

“ But little Mouse, you are not alone,  
In proving foresight may be vain:  
The best laid schemes of mice and men  
Go often askew,  
And leave us nothing but grief and pain,  
For promised joy!

Robert Burns, *To a Mouse*, 1785 ”



[www.animalaid.org.uk](http://www.animalaid.org.uk)

# Alzheimer's Society-funded mouse experiments at Edinburgh University: cruel and without medical benefit

Animal Aid, during the course of researching its *Victims of Charity* report, has discovered an ongoing series of animal experiments conducted at Edinburgh University and funded, in large part, by the Alzheimer's Society. The experimental work involves damaging the brains of mice using various methods, to then study how the animals respond to memory testing, or to analyse their brain biochemistry. The first of the published papers so far unearthed was published in 2006, and the most recent in 2011. The Alzheimer's Society, in the autumn of 2011, granted £335,176 to an Edinburgh researcher to continue brain-damaging mice, for a further three years.

Having examined the experimental rationale and protocols, we conclude that this series of experiments is both without scientific merit and extremely cruel:

## ■ The experiments are superfluous and unnecessary

In general, they attempt to reproduce findings that had already been discovered from clinical, epidemiological or autopsy studies in humans. The overarching common conclusion from much of the work is facile and predictable: brain damage (whether induced through trauma or lack of oxygen) impairs the ability of mice to learn. It has long been known that head injuries and hypoxia in humans are to be avoided for a multitude of reasons, and public health programmes, trauma services, and acute and community medical care are in place to minimise the damage. In addition, drugs like ibuprofen (already proven in clinical trials to have no benefit for Alzheimer's patients, and with dangerous side effects) were tested on injured animal subjects.

## ■ The experiments are highly repetitive and monotonous

Several experiments repeated areas of research recently covered by the same team. The reasons for such close similarities are unclear – perhaps the team wished to allow another experimenter to practise their techniques, or desired more papers for publication.

## ■ The experiments continue to use animal methods and tests that have failed to benefit dementia patients

Information from experiments conducted using transgenic mouse models of Alzheimer's Disease, such as the animals used in the Edinburgh studies, have been central to the design of many failed Alzheimer's drugs. Candidate therapies tested in these models and judged as successful (either biochemically or in behavioural testing) have then failed in human trials.

The experiments employ crude and reductionist cognitive tests, which are supposedly relevant to

Alzheimer's in humans. In particular, the Morris Water Maze (MWM) is used in several experiments. This widely used test forces rodents to swim around a pool of water in order to find an escape route. Mice tend to panic when immersed in water, as, unlike rats, they are not natural swimmers. The test is acknowledged by users as being 'somewhat unpleasant'.

Researchers have justified the use of tests like the MWM via a series of rather absurd inter-species comparisons. For example, they state that mice need certain parts of their brains to be intact in order for the animal to perform well, and these brain areas are disrupted in Alzheimer's sufferers. A 2009 neuroscience text observes that 'it is also important to note that spatial orientation, navigation, learning, and recall (which are used extensively in the MWM) are quite commonly disrupted in patients with dementia'. Such inane explanations fall well short of establishing the MWM as a predictor of human outcomes – which it patently is not.

Lastly, the experimenters investigate the deposition of amyloid protein in response to injury. The role of this substance in the development of Alzheimer's Disease has always been controversial, with causality far from clearly established. Drugs that cleared amyloid from the brains of transgenic mice went on to fail in human trials. However, it is clear that the experimenters consider a biochemical process in a mouse to be, at least in part, an appropriate surrogate for complex neurological deficits in humans. This is even before considering the primitive cognitive testing outlined above.

### ■ The experiments induce severe physical trauma and suffering in the mice

Both the brain damage inflicted on the animals, and the subsequent tests they are forced to undergo, are manifestly cruel.

'Fluid percussion injury' – in other words, high pressure brain damage – was used in several instances. In one protocol, the mice were first anaesthetised, and an 'injury hub' (essentially a small cap) was glued into a hole drilled into their skulls. Experimenters breached the covering overlying the brain in some instances, and these mice were 'excluded from the study' (presumably

killed). The remainder of the hapless mice were then left for a day with a hole in their heads, before being anaesthetised again. A length of high-pressure tubing was then directly connected to the 'injury hub', and fluid used to deliver a blast of intense pressure directly into the animals' skulls. The mice were then placed on their backs, and the time it took them to get up was recorded. Finally, they were anaesthetised for a third time to repair the incision in their scalps and remove the hub. All this took place before the animals were subjected to weeks of cognitive testing.

Fluid percussion injury in rodents has a high mortality rate, as unsurprisingly, many animals stop breathing.

Strokes were induced in other animals via blockage of (usually) the middle cerebral artery using a filament, and animals were subsequently subjected to numerous cognitive tests.

Cerebral hypoperfusion (reduced blood flow) was induced with tiny microcoils (made from piano wire surgically placed around both carotid arteries). These were left in place for the whole of the remainder of the animals' short lives. This procedure has a mortality rate of at least 15 per cent.

All the above procedures must have caused severe pain in the mice. There is, however, no mention of any painkilling medications being administered.

### ■ The experiments are not clinically relevant

As outlined above, mouse models of neurodegenerative diseases have been essentially useless in developing treatments for Alzheimer's. The ever-growing number of clinical trial failures is a testament to this fact. Part of the problem is that the 'experimental Alzheimer's Disease' that researchers produce in animals is emphatically not the same as the human variety.

Inducing strokes in mice, depriving their brains of oxygen and giving them severe head injuries are other 'modelling' techniques performed by the Edinburgh team. This work is basic research, involving the laboratory destruction of animal physiology and anatomy in the hope of finding out

something clinically relevant. However, ischaemic and traumatic damage to the human brain is clearly recognised as undesirable. It is hard to see how any clinical advance could come from demonstrating the same in mice.

The researchers themselves freely admit that their hypoperfusion model does not accurately parallel human disease states. Their stated intention is to examine any causal relationship between cerebral hypoperfusion, white matter pathology and age- or Alzheimer's-related cognitive decline. However, the damage induced in these healthy animals is

fundamentally different from 'the heterogeneity of the human condition in which chronic hypoperfusion and cognitive decline can be influenced by several different factors including ageing, neurodegenerative processes, episodic hypotension, mid-life histories of hypertension and high cholesterol, atrial fibrillation, aortic and carotid atherosclerosis and Type 2 diabetes'. Eliminating these so-called confounding factors does not lead to a better animal model of human disease. Instead, it serves to ensure that these experiments are only relevant to certain kinds of mice injured in certain ways – not to a real-life human population.

## Brief summaries of papers

### **Paper 1 (2006) Humanin is upregulated following *in vivo* brain injury**

Before this experiment, a protein called Humanin had been discovered in the DNA of the brain of an Alzheimer's Disease sufferer. This protein was known to protect cells from certain kinds of damage, including some of the biological changes associated with Alzheimer's. It was also well established from human epidemiological studies that brain injuries are a risk factor for developing the disease.

This experiment used around 60 mice, who either had a nerve toxin (ibotenic acid) directly injected into their brains, or their middle cerebral artery blocked with a filament for one hour. The stated objective was to examine the 'temporal and spatial profile of the endogenous Humanin protein response' in the brains of these mice. The animals survived for possibly random or pre-selected time periods (up to 90 days for the first group, but only up to 71 hours in the second), after which their brains were analysed biochemically.

The researchers concluded that 'similar to Alzheimer's Disease, Humanin is rapidly upregulated in the injured rodent brain and may therefore serve a general survival role following brain injury'.

### **Paper 2 (2008) The effect of *in vivo* focal cerebral ischaemia on amyloid precursor protein processing and amyloid deposition in mutant APP (J9A) mice**

Before this experiment, it was well established from human epidemiological and autopsy studies that cerebrovascular disease (including stroke) occurs in 60-90% of Alzheimer's Disease patients, and can worsen their cognitive decline. It had been suggested that a lack of oxygen to brain tissue (cerebral ischaemia) could cause pathological changes in the brain, which are associated with Alzheimer's. One of these changes, the role of which remains controversial, is the deposition of beta-amyloid protein. It was already established before this experiment that beta-amyloid is formed from the cleavage of amyloid precursor protein (APP).

This study sought to determine whether brain ischaemia alters the expression and processing of APP. To this end, 12 mutant APP mice (a transgenic Alzheimer's model) were given focal brain lesions with middle cerebral artery occlusions. These lesions were, therefore, strokes. After only 24 hours, ten mice were dead

(it is not clear if they were deliberately killed), and their brains were analysed biochemically. The remaining two mice lived for one month, and underwent the same fate.

The researchers found no increased beta-amyloid deposition after 24 hours in ischaemic areas of the mouse brain. In the two mice who lived for a month, APP had been processed into beta-amyloid in the brain areas that had been severely deprived of oxygen. The researchers concluded that 'ischaemia is associated with increased production of beta-amyloid by influencing APP metabolism, which may link the role of ischaemic insults to the pathogenesis of AD'.

### **Paper 3 (2009) Selective axonal damage and long-term spatial reference memory deficits after mild fluid percussion injury in mice**

In the background to this experiment, the researchers set out the pre-existing knowledge gleaned from prior studies. They pointed out how 'widespread damage and dysfunction of axons and myelin tracts that comprise white matter in Alzheimer's Disease (AD) has been demonstrated by numerous neuropathological, imaging and biochemical studies'. They also reported that 'head injury is the major environmental risk factor for AD and can result in persistent cognitive deficits and damage to the white matter in humans'.

The researchers, in an experiment that appeared designed to try to replicate these findings in animals, then damaged the brains of ten mice via 'mild fluid percussion injury'. They then tested for cognitive deficits using the Morris Water Maze at four and eight weeks after injury. All the mice were 'terminated' after eight weeks, by means of an injection of fixative solution into their bloodstream. Their brains were then biochemically analysed.

The experimenters found that 'at four weeks, injured mice demonstrated spatial learning deficits as well as short term spatial memory deficits' in the water maze. On subsequent tissue analysis, the axons of the fluid percussion-injured mice (but not their myelin or neuronal cell bodies) showed a greater level of damage compared with controls. The researchers concluded that their study 'demonstrated that selective changes occur in the axons of white matter in response to mild head injury, which are associated with deficits in cognitive ability' – exactly what was known before the experiment.

### **Paper 4 (2010) Mild Fluid Percussion Injury in Mice Produces Evolving Selective Axonal Pathology and Cognitive Deficits Relevant to Human Brain Injury**

This experiment was extremely similar to Paper 3. In the background to the experiment, the researchers set out the pre-existing knowledge gleaned from prior human studies. They described how mild traumatic brain injury (TBI) in humans 'can result in cognitive impairments that may persist for months to years following the initial injury', and how these impairments 'can be explained in part by progressive damage to the white matter'. They also reported that 'axonal pathology is a feature of human mild TBI, and subtle white matter damage has been detected using magnetic resonance imaging from 1 week following injury and up to 7 years later, even in the absence of grey matter pathology... Moreover, mild TBI predisposes individuals to an increased risk of developing Alzheimer's disease and dementia'.

To this end, 29 mice were brain damaged with 'mild fluid percussion injury' (52 mice were killed altogether, including controls). The injured mice were then extensively 'trained' and 'tested' in a Morris Water Maze, beginning one week after surgery. Mice in the early stages of testing spent nearly a minute in the water, and injured animals were still spending 30 seconds trying to find the hidden platform after many days of testing. Mice were then killed at various times post-injury, up to six weeks. Their brains were then biochemically analysed.

The experimenters found that ‘at 3 weeks post-injury, injured mice showed an impaired ability to learn the water maze task, suggesting injury-induced alterations in search strategy learning’. On subsequent tissue analysis, the axons of the fluid percussion-injured mice (but not their myelin or neuronal cell bodies) showed a significantly greater level of damage compared with controls. The researchers concluded that their mouse model showed that ‘specific and localized axonal damage in the absence of alterations in myelin is sufficient to produce an impairment in spatial learning that is detectable at three weeks after injury’.

#### **Paper 5 (2010)**

##### **Stroke increases oligomeric amyloid and amyloid deposition in mutant APP (J9A) mice**

This experiment covers almost identical ground to Paper 2 above. In the background to this experiment, the researchers set out the pre-existing knowledge gleaned from prior human studies: a history of stroke can increase an individual’s risk of Alzheimer’s Disease, and evidence of stroke is present in one third of Alzheimer’s cases at post mortem.

The researchers stated objective was to ‘determine whether stroke alters the expression and processing of amyloid precursor protein (APP) and amyloid production’. To this end, 35 mutant APP mice were given strokes via middle cerebral artery occlusions. After only 24 hours, 16 were dead (it is not clear if deliberately killed), and their brains were analysed biochemically. The remaining 19 mice lived for one month, and underwent the same fate.

The researchers found no increased beta-amyloid deposition after 24 hours in ischaemic areas of mouse brain, although there was a ‘marked increase in neuronal APP’. In the 19 mice who lived for a month, APP had been processed into beta-amyloid in the ischaemic brain areas. The researchers’ conclusion was identical to that of the Paper 2 experiment: ‘Stroke is associated with increased production of beta-amyloid by influencing APP metabolism, which may link the role of ischaemic insults to the pathogenesis of AD’.

#### **Paper 6 (2010)**

##### **The influence of chronic cerebral hypoperfusion on Alzheimer-like pathology in 3xTg mice**

This was another experiment investigating cerebral oxygen deprivation, and its impact on beta-amyloid deposition. This experiment looked also at tau protein. In the background, the researchers pointed out how ‘cerebrovascular dysfunction, a key feature of Alzheimer’s disease (AD), can result in chronic cerebral hypoperfusion which is suggested to impact on the cognitive decline and degenerative processes’.

The stated aim of this experiment was to investigate ‘whether chronic cerebral hypoperfusion precipitates the development of AD pathology by examining the impact of varying durations and intensity of cerebral hypoperfusion on beta-amyloid and tau pathology’ in transgenic (3xTg) mice at different ages. To this end, the researchers induced a permanent narrowing in both carotid arteries of an unspecified number of mice. This was accomplished via the application of microcoils to the vessels under isoflurane anaesthesia. After either one or six months, the mice were killed (numbers allocated to each group are not specified), and their brains were biochemically analysed.

The researchers concluded that ‘in the 3xTg mice, chronic cerebral hypoperfusion had no major effect on amyloid or tau pathology and only subtle changes in amyloid could be detected in white matter’.

### **Paper 7 (2010)**

#### **Selective white matter pathology induces a specific impairment in spatial working memory**

In the background to this experiment, the researchers set out the pre-existing knowledge gleaned from prior human studies. They report that damage to brain white matter putatively contributes to age-related cognitive decline, and cite post mortem and imaging evidence that this is the case. White matter damage is 'often associated with chronic cerebral hypoperfusion resulting from small vessel pathology'.

However, the researchers were attempting to show a causal relationship between white matter damage and cognitive loss, free from the 'confounding factors' found in human patients such as atherosclerosis, hypertension and diabetes, which influence the presence of white matter lesions. To this end, 'anatomically widespread, diffuse white matter damage was induced, in 3 different cohorts of C57Bl/6J mice, by chronic hypoperfusion produced by bilateral carotid stenosis'. This was performed, as previously, using microcoils. The mice then underwent extensive memory testing in a Morris Water Maze and an eight-arm radial arm maze. Some mice were subjected to the following regime of testing: a maximum of 32 tests per platform, on at least five platform locations, for at least ten days. Others were food-deprived prior to testing. Mice were killed one or two months after the initial surgery, and their brains were biochemically analysed.

The researchers concluded that 'diffuse white matter damage occurred, throughout the brain, in all hypoperfused mice in each cohort and was essentially absent in sham-operated controls. There was a selective impairment in spatial working memory, with all other measures of spatial memory remaining intact, in hypoperfused mice with selective white matter damage'.

### **Paper 8 (2011)**

#### **Activation of Nrf2-Regulated Glutathione Pathway Genes by Ischemic Preconditioning**

The rationale for this experiment was to investigate the role of a transcription factor called Nrf2. This chemical 'turns on' genes responsible for protecting cells against injury from free radicals. This process had already been shown in 'animal models' to limit neurological damage from a stroke. Nrf2 has been investigated extensively for some years, and to date there is no clinical treatment that has emerged from such work.

This experiment had several elements, including the use of mouse cell cultures. In the live animal protocol, researchers gave an unspecified number of mice a stroke via the protocol described below. Six mice survived for a few hours before being killed, and their brains were removed for examination. These mice had been anaesthetised three times – firstly, to have their cerebral arteries blocked with a resinous filament. They were then allowed to recover in an incubator, with the filament still in place and a midline cervical incision still open. They were then anaesthetised for a second time, the filament was removed (after 15 minutes of occlusion), and the wound sutured. After 3.75 hours, during which time the mice would have been suffering the effects of a severe stroke, they were anaesthetised for a third time and decapitated.

Nrf2 target gene expression was then assessed in the brain tissue of these mice, and the researchers found that two genes in particular had been significantly up-regulated. They concluded: 'transient ischemic conditions in vitro and in vivo cause an increase in the expression of Nrf2 target genes associated with the glutathione pathway... these studies indicate that astrocytic Nrf2 may represent an important mediator of endogenous neuroprotective preconditioning pathways'.

# Links to the researchers

## 1 Jill Fowler

Given an Alzheimer's Society Fellowship in 2005, remains a Research Fellow as of 09/12/11.

[http://www.alzheimers.org.uk/site/scripts/documents\\_info.php?documentID=952](http://www.alzheimers.org.uk/site/scripts/documents_info.php?documentID=952)

[http://www.alzheimers.org.uk/site/scripts/documents\\_info.php?documentID=1174](http://www.alzheimers.org.uk/site/scripts/documents_info.php?documentID=1174)

<http://www.ccns.ed.ac.uk/people/postdoc/fowler.html>

<http://www.csbe.ed.ac.uk/academic-partner/dr-karen-horsburgh-dr-jill-fowler>

## 2 Karen Horsburgh

In autumn 2011, given £335,176 grant by the Alzheimer's Society.

<http://www.cnr.ed.ac.uk/Research/horsburgh.html>

[http://www.alzheimers.org.uk/site/scripts/documents\\_info.php?documentID=1742](http://www.alzheimers.org.uk/site/scripts/documents_info.php?documentID=1742)

Background information about the 'mouse hypoperfusion' model can be found here:

<http://www.sinapse.ac.uk/media/events/resources/ti/MBastin.pdf>

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