Project title	**Blood flow and tissue oxygenation in rodents**		
Key words	Haemodynamic response, Imaging, pre-clinical models		
Expected duration of the project (years)	5		
Purpose of the project	Basic research	Yes	
	Translational and applied research	Yes	
	Regulatory use and routine production		No
	Protection of the natural environment in the interests of the health or welfare of humans or animals		No
	Preservation of species		No
	Higher education or training		No
	Forensic enquiries		No
	Maintenance of colonies of genetically altered animals		No
Objectives of the project	In this project we aim to improve our understanding how the haemodynamic response changes in preclinical models of disease. The brain is critically dependent on its blood supply to maintain normal function. Interruption of the blood supply to the brain, as occurs during cerebral ischaemia ('stroke'), causes widespread neuronal death. This neuronal death makes stroke a leading cause of death and disability worldwide. At present, restoration of blood flow by thrombolysis with recombinant tissue plasminogen activator (rtPA) is the only licensed treatment. Since the health and welfare costs associated with stroke represent a significant burden, estimated in the region of £9 billion per annum in the UK alone, there is an urgent need for new treatments. Even after removing the original blockage in the vessel there is a so called "no-reflow" phenomenon in that the oxygen supplied by the larger arteries does not actually reach the brain tissue. With collaborators we want to investigate how neuronal inflammation and /or stroke change the dynamics during the acute phase and the following few hours. Colleagues have recently discovered in a genetically modified mouse model that has an eosinophil deficiency (ΔdblGATA-1 mice) or increased eosinophils (IL5T or IL5 Transgenic) that the changed number of Eosinophils leads to changes in blood flow and blood pressure in the periphery. In this project we want to investigate the effects of eosinophils on		

	blood flow in the brain and in the periphery like the mesentery. This is important to understand as the changes to blood flow induced by different eosinophil concentrations might be a clue why for example patients with inflammation have a worse outcome after stroke.
Potential benefits likely to derive from this project	Outcomes from this research can inform the development of new treatment strategies in stroke patients.
Species and approximate numbers of animals expected to be used, and anticipated period of time	Over 5 years we estimate to use about 300 mice and 100 rats.
Expected adverse effects and the likely/expected level of severity. What will happen to the animals at the end.	All animals will be maintained under general anaesthesia throughout the experiment. The animals will be terminated at the end of the experiment. Therefore the severity of the experiment is non recovery. Animals will be monitored continuously for changes in physiological status together with regular testing for withdrawal responses to maintain surgical levels of anaesthesia and analgesia. Appropriate measures to maintain surgical anaesthesia will be taken immediately if required. Animals will be killed by a Schedule 1 method at the designated establishment. AND/OR Animals will be killed by terminal anaesthesia during which they will be perfused transcardially. OR Decapitation of anaesthetised animals.
Application of the 3 Rs	
1. Replacement Why do animals need to be used, and why non-animal alternatives cannot be used.	The experiments proposed in this work involve the recording of neuronal activation and imaging of intrinsic signals in the rodent cortex. At the moment there is no alternative to in vivo studies to obtain information about the hemodynamic response of the brain and the underlying cortical activity and how this changes with disease. Currently there are no in vitro models or systems that

	replicate the complex interactions and architecture of the CNS and its communication with other potential influencing factors such as the immune and vascular systems.
2. Reduction How the use of minimum numbers of animals will be assured	We have for many years been at the forefront of developing independent component analysis (ICA) methods to separate the signals from noise sources which allows us to reach statistical significance in our experiments with lower numbers of animals. Recently we were the first to demonstrated that these ICA approaches can not only increase the quality of the imaging data but are also capable of identifying the origin of different components in the recorded haemodynamic response. In experiments that compare data between two groups or more a t-test or analysis of variance (ANOVA) will be used respectively. When measurements are taken over several time-points in the same cohort, repeated measures ANOVA will be used. Depending on what form the data take, statistical tests used will either be parametric (equal variance) or non-parametric (unequal variance). If there is any doubt on experimental design or statistical analysis, advice will be sought from a local statistician.
3. Refinement Reasons for the choice of species and why the animal model(s) to be used are the most refined, having regard to the objectives. General measures to be taken to minimise welfare costs (harms) to the animals.	The proposed experiments will be conducted on rats and mice. The mechanisms of the haemodynamic response are very similar in these species to humans. The cerebral and cerebrovascular anatomy is similar in rodents and humans. The proposed studies could not be undertaken in lower species because they do not show such similarities to humans, and in vitro experiments do not allow the study of interactions between different body systems. All experiments will take place under full anaesthesia without recovery which therefore is classified as non recovery throughout.