice of his inspectorate), that the research would involve no more than 'moderate' suffering, for *any* of the marmosets, at *any* stage[5]. In light of the evidence uncovered the BUAV believes this is insupportable. Even by the Home Office's own definitions[6], it should have been quite clear that many of the marmosets would experience substantial suffering. Indeed, at least one of the project licences contemplates that 'severe' effects might be experienced.

  The significance of the Home Office's wrong classification is threefold. First, it meant that the licence applications avoided prior scrutiny by the Animals Procedures Committee ('the APC'), the Government's advisory body – only primate research which is of 'substantial severity' is referred to the APC. Second, it inevitably skewed the cost:benefit test which lies at the heart of the 1986 Act[7]: the lower the level of perceived likely suffering, the easier it is to satisfy the test. Third, it distorts the Home Office's annual statistics, one of the few sources of public information about animal experiments. Other available evidence suggests that the downplaying of suffering is a widespread problem.

- linked to this, the Home Office claims that it does not allow experiments causing severe pain or distress unless it is promptly alleviated (such that it will be only transient). The BUAV evidence shows that the claim is highly dubious. Given that 24-hour care was not provided, it would have been impossible to ensure that

[4] letter from Lord Sainsbury to Sir Alec Broers, vice-chancellor of Cambridge University, dated April 2001

[5] none of the protocols was given a 'severity limit' of more than 'moderate'

[6] see below

[7] section 5(4) of the 1986 Act reads: 'In determining whether and on what terms to grant a project licence the Secretary of State shall weigh the likely adverse effects on the animals concerned against the benefit likely to accrue as a result of the programme to be specified in the licence'. The greater the likely suffering, the greater must be the likely benefit

severe symptoms – which were unquestionably experienced - were always alleviated promptly

- in the event, there can be no doubt that many of the marmosets did suffer very greatly. Several died or had to be euthanased, which is itself strong evidence of substantial or severe suffering. The Home Office claims that it keeps cost:benefit assessments under constant review. Even if its original assessments in this case were defensible – which we would vigorously dispute – it should have revisited them in the light of experience. In addition, the researchers should have reported that the moderate severity limits in the licences were exceeded on numerous occasions.

- there is evidence that procedures which would unquestionably have caused distress to the marmosets – such as water deprivation – were not always taken into account or reported to the Home Office on statistical returns. If this is a widespread practice in UK laboratories – as we suspect it is – the Home Office annual statistics will inevitably underrepresent the amount of animal experimentation which takes place. In addition, the cost:benefit assessment will again be skewed

- under the 1986 Act, a licence can only be granted if a benefit[8] is 'likely' to accrue. Some of the research was acknowledged by the researchers to be fundamental in nature and therefore, almost by definition, not 'likely' to lead to any benefit. Over 800,000 procedures on animals which the Home Office itself categorises as 'fundamental' take place every year in the UK. The lawfulness of its approach to the statutory test is therefore hugely important

- even for research which could properly be categorised as 'applied', it is unclear how the Home Office decided that there was sufficient likelihood of benefit to justify the suffering involved given that it has been going on for a number of years with little apparent progress. In addition, the public has a legitimate interest in knowing the true picture – bad as well as good - about research into diseases which could afflict any of us

- there is considerable doubt as to whether the availability of non-animal alternatives was properly explored by Cambridge University or the Home Office, as is required by section 5(5)(a) of the 1986 Act and Article 7.2 of the EC Directive 86/609 ('the 1986 directive')

- the post-operative care given to brain-damaged marmosets was sometimes wholly inadequate, in part because staffing levels were far too low, particularly at weekends and on public holidays. The Home Office should have been very concerned about this

- the staffing levels were low with only 3 animal technicians to care for 450-500 marmosets. This was totally inadequate and should not have been approved by the Home Office inspectorate.

[8] for a permissible purpose: see section 5(3)

- similarly, the housing conditions for the marmosets were wholly inadequate. Under the 1986 directive housing for laboratory animals has to be appropriate to their health and well-being and any interference with their physiological and behavioural needs has to be kept to the absolute minimum[9] Even a cursory look at the evidence uncovered by the BUAV shows that these legal requirements were not met. In addition, key provisions in the Home Office's codes of practice[10] were clearly not met

5. There are, therefore, strong arguments that the Home Secretary has acted unlawfully in several respects. Unfortunately, numerous other undercover investigations and leaked information[11] have also revealed serious inadequacies in the way in which the Home Office carries out its regulatory role. In addition, it has a poor track record of investigating allegations made following undercover investigations, particularly where (as in this case) there is criticism of its regulatory performance. There is an issue of legitimate public concern. Disclosure of the information revealed by the investigation simply to the Home Office, or any other government body, will clearly not satisfy that concern.

6. The BUAV has been careful only to publish information which is relevant to these considerations of public interest. We have been sensitive to any legitimate considerations of true commercial confidentiality. We have not named any of the individuals concerned, even though the project licence holders have in fact published some of their work.

7. In this report, we describe the research and what it involves for the marmosets; provide a detailed scientific critique; discuss the available non-animal alternatives; explore the licensing issues; and assess the housing conditions and the post-operative care given to the marmosets. Finally, we set out our recommendations.

8. We have no doubt that the evidence reveals a catalogue of appalling suffering and raises very serious issues about the way primate research, and indeed animal research more generally, is regulated in this country. The investigation underlines the need to open up animal research to proper public scrutiny. Put bluntly, the investigation confirms, yet again, that the Government cannot be trusted to fulfil its regulatory functions properly without such scrutiny.

B. SUMMARY OF THE RESEARCH AT CAMBRIDGE UNIVERSITY

9. The research which was the subject of the BUAV undercover investigation involves three project licences, all using marmosets. The projects explore related aspects of brain function, brain disorders and potential treatments.

---

[9] Article 5(a) and (b)

[10] *Code of Practice for the housing and care of animals used in scientific procedures* (1989) and *Code of Practice for the housing and care of animals in designated breeding and supplying establishments* (1995)

[11] see, for example, the BUAV's undercover investigation of Harlan Hillcrest, a multinational company supplying dogs, primates and other animals for vivisection, in 1999; and an article in the *Daily Express* on 21 September 2000 based on leaked documents about xenotransplantation research carried out at Huntingdon Life Sciences for Imutran

10. The three programmes of work are similar in terms of the brain systems being studied; the brain regions and pathways between them; and the neurotransmitters used by the brain cells to communicate. In these senses there is some overlap between the three projects.

11. However each project licence has its own focus, with two or three main aims. The three project licences are as follows:

PPL80/1249: Brain disturbance and repair in primates.

12. The research includes: models of stroke and testing experimental neuroprotective drugs in marmosets; understanding the brain chemistry which underlies cognition; and testing cellular grafts as a method of repairing brain damage. The licence describes this as applied and fundamental research.

PPL80/1326: Neurodegeneration and repair in marmosets.

13. The research includes: models of Parkinson's disease and Huntington's disease in marmosets; testing cellular grafts as a therapy; and comparing these grafts with novel drug treatments and gene therapies. The licence describes this as primarily applied research with fundamental aspects.

PPL80/1344: Neural mechanisms underlying cognition and emotion.

14. The research includes: understanding the roles of specific brain regions in cognition and emotion; and looking for potential targets in the brain for future drug treatments. The licence describes this as mainly fundamental research.

C. ANIMAL SUFFERING

Introduction

15. For a full picture of the suffering endured by marmosets at this laboratory, this section should be read in conjunction with the sections dealing with anaesthesia and animal health, especially post-operative care of brain-damaged animals.

16. The three project licences are based on a similar pattern. Simply put, marmosets are trained to perform a number of cognitive and/or skilled movement tasks, over a period of months. Then the marmosets are brain damaged, and re-tested to determine the specific effects of the damage inflicted. In two of the projects, different experimental therapies are then tried on the animals while monitoring their performance in the tasks.

17. Each experimental protocol in a project licence has a severity *limit* (mild, moderate, or substantial). A number of regulated protocols are licensed for each project as shown in Table 1, but none are designated substantial.

Table 1 Severity of regulated protocols under the three project licences

Project	Number of protocols	Protocols of mild severity	Protocols of moderate severity
PPL80/1249	7	1	6
PPL80/1326	5	0	5
PPL80/1344	4	1	3

18. The Home Office's *Guidance on the Operation of the Animals (Scientific Procedures) Act 1986* ('the Home Office guidance') defines substantially severe protocols as[12]:

> *"Protocols that may result in a major departure from the animal's usual state of health or well-being. These include: ... major surgery; and some models of disease, where welfare may be seriously compromised….*

19. It further says:

> *"If it is expected that even one animal would suffer substantial effects, the procedure would merit a 'substantial' severity limit."*

20. It is perfectly clear from the descriptions that follow that, by these definitions and by basic standards of common sense, substantial suffering occurred and, in many cases, continued for a considerable period. It is important to understand that, by not according any severity limit of more than 'moderate', the Home Office was saying that *no* animal, for *any* period, would suffer more than moderately. We believe that this is completely unsustainable and that it is clear that many of the marmosets did experience substantial suffering.

21. A project licence has an overall severity *banding*, based on the different severity limits of the protocols and the number of animals who are likely to experience that level of severity. Project licence PPL80/1249 had an overall severity banding of moderate, and we believe this to be the case for the other two licences as well.

22. We return to the issues of severity limits and overall severity banding under *Licensing issues* below.

---

[12] ***para. 5.42. There are also definitions of 'unclassified', 'mild' and 'moderate'. 'Moderate' is defined as 'includ[ing] toxicity tests (which do not involve lethal endpoints) and many surgical procedures (provided that suffering is controlled and minimised by effective post-operative analgesia and care). Protocols that have the potential to cause greater suffering but that have controls which minimise severity or terminate the protocol before the animal shows more than moderate adverse effects may also be regarded within the moderate severity limit'. Strictly speaking, the definitions relate to the severity*** **limits** ***which are permitted. However, the definitions must apply equally to the severity*** **bands** ***(see*** *Licensing issues* ***below). The Home Office guidance is issued under section 21(1) of the 1986 Act. It cannot, of course, alter the proper meaning of the provisions in the 1986 Act. .***

C.1. Animal suffering in PPL80/1249

23. This research has two main aims. One is to develop better models of human stroke, and then test experimental neuroprotective drugs[13] and brain grafts as treatment. The second is to explore the fundamental neuropharmacology[14] underlying cognition in marmosets, with possible future relevance to human conditions such as dementia and amnesia. The two areas of research are described separately, below, in terms of animal suffering.

C.1.i) Animal suffering: stroke experiments under PPL80/1249

24. The aim was to produce a 'standard stroke' and then compare different methods of treatment including novel neuroprotective drugs. Brain grafting did not take place during the BUAV's investigation. Marmosets underwent training in cognitive[15] and/or motor tasks before being operated on, and were tested again afterwards.

25. Our investigator witnessed and recorded an operation to produce a stroke. This is major surgery, involving craniotomy (removal of the skull) as can be seen from the description in Box 1.

Box 1
A stroke operation recorded by the BUAV investigator (November 2001)

A marmoset, called **Frank**, was anaesthetised and his scalp cut open from ear to ear. This revealed a layer of muscle, being where the jaw muscles attach to the skull. These were cut and scraped away from the bone.

With an electric saw the skull was cut all round (like the lid of a tin). This took about 20 minutes (damage to the brain must be avoided). The skull was removed or left attached by a small bony 'hinge'. This revealed the brain's meningeal membranes which were cut open, exposing the brain. The marmoset brain is only about 20 millimetres in diameter (not much larger than half a walnut). Lobes of the brain were moved aside, to reveal the artery. Using a microscope for viewing, the artery was occluded causing a stroke.

The meninges were sewn, the skull was replaced and the jaw muscles glued back to it. The skin was pulled back over and stitched.

[13] The purpose of neuroprotective drugs is to limit the death of brain cells in the aftermath of a stroke. Many such drugs have appeared to be effective in animal models but so far none has been safe and effective in human patients.
[14] eg. the roles of cholinergic, glutamatergic and serotonergic systems within the brain.
[15] Cognition refers to higher brain functions such as thought, perception, learning and memory.

Suffering caused by the stroke operation

26. The operation leads to a large one-sided stroke with physical and cognitive effects. As witnessed by our investigator and recorded in laboratory notes, the physical effects after the induced stroke included:

    -- post-operative pain and bruising due to major cranial surgery
    -- inability to use one arm
    -- tendency to rotate and/or turning of the head uncontrollably to one side
    -- tremors
    -- vomiting
    -- reluctance to eat or drink for up to two days afterwards.

27. Cognitive effects of brain damage included:
    -- motor neglect
    -- optic ataxia (difficulty making intentional visually guided movements)
    -- perceptual spatial neglect.

28. According to the project licence, analgesia would be given "if required" for up to 1-2 days. In fact, marmosets were routinely given a single injection of Finadyne subcutaneously at the end of the stroke-inducing operation . According to the post-operative care sheets, that was the only analgesic which many monkeys received, and could not have been continuously effective for a 1-2-day period.

Multiple operations

29. In 2001 and 2002, several marmosets including **Frank**, **Peggy**, **Lennon**, **Reidlem**, **Harkness** and **Tonga** had a stroke operation in the morning. They were anaesthetised again after about three hours, to have three minipumps surgically implanted under the skin at the back of their necks (these are clearly visible as lumps under the skin). The pumps started an infusion of drug into the circulation. The next day the three pumps were surgically removed and three more implanted. These were removed in a fourth operation, on the third day (see also *Licensing issues* below).

Venepuncture problems

30. Marmosets also had intravenous injections of a drug, and blood samples taken to assess drug levels. There is evidence that venepuncture caused welfare problems.

31. Handwritten laboratory notes by the researcher (6.11.01) for **Lennon** stated: "*1ml blood taken from femoral (Rt) after failed saphenous*".

32. For **Reidlem**, the notes (6.11.01) said that the drug was given intravenously via the tail veins: "*...used both sides as 1st vein swelling*." About a blood sample taken the next day: "*Difficulty with femoral, saphenous and tail!*"

33. For **Harkness** (7.11.01) just out of major surgery: "*1ml i.v. NXY injected v. slowly as possibility of seepage into surrounding tissue. Tail slightly swollen, no obvious oedema.*"

34. For **Lore** (19.11.01) "*i.v. took several attempts.*"

35. These notes show that several marmosets experienced swelling due to repeated venepuncture. This should be avoidable by use of good technique, minimising repeated venepuncture, using sufficiently fine needles and applying compression immediately afterwards. It is of great concern that the suffering which is inherent in these experiments should be compounded by avoidable suffering.

Anaesthesia

36. Problems with anaesthesia occurred sometimes. In one particular case, a marmoset called **Frank** was undergoing surgery to induce a stroke (on 20.11.01, as described above in Box 1). Recorded dialogue suggests that the level of anaesthetic was not always deep enough.

37. Despite having been given a dose of anaesthetic a few minutes previously, the marmoset's temperature began to rise. The operating researcher mentioned that this is a sign of the animal "coming round". Then his leg/s "pulled up", which indicates either that muscle tone or the pedal reflex was returning (the animal had just had his artery blocked and was being stitched up). Either interpretation suggests that anaesthesia was too light.

38. Between noting the rise in temperature and injecting another supplementary dose of anaesthetic, it appears that 6-9 minutes elapsed. Even a minute or two *after* this supplementary dose was given, "…a lot of contraction" of Frank's legs was described by the person monitoring the anaesthesia. The marmoset's temperature started to rise again. Frank may have been able to experience pain during some or all of these periods.

39. Additionally, during at least one stroke operation the equipment which displays heart rate and oxygen consumption was not working.

Summary

40. In addition to our concerns about possible problems with anaesthesia, in this stroke research programme some marmosets could suffer all of the following experiences:

    -- major brain surgery (including removal of the whole top of the skull and an induced stroke)
    -- three further operations within three days
    -- swelling/bruising of veins due to repeated needle insertion
    -- one-sided weakness, movement problems, body rotation, tremors, vomiting, loss of appetite, cognitive disabilities
    -- emotional distress such as fear, confusion and anxiety.

41. It defies common sense to claim that this research does not involve substantial or severe suffering.

C.1.ii) Animal suffering: fundamental cognitive experiments under PPL80/1249

42. This basic research is to understand the different roles of transmitter chemicals in the brain (in particular those which underlie cognition), and the effects of damage to different brain regions and to connections between them. The project licence claims that it may have possible future relevance to human conditions such as dementia and amnesia[16].

43. Damage may be done to a specific region of the brain, or to certain kinds of brain cells within a region. This is done mainly by one of three methods: by stereotaxic injection of toxins directly into the brain (the toxins kill selected types of brain cells in that area); by cutting a bundle of brain cell fibres; or removing (usually by suction) an area of brain.

44. Many regions or fibre tracts in the brain were permitted to be damaged in these experiments, and some marmosets endured three or four types of brain damage, with cumulative effects on their wellbeing (see Box 2)

Box 2 Combinations of brain lesions caused in marmosets

Some marmosets had combined lesions, for example:

-- lesions of the nucleus basalis of Meynart (NBM) as well as the vertical limb of the diagonal band of Broca (VDB) (eg. a marmoset named **Tori**).

-- three kinds of brain damage ie. unilateral damage to the VDB + unilateral lesion in the hippocampus + unilateral ablation of the inferotemporal cortex (eg. marmosets **Brighton, Bray, Revenge** and **Vela**).

-- bilateral lesions to the VDB + bilateral transection of the fornix (eg. **Sherwood**, 2.8.01 & 3.9.01).

-- four types of brain damage inflicted in three different operations ie. a lesion to the NBM + suction ablation of the inferotemporal cortex + lesion to the VDB + lesion to the hippocampus.

45. VDB lesions cause large, persistent impairments to certain types of conditional learning; NBM lesions produce smaller, more transient damage to visual discrimination learning and retention. Lesions to both areas are additive and produce a severe and persistent impairment on object discrimination learning in monkeys. These combined lesions also produce delayed and transient hypothermia and hypophagia (poor appetite), requiring special care.

[16] However, the test in section 5(4) of the 1986 Act is whether a benefit is *likely* to accrue. It is difficult to see that this requirement can be met with fundamental research of this nature.

46. Several tests were used to assess marmosets before and after brain damage, as well as to see if any treatments are effective. The marmosets were trained on the tasks for up to six months, before being brain damaged. They were also assessed for spontaneous movements in the home cage.

47. The tasks/tests included tests of skilled movements and tests of cognitive abilities (eg. learning, memory) using an object discrimination task, such as learning to associate a food reward with the colour, shape or position of an object.

48. However, the operations could have other serious, deleterious effects on the marmosets:

    -- Certain lesions cause a delayed, temporary condition in which the animals fail to maintain their temperature and eat very little (hypothermia and hypophagia). They have to be kept in an incubator and hand fed, and may lose weight.
    -- Some of the brain toxins cause fits which have to be prevented by keeping the animal quiet or giving it anti-epileptic drugs.
    -- Kluver-Bucy syndrome is caused by certain lesions. This is a psychological condition, lasting 1-4 weeks, which prevents self-care in the acute phase, because marmosets fail to eat and drink. They appear to experience hallucinations, are sometimes in a trance-like state, have psychic blindness, and have inappropriate and abnormal social interactions (they may ignore other marmosets or indulge in non-stop copulatory activity, and seem very tame towards humans).

49. An illustrative experiment is described below, highlighting the severity of the surgical procedures and their outcomes in terms of animal suffering. Out of 12 lesioned marmosets, two did not complete the experiment: one died unexpectedly from a cerebral artery haemorrhage (stroke) and one developed intractable Kluver-Bucy syndrome and suffered this for four weeks before being killed. Most if not all of the marmosets are believed to have suffered temporary Kluver-Bucy syndrome. Two had epileptic fits, and two had transient hypothermia and hypophagia.

50. In this experiment both inferotemporal lobes of the brain were removed and the projections of the VDB[17] to the hippocampus and entorhinal cortex were destroyed on both sides. The damage to perception and recognition of objects was assessed.

First operation: suction ablation of the inferotemporal lobe (both sides):

51. Marmoset anaesthetised and scalp cut open.
    Jaw (temporal) muscle attachments to the skull were cut away and pulled back
    The whole of the top of the skull was sawn off and removed like a lid.
    The meninges (membranes) were cut open on one side and the brain was lifted to access the floor of the skull.
    The inferotemporal lobe of the brain was sucked out.
    The membranes were sewn up and the meninges on the other side were cut.

[17] VDB is a region of the brain called the vertical limb of the diagonal band of Broca.

The brain was lifted to access the floor of the skull and the second inferotemporal lobe of the brain was sucked out.
The membranes were sewn up, the skull was replaced and the muscles glued back.
The scalp was stitched.
Recovery from operation.
Epileptic fits in two animals.
Kluver-Bucy syndrome (possibly in all marmosets).
Memory damage and "very substantial" learning disability.

Second operation: toxic lesions of VDB projections (both sides):

52. Marmoset anaesthetised.
Head placed in stereotaxic frame (using ear bars, mouth bar and eye-socket hooks).
The skull was drilled, and a needle inserted into the brain and toxin injected into two sites in one hemisphere.
Repeated on the other side.
Recovery.
Temporary drop in temperature, failure to eat, poor movement control (two marmosets).
Memory damage and learning disability.

Additional (undesired) brain damage

53. In some operations which destroy deeper brain structures, damage is inevitably also done to more superficial structures. In this experiment marmosets underwent three kinds of brain damage, bilaterally. In order to transect the temporal stem and amygdala, a suction incision had to be made in the left temporal lobe. This was angled down steeply "*...so that the auditory cortex, rather than visual cortex, was compromised by the surgery.*" Post-mortem histology indeed showed that this had occurred, raising the question: What additional disability and suffering was caused by unwanted (but apparently unavoidable) damage to the auditory cortex of the marmosets?

Post-operative effects

54. The staff had a Standard Operating Procedure (SOP) for post-operative care of marmosets, setting out a grading scale for pain and suffering and how this might be alleviated. This states that "*For more major surgery (ie. that involving craniotomy) most animals will start at score 3 and progress to score 1 although some will start at score 4 mainly on account of the severity of the neurological symptoms.*"

Level 4 is described in Box 3.

Box 3 The Standard Operating Procedure – description of Grade 4 condition in marmosets

"Grade 4
Rather poorly – check temperature, consider whether dehydrated
Lying down or curled up – probably needs analgesics
Needs hand feeding
Consider whether could be infection
Consider whether Named Vet should be called
Serious neurological symptoms:
persistent rotation – consider controlling with neuroleptics
seizures – must be controlled with dark, quiet environment or antiepileptics or Named Vet must be called
'psychotic' behaviour – control with neuroleptics or benzodiazepines
Continue to monitor and hand feed closely."

55. We reproduce this part of the document to illustrate the extent of potential suffering – which includes pain, stereotypic movements, seizures and psychosis (grade 5 is more severe). As SOPs go, it may appear thorough. But it gives a false picture, firstly because there was no 24-hour cover[18]; and secondly because according to visible records the grading scale was not used in practice (there were empty columns for this on the relevant record sheets).

56. An instance of post-operative suffering involved **Belanna**. On 28 June 2001 she weighed 390 grams, and on that day she was lesioned bilaterally in the NBM with a toxin called saporin. On 19 July it was noticed that Belanna was not eating properly and had lost 103 grams (more than 26 per cent of her body weight). She was put in an incubator and given lectade. Laboratory notes say her behaviour was "*Slightly neglectful & 'watching the birdies' wobbly in cage.*"

57. By 23 July, Belanna had regained a little weight but was still neglectful and "*wobbly*", and the next day she had "*Great difficulty putting hands to mouth to hold*." On 26 July she was still underweight (334 grams) and "*…still having problems controlling limbs.*" Her weight dropped again on 27 July and she was thought to have become dehydrated. She was given water and a piece of marshmallow, but "*...she fell a lot whilst eating it*." Despite her condition, the laboratory records indicate that Belanna was used in trials on 30 July. By 2 August, more than a month after her brain lesions, Belanna's coordination was still described as "*peculiar*" and she weighed only 336 grams.

58. For more information on post-operative care, see p 47

[18] researchers occasionally stayed for a short while in the evening

Post-operative deaths

59. Two animals died in the summer of 2000. A brain-damaging operation on **Killimor** had been abandoned on 22 May 2000 because the anaesthetic did not work. Another attempt was made on 1 June 2000. A toxin known as NMDA was injected several times into Killimor's brain, but the last injection had to be abandoned because the animal developed very rapid breathing and began having fits. Killimor was found dead three-quarters of an hour later, according to laboratory notes.

60. On 17 May 2000, **Agar** was anaesthetised for similar lesions to Killimor's. Again according to laboratory notes, Agar was breathing heavily while under anaesthetic and his temperature fell. The next day the marmoset was found dead, with a frothy, pink exudate having come from the mouth or nose.

Emotional distress

61. Clearly, marmosets with neurological symptoms, psychosis, physical disabilities or disrupted social interactions, are undoubtedly suffering emotional distress. For example, lesions of the entorhinal cortex or the amygdala are known to cause emotional impairments.

Distress during training and testing

62. Laboratory notes describing how to train marmosets[19] include the suggestion that incorrect behaviour by a marmoset can be stopped by "*...lowering the shutter very rapidly after the first response, if necessary onto their fingers (but don't drop it!)*." Moreover, the document refers to the fact that "*Some animals get upset at this stage*", that some marmosets are "*miserable*" during training and that some marmosets "*need a lot of shutter banging, they like (sic) you to bang the inside of the shutter before you raise it...*".

63. Even without unacceptable behaviour of this kind, the processes of training and testing obviously cause distress. Marmosets dislike handling and restraint[20], and are sensitive to temporary separation from their family or group. Exposure to an unfamiliar cage causes stress, measured by a 4-5-fold increase in blood pressure[21]. Moreover, the US National Research Council has stated[22] that marmosets "*...appear to have long memories and respond with fearful behaviour to hearing the voice or footsteps of someone who has captured them several months earlier*".

64. This licence, PPL80/1249, states that food deprivation is not required to motivate animals to perform tasks for which a food reward is given. Nevertheless, it

[19] Notes on shaping animals
[20] The UFAW (Universities Federation for Animal Welfare) Handbook on the care and management of laboratory animals 1999 Seventh Edition Volume 1.
[21] Gerber, P et al (1996). Sociophysiological aspects of short-term separation in common marmosets (Callithrix jacchus). EUPREN/EMRG Workshop: The implications of housing and husbandry for scientific quality and wellbeing of non-human primates, Rome.
[22] NRC (1998). The psychological wellbeing of non-human primates. p77, Publ. NAP, USA.

appears that marmosets were food restricted: many were not given breakfast and were kept on half normal rations.

65. These animals have experienced major brain surgery and physical and mental disability. Additionally, if marmosets are considered similar enough to humans to be used in this kind of research, account has to be taken of the mental and emotional suffering they may experience[23]. It appears unlikely that proper account has been taken of the suffering caused by mental and emotional disturbances -- such as confusion, hallucinations, Kluver-Bucy syndrome, social disruption and damage to emotional centres of the brain.

66. It is impossible to believe that this cumulative degree of physical and psychological suffering could properly be described as of 'moderate' (rather than 'substantial') severity, even if judged solely by the definitions in the Home Office guidance (see *Licensing issues*, later).

C.2. Animal suffering in PPL80/1326

67. This research focuses on Parkinson's disease and Huntington's disease, aiming to develop better 'models' in marmosets and using key symptoms which remain stable and measurable over time. Experimental treatments are tested, such as gene therapy in the brain or brain grafts (the latter were not done during the BUAV investigation).

68. Most of the research observed by the BUAV investigator was into Parkinson's disease, using marmosets who have a stereotaxic injection of 6-hydroxydopamine (6OHDA) to a specific part of the brain. Some symptoms (see below) can be seen in the animals' behaviour, or are measured in response to test situations or a drug injection. Some marmosets were used in pilot studies to induce parkinsonism by injecting a viral vector plus a transgene directly into the brain.

69. The behavioural and cognitive deficits were recorded as a measure of the severity and duration of the brain lesion and were also used as an indication of the effectiveness of any experimental treatments.

Suffering due to brain lesions

70. After a one-side brain injection of 6OHDA, there are visible symptoms of brain damage, some of which wane over a period of time:

-- Some marmosets may experience "*severe*" pain post-operatively, according to the project licence. This is expected to be detected by means of a hunched posture, and should be alleviated with stronger painkillers than normal.[24]
-- Marmosets tend to sit with their heads turned over their shoulder on one side.
-- They turn bodily in slow circles (rotation) but are generally less active than normal marmosets.
-- They show perceptual neglect.

[23] No one would doubt that stroke victims can suffer 'substantially' or 'severely'.
[24] It is worth making the point that, since 24-hour care was not provided, the Home Office's requirement that severe suffering should be promptly alleviated could not have been met.

-- They tend to favour one side of the body in certain movements.
-- Their balance is affected, as is their smoothness and speed of movement.
-- They have tremors.
-- It seems inevitable that there will have been psychological suffering both directly as a result of brain damage, and indirectly because of the physical disability which follows.

71. Because undesired damage is done to other brain cells in the track of the needle used to create the targeted lesion, sham-operated animals are used. These have the needle passed into their brains but they do not receive an injection of 6OHDA.

72. Some marmosets were given bilateral injections, which cause dyskinesia (impairment of voluntary movements), tremor and gait disturbance and are more likely to damage their ability to look after themselves, eg. to feed or groom.

73. A subjective scale used by the researchers for assessing marmosets' disabilities – the Disability Rating Scale -- illustrates the severity of suffering. One laboratory document describes the scoring for a marmoset called **Medland**. Medland scored "*Severe reduction in spontaneous movement*" and "*No spontaneous movement*" (the maximum score for akinesia), plus the maximum score for disability, ie. the animal did not climb or jump at all. Plus, Medland's posture was scored as "*Moderately hunched, head down most of the time*", as well as "*Severely abnormal/hunched posture*". The latter was again the maximum severity score for this parameter.

Suffering in training and testing

74. Marmosets were trained in cognitive tasks before a lesion and were re-tested afterwards. Tasks included visual discrimination and other tests, and training and testing could take place over several months. The food intake of marmosets being used in Parkinson's disease experiments was restricted for the purposes of training and testing. Water restriction had been used in the past (for marmosets being trained for research into Huntington's disease) – in which case water was provided only for 2 hours in every 24 hours during the working week. No doubt distress occurs if an animal cannot drink when thirsty, and it is generally recognised that water deprivation is an undesirable method of training.

75. Brain damage was also assessed by recording of spontaneous body rotations (not considered to be a regulated procedure), as well as rotations after an injection of amphetamine or apomorphine (both regulated procedures). For spontaneous rotations and after drug injections, the marmosets were recorded while confined in a very small perspex test box for 30 or 60 minutes.

76. Videotape of marmoset **Shady**, who had had a unilateral 6OHDA lesion, show a distressed animal biting at the air holes of the small box, mouth opened in a scream, and hands feeling round the sides of the container which are progressively obscured by the animal's breathing (August 2001).

77. According to the licence, many of the cognitive tests are also not considered to be regulated procedures (e.g. skilled reaching tasks with tubes or the staircase test),

because they took place in the home cage. But even in these cases the marmoset's cage mate was removed during the task. Marmosets are sensitive to temporary separation from their family or group. Moreover, as we have previously noted the US National Research Council has recognised that marmosets appear to have long memories [25].

Suffering caused during experimental treatments

78. Laboratory records show that in 1998 and 2000, some experiments had been conducted in which fetal pig tissue was grafted into the brains of marmosets. This required high doses of immunosuppressive drugs which caused side effects, described in a laboratory document[26] as "*...an aggressive immunosuppressive regime that was at the limit of tolerance for the long term health of the marmosets.*" Potential side effects described in the licence include infections, weight loss and kidney and liver toxicity.

79. One of the drugs (Neoral) was given daily by gavage. In 1998, a marmoset called **Eagle** was being used to develop the immunosuppressive drug regime. He developed a swollen throat and breathing difficulties. Despite being put in an incubator Eagle was found dead the next day. In the same year a marmoset called **Woodpecker**, who had had pig cells grafted into the brain, developed swellings on the throat and was subsequently found dead. In 2000, **Pongo** who had already endured brain damage and an operation to place pig cells in his brain, suffered throat bleeding as he struggled during gavage.

80. When fetal pig tissue had been grafted into lesioned marmosets, despite the immunosuppression the tissue was rejected. This caused inflammation and damage in the brain ("*extensive gliosis and an inflammatory response*", according to a laboratory document[27]).

81. During the BUAV investigation, a viral vector called adeno-associated virus used with a growth factor gene, was being tested as a therapy. Marmosets were injected into the brain with this prior to a 6OHDA lesion. Conversations with staff indicated that several marmosets (eg. **Cindy**) undergoing this experiment had experienced considerable weight loss.

Venepuncture problems

82. Some marmosets developed bruising from venepuncture for blood sampling. For example, records show that **Emu**, **Swan** and **Pongo** had this problem in 2000. Bruising should be avoidable by use of good technique, minimising repeated venepuncture, using sufficiently fine needles and applying compression immediately afterwards. The suffering inherent in these experiments should not be compounded by avoidable distress.

83. This licence also permits other combinations of protocols not described here, such as the surgical implantation of a brain cannula or electrodes; the surgical removal

[25] NRC (1998). The psychological wellbeing of non-human primates. p77, Publ. NAP, USA.
[26] "Neural transplants of porcine nigra in marmosets with unilateral 6-OHDA lesions".
[27] "Neural transplants of porcine nigra in marmosets with unilateral 6-OHDA lesions".

of brain grafts; injections of neuroactive drugs; injections into the brain of tracer chemicals; or the implantation of dialysis probes. All have the potential to cause even further suffering.

C.3. Animal suffering in PPL80/1344

84. This project is basic (ie. fundamental) brain research not involving an animal model of any human illness, as stated in section 17 of the project licence. Rather it is aimed at understanding the roles of, and connecting circuits between, specific brain regions. This is hoped to be relevant to cognitive and emotional disturbances found in some human disorders (such as dementia, depression or schizophrenia).

85. The research has two aspects:

i] cognitive disturbances (eg. learning, memory etc) and the roles of two brain areas called the prefrontal cortex and the striatum; and
ii] emotional or affective disturbances (related to mood, fearfulness, altered food preferences, changed social interactions), and the roles of two regions of the brain called the orbitofrontal cortex and the amygdala.

86. In both cases, the specific parts of the brains of marmosets were damaged, usually by stereotaxic injections of one of a range of toxins which selectively kill brain cells which use particular neurotransmitters (eg. 5HT or dopamine). The marmosets were pre-trained on specific tasks which measure cognitive or affective aspects of behaviour. After brain damaging injections, the marmosets were re-tested and also required to learn new tasks.

87. Because unwanted damage is done to the brain during the passage of the needle through superficial layers, control animals are also used who are given sham lesions. Thus in these experiments twice as many monkeys were used. In some experiments up to 18 toxin injections were made per animal (see below).

The cognitive disturbance experiments

88. A past experiment carried out by this team illustrates the approach. It used 20 marmosets to study the effect on prefrontal cortical function of lesioning dopamine cells in the caudate nucleus (using 6OHDA injections):

89. Marmosets were trained on various discrimination tasks while confined in a container placed against a touch-sensitive screen. They were also trained to remember which box contained a marshmallow when a delay was imposed between them seeing the marshmallow hidden, and being able to reach for it.

90. All 20 marmosets underwent their first operation. Anaesthetised and in a stereotaxic frame, they received a total of 18 injections to nine different locations on each side of the brain. Only the 10 experimental marmosets received the 6OHDA toxin, the others had control injections.

91. Cognitive testing started after two weeks. Five to eleven months after the first operation, the marmosets were anaesthetised again and a dialysis probe inserted

into the brain to find out whether the damaged brain cells had recovered in any way. This operation lasted 8.5 hours and marmosets were allowed 7-10 days' recovery before testing recommenced.

92. All the marmosets were killed after 18-24 months. Of 20 animals, "*two fell ill and died*" -- 1 control and 1 experimental. No further details were provided. In one animal, the dialysis probe blocked during the second operation.

The emotional disturbance experiments

93. Marmosets were trained and tested in learning and remembering associations which involve emotional aspects. Confined in a dark test box, they used a touch-sensitive screen displaying images such as a marmoset face, a snake, a banana and other symbols. The project licence also envisages analysing how brain lesions affect reactions to social cues such as marmoset vocal calls. A fruit juice or similar liquid reward was used to persuade thirsty animals to work.

94. In order to assess their emotional state, the blood pressure and heart rate of marmosets, both normal and lesioned, were recorded during tests. This was done via an implanted telemetry probe (see later).
The orbitofrontal cortex and amygdala, damaged in these experiments, are key components of brain networks involved in the emotions. They connect to other areas of the brain, and to the motor, endocrine and autonomic systems. Clearly, damage to these brain areas disturbs the animals' emotional wellbeing, as acknowledged by this group: "*...damage to the orbitofrontal cortex in humans and non-human primates can cause inflexibility, impulsiveness and emotional disturbance...*"

95. A student working under this licence was recorded as saying that one of the marmosets, **Sylvester**, was shown an unfamiliar picture on the computer screen during training. The marmoset's blood pressure rose as she darted to the back of the test box. The student acknowledged that Sylvester was frightened: "*It was just frightened, it didn't know what on earth was going on. So that was the most impressive thing we've seen with this animal*."

Post-operative effects

Box 4 (below) provides specific examples of the effects of brain lesions in named monkeys.

Box 4 Laboratory records for some marmosets after brain lesions

Rye was started on water deprivation in October 1997 when training began. He had a one-sided lesion of serotonergic (5HT) brain cells on 25 March 2001. When he recovered consciousness, he had fits and was given valium. By 29 March he still had fits when handled, was not eating normally, and had very dilated pupils; but was considered "*fine*" on 30 March. Later he was discovered to be blind and so was killed on 4 July 2001.

Turks was lesioned one evening in December 2001. According to laboratory records, the surgery was completed at 7pm and Turks had fits just after the operation. She had not regained consciousness by 2am. It appears she was then left overnight unattended. The next day she was conscious but still had not regained her posture. She vomited three times during the day and was very weak at lunchtime. The next day Turks was "*very alert & active, frightened*". She was returned to her home cage on the third day after the operation.

Aukland had a lesion to the medial prefrontal cortex in October 2001. He suffered fits and was given valium. He needed supplementary feeding on the second day. By the third day after surgery, according to laboratory records, he was still "*off balance, still tremors slightly when handled. Very sleepy*."

In general, the following effects occur:

-- post-operative pain (a painkiller is given if obvious signs of pain are observed).
-- temporary loss of appetite and weight loss (requiring special care).
-- physical problems, such as loss of balance and tremors.
-- mental disabilities e.g. memory and learning.
-- epileptic fits when certain toxins are used (see below).
-- mood and other emotional disturbances.

Implantation of telemetry probes

96. Under anaesthetic, a 1.75-inch incision was made through the skin of the abdomen, the muscles were cut and pulled aside, the intestines were lifted upwards and the main abdominal artery tied off. The sensor of the transmitter was placed in the artery via an incision which was sealed with glue and a patch. The artery ligature was released and blood flow restarted. The intestines were replaced, the transmitter was sewn into the abdominal muscle, and the muscle and skin were stitched and glued.

97. Telemetry probes are generally viewed as a means of reducing the suffering which would be caused by other methods of recording blood pressure or heart rate. However during 2000 alone, we know of two marmosets who were so badly damaged in this operation that they had very weak circulation in the lower half of their bodies and poor or no use of their legs, and had to be killed. On 26 April 2000, **Tweety** was unable to move legs or tail after surgery and, barely conscious the next day, was destroyed. **Minnie** had poor movement in the legs after surgery, also on 26 April 2000. Three days later, still unable to move properly, Minnie was weak, refusing solid food and obviously in pain, and was finally killed

98. During the BUAV investigation, five animals who had not yet been given brain lesions but were being trained to respond to images on the screen, were implanted with telemetry probes.

Distress during training and testing

99. The project licence states that "*...as the reward that is used to reinforce the animals' behaviour and thus enable us to test the cognitive and emotional capabilities of marmosets is a liquid reinforce*r", water deprivation is necessary. A liquid reinforcer is considered to be easier than a food reinforcer as there is no problem with satiation, and different liquids (eg. water or milkshake) can be provided via the same automated spout.

100. Animals will work for a preferred food and therefore the use of water restriction appears to be more of a convenience for researchers than a necessity. The marmosets were water deprived for 22 hours a day (with notional respite at weekends and for one week every six months) for up to 2.5 years. Some researchers worked the marmosets at the weekends, and so the required respite was not always given.

101. We were shocked to learn from a laboratory document[28] that the additional use of saline as an aversive stimulus for thirsty monkeys was being considered as part of a "*proposed student project*".

102. The project licence states that "*food deprivation is not necessary since undeprived marmosets will work for preferred foods*", the latter being pieces of marshmallow which were given as rewards for correctly performed tasks. Yet some of the marmosets were water deprived and on dietary restriction: they were fed only in the afternoon, after a testing session (ie. no breakfast), and/or they received only a bland, minimal diet. The researchers admitted that this minimal diet is unpalatable to marmosets. Some marmosets were also denied forage mix, which others were given on Fridays for the weekend.

103. The project licence claims that behavioural tests cause "*relatively little stress*", despite the fact that animals may be separated from their cage mates, confined in a small darkened box and, in some experiments, shown images intended to elicit alarm or fear.

[28] e-mail from licence holder to co-researcher, dated 2.10.2001.

104. These are long-term highly-invasive experiments during which marmosets were deprived of water, food restricted, brain damaged, confined in boxes and taught tasks, underwent second operations, and were subjected to drug injections and blood sampling.

D. CRITIQUE OF RESEARCH

105. Because of the high level of suffering which marmosets will experience – as a result of the totally unsuitable conditions in which they are housed as well as the experiments themselves – the likely benefits have to be correspondingly high if the cost:benefit test in section 5(4) of the 1986 Act is to be met. The quality of the science is therefore of crucial importance.

106. The potential benefits of any medical research are inevitably diminished if the experiments are not reproducible, or cannot be reliably extrapolated to humans. Here, we argue that the value of these research programmes has been overestimated; we believe that this is a wider problem in the licensing of animal experiments which urgently needs to be addressed.

D.1. Critique of research into stroke and cognition under PPL80/1249

Stroke research

107. The immediate and total blockage of a brain artery in an otherwise healthy young animal does not accurately 'model' a human stroke. Human stroke generally occurs in an older person with an underlying condition (such as gradually progressive atherosclerosis). This difference can lead to discrepancies in the outcomes of these two situations.

108. In rodents, the effects of blocking an artery on memory and learning are considered to vary according to species (rats versus mice)[29] . Even using different strains of the same rodent species leads to different degrees of brain damage being caused by the same experimental procedure. Thus the validity of extrapolating marmoset results to human stroke patients has to be questionable.

109. In rodent stroke research, conflicting results from different research groups are caused by: the precise method used to block the brain artery; the duration of occlusion; differences in the extent of brain damage; the timing with respect to circadian rhythms; and the length of post-operative recovery prior to testing[30]. All these variables are likely to affect marmoset experiments too, making the results even more difficult to extrapolate to humans.

110. The marmoset brain is only about 20 millimetres across (0.75 inch). On this scale, even tiny variations in the course of the artery or the site of blockage of the artery would result in significantly different magnitudes of stroke. The researchers' own surgical notes show this:

[29] De Vries, AC et al (2001). Cognitive and behavioural assessment in experimental stroke research: will it prove useful? Neuroscience & Biobehavioural Reviews 25:325-342.
[30] De Vries, AC et al (2001) Ibid.

Frank: "*1 single vessel occluded 1mm medial to olfactory tract*" (20.11.01);
Peggy: "*MCA occluded 1-2mm medial to olfactory tract*" (21.11.01);
Harkness: "*…as well as large artery there appeared to be a 'bundle' of… small vessels at the junction with the olfactory tract*" (7.11.01);
Lore: "*Artery… appeared to be single vessel*" (19.11.01).

111. In stroke research it is uncommon to find a significant correlation between brain histology (the size and nature of the lesion, seen post-mortem) and measured behavioural effects[31]. Reasons can include functional compensation by the brain or recovery, both of which would differ in experimental animals (of different species) with induced strokes compared to human patients. Behaviour is very complex and has built-in redundancy; and there are also large individual differences in the mechanisms underlying behaviour. Therefore measuring behaviour as an indication of stroke and treatment of stroke in one species, as a prediction for another species, is fraught with problems.

112. The lack of reliability and consistency of animal models of stroke have bedevilled research, making it very difficult to judge the value of different treatments -- as well as wasting animals' lives. The debate which started more than ten years ago in the scientific journals is still raging, over the value of animal models to develop neuroprotective drugs to treat stroke[32]. Several such drugs have proved effective in animals, but none has been safe and effective in clinical trials[33] [34].

113. There have been two very recent further failures. One is clomethiazole, used by this research team on marmosets but now found to be of no benefit to patients in two clinical trials[35] [36]. The second is Aptiganel, an NMDA receptor blocker which appeared to be neuroprotective in animals but has now been shown not to improve patient outcomes and even, possibly, to cause harm[37].

Fundamental cognitive experiments

114. This work aims to understand the brain chemistry underlying cognition, by damaging regions of the brain and/or connecting fibre tracts and/or specific brain cell types. The rationale is that this knowledge can be extrapolated to humans and help understanding of conditions such as Alzheimer's disease or amnesia.

---

[31] De Vries, AC et al (2001) Ibid.
[32] Wiebers, DO et al (1990). Animal models of stroke: are they relevant to human disease? Stroke 21:1-3.
[33] Liebeskind, DS & Kasner, SE (2001). Neuroprotection for ischaemic stroke: an unattainable goal? CNS Drugs 15:165-174.
[34] Jonas, S et al (2001). The failure of neuronal protective agents versus the success of thrombolysis in the treatment of ischemic stroke. The predictive value of animal models. Ann N Y Acad Sci 939:257-267.
[35] Wahlgren, NG et al (1999). Clomethiazole acute stroke study (CLASS): Results of a randomized, controlled trial of clomethiazole versus placebo in 1360 acute stroke patients. Stroke 30:21-28.
[36] Lyden, P et al (2002). Clomethiazole acute stroke study in ischemic stroke (CLASS-I): final results. Stroke 33:122-128.
[37] Birmingham K (2002). Future of neuroprotective drugs in doubt. Nature Medicine (news) 8:5.

115. However, the precise location and importance of different kinds of brain cells and fibre tracts are bound to vary between marmosets and humans. Such species differences have already been found by other researchers: for instance, when FMRI[38] studies were conducted to locate the spatial working memory area in humans, it was in a different location from that found previously in monkeys[39]. Other examples of species variations, from research into vision and brain function, illustrate the possibilities:

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

-- The human frontal eye field, associated with eye movements, is located in a different part of the brain in humans compared with monkeys[40].

-- Layer 4A is an important visual area of the brain. In humans, it has different neural inputs from those of macaque monkeys[41]. It also has a unique arrangement of other significant brain cells different even from that of the chimpanzee.

116. Additionally, monkeys with medial temporal lobe ablation (generally considered as models for a form of human amnesia), show different impairments in learning object discrimination tasks compared with amnesic patients[42]. This suggests that monkeys and humans may learn such tasks in different ways.

117. These examples illustrate why the marmoset results cannot reliably be extrapolated to people, and why research to discover the circuitry of the human brain should be conducted non-invasively in human volunteers instead (see section 6 on alternatives).

118. An enormous complexity of interactions underlies functions such as thought, memory, and learning; and there is built-in redundancy. For these reasons, trying to understand the whole by damaging one part and then another and then making behavioural observations is a limited approach, especially when there is individual variation in the location and size of lesions caused.

119. To illustrate, in one experiment by these researchers, five monkeys who had been given the same lesions showed quite different cognitive effects. Two damaged animals performed well at a new concurrent learning task, while the other three were unable to learn the task. Post-mortem analysis of the lesions did not reveal any anatomical basis for the differences in performance between the monkeys.

---

[38] fMRI is functional Magnetic Resonance Imaging.
[39] Courtney, SM et al (1998). An area specialized for spatial working memory in human frontal cortex. Science 279:1347-1351.
[40] Petit, L et al (1997). J. Neurophysiology 77:3386-3390.
[41] Preuss, TM et al (1999). Distinctive compartmental organisation of human primary visual cortex. Proc. Natn. Acad. Sci. 96:11601-11606.
[42] Hood, KL et al (1999). An evaluation of the concurrent discrimination task as a measure of habit learning: performance of amnesic subjects. Neuropsychologia 37:1375-1386.

120. On at least one occasion there were problems with injections of an immunotoxin called saporin into marmosets' brains, apparently because the syringe did not deliver the correct amount. This was only discovered at the end of the experiment when the marmosets were killed and histology was performed on the brain, revealing incomplete cholinergic lesions.

121. The researchers have had problems in creating an identical lesion in each animal, especially when methods of damage such as suction ablation or cutting are used. Lesions may vary in size, completeness, location and effect, and unintended extra damage is sometimes caused to nearby brain tissue, making it more difficult to interpret results (and using even more animals). For example, handwritten laboratory notes reveal:

Ski: "*OK. Difficult to find fornix*[43]*. I think I was a bit too far forward. Am fairly confident I got it but probably did a bit extra damage*" (3.9.01).

Lee: "*Transection less convincing – more messy*" (14.8.01).

122. An experiment carried out by this group further illustrates the difficulties. In this experiment marmosets underwent three kinds of bilateral brain damage. One monkey was subsequently removed from the study because damage had unintentionally been done to an additional area of the brain (the superior temporal cortex). Another monkey did not have complete section of the temporal stem (as it should have), suffered a "*large infarct*" (unintended stroke) and was excluded from analysis. Further details were provided in this paper (section 3) showing that there was considerable variability in the completeness of transections and the extent of damage caused. In another experiment, the degree of damage inflicted by some lesions also varied between individuals.

123. An additional experiment carried out by this group describes how ablation of the inferotemporal cortex could vary. Out of a total of 12 marmosets, "*In one monkey the lesion was small in one hemisphere and in one further monkey the ablation did not quite include all the perirhinal cortex in either hemisphere… There was a very small amount of damage in the amygdala in one hemisphere in each of two monkeys and in the tail of the caudate nucleus in one hemisphere of each of two other monkeys... There was no simple relation between total size of lesion, or that part of the lesion which was bilateral, in each monkey and the total learning score for that monkey.*"

124. Such inconsistencies in lesions make interpretation of results very difficult, and waste more animals' lives. Potential species differences further undermine the value of the work. The costs to animals have been underestimated and the potential human benefits have been exaggerated. We believe it is time for these experiments to stop and the licence to be revoked.

[43] The fornix, inter alia, carries fibres from the VDB to the entorhinal cortex and the hippocampus.

D.2. Critique of Parkinson's disease research under PPL80/1326

125. The 6OHDA-lesion marmoset model resembles human Parkinson's disease superficially. Some scientists in this field believe that marmosets are inherently unsuitable for Parkinson's disease research. The aim here was to select some specific, stable symptoms to enable them to test experimental treatments; yet the researchers cannot know whether or not they have chosen the correct symptoms for this purpose and whether the results will relate to human patients. In the marmoset, the onset of parkinsonism is immediate and caused by a toxin but there is partial and variable recovery over time. In humans onset of Parkinson's disease is gradual, of unknown cause, progressive and there is no spontaneous recovery.

126. The marmoset model is simplistic compared to the complexity of the human condition. For example, in marmosets only dopamine cells are damaged whereas in humans other types and pathways of brain cells are affected too; plus marmosets do not develop Lewy bodies[44] in their brains. Parkinson's disease tends to affect older people, while the marmosets are young.

127. Some behavioural symptoms in marmosets do not correlate with others. For example, some monkeys show complete neglect of a label on their foot (suggesting a severe lesion), but do not rotate at all (suggesting a minor lesion). This variability complicates interpretation. Moreover, given this variability, scientists do not know which symptoms (if any) would be most relevant for the human condition.

128. The researchers do not appear to have succeeded in producing a stable animal model. There are individual differences in the various behavioural symptoms of marmosets after an injection of 6OHDA, and some marmosets partially recover over time, to different extents, even without treatment. Body rotation rates vary between animals (there is no similar feature in the human condition). Compensatory mechanisms in surviving brain cells may be different in lesioned marmosets and in humans with Parkinson's disease. These factors lessen the likelihood of drawing useful conclusions about effective treatments (as well as wasting more animals).

129. One aspect of the current research is to develop a stable "*partial lesion*" model of parkinsonism in marmosets, to test growth factor gene therapies. However, the results from some sham (control) animals overlapped with those from some experimental animals. Lesioned and sham animals did not differ in their overall activity levels. Moreover, so far the partial model has produced transient and selective effects in the animals, with gradual recovery after several weeks.

130. One measure of monitoring the severity of the marmosets' condition was the use of a disability rating scale. Behaviours were observed, both spontaneous and induced (the animals were offered a marshmallow treat and were scored for gait, bradykinesia[45] and balance). The rating was made on a subjective basis and hardly seems a precise enough measure for research of this kind.

[44] Lewy bodies are inclusions in the brains of patients, and are considered to be the pathological hallmarks of Parkinson's disease.
[45] Bradykinesia is abnormal slowness of movement.

131. Brain grafting experiments have been done by this group. However, it is becoming clear that the success of graft survival, integration into the brain and functioning, are all affected by aspects such as housing conditions and the particular behavioural experience and training of the animals. The resulting variation will render the results very difficult to interpret.

D.3. Critique of fundamental cognitive and affective research under PPL80/1344

132. The researchers appear to assume that the role and function of specific brain areas and circuits connecting them, as well as the behavioural effects of lesions, will be sufficiently similar in humans and marmosets to permit useful and precise extrapolations. However, increasingly, studies of the human brain reveal that there are significant species differences in these parameters.

133. For example, differences in the size, location and organisation of the primary visual area of the brain have been found between humans and macaque monkeys (more closely related to humans than marmosets). Comparative studies between eleven primate species whose brain volumes spanned a more than 50-fold range, revealed that the evolution of larger brain sizes involved changes in interhemisphere as well as intrahemisphere connections[46]. In terms of brain volume, marmosets are obviously at the other end of the range to humans. Therefore these connectivity differences are likely to impact the validity of marmoset findings for humans.

134. Studies of patients with specific brain damage have sometimes illustrated species differences in cognition: humans with lesions to the medial temporal lobe cannot perform concurrent discrimination tasks normally, although monkeys with this lesion can[47].

135. Indeed, in the project licence the licensee admits that species differences have already been found, even between macaque monkeys and marmosets, in certain important cells in the amygdala and projections from the thalamus (section 18). Despite the fact that this renders findings from marmosets almost certainly unreliable for human extrapolation, the licensee – appearing to display a complete lack of interest in animal suffering – writes: "*These intriguing differences between the organisation of the rhesus and marmoset amygdalo-prefrontal-thalamic connections require further investigation*".

136. With such significant differences between marmosets and humans in neural circuitry, or in the role of different neurotransmitters in the brain, then not only have the lives of a huge number of animals been wasted but also progress in understanding the human brain will actually have been delayed.

137. In experiments using the toxin 6OHDA, there is often a gradual recovery of function over time even in the absence of treatment. The instability of the lesion means that a larger numbers of animals are used, and subjected to additional

[46] Rilling, JK & Insel, TR (1999). Differential expansion of neural projection systems in primate brain evolution. Neuroreport 10:1453-1459.
[47] Corkin, S (2002). What's new with amnesic patient HM? Nature Reviews Neuroscience 3:153-160.

experiments (ie. a second, lengthy operation for microdialysis studies to confirm the levels of neurotransmitters in the brain).

138. Because of variation in the brains of marmosets, standard brain co-ordinates cannot be used without individual adjustments. This means, as admitted by the licensee, that an "*extra one or two animals*" per group are brain-damaged to ensure that there are enough correctly lesioned marmosets for the experiment.

139. Measuring the effects of lesions by the marmosets' behaviour in certain tasks is compounded by problems. An enormous complexity of interactions underlies functions such as thought, memory, and learning; and there is built-in redundancy - when one part of the brain is lesioned, another area may undertake some of its functions. This reorganisation may not be the same, either qualitatively, quantitatively or temporally, in marmosets as in humans. For these reasons, trying to understand the whole by damaging one part and then another and then making behavioural observations is a limited approach, especially when there is individual variation in the location and size of lesions caused.

E. LICENSING ISSUES

140. This investigation highlights fundamental issues of concern regarding the regulation of animal experiments in Britain, in particular how the cost:benefit assessment is conducted. We summarise the main issues arising out of the research at Cambridge University:

- as we have seen, most of the protocols described in the licences were given a severity limit of 'moderate'. None was given a severity limit of 'substantial'. This means that the Home Office was accepting that not a single animal would ever experience 'substantial' suffering (let alone 'severe' suffering: see below). This is totally unsustainable, even when viewed in terms of the licences themselves (i.e. without taking account of suffering which was inevitable or likely but which was omitted from the licences)

- even had it been reasonable to set severity limits below substantial when granting the project licences, that would not be the end of the matter. A number of standard conditions are attached to licences under section 10(1) of the 1986 Act. Standard condition 6 stipulates that '[f]or any procedure, the degree of severity imposed … shall not exceed the severity limit attached to the procedure'. Under standard condition 8, the project licence-holder must promptly notify the Home Secretary if a severity limit has been, or is likely to be, breached. The Home Office guidance[48] indicates that this is so that cost:benefit can be reassessed. There can be no doubt that many marmosets did experience substantial or severe suffering, such that the Home Office should have been informed. It should then have reassessed cost:benefit.

- the three project licences should have been given an overall severity banding of 'substantial'. According to the Home Office guidance, the overall severity

[48] para 5.44

banding should reflect the 'cumulative effect of each procedure'[49]. The guidance continues[50]:

> 'The assessment of the severity band for the project as a whole reflects the number of animals used on each protocol and the actual suffering likely to be caused as a result. It is based on the overall level of cumulative suffering to be experienced by each animal, not just the single worst possible case. It takes into account the proportion of animals expected to reach the severity limit of the protocol and the duration of the exposure to that severity limit, the nature and intensity of the adverse effects, and the actions to be taken to relieve the suffering'.

In other words, the guidance seems to advocate a global approach. It is questionable whether such an approach is lawful, at least when a project licence contemplates – as do those here – a wide variety of procedures involving large numbers of animals. For example, the effect of including 'mild' procedures in a project licence along with 'substantial' ones might, in a particular case, be that the project is given a 'moderate' banding, despite the fact that some animals will experience substantial suffering. However, in these cases, there was no 'averaging down' (because there were no substantial severity limits): both Cambridge University and the Home Office simply downplayed the level of suffering which many of the marmosets would inevitably experience (and did experience).

- the Home Office guidance also contemplates 'severe' suffering. Paragraph 4.2 says that '[t]he Secretary of State [Home Secretary] will not license any procedure likely to cause severe pain or distress that cannot be alleviated [Section 10(2A)]' This is because of Article 8(3) of the 1986 directive requires member states to ensure that '… in any event the animal is not subject to severe pain, distress or suffering'. In correspondence with the BUAV[51], the Home Office have stated that any severe suffering must be alleviated promptly (if necessary by euthanasia), such that it would only be 'transient'.

  It is not clear how 'severe' suffering is supposed to differ from 'substantial' suffering. The Home Office has been unable to explain the distinction. Most people would regard the level of suffering contemplated by the definition of 'substantial' in the Home Office guidance as 'severe'. As far as the Home Office is concerned, the distinction is not material in the present case (because it claims not to have contemplated either form of suffering). In fact, we believe that common sense dictates that severe suffering should have been in contemplation *and* that it actually occurred, without being promptly alleviated. Indeed, as we shall see, the levels of staffing were such that for long periods there was simply no one on hand to alleviate such suffering.

- effects on the emotional well-being of animals - for example those arising out of the wholly unsuitable conditions in which the marmosets found themselves - are

[49] para 5.47
[50] para 5.48
[51] letter of 24 April 2002

often underestimated or even discounted. This would inevitably skew the cost:benefit assessment

- some protocols which clearly cause distress appear not to be regulated, and presumably therefore are not 'counted' in the cost:benefit assessment

The underplaying of the suffering – both in prospect and in reality – is significant in a number of respects:

- primate experiments of substantial severity are referred, as a matter of policy, to the APC, the Government's advisory body, before they can be granted. By classifying project licences as less than 'substantial', both the licensees and the Home Office inspectorate avoid scrutiny by the APC. This is clearly to the advantage of the researcher. This may be the only logical explanation why the project licences in question were categorised as moderate.

- it inevitably skews the cost:benefit assessment by permitting experiments to take place which might not have been allowed had an accurate classification been made.

- it distorts the Home Office annual statistics (which set out how many procedures there are for each level of severity). The public is thereby misled into believing that fewer animals suffer substantially or severely than is in fact the case[52]. We strongly suspect, from other available evidence, that this is a widespread problem.

E.1. Licensing issues: project licence PPL80/1249

Severity and cost:benefit assessment

141. This licence permits many combinations of interventions: different kinds and causes of brain damage, with up to four different lesions in one animal; desired as well as undesired effects of lesions; and different kinds of experimental treatments, some of which themselves cause toxic outcomes. The cost:benefit test is impossible to apply properly in such circumstances.

142. Although measures are proposed in the licence for treating unwanted symptoms in animals (eg. pain, psychosis, epilepsy), it is unrealistic to imagine that, in a busy laboratory which is not staffed at night and has a skeleton staff at the weekends, the animals' suffering could be continuously alleviated.

143. Marmosets with neurological symptoms, psychosis, physical disabilities or disrupted social interactions, are highly likely to be suffering emotional distress. To take one example, lesions of the entorhinal cortex, as inflicted in this research, are known to cause emotional impairments. Emotional suffering in marmosets,

[52] this will also happen if (in other cases) substantial procedures are lumped together with mild and moderate ones, giving an overall severity banding of 'moderate'

unless it produced overt physical or behavioural symptoms and there were staff present to identify this and take appropriate action, would not receive treatment.

144. The licence reads like a classic exposition of the now-outdated behaviourist school of psychology, which only attended to behavioural outcomes and ignored the subjective experiences of the animals, simply because these could not be measured directly. It is very difficult to see that the animals' emotional suffering could have been adequately considered in the cost:benefit assessment for this work.

145. Several animals only received a single pain-killing injection post-operatively (according to the laboratory records) and were usually left overnight without attention. They must have suffered grievously.

146. This project licence was banded as moderate severity. We believe that this was a gross underestimation of the suffering involved, which was certainly substantial for many animals. As such, the Home Office guidance was not followed in the cost benefit analysis.

Apparent deviations from the licence authority

147. The licence states that "*food deprivation*" is not required. However, food restriction (e.g. no breakfast or half rations) was sometimes used to encourage marmosets to 'work' in training and testing tasks. Having food withheld while seeing other marmosets being fed is likely to be stressful. This should have been included as a regulated procedure in the licence.

148. In this we are supported by the Home Office Guidance (para. 2.15) which states: "*Regulated procedures may be acts of commission (such as dosing or sampling) or of deliberate omission (such as withholding food or water)*."

149. The licence also states that "*an osmotic minipump*" may be implanted and later surgically removed (section 19.4). In fact, some animals had six pumps implanted and removed, involving three surgical operations. This would have exceeded the licence authority unless an amendment was sought and obtained in advance.

E.2. Licensing issues: project licence PPL80/1326

Severity and cost:benefit assessment

150. This licence has a moderate severity banding, yet permits numerous interventions: different kinds and causes of brain damage; and different kinds and combinations of experimental treatments. The injection into the brain of one toxin might have more severe effects than another. Some animals have one-sided lesions and others have bilateral damage.

151. The licence allows the use of unnamed "*other novel toxins*", as well as unnamed viral vectors and unspecified genes for experimental gene therapy – all of whose effects on the marmosets' wellbeing cannot be taken into account if they are not specified in the licence. In the past this research group has used a viral

vector which caused brain inflammation in marmosets, and the same could happen again.

152. As we have seen, it was recognised that some marmosets might experience "*severe*" pain post-operatively, according to the project licence. If severe pain was anticipated as a possibility, given the possibility that it might be overlooked even for a short time the protocol should have had a substantial severity limit.

153. The food intake of marmosets being used in Parkinson's disease experiments was restricted for the purposes of training and testing. Water restriction has also been used for Huntington's disease research, despite the fact that section 19b of the licence states that "*...marmosets will work for preferred foods*." This suggests that using water deprivation to train animals is more of a convenience than a necessity, if food rewards would work. The researcher argues that fluid rewards allow better control and are more convenient to administer automatically. This cannot possibly justify keeping animals without water for 22 out of every 24 hours (with notional respite at weekends and every six months). Distress is inevitable if an animal cannot drink when thirsty (and of course if water restriction were not stressful then there would be no need for respite).

154. It is generally recognised that water deprivation is an undesirable method of training. It is not clear whether water deprivation was counted as a cost in the cost:benefit assessment, but it clearly should have been.

155. Most training and testing procedures are not counted as regulated protocols in this project licence. We argue (in detail, above, under *Animal suffering*) that these do cause distress and should be regulated. Some marmosets used for Huntington's disease experiments started training on tasks in 1998, but proved too "*timid*" or otherwise temperamentally unsuited to training, and did not continue in this project. Nevertheless, these individuals had endured the distress of water deprivation and repeated confinement in the testing apparatus without apparently being included in the Home Office statistics (see below).

156. If animals do not appear in the statistics, despite having endured distress or fear, then confidence is damaged in the accuracy of the Home Office statistics and the numbers of primates used in British laboratories is underestimated.

157. This licence also reads like a document from the discredited behaviourist school of psychology, attending primarily to obvious behavioural outcomes of interventions and ignoring the animals' subjective experiences, simply because these cannot be measured directly. The animals' emotional suffering appears not have been adequately considered in the cost:benefit assessment for this work.

158. The combination of disabilities caused in marmosets – physical, cognitive and emotional – certainly warrant this licence having a substantial severity banding. The fact that it was given a moderate banding is a cause for great concern about an assessment which lies at the heart of the 1986 Act.

Apparent deviations from the licence authority

159. Although water deprivation is mentioned in this licence, it appears from laboratory records that it was not always considered by staff to be a regulated procedure. Animals used under this licence (as part of Huntington's disease research) who had been deprived of water were not always counted in the Home Office Returns. **Xanadu**, for example, was water-deprived for training, although notes on a laboratory document say "*Not HO returned but only had water deprivation.*" Other animals who had been water deprived in 1998 but apparently not entered in the Home Office Returns, according to laboratory records, included **Date, Randall, Mince Pie,** and **Lakeside**.

E.3. Licensing issues: project licence PPL80/1344

Severity and cost:benefit assessment

160. These are long-term experiments during which marmosets were deprived of water, food restricted, brain damaged, confined in boxes and taught tasks, shown fearful stimuli, endured second operations, and were subjected to drug injections and blood sampling. They also suffered physical symptoms such as vomiting, loss of appetite, tremors and epileptic fits.

161. The licence acknowledges that post-operatively some animals have mood alterations. Other sources make it clear that animals with these lesions can experience profound emotional disturbances, as we have shown above. In general, emotional suffering has been underplayed in the project licence and appears not to have been taken adequately into account in the project's severity banding. If marmosets are considered as suitable surrogates for humans in this kind of research, it must be acknowledged that they experience emotional and cognitive suffering similar to that of humans.

162. The licence refers to possible rare problems with telemetry probe implantation in the abdomen. It mentions wound infection, discomfort due to the pressure of the device or peritonitis. There is no mention that animals could be partially paralysed (we know of two who were) and presumably, therefore, this possibility was not considered when the cost:benefit analysis was made and the severity banding assigned.

163. Water deprivation lasted 22 out of 24 hours for 5 in every 7 days, for up to 2.5 years, but is considered to be mild in effect. Some respite was given on the weekends plus free access to water for one week every 6 months. As already stated, distress is inevitable if an animal cannot drink when thirsty, and if water restriction were not stressful then there would be no need for respite. Water deprivation is an undesirable method of training. We suspect that water deprivation was, wrongly, not counted as a significant cost in the cost:benefit assessment.

164. The project licence states: "*All behavioural tests involve relatively little stress*" (section 19), despite the fact that marmosets are known to dislike handling[53] and are sensitive to being separated from peers or taken from a familiar cage. In some tests animals are separated from their cagemates, taken from their home cage, confined in a small, darkened box and shown images intended to elicit alarm or fear. One of the researchers was recorded describing the reaction of a marmoset frightened by an unfamiliar image. "Relatively little stress" is an understatement of the animals' experiences in training and testing.

165. The licence also claims that food deprivation is not necessary, yet in practice food restriction (e.g. no breakfast, and/or a bland, minimal diet) was used to motivate animals to work. Presumably this cost to the animals – being unable to eat while other animals are being fed -- was not counted in the cost:benefit assessment. This appears to be a deviation from the licence authority.

166. It is clear that the project licence banding should have been substantially, not moderately, severe and this underestimation of severity is a very important and significant failure in licensing.

F. NON-ANIMAL RESEARCH

167. The BUAV has always argued that research should be undertaken without animal experiments, especially if the research questions are framed in a slightly different way from the orthodox approach. For example, functional imaging studies in humans may lack a degree of precision (e.g. columns of brain cells versus single brain cells), but the quality and relevance of the results to humans can be far greater (for example there are no species differences).

168. Both the 1986 directive and the 1986 Act prohibit the use of animals where non-animal methods are reasonably available[54]. This requirement should not be interpreted only in the narrow sense of a direct replacement of each animal experiment by an alternative, on a one-to-one basis. This is sometimes feasible, especially when human studies are possible in place of animal experiments; but often a different way of tackling research issues, involving the creative use of combined non-animal methodologies, will be necessary. This must be pursued if the law is to be properly implemented.

169. We are aware that non-animal research, including clinical research, is funded by bodies such as the Wellcome Trust and the MRC. It is against both the letter and spirit of the legislation to allow animal experiments to take place alongside non-animal research which would give the equivalent information.

170. In the following sections some appropriate non-animal methods are described.

[53] The UFAW (Universities Federation for Animal Welfare) Handbook on the care and management of laboratory animals 1999 Seventh Edition Volume 1.

[54] This is under Article 7.2 of the 1986 directive and section 5(5)(a) of the 1986 Act. In addition, under Article 7.3 and section 5(5)(b), a particular animal method must not be used if the equivalent scientific result could be achieved through use of fewer animals or less invasive techniques.

F.1. Alternatives to cognitive and stroke research under PPL80/1249

171. Functional imaging techniques such as fMRI and PET[55] can be used in human volunteers to study how different centres in the brain are connected and what kinds of brain cells are involved in cognitive or motor tasks.

172. Magnetoencephalography (MEG) reveals the chronology of brain activity in real time, showing how different brain regions interact. Combined MEG and fMRI can provide high-resolution localisation of functional responses over time in the human brain.

173. To illustrate, cognitive research using human volunteers and PET imaging has been conducted at the Wellcome Department of Cognitive Neurology in London. It revealed that attentional modulation of cortical activity in object categorisation tasks is linked to two physiologically distinct mechanisms acting on spatially distributed areas[56]. This kind of approach can be applied to some of the questions being asked in the research into cognition under this licence.

174. The neural bases of learning and memory, including large-scale neural networks of brain regions, have also been studied by functional imaging in human volunteers[57] [58] [59]. A recent review of human studies discussed finding about the roles of the prefrontal cortex and amygdala as well as the circuitry involved[60]. Event-related fMRI has been used in volunteers to study areas such as the entorhinal cortex of the hippocampus formation and its connections[61]. These are regions of the brain which have been lesioned in marmoset experiments.

175. A new method called Diffusion Tensor-MRI (DT-MRI) now permits the non-invasive tracking of brain cell connections between different regions of the living human brain in volunteers[62].

176. More resources should also be available for the study of patients who have suffered specific kinds of brain damage. For example, three amnesic patients with damage limited to the hippocampal formation volunteered for intensive neuropsychological studies while they were alive. They also gave permission for their brains to be analysed post-mortem. The combined neuropsychological and

---

[55] fMRI is functional magnetic resonance imaging and PET is positron emission tomography.
[56] Rees, G et al (1997). Two modulatory effects of attention that mediate object categorisation in human cortex. Science 275:835-838.
[57] Cabeza, R & Nyberg, L (2000). Neural bases of learning and memory: functional neuroimaging evidence. Curr. Opin Neurol. 13:415-421.
[58] Nyberg, L et al (2000). Large scale neurocognitive networks underlying episodic memory. J. Cogn. Neurosci. 12:163-173.
[59] Cabeza, R & Nyberg, L (2000). Imaging cognition II: An empirical review of 275 PET and fMRI studies. J. Cogn. Neurosci. 12:1-47.
[60] Davidson, RJ (2002). Anxiety and affective style: role of prefrontal cortex and amygdala. Biol. Psychiatry 51:68-80.
[61] Ploghaus, A et al (2001). Exacerbation of pain by anxiety is associated with activity in a hippocampal network. J. Neurosci. 21:9896-9903.
[62] Conturo, TE et al (1999). Tracking neuronal fiber pathways in the living human brain. Proc. Natn. Acad. Sci. 96:10422-10427.

neuropathological studies contributed important findings about memory and the hippocampus in humans[63].

177. With regard to stroke research, imaging techniques have been used to measure the processes occurring within the brain after a stroke in human patients. Combinations of PET and TMS[64] enable studies of how the brain re-organises after stroke and identifies new brain pathways. Post-mortem fixed brain tissue from stroke patients can be used in biochemical tests to identify the time course of neuronal death after different kinds of stroke. This provides information about the speed with which neuroprotective treatment would need to be applied.

178. For new neuroprotective drugs, mechanisms of action can be demonstrated in in vitro studies, for example effects on calcium and sodium channels and cell receptors. Human blood-vessel cells in culture and long-term aggregating brain cell cultures have also been used to study key molecules, targets and pathological processes in stroke. Such approaches can be combined with human studies at ultra-low, safe doses, with PET imaging to reveal the brain penetration profile and distribution characteristics. Drugs which act by increasing regional cerebral blood flow (a way of limiting stroke damage) can be studied in humans via PET, transcranial Doppler and near infrared spectroscopy[65].

F.2. Alternatives to Parkinson's disease research under PPL80/1326

179. Functional imaging has a key role to play in human studies of Parkinson's disease, thereby avoiding problems of species differences and artificiality of the model.

180. PET imaging has been used to measure levels of dopaminergic activity in the striatum of volunteer Parkinson's patients[66]. This adds to knowledge of the pathophysiology of the condition, and permits direct study of disease progression at a biochemical level. It also enables monitoring of potential effects of new treatments. PET imaging has revealed disturbances of brain functional interactions and cognitive information processing deficits in Parkinson's patients; and has provided direct human evidence for the role of the caudate nucleus in certain cognitive tasks in patients[67] [68].

181. PET has also shown that impaired frontal lobe function in patients with Parkinson's disease is related to low dopaminergic activity in the caudate

[63] Rempel-Clower, NL et al (1996). Three cases of enduring memory impairment after bilateral damage limited to the hippocampal formation. J. Neurosci. 16:5233-5255.
[64] TMS is transcranial magnetic stimulation.
[65] Bonoczk, P et al (2000). Role of sodium channel inhibition in neuroprotection: effect of vinpocetine. Brain Research Bulletin 53:245-254.
[66] Leenders, KL & Oertel, WH (2001). Parkinson's disease: clinical signs and symptoms, neural mechanisms, positron emission tomography, and therapeutic interventions. Neural. Plast. 8:99-110.
[67] Weder, B et al (2000). Disturbed functional brain interactions underlying deficient tactile object discrimination in Parkinson's disease. Hum. Brain Mapp. 11:131-145.
[68] Weder, BJ et al (1999). Impaired somatosensory discrimination of shape in Parkinson's disease: association with caudate nucleus dopaminergic functions. Hum. Brain Mapp. 8:1-12.

nucleus[69]. PET and SPECT[70] allow the non-invasive assessment of changes in dopamine receptor density, and the effect of brain grafts or neuroprotective therapies in Parkinson's patients[71]. A further use of PET is illustrated by research very similar to the rotation tests used by the Cambridge researchers. In volunteer patients with Parkinson's disease, injections of apomorphine provided in vivo evidence that direct dopamine agonists can inhibit the release of endogenous dopamine, possibly by the activation of presynaptic D2/D3 dopamine receptors[72]. fMRI has been applied to Parkinson's patients to study impaired connectivity between frontal cortical regions of the brain[73].

182. Some researchers are studying in cell culture the role of the protein synuclein[74] (found in Lewy bodies in the brains of Parkinson's patients, and used in some of these marmoset experiments). Cell cultures have also been used to study oxidative stress and microglial activation as factors in Parkinson's disease.

183. Interestingly, while the licensee's team has been doing gene therapy experiments with a growth factor on marmosets, clinical trials using an implanted pump to deliver the same growth factor directly to the brain of patients, have already taken place. Clinical trials provide the most relevant method of assessing these kinds of therapies; but some scientists believe that 'high-tech' and highly-invasive approaches such as gene therapy or grafting in the brain are unlikely ever to be practical.

184. On the other hand, more resources clearly should be dedicated to human studies to elucidate the genetic and environmental factors[75] which interact to cause Parkinson's disease. A molecular genetic approach has identified three genes and two or more additional loci in rare familial forms of Parkinson's disease[76]. Strategies for prevention will not be forthcoming until causation is understood, and artificial animal models of parkinsonian symptoms are highly unlikely to reveal the causes of the disease in humans.

F.3. Alternatives to fundamental cognitive and affective research under PPL80/1344

185. Brain lesioning methods in marmosets (or other animals) can identify which brain regions are critical to particular cognitive or affective functions (in those

---

[69] Bruck, A et al (2001). Positron emission tomography shows that impaired frontal lobe functioning in Parkinson's disease is related to dopaminergic hypofunction in the caudate nucleus. Neurosci. Lett. 311:81-84.
[70] SPECT is single photon emission computed tomography.
[71] Thobois, S et al (2001). Contributions of PET and SPECT to the understanding of the pathophysiology of Parkinson's disease. Neurophysiol. Clin. 31:321-340.
[72] de La Fuente-Fernandez, R et al (2001). Apomorphine-induced changes in synaptic dopamine levels: positron emission tomography evidence for presynaptic inhibition. J. Cereb. Blood Flow Metab. 21:1151-1159.
[73] Rowe, J et al (2002). Attention to action in Parkinson's disease: impaired effective connectivity among frontal cortical regions. Brain 125:276-289.
[74] Tofaris, GK et al (2001). alpha-synuclein metabolism and aggregation is linked to ubiquitin-independent degradation by the proteasome. FEBS Letters 509:22-26.
[75] Pezzoli, G et al (2000). Hydrocarbon exposure and Parkinson's disease. Neurology 55:667-673.
[76] Shastry, BS (2001). Parkinson's disease: etiology, pathogenesis and future of gene therapy. Neurosci. Res. 41:5-12.

species), but do not provide any information about the relative timing of activity in various areas. This chronology of activity is important for revealing how different areas functionally interlink.

186. Many of the functional imaging techniques described as alternative approaches to cognitive research under licence PPL80/1249 are equally applicable here. Indeed, one of this licensee's colleagues is using PET imaging with humans in similar work.

187. Magnetoencephalography (MEG) applied to the human brain does record brain activity in real time with an excellent temporal resolution of 1-100 milliseconds. Combining MEG with fMRI imaging (spatial resolution at the millimetre level) can provide localisation of functional responses at very high resolutions, in time and space, in human volunteers. In this way, for example, scientists have recorded cortical activity during semantic processing in the human brain, revealing a wave of activity spreading from the occipital visual cortex to temporal, parietal and frontal areas within 185 milliseconds[77].

188. Transcranial magnetic stimulation (TMS) is used to cause safe, temporary and reversible brain 'lesions' in healthy human volunteers. It thus offers a non-invasive and relevant alternative to many lesioning studies in animals, by providing insight into brain areas which are critical for certain functions. For example, TMS has been used in volunteers to demonstrate the role of the parietal cortex in novel and learned visual conjunction search tasks, and in visual neglect[78]. TMS even permits discrimination of a brain area which is crucial to learning a task but may not be necessary once the task is learned, and it has the potential to be combined with PET and fMRI. The prefrontal cortex, an area of interest to the Cambridge researchers, can be disrupted and its role thus studied by TMS in humans.

189. The neural bases of learning and memory, including large-scale neural networks of brain regions, have also been studied by fMRI and PET in human volunteers[79 80 81]. PET scans with radiolabelled ligands have enabled studies of neurotransmitter fluxes in volunteers during behavioural tasks, for example increased rates of binding and release of dopamine from D2 receptors in the striatum [82], an area of particular interest to the Cambridge scientists.

190. A new method called diffusion tensor magnetic resonance imaging (DT-MRI) offers for the first time ever the opportunity to map, in three dimensions, neuronal

---

[77] Dale, AM et al (2000). Dynamic statistical parametric mapping: combining fMRI and MEG for high-resolution imaging of cortical activity. Neuron 26:55-67.
[78] Stewart, L et al (2001). The role of transcranial magnetic stimulation (TMS) in studies of vision, attention and cognition. Acta Psychologica 107:275-291.
[79] Cabeza, R & Nyberg, L (2000). Neural bases of learning and memory: functional neuroimaging evidence. Curr. Opin Neurol. 13:415-421.
[80] Nyberg, L et al (2000). Large scale neurocognitive networks underlying episodic memory. J. Cogn. Neurosci. 12:163-173.
[81] Cabeza, R & Nyberg, L (2000). Imaging cognition II: An empirical review of 275 PET and fMRI studies. J. Cogn. Neurosci. 12:1-47.
[82] Koepp, MJ et al (1998). Evidence for striatal dopamine release during a video game. Nature 393:266-268.

fibre tracts between different regions of the living human brain without harm to volunteers[83]. This is a potential replacement of invasive and necessarily terminal experiments on marmosets involving the injection of tracers into the brain.

191. In affective processing, the roles of different regions of the brain and circuits between them are also studied in humans. A recent review of human research discussed the roles of the prefrontal cortex and amygdala in anxiety and affect, as well as the circuitry involved[84]. Event-related fMRI has been used to study anxiety and pain exacerbation in volunteers, with emphasis on the entorhinal cortex of the hippocampus formation and its connections[85]. The same technique was applied to research with volunteers in which affective influences on working memory were examined, including the role of the orbitofrontal cortex[86]. These areas of the brain have been lesioned in marmosets as part of this fundamental research.

192. Research into schizophrenia with volunteer patients has used PET imaging. It demonstrated metabolic abnormalities in circuitry in the cortex and correlations with affective flattening and attentional impairment[87]. Further efforts should be made by researchers to collaborate with clinicians in locating patients with brain damage to specific areas, who would be willing to volunteer for neuropsychological and imaging experiments.

193. Such patients have been studied with considerable success, although not in great numbers. For example, probably more has been learned about the <u>human</u> brain and cognition from a single amnesic patient known as HM, who experienced damage – including bilateral lesions of the hippocampus and medial temporal lobe damage -during an operation for epilepsy. HM has been studied for nearly 50 years by various research teams. One particular finding relevant to work under this project licence was that humans with lesions to the medial temporal lobe cannot perform concurrent discrimination tasks normally, although monkeys with this lesion can[88]. HM has agreed that his brain may also be studied in detail after his death.

<u>194. Given the wealth of viable non-invasive techniques available for use in humans (in addition to cell-based research), we believe that fundamental experiments which cause substantial suffering to marmosets and which are only of speculative relevance to humans are completely unjustifiable (both ethically and legally).</u>

[83] Conturo, TE et al (1999). Tracking neuronal fiber pathways in the living human brain. Proc. Natn. Acad. Sci. 96:10422-10427.
[84] Davidson, RJ (2002). Anxiety and affective style: role of prefrontal cortex and amygdala. Biol. Psychiatry 51:68-80.
[85] Ploghaus, A et al (2001). Exacerbation of pain by anxiety is associated with activity in a hippocampal network. J. Neurosci. 21:9896-9903.
[86] Perlstein, WM et al (2002). Dissociation in human prefrontal cortex of affective influences on working memory-related activity. Proc. Natn. Acad. Sci. 99:1736-1741.
[87] Potkin, SG et al (2002). A PET study of the pathophysiology of negative symptoms in schizophrenia. Am. J. Psychiatry 159:227-237.
[88] Corkin, S (2002). What's new with amnesic patient HM? Nature Reviews Neuroscience 3:153-160.

G. HOUSING AND CARE

Introduction

195. The BUAV has serious concerns regarding the housing and care of the colony of breeding and experimental marmosets at Cambridge University. These concerns are raised below and comparisons made with accepted best practice in the field.

196. The Common Marmoset (Callithrix jacchus) occurs naturally in Brazil where they occupy a range of habitat types including swamp forest, tree plantations and scrub. Their behaviour has been well studied. They are active during daylight hours and are almost completely arboreal. They seek security by using dense vegetation as cover and by running up trees. Group sizes range from 3-20 individuals comprising parents, sub-adult and infant offspring. Both parents and the older offspring share the responsibility for carrying new-borns.

197. Common marmosets are territorial. Their territories range in size from 0.5-6ha. A territory must be large enough to contain enough tree species to provide food for the resident marmoset group. Typically this requires at least 50 gum producing trees. Marmosets are omnivorous. Their rich and varied diet includes fruit, leaves, spiders, insects and large amounts of tree gum.

198. It would be clearly impossible to recreate the complexity of the marmosets' forest home in the laboratory. However, a great deal more could be done to provide a stimulating environment than Cambridge University attempted. Much has been written about providing environmental enrichment and there is consensus that primates they need cage furnishings which stimulate natural behaviours and increase activity levels, both of which improve the primates' physical and mental well-being. At Cambridge University some marmosets showed stereotypical behaviour, circling and back-flipping around the cage. Additionally, being an arboreal species, marmosets need tall cages that give them room to climb and flee upward.

199. The Code of Practice for the Housing and Care of Laboratory Animals used in Scientific Procedures ('the Code of Practice') states:[82]

"Size, shape and fittings of pens and cages should be designed to meet the physiological and behavioural needs of the animals. The shape of the cage and the furniture provided may be as important to the animal as the overall size of the cage. ..." (Section 3.21)

"In considering the provision of a suitable laboratory environment for such a widely diverse group, it is best to work from a thorough understanding of the biological, psychological and behavioural needs of the individual species. Primates have high intelligence, most have arboreal habits and all need complex, stimulating environments." (Section 3.43)

[82] Code of Practice for the Housing and Care of Animals used in Scientific Procedures (Home Office sec.21 Animals Scientific Procedures) Act 1986

200. UFAW (Universities Federation for Animal Welfare)[83] states:

"Marmosets and tamarins, even when housed socially, should be provided with a rich and complex within cage environment, as otherwise they show a reduction in social and non-social behaviours (Schoenfield, 1989; Kitchen & Martin, 1996)
The within cage environment should be related to the lifestyle and locomotor habits of the animals. Cage furniture should provide opportunities for a full locomotor repertoire."

H. AREAS OF CONCERN:

H.1. Caging

201. Overall the cages in which the marmosets were kept were small. They did not have access to natural daylight or separate exercise areas. Some of the conditions found by the BUAV investigation contravene best practice in their field.

202. At Cambridge University family groups, stock animals and experimental animals were housed in metal cages. Marmosets undergoing licensed procedures were usually housed in pairs. For animals undergoing experimental procedures, stacked two tier caging was considered acceptable .The marmosets were thus kept at ground level in a single unit caging. Being kept at ground level is likely to be a source of chronic stress for the monkeys as they are almost completely arboreal and need tall cages that give them room to climb and flee upward. The cages were metal with solid sides and a grid floor. There was one shelf inside the cage and a veranda attached to the cage. However, not all cages had verandas.

203. UFAW guidelines state that marmosets should not be housed in tiers, kept at ground level or housed singly. For example:

"...cages should be sufficiently high for them to flee upward, preferably above human eye level, so that they can look down on staff."

"Marmosets should not be housed in tiers, as this restricts the vertical flight response for both upper and lower cages, and those below are effectively trapped on the ground."

204. UFAW guidelines state:

"*Small metal cages with solid walls should be avoided as they stimulate abnormal behaviour and also tend to be damp."*

- Some animals were also housed individually for periods of time and on occasion stock monkeys were kept in cages deemed acceptable only for experimental animals.

[83] The UFAW (Universities Federation for Animal Welfare) Handbook on the care and management of laboratory animals 1999 Seventh Edition Volume 1.

205. UFAW guidelines state:

"Single caging is unsatisfactory for these highly social animals as they soon lose condition, appear nervous, and are often more susceptible to disease, and may even die."

- Nest boxes were metal with a wooden floor.

206. UFAW guidelines state:

"Wooden nest boxes are preferable because they absorb moisture and thus help to keep the inhabitants dry…Unless they are well ventilated, plastic or metal nest boxes are unsatisfactory because, at night, the moisture from the animals' breathing will condense on their fur and walls of the nest box. The marmosets will then have a wet appearance and are particularly prone to losing tail fur."

207. Some marmosets were seen to have a damp appearance; others had matted fur or fur loss. Damp conditions also facilitate the spread of bacterial infections, including pneumonia, which has been reported at Cambridge University. Pneumonia was given as the cause of death for a number of monkeys.

- At Cambridge University there was minimum effort to make the cages interesting and stimulating for the marmosets. Cage furnishings consisted primarily of half a wooden broom stick and the occasional old plastic disinfectant container or branch. Even breeding families who may remain at Cambridge University for many years were denied a stimulating and varied cage environment. The BUAV investigator attempted to introduce additional enrichment by providing (often at her own expense) flower pots and plastic boxes. Once a week a forage mix was put on the floor of the verandas, under the metal grid – although some experimental monkeys on water and food restriction were regularly denied this. According to UFAW, although marmosets will forage from the floor, having foraging containers placed high up in the cage is closer to the height at which they would naturally forage in the wild.

208. However, more imaginative and recommended furnishings as found in the UFAW guidelines were not used. These could include swings, ropes and rope ladders, and other natural destructible material, having an exercise area into which groups are allowed access at different times and using a sap/gum feeder.

- Fights within family groups and within experimental pairs did break out on a fairly regular basis. Fights are rare in the wild and therefore one can assume that a shortage of space and lack of environmental enrichment must have played a role.

209. There is an established relationship between the cage environment and behaviour. According to Wolfensohn and Lloyd (1998)[84], *"A lack of stimulation rapidly results in boredom for most primates"* and *"An impoverished environment*

[84] Wolfensohn, S and Lloyd, M. (1998) Handbook of Laboratory Animal Management and Welfare 2nd Edition. Blackwell Science, Oxford.

leads to a decrease in social behaviours such as grooming, playing and other behaviours such as scent marking and locomotion." (Buchanan-Smith, 1993)[85]

H.2. Animals' diet

210. The marmosets were fed twice daily, Monday to Friday, and once on Saturday and Sunday. They received alternate pieces of fruit (grape, banana or apple), pieces of rusk, monkey nuts or pieces of sandwiches daily.

211. Many of the experimental monkeys were trained (called 'shaping') to carry out a number of skilled motor and cognitive tests prior to being brain damaged. After brain damage, the animals were then required to perform the same tests. Their performance before and after the infliction of brain damage was then assessed. Many monkeys were coerced into performing these tests with the use of water deprivation and food restrictions. Some monkeys were subjected to both regimes. They were then given a 'reward' such as a flavoured milk drink or marshmallows during testing.

212. In the wild, marmosets spend up to 60% of their day foraging for food. Tree gum, an important part of their diet (15% of total diet) was not given to them at Cambridge University during the time of BUAV investigation. The reliance on marshmallows as the main food reward must be a cause of concern. Marshmallows are not nutritious and yet they were fed in large numbers to the marmosets. Several marmosets suffered from tooth abscesses.

213. The Senior Animal Technician told the BUAV investigator that he disagreed with water deprivation and had made his views known to the Home Office inspector. He also said that the conditions in which the monkeys are kept coupled with water and food restrictions stress the animals. In this instance, he is specifically referring to those monkeys held under Project Licence 1344:

"I could say I know marmosets & I know they're stressed…Think of it though, they're water-deprived, they get a diet of pellets, they get no forage mix…basically they're just stuck in an aluminium box."

214. Water deprivation meant that some experimental monkeys were denied water for 22 out of every 24 hours. There was some intermittent respite but the deprivation could continue for the entire length of the experiment which could last for two and a half years. Concerns were expressed by technicians as to whether the intermittent respite was adhered to, particularly at weekends, as no records appeared to be kept by the researchers.

215. Some monkeys were also deliberately kept hungry by being deprived of food (no breakfast), having their food restricted (given half rations) or being given the 'test' diet (a bland, unappealing diet of pellets and carrots, which the researchers themselves recognised as being unpalatable to marmosets) to make animals more receptive to the treats given during testing.

[85] Buchanan – Smith, H.M. (1993) Environmental enrichment for marmosets and tamarins. In: marmosets and tamarins in Captivity: Proceedings of Symposium 17 of the Association of British Wild Animal Keepers, 56-65.

216. An ‘extra’ forage mix was given to the monkeys on a Friday afternoon for the weekend. However, some researchers would even deprive their monkeys of this so that they could ‘work’ them over the weekend or keep them hungry for testing on Monday. For example, instructions such as the following would be left by researchers for the technicians:

“I will be away from 16/8 until 28/8 X will be testing some of my animals. They will remain on water deprivation & testing diet. Please do not give forage mix to animals on water deprivation 17/8 or 24/8. All others (without blue labels) can have sandwiches and fruit.”

217. According to the BUAV investigator, those experimental monkeys on food restriction became very stressed when the monkeys in surrounding cages were fed but they were not. The Code of Practice for the Housing and Care of Animals used in Scientific Procedures states:

“Where ‘withholding of food’ is necessary for experimental or safety reasons as prior to anaesthesia, care should be taken that ‘deprived’ animals are not stressed by exclusion from food whilst other animals around them are fed. This may necessitate removal to another cage or room.” (Section 3.32)

H.3. Staffing:

218. During the period of the BUAV investigation, there were between 440-500 marmosets, including both breeding and experimental animals in nine rooms. Animal technician staff consisted of one senior and two animal technicians (who did the bulk of the work). The two technicians worked 8am – 4pm Monday to Friday. Daily duties included food preparation and feeding the monkeys twice a day, watering, cleaning out cages and rooms, carrying out individual health checks and record keeping. The colony consisted of many brain damaged animals with physical and mental disabilities, as well as those who were sick and injured who may need special individual care and attention. There was no 24 hour cover provided for the monkeys. The BUAV believes that this level of staffing was clearly incompatible with good animal husbandry.

219. In early 2002, the animal technicians had responsibility for the following animals:

Staff member 1 (Senior Technician) had responsibility for 96 monkeys (52 experimental and 44 breeding/stock animals) in 44 cages.

Staff member 2 had responsibility for 190 monkeys (48 experimental and 142 breeding/stock animals) kept in 46 cages.

Staff member 3 had responsibility for 159 monkeys (88 experimental and 71 breeding/stock animals) kept in 58 cages.

220. At weekends and public holidays, animal technician staffing was kept to a minimum. There was only one technician on duty who was expected to feed, water and check all the marmosets, 440 plus animals, within approximately three hours in the morning. Weekend cover did not involve the member of staff returning in the afternoon to carry out a second check on the animals. Some researchers did work part of the weekend but this was often to continue with the animal training and testing. It is unreasonable to expect all tasks, let alone individual health checks, to be done thoroughly in these circumstances by the animal technician staff.

221. Of particular concern is the technician cover that was provided over the Christmas period 2001. For a 12 days period over Christmas and the New Year 21st December 2001 to 2nd January 2002, staff only worked for a few hours each morning as opposed to the full eight-hour working day. However, as seven of those days were either weekends or a public holiday then only one technician worked.

H.4. Breeding and reproduction:

222. Females appear to have been bred at a consistent rate of two litters per year Marmosets naturally produce twins. Triplets and quadruplets have not been recorded in the wild, but do occur in captivity. The third and fourth offspring in a litter will not survive without human intervention.

223. At Cambridge University there was clear acceptance of neonate death. Occasionally, an infant would be given to an outside contact to be hand reared; however, there was no hand-rearing regime in place and babies were often allowed to die or be killed if deemed to be non-thriving. The birth book for 2001 shows that out of a total of 63 litters, 30 were triplets. In total there were 155 births. Out of these 155 infants, 64 died or were killed; 39 of whom were from triplets.

224. Although the facility held between 440-500 marmosets, less than half were used in research at any one time. It would appear therefore that there was an oversupply of monkeys which would argue for slowing the rate of breeding rather than allowing it to continue apace. In the facility, daughters had bred even though caged with their mothers. It is clear that overstocking has been a problem in the past. During 1999/2000, it was reported that the number of marmosets was reaching “critical stocking levels”, which needed to be resolved “with some urgency”.

225. In addition to the acceptance of neonate death, breeding pairs were also killed when their reproductivity levels began to wane. In the wild, marmosets can live to 20, however, at Cambridge University breeding animals could be killed at 10-12 years old.

226. At least two female experimental marmosets became accidentally pregnant and gave birth. Staff had not noticed that the animals were pregnant. One of these, **Lakeside**, was on water deprivation and touch screen training when she gave birth in September 1998. Water deprivation started on 04/06/98 and Lakeside gave birth

on 08/09/98. The other, **Beast** (TN206B) gave birth on 02/07/01 to three infants. Beast had been given an Excitotoxic amyloid lesion (PPL 1344) on 10/01/01. She was being tested and on water deprivation during her pregnancy.

H.5. Animal health

Introduction

227. There are three aspects of animal health that need considering: the general health of the colony; health pre and post surgery and the care of brain damaged animals. These will be considered in turn.

H.5.i) Pre and post surgery

228. Common problems with primate groups include fighting injuries and bacterial and viral infections. This colony has suffered from E.coli, salmonella, pseudomonas and bordetella. The prevention of these diseases depends on good colony hygiene and high standards of management to avoid stress.[89]

229. Diarrhoea was also a problem that occurred with a number of breeding and experimental monkeys, including those on water deprivation and food restriction. Some monkeys lost weight and condition; some were killed due to their deterioration.

230. One breeding family (**Pinks** and **Pegasus**) suffered a bout of illness resulting in a number of deaths; including one of the infants, **Bank**, who died from pneumonia on 22/10/01. The breeding female, Pinks, deteriorated and was killed on 31/10/01. The mortality record showed that she too had pneumonia. Two other youngsters, **Enemy** and **Cube**, suffered weight loss and diarrhoea. We understand that Enemy was subsequently killed.

231. One monkey in particular had deteriorated over a number of months, apparently before action was taken. **Carl** (487), a six year old experimental monkey, was also a breeding male. He lost weight (dropped to below 300g) and fur and generally deteriorated to the point of looking emaciated. The Senior Animal Technician admitted that Carl had been going downhill for a couple of years. Carl was killed in August 2001. The post-mortem diagnosis was given as *"Severe chronic ulcerative enteritis and peritonitis"* in his duodenum:

"A number of factors may predispose to duodenal ulceration including trauma, bacterial infection and stress……but it is also possible that there may have been a pre-existing inflammatory bowel disease present which could have been a contributing factor for the ulceration itself."

232. Another monkey, **Belanna** (676), was given a bilateral NBM on 28/06/01. At this time she weighed 390g. By 19/07/01 Belanna had deteriorated:

[89] The UFAW (Universities Federation for Animal Welfare) Handbook on the care and management of laboratory animals 1999 Seventh Edition Volume 1.

"Monkey noted to be not eating properly in home cage. Had lost a lot of weight when weighed. Slightly neglectful & 'watching the birdies,' wobbly in cage. Put in incubator."

233. On 19/07/01 it was found that Belanna weighed only 287g. She had lost over 100g. She was put in an incubator and hand fed with lectade. However, despite the fact that Belanna was clearly not eating and losing weight, the records show that she was left from 5.25pm on the 19/07/01 until 9.30am on the 20/07/01; a total of 16 hours during which time she was not hand fed, given lectade or other fluids.

234. All animals should be physically assessed prior to surgery to ascertain the likelihood of problems under anaesthesia and other factors predisposing them to prolonged surgery. However, in at least one instance, an unhealthy marmoset – **Pinocchio** – was operated on. He subsequently died on 17/08/01, four days post-surgery. The post-mortem diagnosis for Pinnochio was given as:

"The cause of death here is hepatic necrosis and septicaemia. There is pre-existing chronic active inflammation within the pancreas. This has been present for some time…"

235. Problems with the anaesthetic were reported. Some animals died under anaesthetic or following surgery. Here are some examples:

"Surgery attempt 1 (not finished b/c animal didn't go under well" (**Inch**, 15/08/01)

"Not quite unconscious, needed 5 mins pressure to clot" (**Flaxen**, 28/04/99)

"Has had no surgery – woke up upon first skin incision – was given supp. Saffan but still would not go down again." (**Carina**, 03/05/01)

"Abandoned because would not anaesthetise" (22/05/00). This monkey (**Killimor**) subsequently died under anaesthetic prior to surgery at the second attempt on 01/06/00.

"Died under anaesthetic prior to surgery" (**Kidderpore**, 17/05/00)

"Not becoming sufficiently anaesthetised" "Not deep enough" (**Chestnut**, 08/05/01)

H.5.ii) Post-operative care of brain damaged marmosets

236. The BUAV has concerns regarding the post-operative care that some of the monkeys received. These concerns involved marmosets being left unattended overnight following invasive brain surgery. This included animals with low body temperature, bleeding head wounds, tremors, fits and collapse. A SOP (Standard Operating Procedures) document for post-operative monitoring was attached to one of the project licences (1249). This provided a grading scale for recording the 'well being' of the monkeys from Grades 1 –5 with instructions as to what action should be taken. However, records show examples of marmosets clearly being at Grade 2, 3 or 4 being left unattended for over 12 hours. At these grades, the SOP's instruct "Continue to monitor" which was clearly impossible if the animals were

left unattended overnight. Only once the animals are at Grade 1, can they be returned to their home cage.

237. Furthermore, a column for this grading scale was included on the post-operative record sheets. However, during our investigation we are not aware of the grading scale on these record sheets was not completed.

238. The Code of Practice states:

"The general well-being of all animals must be checked at least once daily. More detailed examinations should be carried out with sufficient frequency to ensure that the health and well-being of the animals is maintained. Animals which are undergoing scientific procedures must be inspected at a frequency commensurate with the severity of the procedure." (Section 3.3)

- **Agar** was operated on 17/5/00 (Bilateral MD/AT thalamus NMDA lesion). According to the record sheet, the surgery finished at 12.00. At 16.25 the entry on the record sheet stated:

"Asleep. Breathing v.fast. 31.7C wrapped in foil blanket. Put in incubator"
(normal daytime temperature for a marmoset is 38.6C)

239. At 17.30 (last entry made that day), Agar's temperature had increased to 34.4C. However, she was then left unmonitored (even though her body temperature was still not normal) until 8.30am the following day (18/5/00) when she was found dead. This was a period of 15 hours. The record sheet stated:

"8.30 Found dead – frothy/dilute blood from mouth/nose"

- **Tonga** underwent surgery to induce an occlusion (stroke) on 7/1/02. During the afternoon of the 7th she was anaesthetised again & had 3 minipumps inserted into her back.

240. On the 8/1/02, the minipumps were removed & fresh ones inserted under anaesthetic. The last entry on the post-op care sheet for the 8th was made at 4.30pm & stated:

"Wobbly but moving around. Still a reluctant drinker"

241. The next entry is the following morning on the 9/01/02 at 8.45am which states

"Nothing eaten. 4arm appears more impaired."

242. On the afternoon of the 9th at 2.16pm, Tonga was anaesthetised again to have the minipumps removed. The last entry on her post-operative care record for that day was made at 4.50pm. It states:

"Moistened mouth with lectade"

243. The next entry is at 8.45am on the morning of the 10th March which states:

"Temp 31.1C. Waistcoat + heatpad + foil blanket, gentle stretching of limbs to encourage circulation & movement"

(Tonga had been left for almost 16 hours again during which time her temperature had plummeted – normal daytime temp is 38.6C & normal nightime temp is 36.3C)

244. The BUAV investigator was told that Tonga was subsequently killed because of her poor condition.

- **Lore** underwent surgery to induce an occlusion (stroke) on the 19/11/01. During the afternoon he was anaesthetised again and had three minipumps implanted into his back.

245. The last entry for that day on his post-operative care sheet was made at 4.35pm.

246. The first entry for the 20th March was made at 8.30am. The records showed that Lore had been left for almost 16 hours without being monitored. The entry states:

"8.30 Tremor of hindquarters. Head – R. Otherwise fine"

247. During the day, Lore was anaesthetised again and had three minipumps removed and replaced. The last entry made at 4.45pm stated:

"OK. 'Stroked' Not very lively, rather quiet."

248. Lore was kept in an incubator for a few days post surgery and hand fed throughout the day with lectade and solid food.

249. However, over the weekend 24th/25th November 2001, Lore was only hand fed three times during the day. He was last seen (and hand fed) at 2.45pm on Sunday 25th and then not seen again until 8.00am the following morning on the Monday. On the Monday, he was fed four times by 12.25pm, six times in total during the day. Why was it considered necessary to hand feed Lore six times on a weekday but only three times at the weekend?

- **Randall**

Randall was operated on the 31/7/01. He was taken out of the frame and put in a recovery cage at 1.40pm. At 4pm, the post-operative record sheet stated:

"Beginning to stir"

At 5.50pm, the last entry stated:

"Head a bit bloody, awake but drowsy"

250. The record then shows that Randall was left for 15 hours overnight even though his head was bloody.

251. Some operations at Cambridge University did not begin until mid-late afternoon. However, we understand that accepted practice is that primates should be starved for 12 hours before being anaesthetised to prevent the risk of vomiting. Therefore, surgery is normally scheduled for first thing in the morning so that the starvation period coincides with night time inactivity. If surgery does not begin until late afternoon, the animal may have been starved for 20 + hours. In a species with as high a metabolic rate as the marmoset, this has health as well as welfare implications.

252. Additionally, late surgery can mean fewer staff on duty to monitor the animals' recovery. However on at least one occasion a number of operations took place on a Friday afternoon. Some of the animals involved were monitored for less than two hours post-surgery before being left unattended.

Examples included:

- **Mulan** was operated on 18/12/01 (Friday). He was not taken out of the surgical frame until 6.10pm. At 7.10pm, the post-operative record sheet stated:

"Twitching limbs, shaking"

253. The last entry was made at 7.30pm and stated:

"Moving about, wobbly"

254. Mulan was then left unmonitored overnight only one hour and 20 minutes after surgery had finished.

- **Anode** was operated on 23/11/01 (Friday). He was taken out of the frame at 5.30pm. The last entry on the post-operative record sheet was made at 7.00pm.

"Up & walking about, fine"

255. The next entry is for 9.30am on the Saturday morning. Anode was left unmonitored overnight only one and a half hours post-surgery.

- **Baloo** was operated on 13/11/01. He was taken out of the frame at 4.22pm. At 4.45pm the entry on the post-operative record sheet stated:

"Twitching & mild shaking"

256. The last entry of the day at 6.10pm stated:

"Awake, quiet, looking left"

257. Baloo was then left overnight less than two hours after surgery had finished.

- **Shazney** was operated on 18/9/01. She was taken out of the frame at 4.05pm. At 5.45pm, the post-operative record stated:

"Trembling & some movement"

258. The last entry was made at 6.15pm:

"Lots of shaking"
"Some bleeding from head wound. Cleaned"

259. Shazney was then left overnight unmonitored despite bleeding from her head wound.

260. On one occasion, surgery was not completed until 7pm. The monkey was then left unattended without having regained consciousness.

- **Turks** was operated on 17/12/01 (Bilateral 5-7 DHT). She was taken off the operating table at 7.00pm. At 23.35pm the post-operative record stated:

"Has not regained consciousness"

261. Other entries included:
"00.25 Responds v.slightly when handled"
"1.40 no change"
"2.00 no change"

262. Turks was then left unattended from 2.00am despite still being unconscious. The next entry on the record is not until 7.45am on the 18/12/01 where it is stated:

"Conscious! Still not regained posture"

263. Turks was sick on a number of occasions throughout the day after being given lectade. It was not until 8.30pm on the 18/12/01 that the post-operative record reports that Turks is:

"Sitting up for the first time" (over 24 hours after surgery had finished).
Turks was then left unmonitored from 9.30pm on the 18/12/01 until 8.25am on the 19/12/01 where it was reported that her:

"Pupils v. dilated"

264. Later at 14.10 that same day, there is an entry that states:

"Very alert & active, frightened"

265. It appears that there had been issues surrounding unattended post-surgical animals at the university on an earlier occasion. In 1999, it was reported that a researcher was leaving rats unattended following surgery late at night and not checking others for two hours post-surgery. According to the Named Veterinary

Surgeon (NVS), the Home Office Inspector had expressed the opinion that someone should remain with the animal constantly until it has regained its righting reflex and was fit to be returned to the animal unit. The monitoring can only be relaxed if it appears that the animal has completely recovered.

266. The following comments made by the NVS regarding post-operative monitoring reflect the potential serious consequences for animal welfare:

"As….himself says, he has rats die approximately one and a half hours after a procedure was completed – so presumably these were just found dead when he checked them."

"There was also the reported incident where the rats overheated – again, this could have been avoided with more regular checking (& perhaps if a thermometer had been left by the rats' cages.)"

If the Home Office Inspector found out that rats are being left unattended for two hour periods after surgery, she would go ape-shit!!"

I. HOME OFFICE VISIT

267. A pre-announced Home Office inspection took place at Cambridge University on 03/10/01. The named Animal Care and Welfare Officer (NACWO) made an announcement to staff. There was a great deal of activity within the unit on the day before (02/10/01) and on the morning of the visit. Staff and researchers were given instructions to make sure that all animals including experimental ones had an identification tag and that records were correct. The Senior Animal Technician stated that *"I want every experimental animal issued with a tag"*. Concern was also expressed by the NACWO and an animal technician about the poor record-keeping of one of the researchers in an adjoining department.

268. On the morning of the visit, staff and researchers kitted themselves out wearing hats and gloves; something which some staff did not normally do. During the inspection, the Home Office inspectorate visited only three rooms: 2, 4 and 9.

269. The warning that a Home Office inspection was imminent allowed the researchers and animal technician staff time and opportunity to get everything "in order" and ensure that certain practices, such as wearing the correct clothing, could be arranged to give the best impression. However, it was a misleading impression of normal practice at Cambridge University and will do little to instil confidence in the Home Office inspectorate in its ability to effectively monitor animal experimentation in the UK.

J. CONCLUSION

270. The investigation has revealed, yet again, a catalogue of appalling animal suffering under a system which the Government claims to be tightly regulated. The grave injustice of what is being done to the marmosets is simply compounded by the questionable nature of the science and the inadequate consideration given to non-animal techniques.

271. The BUAV believes that:

- Cambridge University should lose its certificate of designation (which allows it to carry out animal experiments). These kind of experiments are wholly inappropriate for a seat of civilised learning and culture, particularly one with Cambridge University's worldwide reputation

- the licences in question should be revoked

- the funding bodies (such as the MRC and the Wellcome Trust) should withdraw their funding

- the Home Secretary should set up a public inquiry, wholly independent of the Home Office, into all aspects of the research programmes and primate research in the UK generally. The inquiry should have power to subpoena witnesses and inspect all documents, the marmosets and the housing and research facilities. Regrettably, experience has shown that the Home Office cannot be trusted to investigate impartially the sort of allegations made in this report

- all research using primates under the 1986 Act should be halted. Even if such research represented good science, the cost to the animals is simply too great. In 1997, the Government banned the use of great apes in research because of the level of suffering they inevitably experience. It should apply the same logic to other primates.