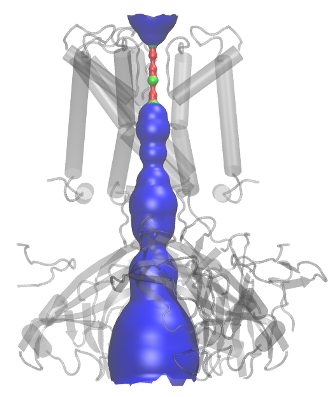
**OXION**

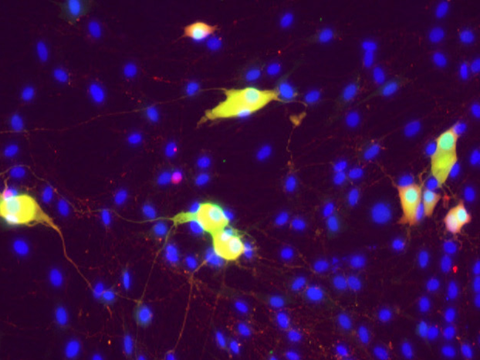
**2012**

**The Wellcome Trust Strategic Award**

**in Ion Channels and Diseases of Electrically Excitable Cells**

****

****



**TABLE OF CONTENTS**

AIMS OF THE INITIATIVE 3

CORE FACILITIES 5

LIST OF OXION MEMBERSHIP 17

OXION GROUPS 18

ASSOCIATE MEMBERS 51

TRAINING PROGRAMME

Training Fellows 61

Graduate Training Programme 65

Graduate Students 73

Vacation Students 81

OXION PUBLICATIONS 84

OXION SEMINARS 89

**Front cover**:

**Figure 1:** Structure of a KirBac potassium channel in an open conformation.

**Figure 2**: A mouse.

**Figure 3**: Immunofluorescent image of cardiac sympathetic neurons after gene transfer with a noradrenergic neuron-specific adenoviral vector Ad.PRS-nNOS. Yellow stained neurons represent overlay of anti-NOS (Fluorescein Streptavitin), anti-TH (Texas-red Streptavitin).DAPI staining visualizes cell nuclei.

**AIMS**

OXION has three main aims:

* To facilitate innovative research programmes that build on an established centre of excellence in integrative ion channel research;
* To train talented young scientists in a range of multidisciplinary skills in integrative physiology, including *in vivo* physiology;
* To strengthen further the links between basic science and the clinic.

The key scientific objectives are to:

* understand the relationship between ion channel structure and function;
* investigate mechanisms involved in targeting and anchoring ion channels at the plasma membrane, especially at the synapse;
* define the roles of different types of ion channels in generating specific patterns of electrical activity and controlling secretion in neuronal and endocrine cells;
* elucidate the role of ion channels in brain, nerve, muscle and endocrine disorders;
* address the role of ion channels in behaviour;
* integrate this information to provide a comprehensive overview of ion channel

function, from molecule to malady.

***Scientific Rationale***

Ion channels play essential roles in the physiology of all cells and defects in ion channel function have profound physiological and behavioural consequences. In many cases, several different organ systems are involved and understanding the clinical disease requires knowledge of molecular and cellular biology, as well as whole organism physiology. Determination of the role of ion channels in health and disease therefore inevitably involves an integrated and multidisciplinary approach. OXION exists to integrate clinical studies with research in human and mouse genetics, and to co-ordinate research on ion channels and electrically excitable cells at all levels: from gene through protein, organelle, cell, system and whole organism to disease.

***OXION Structure and Support***

OXION currently comprises 22 groups, based in Oxford (17), Harwell (2), Cambridge (1) and London (2). We also have 8 Associate Members, based in Oxford (5), Cambridge (1), London (1) and Manchester (1). The group leaders are listed on page 17, and details of their research areas are given on pages 18-60.

The OXION grant supports core facilities in mouse behaviour, *in vivo* mouse physiology, microarray and proteomics and cell imaging. These are led by Drs Rob Deacon, Louise Upton, Sheena Lee, Holger Kramer and Juris Galvanovskis, respectively. There is also support for an animal technician and a part-time administrator, Pippa Cann. All OXION groups have privileged access to the core facilities, while Associate Members pay a reduced fee. Details of the core facilities and how to access them are given in this Handbook.

We currently have 4 training fellows in post: their annual reports can be found in this Handbook. Dr. Mariana Vargas-Caballero has now completed her training fellowship and taken up an appointment to a Research Career Track Lecturership at the University of Southampton. We congratulate her on her success and on the birth of her second daughter. Our sixth (and final) training fellow, Dr. Prafulla Aryal, started in August 2012 and is working with Dr. Stephen Tucker.

This year we welcome three new graduate students: Alexei Bygrave, Antonia Langfelder and Elisa Vergari.

During the past year we hosted 4 vacation students. These placements have been very effective at encouraging undergraduates to consider scientific research as a career and have led to a number of publications. The 2013 vacation studentships will be advertised early next year, and group leaders are requested to submit possible projects for 8-week vacation studentships. Those who know of potential vacation students should also encourage them to submit an application.

We also celebrate the achievements of two of our graduate students, Olivia Shipton and Rebecca Clark. Olivia was awarded the 2012 Jean Corsan Prize for the best scientific paper in neurodegeneration published by a PhD student. Rebecca won second prize as a finalist in the Pursuit Award from the Bloorview Research Institute for 2011. Our warmest congratulations to them both.

***Frances Ashcroft, September 2012***

Imaging Core Facility

# Dr. Juris Galvanovskis

***Department of Physiology, Anatomy and Genetics, OCGF Building, South Parks Road, Oxford OX1 3QX***

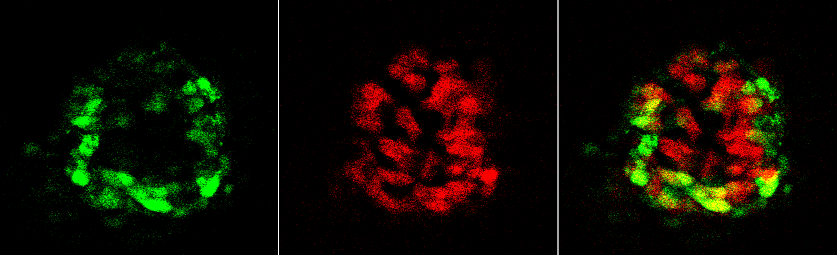
***Tel: 01865 285825 Email: juris.galvanovskis@dpag.ox.ac.uk***

The Imaging Core Facility is run by Dr. Juris Galvanovskis. He is responsible for training graduate students, training fellows and other researchers to use the existing imaging equipment to investigate fluorescent specimens of biological tissue, cells and their sub-cellular structures. An integral part of modern imaging applications in biomedical research is the analysis of acquired images in order to extract quantitative information of interest from original data.

In addition to training on imaging hardware Dr. J. Galvanovskis gives introductory courses on basic analysis software packages that are used for image post-processing. Support in developing novel methods of analysis that may be necessary to solve problems that may arise in someone’s specific research area is also available. Likewise, advice can be provided on the design of imaging experiments and selection of existing imaging equipment in order to achieve the best result and optimize research activities.

The OXION Imaging Core Facility includes some existing imaging equipment held by OXION groups, as well as an Olympus total internal reflection (TIRF) microscope. At present this equipment is located with the corresponding research groups and the TIRF microscope is located in Professor Patrik Rorsman’s laboratory at OCDEM, Churchill Hospital. The Imaging Core Facility also has use of two Carl Zeiss confocal laser scanning systems LSM510 META. One of the scanning modules is arranged on an inverted microscope Axiovert 200, the other identical scanning module is attached to an upright microscope Axioskop 2F. A Coherent infrared laser Chameleon is integrated into both of these confocal systems and allows one to perform imaging with multi-photon excitation. A fluorescence microscope Axio Vert.A1 (Carl Zeiss) equipped with filter sets for viewing cyan, green and red fluorescent proteins and with a digital camera for acquiring images of fluorescent samples is now available for OXION members in OCGF (lab of Pr. Frances Ashcroft). A Leica confocal microscope with an integrated infrared laser for two photon imaging is being set up at Physiology.

Latest examples of images acquired on these imaging systems are seen in Figs. 1 and 2. During the next few years, some of this equipment will be moved to the Department of Physiology, Anatomy and Genetics in order to create a single core facility.

******

***Fig.2.*** *An islet from a control mouse loaded with Fluo-4 detected in the green channel of the confocal microscope; all pancreatic cells are detected in this channel. The same islet is seen in red channel as well. This channel is arranged to detect the emission of the red fluorescent protein and shows α-cells only.*

***Fig. 1.*** *Multivesicular exocytosis detected by confocal imaging in isolated rat β-cells. a) 3-D reconstruction of a single exocytotic event; seen is a ribbon of the β-cell membrane from the rat pancreas; the cell has been exposed for 30 s to FM1-43FX in the presence of 20 mmol/l glucose alone. b) As in a) but showing an example of a large compound event observed in the simultaneous presence of 20 mmol/l glucose and 20 μmol/l carbachol.*



**Collaborations**

Ratiometric measurements of calcium concentration in bovine kidney cells (MDBK cells) by IonOptix Fluorescence Imaging System within a project: Effect of thiazolites on intracellular Ca2+ stores. (Drs. Omodele Ashiru and Terry Butters, Department of Biochemistry, Oxford Glycobiology Institute, Oxford).

Assessment of the binding ability of a fluorescently tagged peptides identified by phage display (and a control peptides) to various cell lines by confocal microscopy. (Dr. Mark Stevenson and Pr. Rajesh Thakker, OCDEM, Churchill Hospital, Oxford).

Imaging of intracellular Ca in various types of cells in pancreatic islets by confocal microscopy. (Prof. Patrik Rorsman, Dr. Quan Zhang, Dr. Orit Braha, PhD student E. Vergari, OCDEM, Churchill Hospital, Oxford).

A single cell tracking in the Zebrafish embryo with the help of photo-convertible fluorescent proteins EosFP and KikGRFP (Prof. Roger Patient & Dr. Stuart Meiklejohn, the Weatherall Institute of Molecular Medicine, Oxford).

Visualization of compound exocytosis in pancreatic β-cells by confocal microscopy and two-photon imaging (Prof. Patrik Rorsman and Dr. Stephan Collins, OCDEM, Churchill Hospital, Oxford).

Studies of exocytosis in pancreatic cells by two-photon imaging (TEPIQ) (Prof. Patrik Rorsman, Dr. Stephan Collins and PhD student David Do, OCDEM, Churchill Hospital, Oxford).

Visualization of ATP concentration in living cells with the help of FRET confocal imaging (Prof. Frances Ashcroft, Dr. Kenju Shimomura and Dr. Heidi de Wet, Department of Physiology, Anatomy and Genetics, Oxford).

Visualization of T-lymphocyte migration within the skin by two-photon imaging of quantum dots attached to the cell surface. (Dr. Graham Ogg, Department of Dermatology, Churchill Hospital, Oxford).

***Publications*** (\*OXION collaborations 2011 – 2012)  
  
1 . \* Hoppa MB, Jones E, Karanauskaite J, Braun B, Collins S, Zhang Q, Clark A, Eliasson L, Genoud C, Macdonald PE, Monteith AG, Barg S, **Galvanovskis J** and **Rorsman P** (2012) Multi-vesicular exocytosis in rat pancreatic β-cells triggered by muscarinic receptor activation. *Diabetologia* **55(4)**:1001-12.

The Rodent Behaviour Facility

**Dr Rob Deacon**

***Department of Experimental Psychology, University of Oxford, South Parks Road, Oxford OX1 3UD Email:*** [***robert.deacon@psy.ox.ac.uk***](mailto:robert.deacon@psy.ox.ac.uk)

Dr. Rob Deacon runs the rodent behaviour core facility and collaborates closely with Dr. David Bannerman. He is responsible for training graduate students, fellows and researchers in the behavioural testing of rats and mice. He is also available to give advice on the design and implementation of novel testing paradigms. He has been working on rodent behaviour in academia and industry since 1974, and in Experimental Psychology at Oxford since 1991.

Rob also undertakes “external” research, taking apparatus and expertise to different departments and testing the animals *in situ.* Although such external work is mainly in Oxford, we have also prepared and tested mice in Switzerland, Chile, Kenya and Russia.

The facilitycentres on behavioural phenotyping, i.e. characterising the behaviour of genetically modified animals, and mice are currently the most widely used animal for this work, as fundamental mammalian genetics has concentrated on this species. But animal behaviourists have traditionally used rats rather than mice. Rats were regarded as more intelligent and reliable, mice as timid and stupid. So developing good behavioural tests for mice was essential to realise the potential of the advances in genetics and molecular biology. We presently run over 40 different behavioural tests, several of which originated in this facility, which can be divided into four main areas: cognition (learning and memory), emotionality (anxiety), motor behaviour (activity, strength and co-ordination) and species-typical behaviours (nesting, burrowing, hoarding etc.).

As well as working with almost all the biomedical departments at Oxford, we have also collaborated with, or advised, pharmaceutical companies (ACADIA, Boehringer Ingelheim, Synaptica); MRC Harwell; the Royal Veterinary College; National Institute for Medical Research, Mill Hill; Veterinary Laboratories Agency, Zvenigorod biological station (Moscow State University), etc. But our main function within the OXION group is to provide training for graduate students, training fellows and other researchers in mouse (and rat) behavioural techniques. We have expertise in CNS lesioning techniques, mainly stereotaxic cytotoxic lesions of the hippocampus (complete or selective dorsal vs ventral) habenula and prefrontal cortex in rats, mice and voles. Currently we are investigating the differential involvement of dorsal and ventral hippocampus in mouse behaviour. Also we have comprehensively phenotyped the naked mole-rat, an extraordinary subterranean rodent which lives up to 30 years and appears to be immune from cancer.

This brief overview illustrates the broad range of behavioural assessments that can be performed to find out “what’s wrong with your mouse”. We are eager to share our expertise in behaviour, so if anyone has a project proposal, especially one involving ion channels, please get in touch. I can be reached via email on [robert.deacon@psy.ox.ac.uk](mailto:robert.deacon@psy.ox.ac.uk), or by phone on 271428.

***Collaborations***

* Learning deficits in hippocampal lesioned and mutant mice (Rawlins, Bannerman).
* The role of the hippocampus in behaviour in mice and voles (collaboration with Zvenigorod Biological Station, Moscow State University).
* Puzzle box studies in mice (University of Zurich).
* Behaviour in mice, drosophila and zebra fish with mutations related to Fragile X syndrome. The degu as a natural model for Alzheimer disease (University of Santiago).
* Phenotyping of the naked mole-rat (University of Nairobi).
* Behavioural functions of dynein (with Fisher group at UCL).
* Functional asymmetry in hippocampal plasticity (collaboration with OXION members from the Paulsen group)
* Synuclein studies (Wade-Martins group).
* T cells, Annexin-A1 and mouse behavior. Collaboration with Fulvio D’Acquisto, William Harvey Research Institute
* Wider publicity for our work. This includes a video for Understanding Animal Research, and eight open access videos for the Journal Of Visualised Experimentation (JOVE), funded by the Wellcome Trust.

***Publications*** (\*Collaborations within OXION: 2011-2012)

* 1. **\* Deacon R** (2012) Assessing burrowing, nest construction and hoarding in mice. *J Vis Exp*

**59**:e2607.

* 1. \* **Deacon RMJ**, Dulu TD, Patel NB (2012) Naked mole-rats: behavioural phenotyping and comparison with C57BL/6 mice. *Behavioural Brain Research* **231**:193-200.
  2. **\*** Murray C, Sanderson DJ, **Barkus C**, **Deacon RM**, **Rawlins JN**, **Bannerman DM**, Cunningham C (2012) Systemic inflammation induces acute working memory deficits in the primed brain: relevance for delirium. *Neurobiol Aging* **33(3)**:603-616.e3.
  3. **\*** Sanderson DJ, **Rawlins JN**, **Deacon RM**, Cunningham C, **Barkus C**, **Bannerman DM** (2012) Hippocampal lesions can enhance discrimination learning despite normal sensitivity to interference from incidental information. *Hippocampus* **22(7)**:1553-66.
  4. **\*** Schneider T, Skitt Z, Liu Y, **Deacon RM**, **Flint J**, Karmiloff-Smith, **Rawlins JN**,

Tassabehji M (2012) Anxious, hypoactive phenotype combined with motor deficits in Gtf2ird 1 null mouse model relevant to Williams syndrome. *Behav Brain Res* **233(2)**:458-73.

The Mouse Neurophysiology Facility

**Dr Louise Upton**

***Department of Physiology, Anatomy and Genetics, Sherrington Building, Parks Road, Oxford. OX1 3PT***

***Tel: 01865 272511 Email: louise.upton@dpag.ox.ac.uk***

### *In vivo* electrophysiology

This includes several different approaches for monitoring neuronal activity in anaesthetised and awake animals, as well as optogenetic techniques for controlling neuronal activity:

1. ***In vivo* extracellular recording** - simultaneously record a) action potentials and b) local field potentials using 16-channel multi-electrodes in anaesthetised animals. A range of sensory stimuli (visual, somatosensory and auditory) are available for testing.
2. ***In vivo* whole-cell recording** – monitor membrane potential changes in single neurons in anaesthetised animals using patch electrodes.
3. **Telemetric EEG measurements** **in** **awake** **mice**. A small transmitter, connected to two skull electrodes is implanted under the skin of the mouse. It can continuously transmit for up to three weeks and allows for long-term recordings in freely-moving animals. Coupled with CCTV monitoring allows behavior to be monitored simultaneously and seizures to be identified unambiguously. The power of oscillations in all frequency ranges (0-200Hz) of the EEG can be monitored, and we are currently working with the manufacturer to develop software to detect seizures automatically.

### Optogenetic control of neuronal activity

1. **Optogenetics**. This powerful tool can be used to control neuronal activity in specified neuronal populations. A light-activated ion channel (channelrhodopsin) or inhibitory chloride and proton pumps (halorhodopsin and archaerhodopsin) can be expressed constitutively in a transgenic mouse or be delivered stereotaxically into the brain encoded by a virus. Optogenetic activation or silencing of neurons can be driven efficiently by an optical fibre cable coupled to an LED light source of a defined wavelength. This can be performed *in vivo* in anaesthetised animals. Alternatively the brain can be removed and used for slice electrophysiology with specific axonal inputs being activated.

### Cardiovascular physiology measurements

1. **Monitoring heart rate, breathing rate and blood oxygenation levels –** performed non-invasively in rats and mice using a pulse oximeter.

### Neuroanatomy

1. **Injections of neuronal tracing agents to study connectivity** –injections of neuronal tracers can be used to identify and map connections between brain areas.
2. **Immunohistochemistry** – antibodies can be used to localise proteins of interest in brain sections.
3. **Quantitative analysis of neuronal morphology –** quantitative measurements of the distribution and morphology of stained neurons can be made usingNeurolucida software and a motorised microscope stage.

### In vivo drug delivery*.*

1. **Osmotic mini-pumps for drug delivery** –miniature infusion pumps (Alzet.com) can be implanted to provide continuous drug infusion in a freely-moving rodent for up to 6 weeks. Delivery can be systemic, or into the cerebrospinal fluid or localised brain regions.
2. **Direct injection in to the CNS –** through an implanted cannula for repeated injections or acutely through a small craniotomy.

Dr Louise Upton runs the core facility in mouse neurophysiology, and is responsible for training graduate students, training fellows and other researchers in neuroanatomical and whole animal recording techniques.

Louise has 15 years’ experience of looking at CNS development in normal and transgenic rodents and uses a variety of anatomical and physiological techniques to do this. She supervises DPhil students, MSc (Neuroscience) student projects and OXION student rotation projects, and contributes to neuroscience teaching and examining for the university.

OXION members thinking of examining neurophysiological parameters in mouse models of disease are encouraged to contact Louise Upton by email.

***Collaborations*** (OXION members in bold)

* Telemetric recording of EEG activity in mice injected with patient sera to investigate auto-immune causes of epilepsy. (**Bethan Lang** and **Angela Vincent**, WIMM, **Julian Bartram** OXION student**)**
* Sensory-motor integration in the mouse whisker system. Extracellular multielectrode and whole cell recording in somatosensory cortex during both optogenetic, electrical and sensory stimulation in anaesthetised mice (**Julian Bartram** OXION student, **Ed Mann**, DPAG)
* The role of the periaqueductal grey in control of the heart (**Goudarz Karimi** OXION student, **David Paterson**, DPAG)
* Network oscillations in cortex: in vivo recordings plus pharmacological blockade of GABA receptors (**Ed Mann,** DPAG).
* Multisensory interaction in auditory cortex: anatomical tracing of connections between visual, somatosensory and auditory cortex; and electrophysiological studies of interactions between sensory stimuli (**Ole Paulsen**, Jonathan Webb and Andrew King, DPAG).
* Topographic order in the mouse auditory thalamocortical system. Using neuronal tracing agents to map the connections between mouse cortex and thalamus; developing tools to allow quantitative analysis of the distribution of labelled cells (**Ian Thompson**, KCL).

### *Publications*

1. \* Li J, Bravo DS, **Upton AL**, Gilmour G, Tricklebank MD, Fillenz M, Martin C, Lowry JP, **Bannerman DM**, McHugh SB (2011) Close temporal coupling of neuronal activity and tissue oxygen responses in rodent whisker barrel cortex. (*Eur J Neurosci* **34**: 1983-96).

The Microarray Facility

**Ms Sheena Lee**

***Department of Physiology, Anatomy and Genetics, OCGF Building, South Parks Road, Oxford OX1 3QX***

***Tel: 01865 272505 Email: sheena.lee@dpag.ox.ac.uk***

**The OXION microarray laboratory** measures genome-wide expression profiles of both protein coding genes and non coding RNAs eg miRNAs and long non coding RNAs. This enables scientists to understand the biological mechanisms of complex processes and diseases.

**Expression microarrays** Affymetrix arrays were chosen as they are a leader in gene expression microarrays. They are high quality oligonucleotide arrays which cover the transcriptome for a wide number of organisms eg human, mouse, Drosophila, C.elegans, bovine etc. They are supported by an extensive range of bioinformatics tools: eg Ensembl, UCSC genome browser, GO-Elite, Ingenuity, David which facilitates the high quality analysis of array data. The arrays are widely used in the academic community, enabling direct comparison of data with that in the literature and public databases.

**Array types** *Affymetrix Gene ST* arrays are the highest quality arrays for measuring protein coding, gene expression profiles, in standard amounts of tissues (10mg) and cells (1x106 cells)-£200/sample for the labelling and the array.

*Laser capture microdissection* is now enabling the measurement of gene expression from as few as 500 individually captured cells on these arrays-Anna Dulneva, Kay Davies.

*MicroRNAs* are relatively newly discovered, short, 22 base RNAs involved in the regulation of gene expression and are being increasingly studied. Agilent produce high quality, highly specific microRNA arrays. Access to a MRC Agilent scanner enables use of the more cost effective Agilent arrays. We have successfully used Agilent arrays to identify the expression of miRNAs in a mouse model of muscular dystrophy and in an FTO knockout mouse.

We can also measure *miRNAs in Laser Capture Microdissected* cells from as little as 1ng starting RNA. We have successfully used this method for identifying miRNAs in motor neurons from the spinal cord of a mouse model of amyotrophic lateral sclerosis, a muscle wasting disease.

*Long non coding RNAs (LincRNAs)* have been recently discovered and are also thought to have a role in controlling gene expression. They and protein coding genes can be measured on the same Agilent 60K array. We have successfully knocked out a long non coding RNA, AK032637 and identified the genes and lincRNAs it regulates.

**RNA-Seq** Microarrays are currently the most cost effective way of measuring gene expression, are easy to analyse and give the same high quality data as RNA-Seq for known genes. Longer term it is planned that a high throughput sequencer will be purchased.

**Bioinformatics** Limma inGeneSpring is used to identify gene expression changes. GO annotation and pathways tools eg GenMAPP’s Go-Elite and Ingenuity and are used to identify collective biological functions in a list of differentially expressed genes as well as to show the interaction between genes.

**Use of the OXION microarray facility** is run on a collaborative basis. The success of an array experiment is very much dependent on the design of the experiment so advice is provided about experimental design, the amount of RNA required, the type of chip to use and the number of replicates needed. RNA samples which pass a quality threshold on the Agilent Bioanalyser are prepared and run on the Affymetrix or Agilent system. Bioinformatics tools are used to identify differentially expressed genes, the collective biological functions of these genes and the interaction between them.

**Sheena Lee**, who took up her post in June 2004, is responsible for running the microarray core facility and for training graduate students, training fellows and other researchers in microarray techniques.

Prior to joining the University of Oxford, Sheena worked for an Oxford Biotech company for 5 years where she set up and ran a gene array core facility.

***Current Collaborations***

* The Fat mass and obesity associated gene (FTO) is associated with obesity. Gene expression changes in tissues from mice that are over expressing FTO and are consequently overweight have been measured-James McTaggart, Myrte Merkestein, Fiona McMurray, Roger Cox, Frances Ashcroft
* miRNA and gene expression in liver from FTO knockout mice-Fiona McMurray, Roger Cox, Frances Ashcroft
* Identification of FTO binding sites and m6A methylation sites on RNA using CLiP-Seq-Myrte Merkestein, Lukasz Stasiak, Martina Helleger, Roger Cox, Frances Ashcroft
* The role of Pauper, a putative large non coding RNA, in controlling gene expression– Keith Vance, Vladislava Chalei, Chris Ponting
* Measurement of gene changes in a SOX4 mutant mouse which has a diabetic phenotype. Genes involved in vesicle formation were found to be altered and are being followed up by Patrik Rorsman's group at the Oxford Centre for Diabetes, Endocrinology and Metabolism-Alison Hough, Roger Cox, Patrik Rorsman
* Lysine (K)-specific demethylase 2B (KDM2B) is selectively recruited to genomic regions associated with up to 70% of promoters. In addition, KDM2B can interact with members of the Polycomb silencing complex, known to be essential for many aspects of development. Microarray data from KDM2B depleted cells will be overlapped with ChIP-Seq data to determine its role in gene expression- Anca Farcas, Rob Klose, Mark Sansom
* Identification of the target genes of linc8, a putative large non coding RNA - Vladislava Chalei, Keith Vance, Chris Ponting
* The role of a putative long non coding RNA which is the evolutionary result of the metamorphosis of previous protein-coding gene to long non coding RNA-Ana Marques (Wellcome Trust funded), Chris Ponting
* Circadian rhythms are coordinated by the suprachiasmatic nuclei (SCN) of the hypothalamus. Direct comparison of gene expression in SCN and whole brain (WB) from the same animals will allow clear confirmation of SCN enriched genes, as well as over-represented gene ontologies and pathways. The group will examine the roles of ion channels and their interacting proteins, using a range of electrophysiological, imaging and behavioural techniques.-Laurence A Brown, Stuart Peirson, Russell Foster, Mark Hankins
* Mutations in glycyl-tRNA synthetase (Gars) have been shown to cause Charcot-Marie-Tooth hereditary neuropathy type 2, an axonal (non-demyelinating) peripheral neuropathy characterized by distal muscle weakness and atrophy. It has been demonstrated that muscle cells secrete glycyl-tRNA synthetase (Gars) and this has an effect on the nerves. Mutant Gars will be added to NSC cells and any gene changes identified- Greg Weir, Zam Cader.
* When the ventral periaqueductal grey (PAG) is stimulated, blood pressure and heart rate decrease. When the dorsal PAG is stimulated blood pressure and heart rate increase, thus indicating a link between the PAG and hypertension. Microarrays will be run of PAG isolated from normal and hypertensive rats-Goudarz Karimi, David Paterson.
* Identification of genes and non coding RNAs affected by knocking out and over expressing a novel non coding RNA- Tom Lickiss, Tamara Sirey, Zoltan Molnar, Chris Ponting
* Gene expression during subependymal zone (SEZ) cell emigration to sites of injury caused by trauma, multiple sclerosis lesions or stroke-Francis Szele
* The transcription factor Er81 is involved in cerebral cortical development. It has been electroporated into embryonic cortical progenitor cells and the patterns of gene expression examined using microarrays- Amanda Cheung, Jamin de Proto, Zoltan Molnar

***Students***

**DPhil students** whohave used the microarray facility and subsequently been awarded a DPhil: Ying Cui, James McTaggart, Chris Church, Mattéa Finelli, Franziska Oeschger, Akshay Bareja, Alison Hough, Olivia Osborn, Anna Hoerder, Dirk Baumer, Lyndsay Murray, Joana Figueiredo (MSc student)

Current DPhil students using the OXION microarray facility**:** Anna Dulneva, Achilleas Livieratos, Anna Farcas

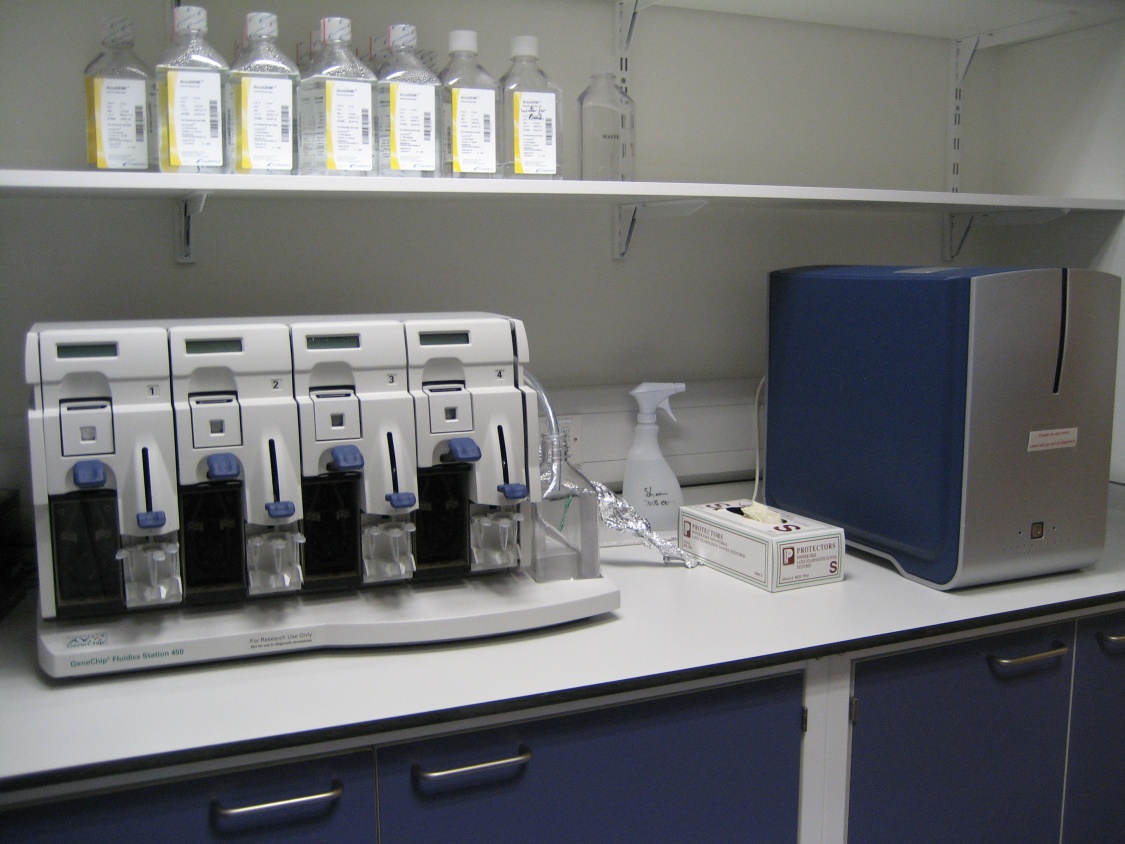
***Publications***

1. Oliver PL, Sobczyk MV, Maywood ES, Edwards B, **Lee S**, Livieratos A, Oster H, Butler R, Godinho SIH, Wulff K, Peirson SN, Fisher SP, Chesham JE, Smith JW, Hastings MH, **Davies KE** and Foster RG (2012) Disrupted circadian rhythms in a mouse model of schizophrenia *Current Biology* **22(4)**:314-9.
2. McTaggart JS, **Lee S**, **Iberl M**, Church C, **Cox RD**, et al. (2011) FTO Is Expressed in Neurones throughout the Brain and Its Expression Is Unaltered by Fasting. *PLoS ONE* **6(11):** e27968. doi:10.1371/journal.pone.0027968
3. Oeschger FM, Wang W-H, **Lee S**, García-Moreno F, Goffinet AM, Arbonés ML, Rakic S, and Molnár Z(2011) Gene Expression Analysis of the Embryonic Subplate *Cereb. Cortex* **22(6)**:1343-59.

***Pricing*** All users purchase their own chips and reagents.Use of the microarray facility is free for OXION members. A charge of £50/sample is made to associate OXION members and £100/sample for non OXION members.

Fluidics station

Scanner



Affymetrix Genechip system

The Proteomics Facility

**Dr Holger Kramer**

***Department of Physiology, Anatomy and Genetics, OCGF Building, Parks Road, Oxford OX1 3QB***

***Tel: 01865 285814 Email:holger.kramer@dpag.ox.ac.uk***

The OXION Proteomics facility is equipped with state of the art equipment for separation and identification of complex biological samples. Fractionation techniques offered in the facility include FPLC (ÄKTA 900) and HPLC (Agilent 1100 series) instrumentation, ultracentrifugation and free flow electrophoresis (FFE, BD Biosciences). Furthermore gel-based approaches for separation by 1D and 2D gel electrophoresis are available. At the core of the analytical capability of the lab are the Bruker Ultrafelx MALDI-TOF/TOF and amaZon ETD Ion Trap LC-MS/MS systems. Both mass spectrometry instruments enable precursor ion fragmentation in order to obtain sequence information from biomacromolecules. With respect to their analytic and instrument characteristic these systems are highly complementary.

The equipment and expertise in the lab allow routine identification of protein/peptide samples, characterization of post-translational and artificial modifications as well as comparative studies. In addition molecular weight determination of expressed and purified proteins can be performed. Protein interaction partner studies are carried out by combining co-immunoprecipitation experiments with identification by tandem mass spectrometry.

An active research interest exists in the lab towards the enrichment and analysis of post-translational modifications (PTMs). This is facilitated by the Ion-Trap LC-MS/MS system which is capable of performing ETD (electron transfer dissociation) fragmentation compatible with sensitive protein modifications that are sometimes lost under conventional fragmentation conditions.

***Collaborative Projects***

Besides providing a general service to the OXION user community (training, sample analysis and data interpretation), Holger participates in the following collaborative projects within and outside OXION:

1. Molecular characterization of KATP ion channels and protein interaction partners (with Gregor Sachse, Heidi de Wet, Fran Ashcroft).

2. Identification of interaction partners and substrates of Human Fat Mass and Obesity Associated Gene product FTO (with Lukasz Stasiak, Myrte Merkestein, Chris Schofield, Fran Ashcroft, Roger Cox).

3. Characterization of targets of autoantibodies with relevance to human neurological disease (with Bethan Lang, Angela Vincent).

4. Quantification of glibenclamide levels in mouse tissues by LC-MS methodology (with Carolina Lahmann, Fran Ashcroft).

5. Proteomics analysis of a hypertensive phenotype in a neonatal rat heart model (with Hege Larsen Rebecca Burton, Gil Bub, David Paterson)

6. Investigation of the phosphorylation status of Nuclear Factor of Activated T-cells, NFAT (with Pulak Kar, Anant Parekh)

***Publications***

1. **Kramer HB**, Lahmann C, Shimomura K, Ashcroft FM (2012) ‘A sensitive and specific LC-MS method for the quantitation of glibenclamide in mouse plasma’; submitted.
2. **Kramer HB**, Nicholson B, Kessler BM, Altun M (2012) ‘Detection of ubiquitin-proteasome enzymatic activities in cells: Application of activity-based probes to inhibitor development’; *Biochim Biophys Acta*. 2012 May 19; [Epub ahead of print]
3. McGouran JF, **Kramer HB**, Mackeen MM, di Gleria K, Altun M, Kessler BM (2012) ‘Fluorescence-based active site probes for profiling deubiquitinating enzymes’; *Org Biomol Chem.* **10(17)**:3379
4. Altun M, **Kramer HB**, Willems LI, McDermott JL, Leach CA, Goldenberg SJ, Kumar KG, Konietzny R, Fischer R, Kogan E, Mackeen M, McGouran J, Khoronenkova SV, Parsons JL, Dianov GL, Nicholson B, Kessler BM (2011) Activity-based chemical proteomics accelerates inhibitor development for deubiquitylating enzymes‘; *Chem Biol.* **18(11)**:1401
5. Ternette N, Wright C, **Kramer HB**, Altun M, Kessler BM (2011) ‘Label-free quantitative proteomics reveals regulation of interferon-induced protein with tetratricopeptide repeats 3 (IFIT3) and 5’-3’-riboexonuclease 2 (XRN2) during respiratory syncytial virus infection’; *Virol J.* **8(1)**:442

**Current OXION Membership**

**OXION Group Leaders**

Dr. Radu Aricescu

Professor Frances Ashcroft

Dr. David Bannerman

Professor David Beeson

Professor Steve Brown

Professor Roger Cox

Professor Kieran Clarke

Professor Kay Davies

Professor Jonathan Flint

Professor Michael Hanna

Professor Dimitri Kullmann

Professor Gero Miesenböck

Professor Anant Parekh

Professor David Paterson

Professor Nicholas Rawlins

Professor Patrik Rorsman

Professor John Ryan

Professor Mark Sansom

Dr. Stephen Tucker

Professor Nigel Unwin

Professor Catherine Vénien-Bryan

Professor Angela Vincent

**OXION Associate Members**

Dr Phil Biggin

Dr Maike Glitsch

Professor Paul Harrison

Dr Bethan Lang

Dr Ed Mann

Professor Ole Paulsen

Professor David Sattelle

Professor Ian Thompson

**Group Members**

**Dr A Radu Aricescu**

***Wellcome Trust Centre for Human Genetics, Division of Structural Biology, University of Oxford Tel: 01865 287564 Email:*** [***radu@strubi.ox.ac.uk***](mailto:radu@strubi.ox.ac.uk)

The ~20-25nm cleft that separates neurons engaged in central nervous system synapses contains an intricate protein network that provides structural support and avenues for communication. Increasingly, the functions and isolated molecular structures of neurotransmitter and other cell surface receptors, adhesion molecules, proteoglycans and secreted proteins that belong to this network are being elucidated. Little is known, however, about the *higher order organization* of molecules in the synaptic cleft, or indeed what might be the functional importance, in normal and pathological circumstances, of such supra-molecular arrangements. To date, technical limitations have hindered the study of such complex systems, despite their biological significance (cellular proteins generally never work "alone").

The aim of my laboratory is to reach a fundamentally different level of knowledge in molecular neuroscience. By exploring trans-synaptic protein complexes of increasing size, we pursue a better understanding of the molecular principles governing synaptic transmission. We currently focus on complexes assembled around, and modulating the function of, ionotropic receptors for glutamate and gamma-amino butyric acid (GABA), the two neurotransmitters that dominate signalling in the vertebrate central nervous system, as well as cell surface receptor enzymes (protein tyrosine phosphatases and kinases). Our work relies on a combination of structural biology techniques: X-ray crystallography, cryo-electron microscopy/tomography and X-ray microscopy. Structurally inspired mechanisms are then validated in the relevant functional context, by live-cell fluorescence microscopy, electrophysiology and studies in model organisms.

***Publications*** (\*collaborations within OXION 2011-2012)

1. Bell CH, **Aricescu AR**, Jones EY, Siebold C (2011) A dual binding mode for RhoGTPases in plexin signalling. *PLoS Biol.* **9**:e1001134.
2. Malinauskas T, **Aricescu AR**, Lu W, Siebold C, Jones EY (2011) Modular mechanism of Wnt signaling inhibition by Wnt inhibitory factor 1. *Nat Struct Mol Biol* **18**:886-893.
3. Seiradake E, Coles CH, Perestenko PV, Harlos K, McIlhinney RA, **Aricescu AR**, Jones EY (2011) Structural basis for cell surface patterning through NetrinG-NGL interactions. *EMBO J*. **30**:4479-4488.

**Professor Frances M Ashcroft**

***Department of Physiology, Anatomy & Genetics, University of Oxford***

***Tel: 01865 285810 Email: frances.ashcroft@dpag.ox.ac.uk***

We are currently experiencing a fast-growing pandemic of type 2 diabetes (T2DM). It affects 336 million people worldwide (many more have impaired glucose tolerance), and it is responsible for 4.6 million deaths each year (one every seven seconds). T2DM increases the risk of heart disease, stroke and microvascular complications such as blindness, renal failure, and peripheral neuropathy. Consequently, it places a severe economic burden on governments and individuals. In the UK alone, ~10% of the NHS budget (£1 million an hour) is spent on treating diabetes and its complications.

It is generally agreed that the primary defect in T2DM lies in the pancreatic beta-cell which fails to secrete sufficient insulin. Age and obesity (which lead to insulin resistance) increase disease risk by placing a greater demand on the beta-cell that it is unable to match. Thus the overall aim of our research is to understand how glucose stimulates insulin secretion from the pancreatic beta-cells, and what goes wrong with this process in both T2DM and monogenic forms of diabetes such as neonatal diabetes. Currently, our primary focus is the ATP-sensitive potassium (KATP) channel, which plays a key role in the physiology and pathophysiology of the beta-cell. We also have an increasing interest in obesity, because of its influence on T2DM.

*Neonatal diabetes*

Insulin secretion results from Ca2+ influx across the beta-cell plasma membrane through voltage-gated Ca2+ channels. In the absence of glucose, KATP channels hold the membrane at a hyperpolarised level, so that voltage-gated Ca2+ channels remain shut and insulin is not secreted. Glucose metabolism generates ATP, which closes KATP channels and thereby stimulates insulin secretion. Similarly, the KATP channel couples metabolism to electrical activity in neurones.

Gain-of-function mutations in either the Kir6.2 or SUR1 subunit of the KATP channel are a common cause of neonatal diabetes (ND), a rare inherited disorder characterised by the development of diabetes within the first six months of life. A very few patients (<3%) experience motor and mental ***d***evelopmental delay, muscle hypotonia, and ***e***pilepsy, in addition to ***n***eonatal ***d***iabetes (DEND syndrome). Rather more (~20%) manifest an intermediate condition consisting of developmental delay, muscle hypotonia and neonatal diabetes (iDEND syndrome). All ND mutations act by reducing the ability of ATP to close the channel, thereby enhancing the KATP current, and preventing membrane depolarization when cell metabolism increases. This leads to impaired insulin secretion from beta-cells and reduced electrical activity in neurones. Sulphonylurea drugs, which close KATP channels directly, stimulate insulin secretion in most patients with neonatal diabetes and have replaced insulin as the therapy of choice.

We have continued to study how mutations affect KATP channel function and so cause either ND (activating mutations) or hyperinsulinism (loss-of-function mutations). The studies have shed light on how the protein functions. We have also investigated why the Kir6.2-V69M mutation, which causes iDEND syndrome, produces a reduced sensitivity to general anaesthesia. This effect was not reversed by sulphonylurea (glibenclamide) therapy suggesting the drug either cannot access the brain at concentrations high enough to shut the channel or that KATP channel function is needed during development. With Holger Kramer (OXION proteomics) we developed a sensitive method for assaying plasma glibenclamide concentrations and found that female mice have much higher plasma glibenclamide levels than males treated with the same

dose. Interestingly, Kir6.2-V69M animals showed no difference in susceptibility to injected anaesthetics.

We found that selective activation of the Kir6.2-V69M mutation in pancreatic beta-cells in adult life causes acute diabetes. This could be rapidly reversed by glibenclamide or insulin therapy. As little as two weeks of severe hyperglycaemia reduced insulin content by 50%, and caused a small decrease in islet number, size and number of beta-cells/islet. Insulin therapy was not as effective as glibenclamide at protecting the islets from the effects of KATP channel activation. With Kieran Clarke, we found that the Kir6.2-V69M mutation has no obvious effect on cardiac function, despite the high density of KATP channels in the heart. Functional studies suggest this is because the cardiac and beta-cell KATP channels handle nucleotides differently. We have now begun to study the effects of the Kir6.2-V69M mutation on glucagon secretion from pancreatic alpha cells (with Patrik Rorsman).

In separate studies, we have continued to work towards obtaining high-resolution structures of SUR1 and the complete KATP channel complex. We also analysed the complex way in which nucleotides interact with glibenclamide to block the KATP channel.

*FTO and obesity*

Type 2 diabetics are often obese. There is good evidence that single gene polymorphisms in the fat mass and obesity related protein (FTO) are associated with an enhanced risk of obesity. Collaborative studies with Roger Cox have shown that enhanced FTO expression increases food intake, fat mass and obesity; and conversely that loss of activity, or a reduction in activity, results in reduced fat mass and increased energy expenditure, but unchanged physical activity in mice. We also found that FTO is widely expressed throughout the brain and that neither its expression nor nuclear localization changes on fasting. In collaboration with Sheena Lee (microarray facility), we have identified changes in gene expression that result from enhanced expression of FTO in different tissues.

*OXION collaborations*

This year we have had extensive collaborations with Profs Clarke, Cox, Sansom and Rorsman and also benefitted from collaborations with Sheena Lee (microarrays) and Holger Kramer (proteomics).

***Publications*** (\*collaborations within OXION 2011-2012)

# \* Ashcroft FM, Rorsman P (2012) Diabetes and the beta-cell: the last ten years. *Cell* 148:1160-1171.

1. \* **de Wet H**, Shimomura K, Aittoniemi J, Nawaz A, **Lafond M**, **Sansom M**, **Ashcroft FM** (2012) A universally conserved residue in the SUR1 subunit of the KATP channel is essential for translating nucleotide binding at SUR1 into channel opening. *J Physiol* *in the press.*

# \* Clark R, Mannikko R, Stuckey D, Iberl M, Clarke K, Ashcroft FM (2011) Mice expressing a human KATP channel mutation have altered channel ATP sensitivity, but no cardiac abnormalities. *Diabetologia* 55:1195-1204*.*

# \* McTaggart JS, Lee S, Iberl M, Church C, Cox RD, Ashcroft FM (2011) FTO is Expressed in Neurons Throughout the Brain and its Expression is Unaltered by Fasting. *PLoS One* 6:e27968.

1. Ashcroft FM (2012) *The Spark of Life*. Penguin 339 pages

**Professor David Bannerman**

***Department of Psychology, University of Oxford, South Parks Road, Oxford OX1 3UD***

***Tel: 01865 271426 Email: david.bannerman@psy.ox.ac.uk***

We carry out behavioural neuroscience research in the Department of Experimental Psychology. We are particularly interested in the contribution that different AMPA and NMDA glutamate receptor subtypes and their subunits make to learning and memory in medial temporal lobe structures like the hippocampus. I am funded by a Wellcome Trust Senior Research Fellowship which runs until 2014 and joined the OXION consortium in the summer of 2004.

During the last year we have extended our characterisation of the behavioural phenotype of glutamate receptor sub-unit knockout mice. For example we have continued to investigate and characterise the role of GluA1 AMPA receptors in various forms of short-term memory. We have also conducted experiments assessing the importance of NMDA receptors, specifically in the granule cells of the dentate gyrus and the pyramidal cells of the CA1 hippocampal subfields for learning and memory. In addition, we also aim to identify (i) which brain areas and (ii) which neural mechanisms underlie different aspects of behaviour by recording electrophysiological and haemodynamic signals in behaving animals.

***Publications*** (\*collaborations within OXION 2011-2012)

1. \* Allen K, **Rawlins JNP**, **Bannerman DM**, **Csicsvari J** (2012). Hippocampal place cells can encode multiple trial-dependent features through rate modulation. *Journal of Neuroscience,* in press.
2. **\* Bannerman DM**, Bus T, Taylor AM, Sanderson DJ, Schwarz I, Jensen V, Hvalby Ø, **Rawlins JNP**, Sprengerl R, Seeburg PH (2012). Dissecting spatial knowledge from spatial choice by hippocampal NMDA receptor deletion. *Nature Neuroscience* **15(8)**:1153-9.
3. \* **Barkus C**, Dawson LA, Sharp T, **Bannerman DM** (2012) [GluN1 hypomorph mice exhibit wide-ranging behavioral alterations.](http://www.ncbi.nlm.nih.gov/pubmed/22300668) *Genes Brain Behav,* 3:342-351.

1. \* GoodsonM, RustMB, Witke W, **Bannerman DM**, Mott R, Ponting CP, **Flint J** (2012) Cofilin-1: a modulator of anxiety in mice. *PLoS Genetics,* in press.
2. \* Laatikainen LM, Sharp T, **Bannerman DM**, **Harrison PJ**, Tunbridge EM (2012) Modulation of hippocampal dopamine metabolism and hippocampal-dependent cognitive function by catechol-O-methyltransferase. *J Psychopharmacology,* in press*.*
3. \* Li J, Bravo DS, **Upton AL**, Gilmour G, Tricklebank MD, Fillenz M, Martin C, Lowry JP, **Bannerman DM**, McHugh SB (2011). Close temporal coupling of neuronal activity and tissue oxygen responses in rodent whisker barrel cortex. *Eur J Neuroscience,***34**:1983-1996.
4. \* Pritchett D, Wulff K, Oliver PL, **Bannerman DM**, **Davies KE**, **Harrison PJ**, Peirson SN, Foster RG (2012) [Evaluating the links between schizophrenia and sleep and circadian rhythm disruption.](http://www.ncbi.nlm.nih.gov/pubmed/22569850) *J Neural Transm.* May 10. [Epub ahead of print].
5. \* Sanderson DJ, **Rawlins JNP**, **Deacon RMJ**, Cunningham C, **Barkus C**, **Bannerman DM** (2011). Hippocampal lesions can enhance discrimination learning despite normal sensitivity to interference from incidental information. *Hippocampus* **22(7)**:1553-66.

**Professor David Beeson**

***Weatherall Institute for Molecular Medicine*, *John Radcliffe Hospital, University of Oxford Oxford OX3 9DS***

***Tel: 01865 222311 Email: dbeeson@ndcn.ox.ac.uk***

We study inherited diseases that affect neuromuscular transmission, with the major focus on mutations of muscle acetylcholine receptors (AChR) and of proteins that govern synaptic structure. The neuromuscular synapse is both well understood and accessible for study. Functional analysis of mutations at the molecular level can be directly correlated with measurements of defective synaptic transmission in vivo and with the clinical features of the patients. The work ranges from the studies of single channels, through to animal models of disease, to phenotypic characterisation of the patients. It provides translational research of bedside to bed and back, with the bench research generating data directly relevant to patient treatment regimes. Moreover, a detailed knowledge of inherited dysfunction of neuromuscular transmission forms a paradigm for investigation of other neurological syndromes that may result from defective synaptic transmission in the CNS.

***Publications*** (\*collaborations within OXION: 2011-2012)

1. \* **Belaya K**, Finlayson S, Slater CR, Cossins J, Liu WW, Maxwell S, McGowan SJ, Maslau S, Twigg SR, Walls TJ, Pascual SI, Palace J, **Beeson D** (2012) [Mutations in DPAGT1 Cause a Limb-Girdle Congenital Myasthenic Syndrome with Tubular Aggregates.](http://www.ncbi.nlm.nih.gov/pubmed/22742743) *Am J Hum Genet* **91(1)**:193-201.
2. \* Cossins J, Liu WW, **Belaya K**, Maxwell S, Oldridge M, Lester T, Robb S, **Beeson D** (2012) [The spectrum of mutations that underlie the neuromuscular junction synaptopathy in DOK7 congenital myasthenic syndrome.](http://www.ncbi.nlm.nih.gov/pubmed/22661499) *Hum Mol Genet* **21(7)**:3765-75.
3. \* Webster R, Maxwell S, Spearman H, Tai K, Beckstein O, **Sansom M**, **Beeson D** (2012) [A novel congenital myasthenic syndrome due to decreased acetylcholine receptor ion-channel conductance.](http://www.ncbi.nlm.nih.gov/pubmed/22382357) *Brain* **135(Pt 4)**:1070-80.
4. Chaouch A, **Beeson D,** Hantaï D, Lochmüller H (2012) [186th ENMC international workshop: congenital myasthenic syndromes 24-26 June 2011, Naarden, The Netherlands.](http://www.ncbi.nlm.nih.gov/pubmed/22230109) *Neuromuscul Disord*. **22(6)**:566-76.
5. Maselli RA, Fernandez JM, Arredondo J, Navarro C, Ngo M, **Beeson D**, Cagney O, Williams DC, Wollmann RL, Yarov-Yarovoy V, Ferns MJ (2012) LG2 agrin mutation causing severe congenital myasthenic syndrome mimics functional characteristics of non-neural (z-) agrin. *Hum Genet*. **131(7)**:1123-35.
6. \* Guergueltcheva V, Müller JS, Dusl M, Senderek J, Oldfors A, Lindbergh C, Maxwell S, Colomer J, Mallebrera CJ, Nascimento A, Vilchez JJ, Muelas N, Kirschner J, Nafissi S, Kariminejad A, Nilipour Y, Bozorgmehr B, Najmabadi H, Rodolico C, Sieb JP, Schlotter B, Schoser B, Herrmann R, Voit T, Steinlein OK, Najafi A, Urtizberea A, Soler DM, Muntoni F, **Hanna MG**, Chaouch A, Straub V, Bushby K, Palace J, **Beeson D**, Abicht A, Lochmüller H (2011) [Congenital myasthenic syndrome with tubular aggregates caused by GFPT1 mutations.](http://www.ncbi.nlm.nih.gov/pubmed/21975507) *J Neurol*. Oct 6. [Epub ahead of print]
7. Palace J, Lashley D, Bailey S, Jayawant S, Carr A, McConville J, Robb S, **Beeson D** (2012) [Clinical features in a series of fast channel congenital myasthenia syndrome.](http://www.ncbi.nlm.nih.gov/pubmed/21940170) *Neuromuscul Disord*. **22(2)**:112-7.

**Professor Kieran Clarke**

***Department of Physiology, Anatomy & Genetics, Parks Road, Oxford OX1 3PT***

***Tel: 01865 282248 Email: kieran.clarke@dpag.ox.ac.uk***

**OXION project: The role of Nnt in the heart**

*With Professors Frances Ashcroft and Roger Cox*

Nicotinamide nucleotide transhydrogenase (Nnt) is a nuclear-encoded mitochondrial protein thought to be involved in free radical detoxification. In heart, Nnt’s function has yet to be defined although it is present at high levels. The aim of this project is to determine whether the loss of Nnt from the mitochondria affects contractile function and/or energy metabolism in the heart. *In vivo* cardiac function will be measured in *Nnt* mutant and control mice using non-invasive magnetic resonance imaging (MRI). Cardiac high energy phosphate levels will be measured using 31P MR spectroscopy in isolated, perfused hearts. Mitochondrial dysfunction in cardiac and skeletal muscle will be characterised by measuring oxygen utilisation during respiration in isolated mitochondrial preparations in the presence of different substrates. This will allow sensitive measurements to be made of respiration rates and respiratory coupling of oxidation and ATP synthesis. *In vitro* analysis of the activities of complexes I-IV of the electron transport chain, and key enzymes of the TCA cycle, will complement respiratory data by allowing a complete characterisation of metabolic changes at the mitochondrial level with and without high-fat feeding. Cardiac mitochondrial uncoupling proteins (UCPs) and *Nnt* protein levels will be measured using immunoblotting.

***Publications*** (*\** collaborations within OXION 2011-2012*)*

1. Chan HH, Meher Homji Z, Gomes Rs, Sweeney D, Thomas GN, Tan JJ, Zhang H, Perbellini F, Stuckey DJ, Watt SM, Taggard D, **Clarke K**, Martin-Rendon E, Carr CA (2012) Erratum to: human cardiosphere-derived cells from patients with chronic ischaemic heart disease can be routinely expandedfrom atrial but not epicardial ventricular biopsies. *J Cardiovasc Transl Res* July 20 [Epub ahead of print].
2. Chan HH, Meher Homji Z, Gomes Rs, Sweeney D, Thomas GN, Tan JJ, Zhang H, Perbellini F, Stuckey DJ, Watt SM, Taggard D, **Clarke K**, Martin-Rendon E, Carr CA (2012) Human cardiosphere-derived cells from patients with chronic ischaemic heart disease can be routinely expandedfrom atrial but not epicardial ventricular biopsies. *J Cardiovasc Transl Res* July 3 [Epub ahead of print].
3. Dodd MS, Ball DR, Schroeder MA, Le Page LM, Atherton HJ, Heather LC, Seymour AM, Ashrafian H, Watkins H, **Clarke K**, Tyler DJ (2012) In vivo alterations in cardiac metabolism and function in the spontaneously hypertensive rat heart. *Cardiovasc Res* **95(1)**:69-76.
4. Edwards LM, Tyler DJ, Kemp GJ, Dwyer RM, Johnson A, Holloway CJ, Nevill AM, **Clarke K** (2012) The reproducibility of 31-phosphorus MRS measures of muscle energetics at 3 Tesla in trained men. *PLoS One* **7(6)**:e37237.
5. Heather LC, Cole MA, Tan JJ, Ambrose LJ, Pope S, Abd-Jamil AH, Carter EE, Dodd MS, Yeoh KK, Schofield CJ, **Clarke K** (2012) Metabolic adaptation to chronic hypoxia in cardiac mitochondria. *Basic Res Cardiol* **107(3)**:268.
6. Holloway CJ, Dass S, Suttie JJ, Rider OJ, Cox P, Cochlin LE, Jackson H, Fast AM, Johnson Aw, Karamitsos TD, Neubauer S, **Clarke K** (2012) Exercise training in dilated cardiomyopathy improves rest and stress cardiac function without changes in cardiac high energy phosphate metabolism. *Heart* **98(14)**:1083-90.
7. Lydgate CA, Bohl s, Ten Hove M, Faller KM, Ostrowski PJ, Zervou S, Medway DJ, Aksentijevic D, Sebag-Montefiore L, Wallis J, **Clarke K**, Watkins H, Schneider JE, Neubauer S (2012) *Cardiovasc Res* Aug 21 [Epub ahead of print].
8. Rider OJ, Holloway CJ, Emmanuel Y, Bloch E, **Clarke K**, Neubauer S (2012) Increasing plasma free fatty acids in healthy subjects induces aortic distensibility changes seen in obesity. *Circ Cardiovasc Imaging*  **5(3)**:367-75.

**Professor Roger D Cox**

***Head of Metabolism and Inflammation, MRC Harwell Mammalian Genetics Unit, Harwell Science and Innovation Campus, Oxfordshire OX11 0RD  
Tel: 01235 841184 Email:*** [***r.cox@har.mrc.ac.uk***](mailto:r.cox@har.mrc.ac.uk)

Our overall aim is to develop new mouse models for type 2 diabetes that will allow the identification of key genes and/or pathways for a systematic analysis of the process of disease development and the effect of environmental factors. This may help further the understanding of the biology of diabetes and ultimately identify new targets for therapeutic intervention.

The programme is currently focussed on characterising new models of insulin resistance and secretion, arising from our past work (*Sugarlump, Dipdab, Treacle, Nish, IGT10* and *Sweet P*!, BigBoy), which includes detailed physiological and molecular phenotyping, and mapping and cloning of the underlying mutations. Once cloned selected novel genes are investigated in more detail to determine their function particularly where their role in diabetes and/or their mechanism of action was not previously known (*Sox4, Mll2(Wbp7) and FTO*). In selected models we are undertaking analysis of disease processes and translational studies around particular genes and models.

We are also beginning a new screen for metabolic phenotypes and complications of metabolic disease in aged mice from recessive ENU mutaganised pedigrees. The aim is to follow mice to 18 months of age and to have sufficient sized cohorts to allow chromosomal mapping that will be followed by NGS sequencing for identification of the underlying gene.

Finally, genes identified in human genome wide association studies are being screened through the MRC-Harwell ENU-mutated DNA resource in order to generate mouse models of diabetes and metabolic disease for functional validation and analysis. Additional alleles, such as overexpression of a gene, are then constructed for further analysis.

***Publications***(\*collaborations within OXION: 2011-2012)

1. \* Esapa C, Hough T, Testori S, Head R, Crane E, Chan C, Evans H, Bassett J, Tylzanowski P, McNally E, Carr A, Boyde A, Howell P, Clark A, Williams G, Brown M, Croucher P, Nesbit M, **Brown S**, **Cox R**, Cheeseman M, Thakker R (2011) A mouse model for spondyloepiphyseal dysplasia congenita with secondary osteoarthritis due to a Col2a1 mutation. *J Bone Miner Res* doi: 10.1002/jbmr.547. [Epub ahead of print] PMID:22028304.
2. \* Karunaratne A, Davis GR, Hiller J, Esapa CT, Terrill NJ, **Brown SD**, **Cox RD**, Thakker RV, Gupta HS (2012) Hypophosphatemic rickets is associated with disruption of mineral orientation at the nanoscale in the flat scapular bones of rachitic mice with development. *Bone* May 15. [Epub ahead of print] PMID: 22609228
3. \* Karunaratne A, Esapa C, Hiller J, Boyde A, Head R, Bassett J, Terrill N, Williams G, Brown M, Croucher P, **Brown S**, **Cox R**, Barber A, Thakker R, Gupta H (2011)

Significant deterioration in nanomechanical quality occurs through incomplete extrafibrillar mineralization in rachitic bone: evidence from in-situ synchrotron X-ray scattering and backscattered electron imaging. *J Bone Miner Res* Dec 8. doi: 10.1002/jbmr.1495. [Epub ahead of print] PMID: 22161748.

1. Lee AWS, HengstlerH, SchwaldK, Berriel-DiazM, LorethD, KirschM, KretzO, HaasCA, Hrabě de AngelisM, HerzigS, Brümmendorf T, KlingensporM, RathjenFJ, RozmanJ, NicholsonG, Cox\*RD and Schäfer\*MKE (2012) Functional Inactivation of the Genome-wide Association Study Obesity Gene Neuronal Growth Regulator 1 in Mice Causes a Body Mass Phenotype. *PLoS One* (in press). \* these authors contributed equally.
2. McMurray F, Cox RD (2011) Mouse models and type 2 diabetes: translational opportunities. *Mamm Genome* **22(7-8)**:390-400. PMID: 21713584.

1. \* McTaggart JS, **Lee S**, **Iberl M**, Church C, **Cox RD,** **Ashcroft FM** (2011) FTO Is Expressed in Neurones throughout the Brain and Its Expression Is Unaltered by Fasting. *PLoS One*. **6(11)**:e27968. Epub 2011 Nov 30. PMID: 22140494.
2. Ripoll VM, Meadows NA, Bangert M, Lee AW, Kadioglu A, **Cox RD** (2012)

Nicotinamide nucleotide transhydrogenase (NNT) acts as a novel modulator of macrophage inflammatory responses. *FASEB J* May 16. [Epub ahead of print]. PMID: 22593545

**Professor Kay Davies**

**Molecular analysis of neuromuscular and neurological disorders**

***MRC Functional Genomics Unit, Department of Physiology, Anatomy and Genetics, Oxford***

***Tel. 01865 285880 Email.*** [***kay.davies@dpag.ox.ac.uk***](mailto:kay.davies@dpag.ox.ac.uk)

The Davies group’s research programme has two major strands. The first is focused on the development of an effective treatment for the muscle wasting disease, Duchenne muscular dystrophy (DMD). The second strand is focused on using the mouse as a model to determine the molecular basis of psychiatric, neurodegenerative and neurodevelopmental diseases.

***Muscle disease***

DMD is a progressive muscle wasting disease caused by the absence of the large protein, dystrophin in all muscle cells of patients. At present there is no effective treatment for the disorder although there are various promising approaches. Some strategies can only be used for certain mutations and the challenge of many therapeutic approaches is the targeting of all muscles including the heart. We have focused our efforts on the development of an effective therapy which would be applicable to all patients and potentially target all muscles. This approach involves increasing the levels of the dystrophin-related protein utrophin in muscle through the oral administration of a small molecule drug. We have already shown that increased levels of utrophin can functionally replace dystrophin in the mouse and dog models of the disease. We developed the drug SMT C1100 with Summit plc who are currently conducting a Phase I clinical trial. This work shows proof of principle of the approach and SMT C1100 is a first in class compound. We have now developed a new screen to find best in class drug candidates as a follow on to this programme of utrophin upregulation.

***Neurological disorders***

The aim of this programme is to identify and characterise new models of human neurological disease using mice from the large-scale ENU mutagenesis screen at the MRC MGU. We have focused on the characterisation of four new mutant lines selected for their potential for providing novel links between ion channel function and cerebellar development, lysosome storage, oxidative stress and neurodegeneration, as well as synaptic vesicle trafficking and psychiatric disease. The long term objective is to focus on those mutants which are most likely to have an impact on our understanding of human neurological disorders.

***Publications*** (\*Collaborations within OXION 2011-2012)

1. Fairclough RJ, Bareja A, **Davies KE** (2011) Progress in therapy for Duchenne muscular dystrophy. *Experimental Physiology* **96**: 1101-1113.
2. Fairclough RJ, Perkins KJ, **Davies KE** (2012) Pharmacologically targeting the primary defect and downstream pathology in Duchenne muscular dystrophy. *Current Gene Therapy* **1**: 206-244.
3. Gehrig SM, van der Poel C, Sayer TA, Schertzer JD, Henstridge DC, Church JE, Lamon S, Russell AP, **Davies KE**, Febbraio MA, Lynch GS (2012) HSP72 preserves muscle function and slows progression of severe muscular dystrophy. *Nature* **484**: 394-398.
4. Goyenvalle A, Crisp A, **Davies KE** (2012) Novel delivery of molecular therapetuics to the heart using non-biologic constructs. *Molecular and Translational Cardiology*, in press.
5. Goyenvalle A, **Davies KE** (2012) Engineering multiple U7snRNA constructs to induce single and multiexon-skipping for Duchenne muscular dystrophy. *Molecular Therapy*. doi: 10.1038/mt.2012.26.
6. Goyenvalle A, Babbs A, Wright J, Wilkins V, Powell P, Garcia L, **Davies KE** (2012) Rescue of severely affected Dystrophin/Utrophin deficient mice through scAAV-U7snRNA mediated exon skipping. *Human Molecular Genetics* **21**: 2559-2571.
7. \* Oliver PL, Finelli MJ, Edwards B, Bitoun E, Butts DL, Becker **EBE**, Cheeseman MT, Davies B, **Davies KE** (2011) Oxr1 is essential for protection against oxidative stress-induced neurodegeneration. *PLoS Genetics* Sep:7(10):e1002338.
8. \* Oliver PL, Sobczyk MV, Maywood ES, Edwards B, **Lee S**, Livieratos A, Oster H, Butler R, Godinho SIH, Wulff K, Peirson SN, Fisher SP, Chesham JE, Smith JW, Hastings MH, **Davies KE**, Foster RG (2012) Disrupted circadian rhythms in a mouse model of schizophrenia*. Current Biology* **22**: 1-6.
9. \* Pritchett D, Wulff K, Oliver PL, **Bannerman DM**, **Davies KE**, Harrison PJ, Peirson SN, Foster RG. (2012) Evaluating the links between schizophrenia and sleep and circadian rhythm disruption*. Journal of Neural Transmission* PMID 22569850.
10. Ravenscroft G, Jackaman C, Sewry CA, McNamara E, Squire SE, Potter AC, Papadimitriou J, Griffiths LM, Bakker AJ, **Davies KE**, Laing NG, Nowak KJ (2011) Actin nemaline myopathy mouse reproduces disease, suggests other actin disease phenotypes and provides cautionary note on muscle transgene expression. *PLOS ONE*. 2011:**6**(12):e28699.
11. \* Stuckey D, Carr C, Camelliti P, Tyler D, **Davies K**, **Clarke K** (2012) In vivo MRI characterization of progressive cardiac dysfunction in the mdx mouse model of muscular dystrophy *PloS ONE* **7**: e28469.
12. \* Tan SC, Carr CA, Yeoh KK, Schofield CJ, **Davies KE**, **Clarke K** (2011) Identification of valid housekeeping genes for quantitative RT-PCR analysis of cardiosphere-derived cells preconditioned under hypoxia or with prolyl-4-ydroxylase inhibitors. *Molecular Biology Report* 9th Nov Epub ahead of publication PMID:22065248.

For more information on research in the Davies lab click [here](http://www.mrcfgu.ox.ac.uk/research/kay-e-davies).

**Dr Jonathan Flint**

***Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford OX3 7BN***

***Tel: 01865 287512 Email:*** [***jf@well.ac.uk***](mailto:jf@well.ac.uk)

Jonathan Flint works on the genetic basis of behaviour, in both rodents and humans. His group has developed new strategies to identify alleles contributing to variation in anxiety and depression. His laboratory carries out high throughput genotyping, using the core genomics facility at the Wellcome Trust Centre, on mouse and human DNA. His group has established new approaches for analysing genetic variation in the mouse and has pursued this methodology to the point where it is possible to identify genetic effects contributing to a few per cent of the total variance of a phenotype. Using this approach, he is currently mapping genes involved in fear related behaviour in mice and has preliminary evidence that regulators of G protein signalling (RGS genes) downstream of G-protein coupled receptors, are important regulators of anxiety.

***Collaborations***

Jonathan Flint’s group works closely with the laboratory of Professor Rawlins. Together they have established a laboratory for behavioural analysis of mice, using a series of automated tasks to measure fear-related traits and learning and memory. In addition they are able to take a wide variety of physiological measures in mice, including haematological and immunological parameters that together with the behavioural analysis, provide a detailed characterisation of a mutant’s phenotype. The system is currently able to collect data on up to 50 animals per week. Together with Drs Mott and Gaugier and Professor Cookson, they have received a supplementary two year grant from The Wellcome Trust for simultaneous fine mapping of quantitative trait loci for multiple phenotypes.

***Publications*** (\*collaborations within OXION 2011-2012)

1. \* Goodson M, Rust MB, Witke W, **Bannerman DM**, Mott R, Ponting CP, **Flint J** (2012) Cofilin-1: a modulator of anxiety in mice. *PLoS Genetics* (in press).

**Professor Michael G Hanna**

***MRC Centre for Neuromuscular Diseases, Department of Molecular Neuroscience, UCL Institute of Neurology, London***

***Tel: 020 3448 8013 Email: m.hanna@ucl.ac.uk***

**Clinical and Genetic Studies on Human Neurological Channelopathies**

My group has a long standing clinical and genetic research interest in human neurological channelopathies. Over the last ten years we have established one of the largest databases in the world of human patients with genetic channelopathies affecting skeletal muscle and brain. We are now recognised by the UK Department of Health and received permanent DoH funding through National Commissioning Group [NCG] to provide the National UK clinical and DNA diagnostic and advisory service for patients with channelopathies.

We undertake genetic research programmes identifying the molecular genetic basis of periodic paralysis, non-dystrophic myotonia, certain types of epilepsy and episodic ataxia. We have established dysfunction of the neuronal potassium channel Kv1.1 as a cause of human epilepsy and we have provided evidence that the brain P/Q-type calcium channel CaV2.1 is implicated in certain forms of human epilepsy. We completed the largest clinical genetic and molecular expression study in over 300 patients with the muscle chloride channelopathy- myotonia congenita. Recently we have shown that genetic mutations predicted to cause gating pore currents in the muscle sodium and calcium channels are the major cause of periodic paralysis opening new avenues for therapy.

**MRC Centre for Translational Research in Neuromuscular Diseases**

We were successful in obtaining an MRC translational research Centre grant for £3m for 5 years from February 2008. Professor Hanna is the Centre Director, and this is a joint initiative between the Centre for Neuromuscular Diseases at the institute of Neurology UCL and colleagues in Newcastle and at the Institute of Child Health at UCL. The main aim of the centre is to translate basic science work into clinical trials and treatments for adults and children with neuromuscular diseases. The five core areas of the centre are a neuromuscular clinical trials unit, a neuromuscular animal model unit, neuromuscular MRI in humans and animals, a UK neuromuscular biobank and neuromuscular clinical and non-clinical PhD programmes.

The facilities of the centre will have a major benefit in taking forward clinical trials in muscle channelopathies including a recently funded NIH trial of carbonic anhydrase inhibitors in periodic paralysis. Other activities in the centre will have value for channelopathy research, e.g. the biobank will have the potential to make human muscle cell cultures from channelopathy patients available for basic research.

***Collaborations***

We collaborate closely with OXION member Professor Kullmann at the Institute of Neurology, and also with Professor Nick Wood and Dr Stephanie Schorge to perform molecular expression studies of mutant channels identified in patients. We have NIH [USA] funding to undertake current large-scale natural history studies and treatment trials in various human skeletal muscle channelopathies. We are interested in translational channelopathy research.

Funding: DoH [NCG], MRC, NIH [USA] and Action Research.

***Publications*** (**\***collaborations within OXION 2011-2012)

1. Burge JA, **Hanna MG** (2012) Novel insights into the pathomechanisms of skeletal muscle channelopathies. *Curr Neurol Neurosci Rep* **12(1)**:62-9.
2. \* Guergueltcheva V, Müller JS, Dusl M, Senderek J, Oldfors A, Lindbergh C, Maxwell S, Colomer J, Mallebrera CJ, Nascimento A, Vilchez JJ, Muelas N, Kirschner J, Nafissi S, Kariminejad A, Nilipour Y, Bozorgmehr B, Najmabadi H, Rodolico C, Sieb JP, Schlotter B, Schoser B, Herrmann R, Voit T, Steinlein OK, Najafi A, Urtizberea A, Soler DM, Muntoni F, **Hanna MG**, Chaouch A, Straub V, Bushby K, Palace J, **Beeson D**, Abicht A, Lochmüller H (2011) Congenital myasthenic syndrome with tubular aggregates caused by GFPT1 mutations. *J Neurol* Oct 6 [Epub ahead of print].
3. Matthews E, Plotz PH, Portaro S, Parton M, Elliott P, Humbel RL, Holton JL, Keegan BM, **Hanna MG** (2012) A case of necrotizing myopathy with proximal weakness and cardiomyopathy. *Neurology* **78(19)**:1527-32.
4. Matthews E, Portaro S, Ke Q, Sud R, Haworth A, Davis MB, Griggs RC, **Hanna MG** (2011) Acetazolamide efficacy in hypokalemic periodic paralysis and the predictive role of genotype. *Neurology* **77(22)**:1960-4.
5. Pitceathly RD, Murphy SM, Cottenie E, Chalasani A, Sweeney MG, Woodward C, Mudanohwo EE, Hargrreaves I, Heales S, Land J, Holton JL, Houlden H, Blake J, Champion M, Flinter F, Robb SA, Page R, Rose M, Palace J, Crowe C, Longman C, Lunn MP, Rahman S, Reilly MM, **Hanna MG** (2012) Genetic dysfunction of MT-ATP6 causes axonal Charcot-Marie-Tooth disease. *Neurology* **79(11)**:1145-54.
6. Pitceathly RD, Rahman S, **Hanna MG** (2012) Single deletions in mitochondrial DNA – molecular mechanisms and disease phenotypes in clinical practice. *Neuromuscul Disord* **22(7)**:577086.
7. Pitceathly RD, Tomlinson SE, Hargreaves I, Bhardwaj N, Holton JL, Morrow JM, Evans J, Smith C, Fratter C, Woodward CE, Sweeney MG, Rahman S, **Hanna MG** (2012) Distal myopathy with cachexia: an unrecognized phenotype caused by dominantly-inherited mitochondrial polymerase y mutations. *J Neurol Neurosurg Psychiatry* Aug 29 [Epub ahead of print].
8. Portaro S, Musumeci O, Rizzo V, Rodolico C, Sweeney MG, Buccafusca M, **Hanna MG**, Toscano A (2012) Stiffness as a presenting symptom of an odd clinical condition caused by multiple sclerosis and myotonia congenital.  *Neuromuscul Disord* Aug 23 [Epub ahead of print].
9. Pulkes T, Dejthevaporn C, Apiwattanakul M, Papsing C, **Hanna MG** (2012) Paroxysmal neuromyotonia: a new sporadic channelopathy. *Neuromuscul Disord* **22(6)**:479-82.
10. Raja Rayan DL, Haworth A, Sud R, Matthews E, Fialho D, Burge J, Portaro S, Schorge S, Tuin K, Lunt P, McEntagart M, Toscano A, Davis MB, **Hanna MG** (2012) A new explanation for recessive myotonia congenital: exon deletions and duplications in CLCN1. *Neurology* **78(24)**:1953-8.
11. Rajakulendran S, Kaski D, **Hanna MG** (2012) Neuronal P/Q-type calcium channel dysfunction in inherited disorders of the CNS. *Nat Rev Neurol* **8(2)**:86-96.

**Professor Dimitri M Kullmann**

***Department of Clinical and Experimental Epilepsy, Institute of Neurology, UCL, London WC1N 3BG***

***Tel: 020 3448 4100 Email:*** [*d.kullmann@ion.ucl.ac.uk*](mailto:d.kullmann@ion.ucl.ac.uk)

My laboratory studies synaptic transmission, inherited and acquired disorders of ion channel function in neurological diseases, and the computational properties of simple neuronal circuits. We apply a combination of electrophysiology, optical methods and viral manipulation of ion channels, and are using these to test novel treatments for focal epilepsy. My group collaborates with MC Walker, S Schorge, K Volynski, and MG Hanna (OXION member) at the Institute of Neurology, and with several groups in Oxford including A Vincent (OXION member) and K Lamsa (Pharmacology).

***Publications*** (\*collaborations within OXION 2011-2012)

1. Akam T, Oren I, Mantoan L, Ferenczi E, **Kullmann DM** (2012) Oscillatory dynamics in the hippocampus support dentate gyrus–CA3 coupling. *Nat Neurosci* **15**:763-8.
2. \* Hanna MG**, Kullmann DM** (2012) Channelopathies. In: *Neurogenetics* (Ed. NW Wood) CUP.
3. Oren I, **Kullmann DM** (2012) Mapping out hippocampal inhibition*. Nat Neurosci* **15**:346-7.
4. **Kullmann DM** (2012) The Mother of All Battles 20 years on: is LTP expressed pre- or postsynaptically? *J Physiol* **590**:2213-6.
5. Vivekananda U, Hirsch NP, **Kullmann DM**, Alvarez D, Phadke R, Howard RS (2012) Vasculitis of the central and peripheral nervous system mimicking brain death. *Clin Neurol Neurosurg* **114**:399-401.
6. Walker MC, **Kullmann DM** (2011) Tonic GABAA receptor-mediated signaling in epilepsy. In: Jasper's Basic Mechanisms of the Epilepsies 4th Ed. Eds: JL Noebels, M Avoli, RW Olsen, AV Delgado-Escueta (OUP).
7. Mantoan L, **Kullmann DM** (2011) Evaluating first seizures in adults in primary care. *Practitioner* **255**:25-8, 2-3.
8. **Kullmann DM** (2011) What's wrong with the amygdala in temporal lobe epilepsy? *Brain* **134**:2800-1.

**Most significant papers in last year: 1,13**

**Gero Miesenböck**

***Centre for Neural Circuits and Behaviour, University of Oxford, Tinsley Building, Mansfield Road, Oxford, OX1 3TA***

***Tel: 01865 282261 Email: gero.miesenboeck@dpag.ox.ac.uk***

Gero Miesenböck is the principal architect of the emerging field of "optogenetics", which develops genetic strategies for observing and controlling the function of brain circuits with light. He developed the first genetically encoded optical sensors of neuronal activity and reported the first use of such probes for imaging signal processing in an intact nervous system.

He was also the first to introduce the notion of remotely controlling neural circuits with the help of optically gated ion channels, and was the first to use such tools to control an animal’s behaviour.

The Miesenböck group uses these optical approaches to read and change the minds of fruit flies and other species; their current research focuses on the structure and dynamics of circuits involved in sensory processing, memory, action selection, and motor pattern generation.

***Publications*** (\*collaborations within OXION 2011-2012)

1. Denk W, **Miesenböck G** (2012) Neurotechnology: summa technologiae. *Curr Opin Neurobiol* **22(1)**:1-2.
2. **Miesenböck G** (2012) Synapto-pHluorins: genetically encoded reporters of synaptic transmission. *Cold Spring Harb Protoc* **2012(2)**:213-7.
3. **Miesenböck G** (2011) Optogenetic control of cells and circuits. *Annu Rev Cell Dev Biol* **27**:731-58.

**Anant B Parekh**

***Professor of Cell Physiology, Department of Physiology, Anatomy and Genetics, Parks Road, Oxford OX1 3PT***

***Tel: 01865 272439 Email: anant.parekh@dpag.ox.ac.uk***

**Calcium channels and cell function in health and disease**

Anant Parekh’s research interests revolve around calcium channels, intracellular calcium signalling and cell function in health and disease with particular emphasis on allergy and asthma. The work focuses on store-operated calcium channels in the plasma membrane, structure-function relationship of the channels, how these channels interact with intracellular organelles especially the nucleus and how these fundamental elements go awry in allergic rhinitis and nasal polyposis. His laboratory has developed the concept that calcium microdomains near open store-operated channels activate spatially and temporally distinct processes (secretion, metabolism and gene expression) and has dissected out the underlying signal transduction pathways, leading to the important discoveries including memory and facilitation to regulated gene expression. The group has uncovered a new mechanism for intercellular signalling, involving a feed forward loop between store-operated channels and secreted leukotrienes which leads to a wave of excitation that spreads through the entire cell population. Recent work in the laboratory on acutely isolated nasal mast cells from patients with allergic rhinitis and polyposis has revealed that a similar positive feedback loop operates in humans and this has led to a new proposed therapy for these disorders.

***Publications*** (\*collaborations within OXION 2011-2012)

1. Kar P, Bakowski D, Di Capite J, Nelson C, **Parekh AB** (2012) [Different agonists recruit different stromal interaction molecule proteins to support cytoplasmic Ca2+ oscillations and gene expression.](http://www.ncbi.nlm.nih.gov/pubmed/22509043) *Proc Natl Acad Sci U S A.* **109**:6969-74.
2. Kar P, Nelson C, **Parekh AB** (2012)[CRAC channels drive digital activation and provide analog control and synergy to Ca(2+)-dependent gene regulation.](http://www.ncbi.nlm.nih.gov/pubmed/22245003) *Current Biology* **22**:3242-7.
3. Ng SW, Bakowski D, Nelson C, Mehta R, Almeyda R, Bates G, **Parekh AB** (2012)

[Cysteinyl leukotriene type I receptor desensitization sustains Ca2+-dependent gene expression.](http://www.ncbi.nlm.nih.gov/pubmed/22230957) *Nature* **482**:111-5.

**Professor David J Paterson**

***Department of Physiology, Anatomy & Genetics, University of Oxford, Parks Road, Oxford OX1 3PT***

***Tel: 01865 272518 Email:*** [***david.paterson@dpag.ox.ac.uk***](mailto:david.paterson@dpag.ox.ac.uk)

***Cardiac-Neural coupling and Sudden Cardiac Death***

***[Featured on BBC4 10th July 2012 at 9pm. Heart vs Mind:what makes us human.]***

[***http://www.bbc.co.uk/programmes/b01kpvj1***](http://www.bbc.co.uk/programmes/b01kpvj1)

Many cardiovascular diseases (eg heart failure, hypertension, post myocardial infarction) are also diseases of the autonomic nervous system. Neurohumoral activation and high levels of circulating catecholamines is a negative prognostic indicator for sudden cardiac death and a strong independent predictor of mortality. A significant component of the autonomic derangement occurs within the peripheral nervous system and results from enhanced cardiac sympathetic and impaired vagal neurotransmission. A second site of impairment occurs post-junctionally at the level of the β-adrenoceptor, since the arrhythmic action of isoprenaline is enhanced in pacemaker cells and papillary muscle. The mechanism responsible for defective transmission and hyper-responsiveness of post synaptic channels may be related to free radical damage that is secondary to oxidative stress. Soluble guanylate cyclase (sGC), the key pre-cursor of cGMP-dependent effects of nitric oxide (NO) is down-regulated in diseased hearts as is the bioavailability of NO itself. Recently, large genome wide association studies have linked variation in a little known gene encoding NOS1 activator protein (NOS1-AP) to risk of sudden cardiac death in humans. We hypothesize that NOS1-AP can influence cardiac autonomic neural signaling and control of arrhythmogenesis. Therefore our aim is to (i) understand the role played by free radicals, in particular NOS1-AP in the autonomic control of cardiac excitability, and (ii) develop a strategy to rescue impaired neural phenotypes.

***Gene transfer strategy*** Adenoviral gene transfer is an effective way of modifying gene expression and has been used with good effect to understand whether changes in proteins or enzyme activity play a key role in physiological function. We have found that neuronal NOS (nNOS/NOS1) gene transfer into cholinergic intracardiac ganglia can facilitate Ach release, and more recently in the hypertensive rat (SHR), bring the heart rate responsiveness to vagal activation to similar levels seen in the normotensive control (WKY). Hypertensive animals have also enhanced cardiac noradrenaline (NA) release indicating that a component of the autonomic dysfunction resides at the level of the sympathetic varicosity. Our next step is to target these neurones with nNOS and study the ion channels involved in transmitter release. There are however, several significant limitations in using a viral gene transfer approach. Specifically, adenovirus can transfect a broad range of cells, transduction is promiscuous and the gene of interest (in our case the nNOS gene), can be placed into cells that may not constitutively express them thereby leading to unwanted effects and confounding the interpretation of the data. This problem can be circumvented by targeting nNOS to selected cellular populations using cell-type specific viral vectors. To this end we have re-engineered a viral vector for nNOS or reporter gene which only targets sympathetic neurons (Wang et al. 2007). Importantly we have established efficacy of this vector and shown in normotensive rats that we can attenuate NA release, and that this response is sensitive to NOS inhibition. We now plan to apply this vector to the SHR and normotensive WKY rat to establish whether we can rescue the impaired peripheral neural phenotype over the long with our new lenti viral vector (Wang et al. 2009).

***Impaired post synaptic beta adrenergic signalling***

Under normal conditions NO is thought to attenuate myocardial responsiveness to β-adrenergic stimulation, via activation of cGMP-stimulated phosphodiesterase II and subsequent inhibition of the L-type Ca2+- current (ICaL). Ventricular myocytes from the nNOS knockout mouse show an enhanced inotropic response to β-adrenergic stimulation and an associated increase in calcium current density, suggesting that nNOS-derived NO plays a role in the negative feedback modulation of calcium entry. This is significant, since nNOS has been localised to the sarcoplasmic reticulum (SR) in ventricular myocardium of rabbit, mouse, and human. Although there are no data regarding the precise role of nNOS in modulating pacemaking currents in the sino-atrial node (SAN), evidence linking SR calcium release to the generation of pacemaker rate and the positive chronotropic action of β-agonists suggests that nNOS-mediated regulation of ICaL could modulate the chronotropic response of the heart to β-adrenergic stimulation.

We now propose to test the idea that impaired NO-cyclic nucleotide signalling caused by hypertension removes a “brake” on the β-adrenergic signalling cascade, resulting in hyper-responsiveness to NA via cAMP-mediated activation of ICaL. Our electrophysiology evidence convincingly shows that nNOS attenuates the response of ICaL to NA in the SHR (Heaton et al 2006). These data suggest NO can play a significant autocrine role in the modulation of calcium channels involved in pacemaking. We now need to establish whether this response is cGMP dependent or whether it can be blocked by NOS inhibitors or inhibitors of soluble guanylate cyclase. In addition we also need to determine whether phosophodiesterases (PDE) and cyclic nucleotides are different between strains, and whether nNOS gene transfer alters the levels of PDE and cyclic nucleotides. These results have underpinned our recently published two-cell model (neuron-myocyte) to help explain enhanced sympathetic neurotransmission in hypertension (Tao et al. 2011). We have also recently developed a cardiac-neural co-culture preparation to study the interaction between these cells using imaging and patch clamp techniques.

***Summary of Approach***

A multi-disciplinary approach will be used incorporating (i) electrophysiological patch-clamp measurements to record channel activity, (ii) *in-vivo* gene transfer to deliver genetic material with adenovirus, (iii) *in-vitro* organ bath atria preparations with intact sympathetic nerves to measure function (heart rate) and (radio-labelled) noradrenaline release (iv) molecular/histological techniques to measure transgene protein expression (nNOS, eGFP) NOS activity, cAMP, cGMP; and confocal microscopy to identify localisation of nNOS and reporter gene in sympathetic nerves and SAN cells/atrial tissue, (v) computational physiology.

***Publications*** (\*Collaborations within OXION 2011-2012)

1. \* Li D, Lee CW, Buckler K, **Parekh A**, Herring N and **Paterson DJ** (2012) Abnormal Intracellular Calcium Homeostasis in Sympathetic Neurons From Young Prehypertensive Rats. *Hypertension* **59(3)**:642-9

See http://paterson.physiol.ox.ac.uk/publications/

**Professor Patrik Rorsman**

***OCDEM, Churchill Hospital, University of Oxford, Oxford OX3 7LJ***

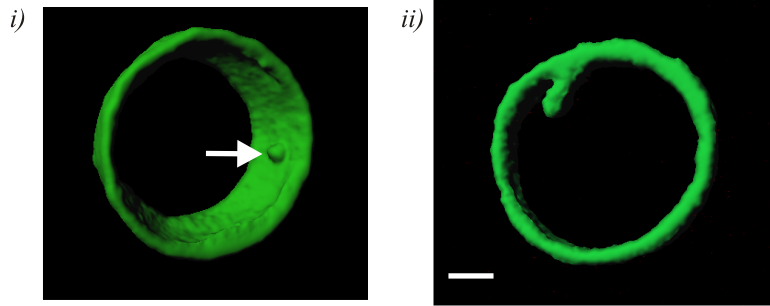
***Tel: 01865 857348 Email: patrik.rorsman@ocdem.ox.ac.uk***

When exposed to high glucose levels, pancreatic β-cells start generating action potentials. The objective of this electrical activity is to generate the signal that triggers exocytosis of the insulin-containing secretory granules (i.e. an elevation of the cytoplasmic Ca2+-concentration; [Ca2+]i).

We are interested in mechanisms that determine the magnitude of the exocytotic response in β-cells and how it is regulated. The traditional view is that the secretory granules fuse individually with the plasma membrane. We have developed an electrophysiological technique to detect single exocytotic events that is based on the expression of purinergic P2X2 receptors at high density in the plasma membrane (Karanauskaite et al. 2009). Like other types of secretory vesicles, insulin granules contain ATP at fairly high concentrations and it is co-released with insulin during β-cell exocytosis (Galvanovskis et al. 2011). In β-cells engineered to overexpress P2X2 receptors, insulin granule exocytosis can accordingly be monitored as transient inward currents due to ATP co-released with the peptide hormone. When exocytosis is triggered by weak stimulation (e.g. [Ca2+]i just above threshold for exocytosis), these events are fairly uniform and their amplitude distribution is well described by a single Gaussian. However, during more intense stimulation (high [Ca2+]i), many (10-20%) of the events are much (5-10) bigger than that expected for unitary events (Hoppa et al. 2012). The currents associated with these events rise monotonically and as quickly as the smaller events, suggesting they do not reflect the rapid sequential release of several individual granules but rather exocytosis of a multivesicular complex preformed within the β-cell that undergoes exocytosis as a single entity (*compound exocytosis*).

Physiologically, these multivesicular events form during muscarinic stimulation (by carbachol) of insulin secretion; a condition associated with a uniform elevation of [Ca2+]i by mobilization of intracellular Ca2+ stores. Optical/ultrastructural evidence for the occurrence of multivesicular exocytosis in the presence of carbachol was obtained by multiphoton live-cell imaging using the extracellular space marker sulforhodamine B (SRB) (Takahashi et al. 2002), confocal imaging of fixed cells using the FM1-43FX (Fig. 1) and electron microscopy. Statistical analysis of the electrophysiological and optical data combined with insulin secretion measurements suggest that the carbachol-induced stimulation of insulin secretion is almost entirely accounted for by compound exocytosis.

We conclude that compound exocytosis, although being a fairly rare event, is functionally important under certain physiological conditions associated with high insulin requirements. We speculate that compound exocytosis might be particularly relevant in a cell with a low Ca2+-channel density (like the β-cell; (Barg et al. 2001)) by enabling Ca2+-influx through individual Ca2+-channels to induce several granules worth of insulin secretion.

**Fig. 1.** Multivesicular exocytosis detected by confocal imaging in isolated rat beta cells. *i*) 3-D reconstruction of exocytotic events observed in rat beta cells captured during 30 s exposures to FM1-43FX in the presence of 20 mmol/l glucose alone. The event highlighted by the arrow has a diameter of ~0.6 μm attached to the plasma membrane. *ii*) As in a but showing an example of a large rod-like event observed in the simultaneous presence of 20 mmol/l glucose and 20 μmol/l carbachol. The ‘rod’ extending into the cell from the plasma membrane has a length of 2 μm, suggesting it involves the fusion of 5 individual granules. Scale bar: 2 μm.

***References***

1. Barg S, Ma X, et al (2001). "Fast exocytosis with few Ca(2+) channels in insulin-secreting mouse pancreatic B cells." *Biophys J* **81**:3308-3323.
2. Galvanovskis J, Braun M et al (2011) Exocytosis from pancreatic beta-cells: mathematical modelling of the exit of low-molecular-weight granule content. *Interface Focus* **1**:143-152.
3. Hoppa MB, Jones E et al (2012) Multivesicular exocytosis in rat pancreatic beta cells. *Diabetologia* **55**:1001-1012.
4. Karanauskaite J, Hoppa MB et al (2009) Quantal ATP release in rat beta-cells by exocytosis of insulin-containing LDCVs. *Pflugers Arch* **458**:389-401.
5. Takahashi N, Nemoto T et al. (2002) Two-photon excitation imaging of pancreatic islets with various fluorescent probes. *Diabetes* **51**:Suppl 1:S25-28.

***Publications*** (\*collaborations within OXION: 2011-2012)

1. \* **Ashcroft FM** and **Rorsman P** (2012). Diabetes mellitus and the beta cell: the last ten years. *Cell* **148**:1160-1171.
2. \* **Galvanovskis** J, Braun M and **Rorsman** P (2011) Exocytosis from pancreatic beta-cells: mathematical modelling of the exit of low-molecular-weight granule content. *Interface Focus* **1**:143-152.
3. \* Hoppa MB, Jones E, Karanauskaite J, Ramracheya R, Braun M, Collins SC, Zhang Q, Clark A, Eliasson L, Genoud C*,* Macdonald PE, Monteith AG, Barg S, **Galvanovskis J**, **Rorsman P** (2012) Multivesicular exocytosis in rat pancreatic beta cells. *Diabetologia* **55**:1001-1012.
4. **Rorsman P**, Braun M and Zhang Q (2012) Regulation of calcium in pancreatic alpha- and beta-cells in health and disease. *Cell Calcium* **51**:300-308.
5. Rosengren AH, Braun M, Mahdi T, Andersson SA, Travers ME, Shigeto M, Zhang E, Almgren P, Ladenvall C, Axelsson AS*, et al.* (2012) Reduced Insulin Exocytosis in Human Pancreatic beta-Cells With Gene Variants Linked to Type 2 Diabetes. *Diabetes* **61**:1726-1733.

**Professor Mark Sansom**

***Structural Bioinformatics and Computational Biochemistry Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU***

***Tel: 01865 613306 Email:*** [*mark.sansom@bioch.ox.ac.uk*](mailto:mark.sansom@bioch.ox.ac.uk)

The overall theme of work in our group is to apply biomolecular modelling and multiscale simulations to membrane protein systems. Membrane proteins play key roles in cell biology and have been estimated to account for ~25% of genes. There are two ways in which computational methods are valuable: (i) to probe the relationship between static/average structures and the functional dynamics of a protein; and (ii) to analyse known membrane protein structures in order to reveal underlying principles of membrane protein structure and stability. The research in my group can be grouped into the following themes. For more details see [http://sbcb.bioch.ox.ac.uk](http://sbcb.bioch.ox.ac.uk/)

1. Molecular simulations: from structure to dynamics

We are extending simulation studies of membrane proteins in three directions: (i) increasing the depth of our simulations to increase our understanding of e.g. the physics of ion permeation through channels; (ii) increasing the range of simulations to perform comparative studies of the dynamics of membrane proteins; and (iii) increasing the complexity of simulations to explore structural dynamics in multi-component transport systems.

2. Ion channels

Simulation and modelling studies are being used to explore structural dynamics in a wide range of ion channels and related pore-like transporters. These studies include: (i) ion permeation mechanisms in K channels, exemplified by KcsA and KirBac; (ii) K (Kir and Kv) channel gating via multi-scale molecular dynamics simulations; and (iii) dynamic interactions of ion channels with their membrane lipid environment.

3. Pore-like transporters

We have extended our simulation studies to both passive pore-like transporters (e.g. OprP from bacterial outer membranes) to more complex transporters of the ABC, MFS, and Na+-coupled transporter families. Simulations are being used to explore selectivity of solute binding and transport, and also conformational changes underlying transport mechanisms.

4. Signalling in membranes

Simulation methods are also being used to explore a wider range of signalling systems within membranes, including PIP2-binding proteins, and the ErbB and related receptor families.

***Collaborations***

My group has a number of collaborations with other members of OXION. For example, with Frances Ashcroft we are studying structure/function relationships in Kir6.2, SUR1 and related proteins; with Stephen Tucker we are using molecular modelling and simulations to explore gating models in a number of mammalian and prokaryotic channels. We are also collaborating with colleagues in London on modelling clinically important voltage activated channel mutations.

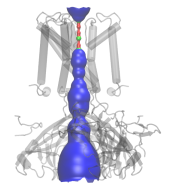
***Publications***(*\**collaborations within OXION: 2011-2012*)*

1. \* Andres-Enguix I, Shang L, Stansfeld PJ, Morahan JM, **Sansom MS**, Lafrenière Rg, Roy B, Griffiths LR, Rouleau GA, Ebers GC, Cader ZM, **Tucker SJ** (2012) Functional analysis of missense variants in the TRESK (KCNK18) K channel. *Sci Rep* **2**:237.
2. \* Bavro VN, De Zorzi r, Schmidt MR, Muniz JR, **Zubcevic L**, **Sansom MS**, **Vénien-Bryan C**, **Tucker SJ** (2012) Structure of a KirBac potassium channel with an open bundle crossing indicates a mechanism of channel gating. *Nat Struct Mol Biol* **19(2)**:158-63.
3. Dahl AC, Chavent M, **Sansom MS** (2012) Bendix: intuitive helix geometry analysis and abstraction. *Bioinformatics* **28(16)**:2193-4.
4. \* **de Wet H**, Shimomura K, Aittoniemi J, Ahmad N, **Lafond M**, **Sansom M**, **Ashcroft FM** (2012) A universally conserved residue in the SUR1 subunit of the KATP channel is essential for translating nucleotide binding at SUR1 into channel opening. *J Physiol* Sept 10 [Epub ahead of print]
5. García-Fandiño R, **Sansom MS** (2012) Designing biomimetic pores based on carbon nanotubes. *Proc Natl Acad Sci USA* **109(18)**:6939-44.
6. Hall BA, Armitage JP, **Sansom MS** (2011) Transmembrane helix dynamics of bacterial chemoreceptors supports a piston model of signalling. *PLoS Comput Biol* **7(10)**:e1002204.
7. Kalli AC, Hall BA, Campbell ID, **Sansom MS** (2011) A helix heterodimer in a lipid bilayer: prediction of the structure of an integrin transmembrane domain via multiscale simulations. *Structure* **19(10)**:1477-84.
8. Lumb CN, **Sansom MS** (2012) Finding a needle in a haystack: the role of electrostatics in target lipid recognition by PH domains. *PLoS Comput Biol* **8(7)**:e1002617.
9. Norimatsu Y, Ivetac A, Alexander C, Kirkham J, O’Donnell N, Dawson DC, **Sansom MS**  (2012) Cystic fibrosis transmembrane conductance regulator: a molecular model defines the architecture of the anion conduction path and locates a “bottleneck” in the pore. *Biochemistry* **51(11)**:199-212.
10. Norimatsu Y, Ivetac A, Alexander C, O’Donnell N, Frye L, **Sansom MS**, Dawson DC (2012) Locating a plausible binding site for an open channel blocker, GlyH-101, in the pore of the cystic fibrosis transmembrane conductance regulator. *Mol Pharmacol* Aug 24 [Epub ahead of print]
11. Parton DL, Akhmatskaya EV, **Sansom MS** (2012) Multiscale simulations of the antimicrobial peptide maculatin 1.1: water permeation through disordered aggregates. *J Phys Chem B* **116(29)**:8485-93.
12. Pongprayoon P, Beckstein O, **Sansom MS** (2012) Biomimetic design of a brush-like nanopore: simulation studies. *J Phys Chem B* **116(1)**:462-8.
13. Stansfeld PJ, **Sansom MS** (2011) Molecular simulation approaches to membrane proteins. *Structure* **19(11)**:1562-72.
14. \* Webster R, Maxwell S, Spearman H, Tai K, Beckstein O, **Sansom M**, **Beeson D** (2012) A novel congenital myasthenic syndrome due to decreased acetylcholine receptor ion-channel conductance. *Brain* **135(Pt 4)**:1070-80.

**Dr Stephen J. Tucker**

***Biological Physics Group, Department of Physics, Clarendon Laboratory, Oxford***

***Tel: 01865 272382 Email: stephen.tucker@physics.ox.ac.uk***

Our research is focused on understanding the relationship between ion channel structure and function. The objectives are to understand their molecular mechanism of operation at an atomic level as well as understanding their role in physiology and disease. As a model system we work primarily with the inwardly-rectifying family of potassium channels or ‘Kir’ channels. We are particularly interested in understanding how Kir channels respond to changes in intracellular pH and also phosphoinositides such as PIP2, and why defects in this regulation give rise to inherited diseases such as Type II Bartter’s Syndrome (Kir1.1), Andersen’s Syndrome (Kir2.1) and SeSAME/EAST Syndrome (Kir4.1/Kir5.1). We are also using prokaryotic homologs of these channels (KirBac channels) and have most recently used X-ray crystallography to determine the structure of a KirBac channel in the open state. In addition to studies of the Kir/KirBac family we also study the mechanism of gating in the K2P family of K+ channels and their regulation by a variety of different drugs and natural lipids. We use a wide variety of techniques including molecular biology, electrophysiology, protein biochemistry and fluorescence techniques to study these channels.

***Collaborations***

Collaborations within the initiative include projects with Prof. Mark Sansom to model potassium channel structures, with the Mary Lyon Centre at MRC Harwell to investigate genetic models of Kir channel function and with Dr Catherine Venien-Bryan (Dept Biochemistry) to study the conformational dynamics of the KirBac3.1 channel.

***Publications*** (\*collaborations within OXION 2011-2012)

1. \* Andres-Enguix I, Shang L, Stansfeld PJ, Morahan JM, **Sansom MSP**, Lafrenière RG, Roy B, Griffiths LR, Rouleau GA, Ebers GC, Cader MZ and **Tucker SJ** (2012) Functional analysis of missense variants in the TRESK (*KCNK18*) K+ channel.  
   *Scientific Reports* **2**:237.
2. \* Bavro VN, De Zorzi R, Schmidt MR, Muniz JRC, Zubcevic L, **Sansom MSP**, **Vénien-Bryan C** and **Tucker SJ** (2012) Structure of a KirBac potassium channel with an open bundle-crossing indicates a mechanism of channel gating. *Nature Structural and Molecular Biology* **19**:158-63.

**Dr Catherine Vénien-Bryan**

***Laboratory of Molecular Biophysics, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU***

***Tel: 01865 613295 Email:*** [***catherine.venien@bioch.ox.ac.uk***](mailto:catherine.venien@bioch.ox.ac.uk)

Many signals in the cell are conveyed by interacting protein molecules. How do protein-protein interactions lead to a response? The most likely explanation is through changes in structure. We study protein-protein interactions in the control of signalling processes using cryo-electron microscopy and combining the results with information from X-ray diffraction studies.

Ion channels regulate the flow of ions across an otherwise impermeable cell membrane. They are crucial for a wide range of biological processes and mutations in their genes cause multiple human diseases. The inwardly rectifying potassium (Kir) channels comprise a superfamily of K+ channels that regulate membrane potential and K+ transport in many cell types. Opening and closing (gating) of Kir channels may occur spontaneously but is modulated by numerous intracellular ligands that bind to the channel itself. In order to understand how Kir channels open and close it is essential to obtain a structure for the same channel in both the open and closed states. Three-dimensional (3-D) crystals of KirBac1.1 (or KirBac3.1) in the open state have been difficult to obtain. This may be because the open-state conformation is too flexible for crystallization, or because it requires a lipid bilayer to stabilise the open conformation. In this respect, 2-D crystals obtained in the presence of a lipid bilayer, and analysis of cyo-electron microscope (EM) tilted images may be a better stategy.

We have also studied the gating mechanism of this channel using radiolytic footprinting. (Gupta et al 2010). The purified protein stabilized in the open or the closed conformations was exposed to focused synchrotron X-ray beams to modify solvent accessible amino acid side chains. These modifications were identified and quantified using high-resolution mass spectrometry. The comparison of the open and closed state directly provided support for a proposed gating mechanism of the Kir channel.

*Specific objectives of our research are:*

-To obtain the 3D structure of KirBac3.1 in the open state using cryo electron microscopy images of 2D crystals and image analysis.

-To understand the gating mechanism of KirBac3.1, how does the pore open and closed?

***Collaborations***

With Dr Tucker, I am studying the 3D structure of KirBac3.1 in the open state using tilted images of 2D crystals taken with an electron microscope. With Prof. Ashcroft, I am studying the structure of SUR protein and the change of conformation upon binding of ligands.

***Publications***(\*collaborations within OXION 2011-2012)

1. \* Bavro VN, De Zorzi R, Schmidt MR, Muniz JR, Zubcevic L, **Sansom MS**, **Vénien-Bryan C**, **Tucker SJ** (2012) Structure of a KirBac potassium channel with an open bundle crossing indicates a mechanism of channel gating. *Nat Struct Mol Biol* **19(2**):158-63.
2. Sinclair JC, Davies KM, **Vénien-Bryan C**, Noble ME (2011) Generation of protein lattices by fusing proteins with matching rotational symmetry. *Nat Nanotechnol* **6(9)**:558-62.

**Professor Angela Vincent**

***Neurosciences Group, Nuffield Department of Clinical Neurosciences, West Wing and Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU***

***Tel: 01865 234630 Email: angela.vincent@clneuro.ox.ac.uk***

The work of the Neurosciences Group, now based mainly in the new West Wing of the John Radcliffe Hospital, concentrates on the identification and investigation of antibody-mediated disorders of the nervous system. We identify, characterize and investigate the pathogeneses of diseases that are associated with specific autoantibodies to neuronal or muscle ion channels or receptors. We use a combination of radioimmunoprecipitation and cell-based assays (antibodies to HEK cells transfected with cDNAs for glycine receptors, NMDA receptors, AMPA receptors, Aquaporin-4, CAPR2, LGI1, MOG) to identify the patients. We perform a large number of clinical assays for the UK and elsewhere. We have also established neuronal culture systems and proteomic methods (with Dreger/Holger, OXION) for identifying patients with antibodies to previously undefined targets. We combine these approaches with clinical characterization of the patients via questionnaires and follow-up of individual patients and patient cohorts. Finally, we try to demonstrate the pathogenicity of the antibodies by a range of in vitro cell-based assays, in vitro cultures, acute application to brain slice, and in vivo injections (with Deacon, Bannerman, Rawlins OXION). We have begun to establish telemetric recordings of EEG in mice (Upton, OXION).

Over the last years, these approaches have enabled us to dissect antibodies to VGKC-complexes demonstrating that most of the antibodies are against the complexed proteins CASPR2, LGI1 or Contactin-2 (S Irani, S Alexander (OXION Student, joint first authors) et al 2010), and to identify over 50 patients with NMDAR antibodies (S Irani, K Bera (OXION Student) et al 2010). These two studies, published in *Brain*, have been cited more than 40 times each over the last two years. The VGKC-complex antibodies have also been characterised in patients with Morvan’s syndrome and in a novel form of epilepsy (S Irani et al 2011;2012). CASPR2 antibodies were also identified by immunoprecipitation and mass spectroscopy in patients with a subacute progressive form of cerebellar ataxia (collaboration with Holger, Becker et al 2012). The purified IgG antibodies from these patients have been applied to brain slices (M Capogna, MRC ANU), to neuronal slices (with Mark Cunningham in Newcastle), and injected intrathecally (P Pettingill with Deacon). These results suggest that the antibodies are directly pathogenic and reduce NMDAR numbers (K Bera OXION DPhil awarded 2011). We are also investigating the presence of antibodies to neuromuscular junction components such as agrin and the recently discovered LRP4 (K Belaya Postdoc OXION with Beeson). We have now identified over 50 patients with the glycine receptor antibodies that we discovered and are defining the clinical presentation and pathogenic mechanisms (MI Leite, A Carvajal et al in preparation). We have completed and published a study on the effects of serum from a severe pain syndrome on mouse behavior (Goebel et al; Deacon OXION). Finally, all of these investigations are now being addressed in the paediatric population where various forms of encephalopathy are understudied.

***Publications*** (\*collaborations within OXION 2011-2012)

1. Lalic T, Pettingill P, **Vincent A**, Capogna M (2011) Human limbic encephalitis serum enhances hippocampal mossy fiber-CA3 pyramidal cell synaptic transmission. *Epilepsia* **52(1)**:121-31.
2. \* **Becker EB**, Zuliani L, Pettingill R, **Lang B**, Waters P, Dulneva A, **Sobott F** et al (2012) Contactin-associated protein-2 antibodies in non-paraneoplastic cerebellar ataxia. *J Neurol Neurosurg Psychiatry* **83(4)**:437-40.
3. Hacohen Y, Wright S, Siddiqui A, Pandya N, Lin JP, **Vincent A** et al (2012) A clinico-radiological phenotype of voltage-gated potassium channel complex antibody-mediated disorder presenting with seizures and basal ganglia changes. *Dev Med Child Neurol* Jul 22.
4. \* Irani SR, Pettingill P, Kleopa KA, Schiza N, Waters P, Mazia C, Zuliani L, Watanabe O, **Lang B**, Buckley C, **Vincent A** (2012) Morvan syndrome: Clinical and serological observations in 29 cases. *Ann Neurol*. Mar 9.
5. Jacob S, Viegas S, Leite MI, **Webster R**, Cossins J, Kennett R, Hilton-Jones D, Morgan BP, **Vincent A** (2012) Presence and pathogenic relevance of antibodies to clustered acetylcholine receptor in ocular and generalized myasthenia gravis. *Arch Neurol*. **69(8)**:994-1001.
6. Kitley J, Leite MI, Nakashima I, Waters P, McNeillis B, Brown R, Takai Y, Takahashi T, Misu T, Elsone L, Woodhall M, George J, Boggild M, **Vincent A**, Jacob A, Fujihara K, Palace J (2012) Prognostic factors and disease course in aquaporin-4 antibody-positive patients with neuromyelitis optica spectrum disorder from the United Kingdom and Japan. *Brain* **135(Pt 6)**:1834-49.
7. Viegas S, Jacobson L, Waters P, Cossins J, Jacob S, Leite MI, Webster R, **Vincent**  (2012) Passive and active immunization models of MuSK-Ab positive myasthenia: electrophysiological evidence for pre and postsynaptic defects. *Exp Neurol* **234(2)**:506-12.
8. Waters PJ, McKeon A, Leite MI, Rajasekharan S, Lennon VA, Villalobos A, Palace J, Mandrekar JN, **Vincent A**, Bar-Or A, Pittock SJ (2012) Serologic diagnosis of NMO: a multicenter comparison of aquaporin-4-IgG assays. *Neurology* **78(9)**:665-71.

**Associate Members**

**Dr Phil Biggin**

***Structural Bioinformatics and Computational Biochemistry, Department of Biochemistry, South Parks Road, Oxford OX1 3QU***

***Tel: 01865 613305 Email: philip.biggin@bioch.ox.ac.uk***

We are particularly interested in developing and applying computational methods including docking and molecular dynamics simulations to receptor proteins such as the ligand-gated ion channels.  These are receptors that upon binding of a ligand change their conformation such that ions can pass through a central pore and down their electrochemical gradient. We are particularly interested in two distinct families of these receptors: 1. The ionotropic glutamate receptors and 2. The nicotinic acetylcholine receptor. Although there has been a recent increase in the amount of structural information available, many questions still remain concerning the dynamics associated with these processes. An understanding of these processes should be useful in the design of new drug treatments for a range of diseases including Alzheimer's, Parkinsons's, and epilepsy.

***Collaborations***

We have collaborations with Professor David Sattelle (Manchester/OXION), Dr Mark Mayer (NIH), Nicole Zitzmann (Oxford), Prof Lucia Sivilotti (UCL) and Professor Isabel Bermudez (Oxford Brookes).

***Publications*** (\*Collaborations within OXION 2011-2012)

1. Heifetz A, Morris GB, **Biggin PC**, Barker O, Fryatt T, Bentley J, Hallett D, Manikowski D, Pal S, Reifegerste R, Slack M, Law R (2012) [Study of human Orexin-1 and -2 G-protein-coupled receptors with novel and published antagonists by modeling, molecular dynamics simulations, and site-directed mutagenesis.](http://www.ncbi.nlm.nih.gov/pubmed/22448975) *Biochemistry* **51**:3178-97.
2. Moroni M, Biro I, Giugliano M, Vijayan R, **Biggin PC**, Beato M, Sivilotti LG (2011) [Chloride ions in the pore of glycine and GABA channels shape the time course and voltage dependence of agonist currents.](http://www.ncbi.nlm.nih.gov/pubmed/21976494) *J* *Neurosci*. **31**:14095-106.
3. Munz M, **Biggin PC** (2012) JGromacs: a Java package for analyzing protein simulations. *J Chem Inf Model* **52**:255-9.
4. Ross GA, Morris GM, **Biggin PC** (2012) Rapid and accurate prediction and scoring of water molecules in protein binding sites. *PLoS One* **7**:e32036.

**Dr. Maike Glitsch**

***Department of Physiology, Anatomy and Genetics; Sherrington Building; Parks Road, Oxford University; Oxford OX1 3PT***

***Tel 01865 282491 Email: maike.glitsch@dpag.ox.ac.uk***

My group is interested in signalling in the brain in health and disease. We focus on signalling cascades mediated by G protein coupled receptors linking to classic Transient Receptor Potential (TRPC) channels using electrophysiology, fluorescent calcium imaging and molecular biology. We have previously shown that TRPC channels in the cerebellum are differentially up- and down-regulated during early postnatal development and that cerebellar tumours couple to activation of TRPC channels following stimulation of G protein coupled receptors in response to a drop in extracellular pH, resulting in gene expression. External acidosis, which is common to a number of diseases including tumours, ischemia and inflammation (which also occurs in neurodegenerative diseases such as Alzheimer’s Disease) hence can trigger signalling cascades that may lead to changes in proteins expressed in a given cell. We now look at effects of extracellular acidosis on cerebellar development since cell proliferation in normal development can also be accompanied by external acidosis as well as impact of external acidosis on calcium signalling in microglia, the immune cell of the brain. Another aspect of my group is the physiology of TRPC channels in healthy cerebellar tissue: activation of parallel fibres leads to the generation of a slow excitatory postsynaptic potential in Purkinje cells that is mediated by TRPC channels. We are looking at the activation and regulatory mechanisms controlling these channels in rodent cerebellar slices.

***Publications***(\*collaborations within OXION 2010-2011)

1. Nelson C, **Glitsch MD** (2012) Lack of kinase regulation of canonical transient receptor potential 3 (TRPC3) channel-dependent currents in cerebellar Purjinje cells. *J Biol Chem* **287(9)**:6326-35.

**Professor Paul Harrison**

***Department of Psychiatry, Neurosciences Building, Warneford Hospital, Oxford OX3 7JX.***

***Tel 01865 223730. Email:*** [***paul.harrison@psych.ox.ac.uk***](mailto:paul.harrison@psych.ox.ac.uk)

My group’s core interest is the molecular and translational neurobiology of psychosis (schizophrenia and bipolar disorder), currently focusing on the mechanisms by which candidate genes and polymorphisms that have been reportedly associated with these disorders may operate, with particular focus on glutamatergic mechanisms. Genes of interest include D-amino acid oxidase (*DAO*), type II metabotropic glutamate receptors (*GRM2, GRM3*), catechol-O-methyltransferase (*COMT*), and *ZNF804A*. For example, led by Liz Tunbridge, COMT is being studied using pharmacological and genetic mouse models, utilising slice electrophysiology, in vivo microdialysis, behavioural testing, and gene expression analyses, as well as investigating the correlates of functional COMT polymorphisms in human brain using magnetoencephalography. As a second example, led by Phil Burnet, we found increased expression and activity of DAO in the brain in schizophrenia, and have gone on to study the molecular, neurochemical, electrophysiological, and behavioural consequences of over-expression, knockdown, and knockout of DAO.

**Collaborators in OXION: David Bannerman, Ed Mann.**

***Publications*** (\*collaborations within OXION: 2011-2012)

1. \* Deakin IH, Nissen W, Law AJ, Lane T, Kanso R, Schwab M, Nave K-A, Lamsa K, **Paulsen O**, **Bannerman DM**, **Harrison PJ** (2012) Transgenic over-expression of neuroregulin 1 (NRG1) type I affects working memory and hippocampal oscillations but not long term potentiation. *Cerebral Cortex* **22**:1520-1529.
2. \* **Harrison PJ**, Pritchett D, Stumpenhorst K, Betts JF, Nissen W, Schweimer J, Lane T, Burnet PWJ, Lamsa K, Sharp T, **Bannerman DM**, Tunbridge EM (2012) Genetic mouse models relevant to schizophrenia – taking stock and looking forward. *Neuropharmacology*  **62**:1164-1167.
3. \* Laatikainen LM, Sharp TS, **Bannerman DM,** **Harrison PJ**, Tunbridge EM (2012) Modulation of hippocampal dopamine metabolism and hippocampal-dependent cognitive function by catechol-O-methyltransferase inhibition. *Journal of Psychopharmacology* (in press)
4. Law AJ, Wang Y, Sei Y, O’Donnell P, Piantadosi P, Papaleo F, Huang W, Thomas CJ, Vakkalanka R, Besterman A, Lipska B, Hyde TM, **Harrison PJ**, Kleinman JE, Weinberger DR (2012) NRG1-ErbB4-p110d signaling in schizophrenia and p110d inhibition as a potential therapeutic strategy. *Proceedings of the National Academy of Sciences USA* (AOL 11 June 2012)
5. Pritchett D, Wulff K, Oliver PL, **Bannerman DM,** Davies KE, **Harrison PJ**, Peirson SN, Foster RG (2012) Evaluating the links between schizophrenia and sleep and circadian rhythm disruption. *Journal of Neural Transmission* (in press)

**Dr Bethan Lang**

***Neurosciences Group, Level 5/6 West Wing John Radcliffe Hospital, Department of Clinical Neurology, University of Oxford, Oxford***

***Tel: 01865 222321 Email: bethan.lang@imm.ox.ac.uk***

For many years my group has been involved in autoimmune disorders of the peripheral, autonomic and central nervous system. In the Lambert-Eaton myasthenic syndrome (LEMS), patients develop autoantibodies to voltage-gated calcium channel (VGCC) present on the presynaptic neuromuscular junction. Around 60% of LEMS patients have an associated lung carcinoma, the small cell lung carcinoma (SCLC) and it is thought, that in these cases, the VGCC antibodies are directed against channels on the tumour surface. We are very interested in why these LEMS/SCLC patients have a better prognosis than patients with SCLC alone and why some of these LEMS/SCLC patients develop cerebellar ataxia.

As well as the peripheral nervous system, the autonomic nervous system is also affected in a proportion of patients with LEMS. This autonomic dysfunction may also be induced by specific anti-VGCC antibodies however in autoimmune autonomic neuropathy antibodies are detected against a different target, namely a neuronal form (α3) of the acetylcholine receptor found on the autonomic ganglia. The pathogenicity and interrelationship of these antibodies in the autonomic nervous system is not yet fully understood.

More recently, in collaboration with Professors Vincent, Beeson and Dr Upton groups within the OXION initiative, we have been investigating whether antibodies to a number of different ion channels and receptors have a role in epilepsy. We have demonstrated antibodies to different proteins of the voltage-gated potassium channel complex and to NMDA receptors in certain groups of patients with epilepsy and are currently investigating other putative antigens including members of the AMPA receptor family and looking to establish the pathogenicity of the detected antibodies and in collaboration with Dr Holger Kramer (OXION) we are looking for antibodies to novel antigens using proteomic techniques

***Publications*** (\*collaborations within OXION 2011-2012)

1. \* **Becker EB**, Zuliani L, Pettingill R, **Lang B**, Waters P, Dulneva A, **Sobott F**, Wardle M, Graus F, Bataller L, Robertson NP, **Vincent A** (2012) Contactin-associated protein-2 antibodies in non-paraneoplastic ataxia. *J Neurol Neurosurg Psychiatry* **83(4)**:437-40.
2. Dale RC, **Lang B**, Brilot F, Polfrit Y, Smith GH, Wong M (2011) Treatment-responsive pandysautonomia in an adolescent with ganglionic α3-AChR antibodies. *Eur J Paediatr Neurol*.
3. Irani SR, Bien CG, **Lang B** (2011) Autoimmune epilepsies. *Curr Opin Neurol* **24**:146-53.
4. \* Suleiman J, Brenner T, Gill D, Troedson C, Sinclair AJ, Briolot F, **Vincent A**, **Lang B**, Dale RC (2011) Immune-mediated steroid-responsive epileptic spasms and epileptic encephalopathy associated with VGKC-complex antibodies. *Dev Med Child Neurol* **53**:1058-60.
5. Titulaer MJ, **Lang B**, Verschuuren JJ (2011) Lambert-eaton myasthenic syndrome: from clinical characteristics to therapeutic strategies. *Lancet Neurol* **10**:1098-1107.
6. \* Titulaer MJ, Maddison P, Sont JK, Wirtz PW, Hilton-Jones D, Klooster R, Willcox N, Potman M, Sillevis Smitt PA, Kuks JB, Roep BO, **Vincent A**, van der Maarel SM, van Dijk JG, **Lang B**, Verschuuren JJ (2011) Dutch-English Lambert-Eaton Myasthenic syndrome (LEMS) tumor association prediction score accurately predicts small-cell lung cancer in the LEMS. *J Clin Oncol* **29**:902-8.
7. \* **Vincent A**, Irani SR, **Lang B** (2011) Potentially pathogenic autoantibodies associated with epilepsy and encephalitis in children and adults. *Epilepsia* **52(8)**:8-11.

**Dr Edward Mann**

***Department of Physiology, Anatomy & Genetics, Sherrington Building, Parks Road, Oxford, OX1 3PT***

***Tel: 01865 285835 Email: ed.mann@dpag.ox.ac.uk***

**Cellular mechanisms underlying cortical network oscillations and plasticity**

Neuronal activity in cortical networks is orchestrated within a variety of brain rhythms. The frequency and spatial scale of these network oscillations vary with behavioural state, and display characteristic disturbances in numerous brain disorders, including epilepsy and schizophrenia. Resolving the mechanisms underlying the generation of cortical oscillations, and how they influence cortical circuit processing and plasticity, is critical for translating our understanding of brain function between the cellular and behavioural levels.

Our research focuses on how excitatory cortical neurons and inhibitory GABAergic interneurons interact to naturally synchronize network activity. The population of GABAergic interneurons constitutes an array of distinct neuronal subtypes, and it is becoming increasingly clear that different GABAergic loops are recruited during different brain rhythms. We use a combination of electrophysiological, optical imaging and optogenetic techniques to dissect these circuits in rodent cortex. The specific goals of our research are to understand: (i) how cortical networks can switch dynamically between oscillations at different frequencies, (ii) how the mechanisms of rhythmogenesis are continuously tuned to yield a stable repertoire of network oscillations despite learning-related plasticity in the underlying circuitry, and (iii) how different patterns of physiological and pathological GABAergic synchronization affect the behavioural output of cortical networks.

***Collaborations***

We have collaborations with Professor Ole Paulsen (Cambridge/OXION), Dr Louise Upton (Oxford/OXION), and Professor Paul Harrison (OXION/Oxford).

***Publications*** (\*collaborations within OXION 2011-2012)

1. Verret L, **Mann EO**, Hang GB, Barth AM, Cobos I, Ho K, Devidze N, Masliah E, Kreitzer AC, Mody I, Mucke L and Palop JJ (2012) Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in Alzheimer model. *Cell* **149**:708-21.

**Professor Ole Paulsen**

***Department of Physiology, Development and Neuroscience, University of Cambridge, Downing Street, Cambridge CB2 3EG***

***Tel: 01223 333804 Email: op210@cam.ac.uk***

The Neuronal Oscillations Group (NOG) aims to understand how ion channels control network activity in the brain. We investigate how different kinetic properties of ligand-gated and voltage-gated ion channels confer specific frequency properties on neurons and networks of neurons. The main hypotheses are that network oscillations emerge from these frequency preferences, and that these oscillations control the timing of action potentials during spike timing-dependent plasticity, a phenomenon widely believed to underlie memory formation. Insights into these mechanisms might also be important for the understanding of brain disorders, including memory impairment, schizophrenia, and epilepsy.

***Publications*** (\*collaborations within OXION 2011-2012)

1. \* Botcherby EJ, Smith CW, **Kohl MM**, Debarre D, Booth MJ, Juskaitis R, **Paulsen O** and Wilson T (2012) Aberration-free three-dimensional multiphoton imaging of neuronal activity at kHz rates. *Proc Natl Acad Sci USA* **109**:2919-24.
2. \* Reeve JE, **Kohl MM**, Rodriguez-Moreno A, **Paulsen O** and Anderson HL (2012) Addendum: Caged intracellular NMDA receptor blockers for the study of subcellular ion channel function. *Commun Integrat Biol* **5**:3,1-3.
3. \* Deakin IH, Nissen W, Law AJ, Lane T, Kanso R, Schwab MH, Nave K-A, Lamsa KP, **Paulsen O**, **Bannerman DM** and **Harrison PJ** (2012) Transgenic overexpression of the type I isoform of neuregulin 1 affects working memory and hippocampal oscillations but not long-term potentiation. *Cereb Cortex* **22**:1520-9.

**Professor David Sattelle**

***Professor of Molecular Neurobiology, Faculty of Life Sciences, AV Hill Building, University of Manchester, Oxford Road, Manchester M13 9PT, UK (formerly MRC FGU, Oxford)***

***Tel: 0161-2755792 Email:*** [*david.sattelle@manchester.ac.uk*](mailto:david.sattelle@manchester.ac.uk)

Transgenic lines and mutants of the nematode genetic model organism *Caenorhabditis elegans* can mimic aspects of human channelopathies and other nervous system and neuromuscular disorders. Such models can be used in genetic suppressor / enhancer screens, in the search for new therapeutic targets. They also permit rapid screening for chemical modifiers, thereby highlighting possible drug candidates. A device for low-cost, automated, high-throughput, *in vivo*, drug screening has been constructed for library-scale, chemical screening on *C. elegans* disease models. By this means, we are pursuing the re-profiling of drugs already approved for human use as well as the discovery of new chemical leads. Current collaborations on chemical libraries involve Dr Angela Russell (Oxford), Dr Barbara Saxty (MRCT) as well as colleagues in Industry. *C. elegans* models mimicking aspects of familial and sporadic Alzheimer’s disease, fronto-temporal dementia, congenital myasthenia syndrome channelopathies and spinal muscular atrophy are being explored.

We are collaborating with Conformetrix, a University of Manchester spinout Company, to study the actions of newly generated positive allosteric modulators (PAMs) of human α7 nAChRs. We have also exploited the striking differences between the allosteric modulator actions on the human α7 nicotinic acetylcholine receptor (nAChR) and ACR-16, its evolutionarily remote *C. elegans* homologue to better understand the allosteric drug binding site. A gain of function mutation in ACR-16 is lethal, suggesting the potential as anthelmintics of PAMs targeting ACR-16. Some parasitic nematode nAChR subunit classes, along with L-glutamate-gated chloride channels (GluCls) have no known counterpart in vertebrate host species. Access to invertebrate genomes is fast uncovering more such candidate animal health drug targets. Understanding their functions and pharmacology will help improve the design and safety of the next generation of anti-parasitic drugs. This work has been greatly facilitated by the Hibbs / Gouaux structure of the first eukaryotic ligand-gated ion channel, the GluCl of *C. elegans* complexed with ligands acting at the neurotransmitter binding site, the allosteric site and the ion channel.

A range of current collaborations with OXION colleagues are proving fruitful and include: Philip Biggin (Biochemistry, Oxford) - modeling receptor-ligand interactions; Kay Davies, (MRC FGU, Oxford) - model organisms in the study of neuromuscular diseases. Past collaborations with Bethan Lang, David Beeson and Angela Vincent (WIMM) on nAChR-related disorders were also fruitful.

***Publications***  (\*collaborations within OXION 2011-2012)

1. Grice S, Sleigh JN, Liu J-L and **Sattelle DB** (2011) Invertebrate models of spinal muscular atrophy: insights into mechanisms and potential therapeutics. *Bioessays* **33**: 956-965.
2. Lees K, Sluder A, Shannan N, Hammerland L and **Sattelle DB** (2012) Ligand

Gated Ion Channels as Targets for Anthelmintic Drugs: Past, Current and Future Perspectives. InAntiparasitic and Antibacterial Drug Discovery: from Molecular Targets to Drug Candidates(ed Conor Caffrey) pp3-21.Wiley – VCH Verlag GmbH & Co. KGaA*.*

**Professor Ian D Thompson**

***MRC Centre for Developmental Neurobiology, Guy’s Campus, King’s College London, SE1 1UL***

***Tel: 0207 848 6747 Email: ian.thompson@kcl.ac.uk***

Here at KCL, my group’s interests have focused on the more developmental aspects of visual system research using the mouse as our main experimental model. In particular, we want to use quantitative measures (both morphological and physiological) of topography to dissect how the various molecularly-based and activity-based cues interact to form the retinotopic maps that underly visual function. Arising from this characterisation is an interest in how the Receptive Fields (RFs) of individual neurons are constrained by the underlying topography.

The work on topography is funded by a Wellcome Trust Programme Grant, *“Measuring and modelling the dynamics of retinotopic map formation”* with Uwe Drescher (MRC Centre), David Willshaw (Edinburgh) and Stephen Eglen (Cambridge). The grant was awarded in November 2007 and activated in September 2008 with the appointment of Dr. Andrew Lowe as RA. Andrew has been refining our mouse electrophysiology and is implementing multi-electrode recording and intrinsic signal optical imaging. The whole animal homeostasis has been greatly improved to extend the recording and imaging sessions. We have undertaken quantitative investigations of RF organisation in mouse superior colliculus (SC), which will parallel the investigations done in striate cortex in collaboration with Dr. Louise Upton. Dr. Lowe has rewritten our stimulus presentation and neuronal response analysis software to optimise presentation schedules and data acquisition, the latter has been greatly enhanced by the use of the multi-electrode array. Two PhD students joined the group in October 2009, initially using anatomical tracers to examine the development of projections within the mouse visual system.

Our use of multiple fluorescent retrograde neuronal tracers to quantify retinotopic map order and precision in neonatal rodents has been further refined since the publication with Dr. Upton on the hamster SC (Upton et al., 2007). We now have a thorough description of the development of the projection in the neonatal mouse which has generated key ‘probe’ ages and injection separations that define crucial stages in the elaboration of the map. Applying the approach to mutant mice with disrupted patterns of spontaneous activity is leading us to question the common view that the basic map is established by molecular gradients and is subsequently refined by neuronal activity. The experimental data on normal and mutant animals are being incorporated into theoretical models of map formation, which have been generated in Cambridge and in Edinburgh. A collaboration with Dr. David Sterratt in Edinburgh has investigated the topological consequences of flat-mounting mouse retina and has enabled us to re-fold the retinae and describe topology in polar co-ordinates using standard profiles (now submitted for publication).

The first publication from an MRC Pathfinders award, in collaboration with Dr. Nicola Sibson and Dr. Andrew Lowe, is on the optimisation of MRI imaging of retinal projections using Mn++ as an MRI tracer (Lowe et al., 2008). Dr. Lowe is using his great experience in MRI and fMRI to optimize strategies for intrinsic signal optical imaging, which will also use our Local Field Potential data from the collicular electrophysiology. We are moving our quantitative visual electrophysiology approach (established with long-standing collaboration with Dr. David Tolhurst, Cambridge: eg Tolhurst et al., 2009) but moving from ferret to mouse and from visual cortex to superior colliculus. Collaborations initiated in the MRC with Martin Meyer are using genetically-encoded Ca++ reporters to characterise the visual responses of ganglion cells targeting the optic tectum of zebrafish (paper in press)

***Collaborations within Oxion***

***Publications*** (\*collaborations within OXION 2011-2012)

1. Nikolaou N, Lowe AS, Walker AS, Abbas F, Hunter PR, **Thompson ID**, Meyer MP (2012) Parametric functional mapping of visual inputs to the tectum. *Neuron* (in press)

**TRAINING PROGRAMME**

The Training Programme has two inter-related aims (i) to provide training in new techniques for any member of the consortium, from graduate student to group leader and (ii) to promote collaboration between the members of the consortium. These aims are achieved by the provision of core facilities (equipment and research staff) funded by the Strategic Award and by the presence of Training Fellows and OXION graduate students, whose projects must involve collaboration between at least two groups in the consortium. Finally, we run a Graduate Training Progamme for first year OXION graduates that is a mixture of taught course work, demonstrations of techniques and laboratory rotations.

**Training Fellows**

**Katsiaryna Belaya** (2010-2013)

I am interested in the diseases of the neuromuscular junction, and I’m currently working on two projects:

1. The search for new genes in which mutations can lead to the development of congenital myasthenic syndromes (CMS).

2. The search for novel antigenic targets in patients with autoimmune myasthenia gravis (MG).

**1. The search for new genes in CMS.**

Congenital myasthenic syndromes are inherited disorders of neuromuscular transmission that are characterised by the weakness of ocular, bulbar and limb muscles. To date, mutations in 15 different genes have been shown to lead to impaired neuromuscular transmission although some are limited to single case reports. Additionally, there is still a number of patients with typical CMS symptoms, where the underlying mutations have not been found. To identify new genes that may lead to the development of CMS, I have performed whole exome capture and next generation sequencing from four patients with CMS. Analysis of the obtained data showed that two patients had mutations in *DPAGT1* gene, while the other two patients had mutations in another novel gene. Interestingly, both identified proteins are involved in the same cellular process – protein glycosylation.

Sanger sequencing of a further cohort of CMS patients with varying symptoms revealed six more patients with mutations in *DPAGT1* gene, bringing the total number of patients with mutations in *DPAGT1* gene to eight. Sequencing of family members of all patients revealed that all mutations are inherited in a Mendelian pattern and segregate with the CMS symptoms. All patients with mutations in *DPAGT1* gene share a number of common clinical features which distinguish them from the majority of other CMS patients. In terms of treatment, all patients respond well to pyridostigmine, and two benefited from taking 3,4-diaminopyridine.

I have also performed functional analysis of DPAGT1 to establish how the mutations in this gene lead to the development of CMS. Using the DPAGT1-specific inhibitor tunicamycin, I showed that DPAGT1 is required for efficient glycosylation of acetylcholine receptor (AChR) subunits and for efficient export of acetycholine receptors to the cell surface. This is consistent with the defects observed in the muscle biopsies from the patients which display a drastic reduction in the amount of AChR present in the NMJ region. Thus we suggest that the primary pathogenic mechanism of *DPAGT1* mutations is reduced levels of AChRs at the endplate region.

To conclude, to date we have discovered two new genes in which mutations lead to the development of CMS. We also propose a pathogenic mechanism of how these mutations lead to the development of the disease. In future, we plan to study in greater detail how the newly identified proteins contribute to the normal function of the NMJ. Additionally, we plan to perform next generation sequencing on a further cohort of CMS patients to identify additional genes that may lead to the development of this disorder.

**2. The search for novel autoantibodies in patients with autoimmune MG.**

In my second project, I’m looking for new potential targets for autoantibodies in autoimmune myasthenia gravis. This is the most common autoimmune disorder of the NMJ with the prevalence of the disease of approximately 10 in 100,000 people. In the majority of patients, the disease is caused by autoantibodies to the AChR receptor, while a smaller fraction of patients have antibodies to the muscle specific kinase (MuSK). However, in 5-10% of the patients, no autoantibodies to these proteins can be detected, suggesting that other proteins can serve as antigenic targets in this disorder. One candidate protein is Agrin. Agrin is an extracellular signalling molecule that is essential for the formation and maintenance of neuromuscular junctions. To date, I have screened 424 serum samples from patients with suspected myasthenia gravis, and I have identified 35 Agrin-positive samples. Thus, it is likely that Agrin can indeed be an antigenic target in myasthenia gravis patients. In future, I plan to perform experiments to establish whether anti-Agrin antibodies are pathogenic and how they might contribute to the development of the disease.

**Publications**

1. **\* Belaya K**, Finlayson S, Slater CR, Cossins J, Liu WW, Maxwell S, McGowan SJ, Maslau S, Twigg SR, Walls TJ, Pascual Pascual SI, Palace J, **Beeson D** (2012) [Mutations in DPAGT1 Cause a Limb-Girdle Congenital Myasthenic Syndrome with Tubular Aggregates.](http://www.ncbi.nlm.nih.gov/pubmed/22742743) *Am J Hum Genet.* **91(1)**:193-201.
2. Cossins J, Liu WW, **Belaya K**, Maxwell S, Oldridge M, Lester T, Robb S, **Beeson D** [The spectrum of mutations that underlie the neuromuscular junction synaptopathy in DOK7 congenital myasthenic syndrome.](http://www.ncbi.nlm.nih.gov/pubmed/22661499) *Hum Mol Genet.* **21(17)**:3765-75.

**Paul Miller (**2010-2013)

Project: Structural studies of GABA-A and glycine receptors

Since 2001 my PhD and postdoctoral research has focused on understanding the molecular mechanisms of function of membrane protein receptors. Specifically, I’ve studied one type of ligand-gated ion channel within the central nervous system, the glycine receptor. These receptors are part of a larger conserved protein family, the pentameric ligand-gated ion channel super-family, which includes nicotinic acetylcholine receptors, serotonin type-3A receptors and GABA-A receptors. These receptors are distributed throughout the central nervous system at synaptic junctions and mediate fast communication between neurons upon release of neurotransmitter. As such they perform a vast array of pivotal functions in central nervous system communication. For example, GABA-A receptors mediate the majority of rapid inhibitory neurotransmission in the brain and so control brain excitability. They are targets for clinically important drugs including benzodiazepines, barbiturates and anesthetics in treatments for disorders such as epilepsy, stress, pain and insomnia.

Despite the obvious importance of this protein super-family in neuropathology, as yet no atomic resolution structures are available for either serotonin type-3A, GABA-A or glycine receptors. High resolution structures are of vital importance because without them it is not possible to understand the relationships drugs share with their receptor targets. Specifically, to understand the molecular rules governing affinity, action and selectivity. With accurate structures it should be possible to rationally design improved or novel medications that offer new treatments or exhibit better selectivity and have reduced side effects.

The OXION training fellowship has given me the opportunity to transfer my research from the functional analysis of measuring drug pharmacology of ligand-gated ion channels using a technique known as whole-cell patch-clamp electrophysiology, into a more structural analysis of these proteins to physically see how drugs bind. So far this has allowed me to develop a robust protein purification process for large-scale production of GABA-A receptors which I am currently analyzing using X-ray crystallography, electron microscopy and solid-state NMR to obtain high-resolution structural data. My hope in the future is to combine these functional and structural techniques to come to a far deeper understanding of how pentameric receptors operate and respond to drugs and to facilitate the rational development of novel or enhanced therapeutics.

**Mariana Vargas-Caballero** (2009-2012)

During my Biology degree in Toluca, Mexico, I became fascinated by synapses, and this led me to start my training as an electrophysiologist at the Institute of Cellular Physiology at the UNAM in Mexico City. A PhD followed at the laboratory of Hugh Robinson at the University of Cambridge, and I focused on the study of NMDA receptors, which are crucial players in synaptic plasticity. Then, a postdoctoral Wellcome Trust Fellowship gave me the opportunity to consolidate my training in electrophysiology in leading laboratories in North America and back in the UK, and to initiate my training in additional techniques during the intensive Neurobiology training course at the MBL in Woodshole.

The first part of my OXION Training Fellowship at the laboratory of Prof. Ole Paulsen focused on the study of synaptic plasticity in the hippocampus and, in particular, how plasticity molecules involved in Alzheimer's disease affect this plasticity. My electrophysiology-based work, together with further molecular biology training at the laboratory of Prof. Paul Harrison, led to my first senior-author paper in the Journal of Neuroscience. We showed that Tau protein is required for amyloid-β-mediated inhibition of synaptic plasticity. My productive and exciting collaboration with Prof. Ole Paulsen and Dr. Richard Wade-Martins continues and we now aim to understand the underlying mechanisms by which amyloid beta inhibits long term potentiation, and the specific role of Tau protein phosphorylation in synaptic pathology.

Many Alzheimer’s mouse models exist, and their cognitive and synaptic impairment has been relatively well characterized in older mice, once the phenotype is well established. However, to be able to intervene early at a clinical level in human patients, we must understand how the disease starts. With this in mind, my current project focuses on elucidating the earliest time points of cognitive impairment in an Alzheimer’s mouse model. For this I am training in behavioural techniques at the laboratory of Dr. David Bannerman working with young Alzheimer’s mice to be able to detect the emergence of cognitive impairment. By building upon this initial characterisation, I aim to identify the synaptic correlates of early cognitive impairment in further work.

At the end of this academic year – my last one as an OXION Training Fellow, I will be moving to the University of Southampton, where I have been appointed a Research Career Track Lecturer at the Institute for Life Sciences. Warmest thanks to OXION for bridging the gap between my postdoctoral training and my independent research group and for allowing me the extremely productive interaction with established researchers and fantastic students, postdocs and staff at the University of Oxford.

**Melissa Brereton** (2011-2014)

My research interests are centred on understanding how KATP channels regulate pancreatic hormone secretion in health and disease. I am currently working on two research projects:

**Electrophysiological characterisation of KATP channel mutations that cause neonatal diabetes**

Neonatal diabetes (ND) is a rare monogenic form of diabetes with an incidence of 1 in 200, 000. Patients present with hyperglycaemia within the first 6 months of life and 50% of all ND cases result from gain-of-function mutations in KATP channels. ND mutations impair the ability of ATP, a product of glucose metabolism, to inhibit the channel resulting in an increase in KATP current. In pancreatic β-cells, KATP channels link glucose metabolism to insulin secretion and an increase in KATP current hyperpolarises the β-cell membrane, preventing insulin release in response to hyperglycaemia. Pharmacological agents, known as sulphonylureas (SUs), inhibit KATP channels and are the current therapy of choice for ND patients. Identifying ND patients whose disease phenotype arises due to KATP mutations enables switching from daily insulin injections to oral SU tablets. This allows for better glycaemic control and an improved quality of life for the patients. Through close collaboration with clinical colleagues, this project helps determine the severity of ND mutations using electrophysiology and the *Xenopus laevis* oocyte expression system. These experiments allow ND mutations to be characterised and the degree of reduced ATP sensitivity quantified to gain a better understanding of the role of KATP channels in this disease. Studying these physiological mutations can also help elucidate key residues in the KATP channel complex that are important for gating and ATP binding and therefore correct functioning of the channel.

**Investigating the impact of hyperglycaemia on α-cell function in a mouse model of diabetes expressing a gain-of-function mutation in β-cell KATP channels**

Glucagon is secreted from pancreatic α-cells in response to low blood glucose and acts primarily on the liver to increase hepatic glucose production. KATP channels are expressed and functional in α -cells where they have been suggested to directly sense a fall in plasma glucose and increase glucagon secretion. Glucagon secretion is also under paracrine regulation from neighbouring β - and δ-cells which secrete insulin and somatostatin respectively. In this way, a rise in glucose promotes insulin and somatostatin release which in turn acts upon α-cells to inhibit glucagon secretion. In type 2 diabetes, glucagon secretion is inappropriately elevated at high glucose concentrations and secretion impaired in response to hypoglycaemia. It is evident that α-cell function is altered in type 2 diabetes but it is unclear whether this reflects hyperglycaemia *per se* or a paracrine effect due to reduced insulin secretion. In collaboration with Professor(s) Frances Ashcroft and Patrik Rorsman, this project utilises a mouse model characterised by reduced insulin secretion and hyperglycaemia which expresses a physiological gain-of-function mutation in KATP channels (KIR6.2-V59M) specifically within the pancreatic β-cells. Employing both *in vivo*; fasting plasma glucagon measurements, hypoglycaemia-induced tolerance test / insulin-tolerance test and *in vitro* experiments; glucagon secretion measurements in the intact perfused pancreas and isolated islets the impact of acute and chronic hyperglycaemia on α-cell function is being investigated. It is hoped that the results from this study will provide novel insights into the mechanisms underlying hyperglucagonemia in type 2 diabetes and also provide a greater understanding of how glucagon is regulated in neonatal diabetes as KIR6.2-V59M is a common mutation in this condition.

**Graduate Training Programme**

**OXION grant 2003-2008**

**Sian Alexander (2003) Brittany Zadek (May 2005)**

**(Submitted DPhil 2007 - (Submitted DPhil 2008 -**

**Awarded) Awarded)**

**Angela Cohen (2003 – left 2004)**  **Michael Kohl (2005)**

**(Submitted DPhil 2010 -**

**Awarded)**

**Adrian Hon (2003 – left 2004) Katarzyna Bera (2006)**

**(Submitted DPhil 2010 -**

**Awarded)**

**Tommas Ellender (2004)**

**(Submitted DPhil 2009 -** **Michael Craig (2006)**

**Awarded)** **(Submitted DPhil 2011 –**

**Awarded)**

**Stephan Kaizik (2004) Rebecca Clark (2006)**

**(Submitted DPhil 2010- (Submitted DPhil 2010 –**

**Awarded) Awarded)**

**Amy Hoon (née Schou) (2006)**

**(Submitted DPhil 2011 - Awarded)**

**OXION grant 2009-2014**

**Carolina Lahmann (2009)**

**Olivia Shipton (2009)**

**Goudarz Karimi (2010)**

**Aletheia Lee (2010)**

**Lukasz Stasiak (2010)**

**Gauri Ang (2011)**

**Julian Bartram (2011)**

**Hege Larsen (2011)**

**Conor McClenaghan (2011)**

**Christoph Treiber (2011) – moved to a different course after 4 months**

**Alexei Bygrave (2012)**

**Antonia Langfelder (2012)**

**Elisa Vergari (2012)**

We organise a formal training course for the first year of the graduate student’s 4-year studentship, after which they proceed to a full research project that must be based in at least two laboratories within the consortium. In the first half of the first term, students attend selected modules from the MSc courses in Neuroscience or in Structural Biology together with two courses specifically tailored to the needs of the OXION programme (‘Mouse Neurobiology’ and ‘Genes to Clinic’). One of the features of the tailored course is the active involvement of the Core Facility scientists and of the Training Fellows in the teaching. In each of the next two terms, the students undertake a 4 month research project and write a 3000 word report on each and also study an advanced module from the MSc in Neuroscience, for which they have to write a 1500 word essay.

**OXION courses: Mouse Neurobiology**

1. Background techniques and Home Office training modules
2. Mouse genetic models
3. Basic screen for phenotyping
4. Mouse neuroanatomy
5. Mouse *in vivo* neurophysiology
6. Mouse *in vitro* neurophysiology
7. Analytical testing of mouse behaviour

**OXION courses: Genes to clinic**

1. Genetic model organisms and ion channels
2. Functional genomics
3. Analysis of ion channel structure and function
4. Analysis of human neurological diseases
5. Imaging

***OXION Training Fellows***

1. Mariana Vargas Caballero (Paulsen/Bannerman/Rawlins): Regulation of AMPA channels by persistent kinase activity: implications for the maintenance of spatial memory
2. Kate Belaya (Beeson/Vincent): Pathogenic molecular mechanisms of mutations underlying MuSK and DOK7 synaptopathies
3. Paul Miller (Aricescu/Ashcroft): Structural studies of GABA-A and glycine receptors
4. Melissa Brereton (Ashcroft/Cox): KATP channelopathies
5. Prafulla Aryal (Tucker/Sansom): Structural biology of KIR/KIRBAC potassium channel. (*Started August 2012)*

***OXION Graduate Students***

1. Ms Gauri Ang
2. Mr Julian Bartram
3. Alexei Bygrave
4. Mr Goudarz Karimi
5. Ms Caroline Lahmann
6. Ms Antonia Langfelder
7. Ms Hege Larsen
8. Ms Aletheia Lee
9. Mr Conor McClenaghan
10. Ms Olivia Shipton
11. Mr Lukasz Stasiak
12. Ms Elisa Vergari

# *Research Projects*

**Siân Alexander** *Supervisor: Stephen Tucker*

‘Comparing the interactions of Kir5.1 homologues in Xenopus tropicalis and Anguilla japonica with XT4.1: localisation of a 43-reidue domain critical for functional heteromerisation’

*Supervisor: Angela Vincent*

‘Investigating the potential involvement of the nicotinic acetylcholine receptor alpha-7 subunit in Rasmussen’s Encephalitis’

Main DPhil Project *Supervisors: Angela Vincent/Stephen*

*Tucker/Ole Paulsen*

‘Modulation of voltage-gated potassium channels: a pathophysiological mechanism of potassium channel antibodies in limbic encephalitis?’

**Angela Cohen** *Supervisor: David Sattelle*

‘Cloning of candidate C. elegans 5-HT3R subunits’

*Supervisor: Mark Sansom*

‘Modelling of candidate 5-HT3 receptor subunits in C. elegans.’

**Adrian Hon** *Supervisor: Louise Upton*

‘Organisation of on/off cells in the mouse lateral geniculate nucleus’

*Supervisor: Angela Vincent*

‘Animal models of autoimmune limbic encephalitis.’

**Tommas Ellender**  *Supervisor: Mathias Dreger*

‘Searching for binding partners of the ion channels TRPV3 and Kir5.1’

*Supervisor: Dimitri Kullmann*

‘Role of NR2B-containing NMDA receptors in silent synapses’

Main DPhil Project *Supervisors: Ole Paulsen/*

*Joszef Csicsvari*

‘The cellular mechanisms underlying sharp wave and ripple oscillations in the

hippocampus and their role in cooperative long-term potentiation’

**Stephan Kaizik** *Supervisor: David Sattelle*

‘Investigation of store operated calcium entry in Drosophila S2 cells by knocking

down TRP channels with dsRNA’

*Supervisor: Angela Vincent*

‘How do antibodies get through the blood brain barrier?’

Main DPhil Project: *Supervisors: Frances Ashcroft/Cox*

‘Analysis of mouse models of insulin secretion disorders’

**Michael Kohl** *Supervisors: Mark Sansom/ Fran Ashcroft*

‘Structural simulations of the K-ATP channel’

*Supervisor: Dimitri Kullmann*

‘Plasticity in feed-forward interneurons in the hippocampus’

Main DPhil project: *Supervisors: Ole Paulsen/*

*Mark Sansom*

‘The origin of cortical network bistability’

**Brittany Zadek**

**M**ain DPhil project *Supervisors: Frances Ashcroft/*

‘Inward rectifier K+ channel structure and’ *Catherine* *Venien-Bryan*

function’

**Katarzyna Bera** *Supervisors: Fran Ashcroft/*

*Mathias Dreger*

‘Proteomic analysis of mitochondria and insulin-containing secretory vesicles in the β-cell related cell line Ins-1’

*Supervisors: David Sattelle*

‘Electrophysiological studies on α7 nicotinic receptors, a key drug target for Alzheimer’s disease’

Main DPhil project: *Supervisors: Angela Vincent/Dimitri Kullmann*

‘Autoantibodies to NMDAR in a novel type of encephalitis’

**Rebecca Clark** *Supervisor: Stephen Tucker*

‘The functional characterization of a novel microbial inwardly rectifying

potassium channel, KirBac9.1’

*Supervisor: Ole Paulsen*

‘The role of astrocytes and their associated presynaptic NMDA receptors in long term depression’

Main DPhil project: *Supervisors: Fran Ashcroft/*

*Patrik Rorsman*

‘Physiological studies of mutant KATP channels causing diabetes and neurological symptoms’

**Michael Craig** *Supervisors: Steve Buckingham/*

*David Sattelle*

‘Fyn, Nicotinic Acetylcholine Receptors (nAChRs) and protection against

β-amyloid neurotoxicity’’

*Supervisors: Mathias Dreger/*

*Ole Paulsen*

‘The role of the transient receptor potential ion channel TRPV3 in hippocampal neural circuits’

Main DPhil project: *Supervisors: Louise Upton/*

*Ole Paulsen*

‘In vitro and in vivo characterisation of GABAergic interneurons in layer 1 of the

rodent barrel cortex’

**Amy Hoon** *Supervisor: David Paterson*

‘Protocol development for isolation and culturing of neurons of the sympathetic

chain’

*Supervisors: David Bannerman/*

*Nick Rawlins*

‘A sex difference in the intelligence of NR2B overexpressing mice?’

Main DPhil project: *Supervisors: David Bannerman/*

*Ole Paulsen*

‘The role of NMDA receptors in associative and non-associative forms of hippocampal-dependent learning’

**Caroline Lahmann** *Supervisor: Fran Ashcroft*

‘Functional characterization of ATP-sensitive potassium channels’

*Supervisor: Radu Aricescu*

‘Structural characterization of the sulphonylurea receptor nucleotide-binding domains’

Main DPhil project: *Supervisors: Fran Ashcroft/Roger Cox*

‘Characterization of a mouse model of iDEND syndrome’

**Olivia Shipton** *Supervisors: Mariana Vargas- Caballero/Ole Paulsen*

’Tau protein is required for amyloid β-induced impairment of hippocampal long-term

potentiation’

*Supervisor: Nick Rawlins*

‘Cell type specific optical stimulation in the lateral amygdala’

Main DPhil project: *Supervisors: David Bannerman/Ole Paulsen/Stephen*

*Tucker*

‘Synaptic plasticity in the mouse lateral amygdala in vitro and in vivo'

**Aletheia Lee** *Supervisor: Radu Aricescu*

*‘*Investigating trans-synaptic protein interaction*’*

*Supervisor: David Bannerman*

‘Behavioural phenotyping of GluA1-deficient mice’

Main DPhil project: *Supervisors: David Bannerman/*

*Radu Aricescu*

‘Investigating the role of iGluRs in cognitive function’

**Goudarz Karimi** *Supervisor: Fran Ashcroft*

‘Functional analysis of Kir6.2 mutation, S331P, causing neonatal diabetes’

*Supervisor: Ed Mann*

‘Mechanisms of GABAergic inhibition and network synchronization by

hippocampal parvalbumin-expressing interneurons’

Main DPhil project: *Supervisors: David Paterson/Ed*

*Mann*

‘Neurocircuitry regulating cardiac excitability during exercise and disease’

**Lukasz Stasiak** *Supervisor: David Beeson*

‘Purinergic modulation of vesicular release at the neuromuscular junction’

*Supervisors:Angela*

*Vincent/Louise Upton*

‘Immunity in neurological/neurodevelopmental disorders’

Main DPhil project: *Supervisors: Frances Ashcroft/*

*Roger Cox*

‘Functional analysis of the fat mass and obesity–associated gene *FTO’*

**Gauri Ang** *Supervisor: Angela Vincent*

‘Antibodies in women who develop postpartum psychosis’

*Supervisor: David Bannerman*

‘Investigating learning and memory in GluA1 knockout mice’

Main DPhil project: *Supervisors:David Bannerman/*

*Kay Davies*

‘Circadian rhythms in potential models of psychosis’

**Julian Bartram** *Supervisors: Ed Mann/Louise Upton*

‘The role of two distinct regions in M1 for sensory-motor integration’

*Supervisor: Angela Vincent*

‘Histological and EEG studies of potential autoimmune causes of epilepsy’

Main DPhil project: *Supervisors:Ed Mann/Patrik Rorsman*

‘Synaptic plasticity during cortical Up-Down state oscillatory activity’

**Hege Larsen** *Supervisor: David Paterson*

‘Neuronal control of the heart’ *Supervisors: Fran Ashcroft/Kieran Clarke*

‘The use of magnetic resonance for the assessment of cardiac metabolism’

Main DPhil project: *Supervisors: David Paterson/EdMann*

‘Neuronal control of cardiac excitability’

**Conor McClenaghan** *Supervisor: Fran Ashcroft*

‘The double mutation E322A/D323A in Kir6.2 reduces ATP sensitivity of KATP

Channels’

*Supervisor: Stephen Tucker*

‘Investigating Modulation of Two-Pore Potassium Channels’

Main DPhil project: *Supervisors: Stephen Tucker/Fran Ashcroft*

‘Studies of potassium channel gating and pharmacology’

# *Essay topics*

**Sian Alexander**

* ‘How much can computational neuroscience tell us about the potential biological utility of spike-timing dependent plasticity?’
* ‘What is understood of the contribution of dendritic integration to neuronal signalling and network processing?’

**Angela Cohen**

* ‘All the king’s horses and all the king’s men: is there a Happy ending for patients with spinal cord injuries? A review of recent advances’
* ‘Double Trouble – Can unusual DNA structures explain genetic anticipation?

**Adrian Hon**

* ‘Spatial Navigation and Theta Oscillations’
* ‘Sound Localisation in Mammals’

**Tommas Ellender**

* ‘Silencing the brain’
* ‘The silent synapses: from mechanism to function’

**Michael Kohl**

* ‘How is information encoded by neuronal activity?’

**Katarzyna Bera**

* ‘The neuronal crusade during cerebral cortex development’
* ‘Multiple sclerosis – insights from animal models’

**Rebecca Clark**

* ‘The perirhinal cortex: unravelling the memory vs. perception debate’
* ‘Voltage gated potassium channels – assessing the evidence for the sliding helix, transporter and paddle models’

**Michael Craig**

* ‘Implantation of olfactory ensheathing cells as a strategy for the promotion of CNS regeneration’
* ‘Can animal models ever be a truly accurate paradigm for the study of human disease’

**Amy Hoon**

* ‘What do the residual functions in neglect tell us about attention?’
* ‘Is there a common cognitive impairment in schizophrenia?’

**Caroline Lahmann**

* Voltage-gated sodium channels: important targets for the treatment of pain’
* ‘Genetic models of Parkinson’s disease’

**Olivia Shipton**

* ‘How useful are in vivo compared to in vitro electrophysiological recordings for understanding the neuronal basis of fear conditioning in the amygdale?’
* ‘The molecular basis of cholinergic transmission: how a receptor can determine a role’

**Goudarz Karimi**

* ‘Exercise and neurogenesis, what are the prospects?’
* ‘Neonatal programming of differences in social behaviour through epigenetic marking of ERα’

**Aletheia Lee**

* ‘Emergence of the cortex: evolution and neurogenesis’
* ‘The genetic basis of behaviour: fishing insights from the zebrafish’

**Lukasz Stasiak**

* ‘ Long non-coding RNA in CNS development’
* ‘Examination of structure-function relationship in rapsyn and related myasthenic syndromes’

**Gauri Ang**

* ‘The role of glutamate receptor subunits in learning and memory in genetically modified mice’
* ‘Targetting NMDA receptors in the treatment of anxiety disorders’

**Julian Bartram**

* ‘The role of up-down states in memory consolidation’
* ‘Molecular mechanisms underlying the pathophysiology of anti-ionotropic glutamate receptor encephalitis’

**Hege Larsen**

* ‘Surface plasmon resonance (SPR) and its role in drug discovery’
* ‘Single vs dual/multiple action drug treatment of depression’

**Conor McClenaghan**

* ‘Molecular mechanisms underlying axonal growth
* ‘Neuromuscular junction synaptopathy’

**COMMENTS FROM OUR CURRENT GRADUATE STUDENTS:**

**Carolina Lahmann (2009)**

My time as an OXION student is quickly coming to an end. I am about to start my final year as a DPhil student under the joint supervision of Prof. Fran Ashcroft and Prof. Patrik Rorsman. For my DPhil project, I have been using a mouse-model of intermediate DEND syndrome, which is caused by a gain-of-function mutation on the ATP-sensitive potassium channel (KATP channel). This condition is characterized by diabetes diagnosed within the first six months of life in addition to various neurological symptoms, including developmental delay, muscle weakness and epilepsy in some patients.

I have been studying the effect of the most common iDEND mutation, V59M, on neurological function both at the *in vivo­* and ­cellular level. In addition, I have been trying to determine whether sulphonylureas, drugs that inhibit the KATP channel, are able to significantly ameliorate the neurological symptoms associated with iDEND syndrome. This has involved work with Dr. David Bannerman’s group as well as with Dr. Louise Upton.

Additionally, I have been working with the Rorsman group to understand the effect of the V59M mutation on the function of pancreatic alpha-cells, which are in charge of glucagon secretion. This has allowed me to learn new techniques including the *in situ* perfusion of the pancreas.

There are still plenty of experiments that I would like to do and so I am hoping this last year will be a very fruitful one.

**Publications**:

1. Moroni M, Meyer JO, **Lahmann C** & Sivilotti LG (2011) In Glycine and GABAA Channels, Different Subunits Contribute Asymmetrically to Channel Conductance via Residues in the Extracellular Domain *The Journal of Biological Chemistry* **286(15**):13414–13422.

**Olivia Shipton (2009)**

I have now completed the second year of my DPhil investigating the role of glutamate receptor channels in Alzheimer’s disease, synaptic plasticity and learning and memory.

To study the mechanisms involved in Alzheimer’s disease, which is the most common form of neurodegenerative dementia, I use an *in vitro* brain slice model of the cognitive impairments that result from synaptic dysfunction early in the disease process. These pathological changes are thought to be triggered by amyloid beta (Aβ) and tau protein, which are the molecular components of the two hallmark pathologies of Alzheimer’s disease, amyloid plaques and neurofibrillary tangles respectively. However, whether and how these molecules interact to cause synaptotoxicity is unknown. In the first year of my DPhil I showed that tau protein is required for the robust phenomenon of Aβ-induced impairment of hippocampal long-term potentiation (LTP), a widely accepted cellular model of memory. I used wild-type mice and mice with a genetic knockout of tau protein (*Tau-/-*) and recorded field potentials in an acute hippocampal slice preparation. I found that the absence of tau prevented the Aβ-induced impairment of LTP. Moreover, Aβ increased tau phosphorylation and a specific inhibitor of the tau kinase glycogen synthase kinase 3 both blocked this increased tau phosphorylation and prevented the Aβ-induced impairment of LTP in wild-type mice.

In my second year I have investigated the mechanisms underlying the Aβ-induced LTP impairment. Given the importance of NMDA receptors channels (NMDAR) for LTP, I used voltage-clamping to measure AMPA and NMDA receptor-mediated currents in CA1 pyramidal neurons. I found a reduction in the NMDA/AMPA receptor-mediated current ratio in wild-type mice following Aβ exposure, due to a specific reduction in GluN2B subunit-containing NMDARs. However, this reduction was not present in *Tau-/-* mice, potentially explaining why they do not show an Aβ-induced LTP impairment. I am currently using an optogenetic tool that enables me to access hippocampal synapses expressing different levels of GluN2B-subunit containing NMDARs (Kohl et al. 2011) to study this further and establish whether a certain type of synapse is more vulnerable to Aβ. In addition, I will explore why tau protein is critical to see the Aβ-induced synaptotoxicity.

In parallel, I am investigating the roles that different types of hippocampal synapses normally play in learning and memory. I am using optogenetics to silence the excitatory neurons of the left or right CA3 while mice perform hippocampus-dependent behaviour tasks.

I hope that these two parts of my project will complement each other to provide an insight into the role that GluN2B-subunit containing NMDARs play in synaptic plasticity and what happens when this is affected by disease.

***Publications***

1. **Shipton OA**, Leitz JR, Dworzak J, Acton CE, Tunbridge EM, Denk F, Dawson HN, Vitek MP, Wade-Martins R, **Paulsen O**, **Vargas-Caballero M** (2011) **Tau protein is required for amyloid β-induced impairment of hippocampal long-term potentiation.** J. Neurosci. **31(5):**1688-1692.
2. **Kohl MM**, **Shipton OA**, **Deacon RM**, **Rawlins JNP**, Deisseroth K, **Paulsen O** (2011) Hemisphere-specific optogenetic stimulation reveals left-right asymmetry of hippocampal plasticity. *Nat. Neurosci.* **14(11***):*1413-5*.*

**Goudarz Karimi (2010)**

The second year of my OXION DPhil was very exciting and has contributed greatly to my development as a scientist. With my main DPhil project I am able to integrate the fields of neuroscience and cardiovascular science, under the supervision of Prof. David Paterson and Dr. Ed Mann. In my project I study the central nervous system regulation of blood pressure and heart rate. It is well known that HCN ion channels and the midbrain periaqueductal grey (PAG) play essential roles in the physiology of the cardiovascular system. The goal of my research is to understand to what extent HCN ion channels are involved in the cardiovascular autonomic phenotype during hypertension.

This year I spent most of my time on examining the histological distribution and quantifying the protein levels of HCN ion channels within the PAG. Using Spontaneous Hypertensive Rats and the normotensive controls Wistar Kyoto, I studied the distribution of HCN1, HCN2, HCN3 and HCN4 channels in the PAG by doing classical Horseradish Peroxidase staining of midbrain slices of these animals. Since the development of hypertension is age dependent I also investigated whether the distribution of HCN channels is age dependent by comparing 4 week old and 16 week old animals. In addition to the histological studies, I performed Western Blots of PAG derived proteins to examine whether there are quantitative differences in HCN channel protein levels between hypertensive and normotensive animals.

My year was very fruitful in terms of gaining experience in new techniques, like immunocytochemistry and Western Blot, which I was previously unfamiliar with. Besides practical techniques I have also gained more insight in how to plan a study and in the general organization and execution of research projects. At the moment I am in the process of doing a study consisting of stereotactic injections in the PAG and I will be working with Dr. Louise Upton to record and analyze cardiovascular parameters in response to pharmacological manipulations of HCN channels within the PAG. For the rest of my DPhil, using *in vitro* and *in vivo* electrophysiology, I will be focusing on whether HCN channels mediate differences in PAG neuron excitability between hypertensive and normotensive animals. In addition I will investigate whether manipulating HCN channel expression or activity can lead to changes in cardiac phenotype.

**Aletheia Lee (2010)**

My second year as an OXION student has been directed at laying the necessary groundwork for the main DPhil research, which involves collaboration between Professor David Bannerman at Experimental Psychology and Dr. Radu Aricescu at Structural Biology. We are interested in the study of the role of specific glutamate AMPA-type receptor subtypes in the neural dynamics of particular circuits in the brain, and how this contributes to adaptive behaviour. To this end, we seek to employ different methods of manipulation that target precise AMPA receptor subunits, so as to perturb the receptors and investigate the impact on physiological and behavioural function.

For the first part of the year I spent time examining the behaviour of knockout mice, which lack the expression of the AMPA receptor GluA1 subunit throughout the brain, by observing their performance on a spatial learning task. This experiment was conducted alongside a pilot run of *in vivo* amperometric and electrical recordings to obtain readouts of tissue oxygen and local field potential responses from the animal in a behavioural context.

Another portion of time was devoted to the design and screening of a novel tool we are striving to establish, as an alternate technique for subunit-specific disruption. In contrast to the ongoing, global deletion in knockout mouse models, we would like to target synthetic antibodies against the subunit of interest to alter its function in a transient, localized manner. At present, this strategy is still work in progress, but preliminary screens have indicated some promising samples to be verified using cellular constructs.

On the whole, the second year has been rewarding in various aspects of research, particularly in the honing of skills required for independent work in each of the labs. With better understanding of principles and greater confidence when conducting procedures, I am more equipped to think critically as I proceed.

**Lukasz Stasiak (2010)**

At the beginning of the second year as an OXION student, I started my main DPhil project in a collaboration with Prof. Frances Ashcroft (Oxford) and Prof. Roger Cox (Harwell) to study diabetes and obesity and I will be working on this subject for the remaining component of my DPhil at the University of Oxford. This collaboration is a result and another great example of the unique OXION network of scientists and researchers studying ion channels and integrative physiology. For me it is an amazing opportunity to work at two exceptionally good institutions and benefit from working with the best people in their field.

In my DPhil project I decided to focus on Fat Mass and Obesity Associated (FTO) gene and protein. Genome wide association scans have shown that common variants in the human FTO gene predispose to obesity and increased fat mass. Around 16% of the population is homozygous for the first risk allele to be identified (rs9939609; A) and has a ~1.67-fold increased risk of obesity, weighing on average ~3kg more than controls. The increase in weight is entirely due to an increase in fat mass. However, the mechanism by which FTO modulates fat mass remains unclear.

The overall aim of my project is to understand how the fat mass and obesity-related (FTO) gene regulates fat mass and body weight. The specific aims are to determine how FTO functions at the molecular and cellular level and which tissues are involved in the effects of FTO on body mass index. For the last year I have been searching for proteins interacting with mFTO. I utilized an mFTO antibody to co-immunoprecipitate native mFTO and associated proteins from mouse liver cells. I have successfully precipitated FTO and potential binding partners which were further identified by tandem mass spectrometry at the OXION Mass Spectroscopy facility with the help of Dr. Holger Kramer. Also, since recent microarray studies performed at the OXION Microarray facility run by Sheena Lee have shown unique and great fold changes of mRNA levels in Cerebellum, Hupothalamus and Muscle. I currently test whether the muscle is key to the body composition changes by knocking out FTO using MCK-Cre recombinase transgenic mouse. Mice are subjected to detailed *in vivo* metabolic phenotyping at Harwell MRC and these data should reveal if food intake, energy expenditure and metabolic rate are affected and help define the physiological process underlying the BMI phenotype.

For the rest of my DPhil I will continue working on co-immunoprecipitation in order to find weak interaction partners as well as use mice overexpression, knock-out and native tissue. Moreover, I will study several more FTO mice models including brain knock-out, muscle and brain over-expression mice models.

**Gauri Ang (2011)**

The first year in the OXION programme has been an enjoyable and enriching one. The two lecture modules I have attended this academic year were under the Neuroscience programme and they have given me the opportunity to be exposed to the different types of research that is ongoing in the different sub-fields of Neuroscience. For my first lab rotation, I worked under the supervision of Professor Angela Vincent as I was interested in gaining experience in clinical research. The lab focuses on looking for auto-antibodies in patients suffering from neurological diseases. In my project, I looked for the presence of auto-antibodies in blood sera of women who had developed postpartum psychosis. The results of the study would have important clinical relevance to women with postpartum psychosis as they may respond well to immunotherapy if their condition was mediated by auto-antibodies.

I am currently in the middle of my second lab rotation project under Professor David Bannerman. I am training wild-type mice to associate an auditory cue to a visual cue. The aim of the project is to look at how the number of training sessions in this associative learning task affects memory. The next part of this mini-project will be to use genetically-modified mice in the same task, to help us understand the role of NMDA receptors in associative learning. At the moment, we are also performing a meta-analysis of previous studies to review whether the hippocampus is needed in object recognition, with the help of Professor Jonathan Flint.

For my DPhil project, I will be working under the supervision of Professor Kay Davies and Dr. David Bannerman. My project will involve using mouse models to determine the impact of defects in candidate neuropsychiatric genes on sleep/circadian biology and cognition. This will be done by exploiting mouse models in combinations with cellular, physiological and behavioural assays, and environmental stressors. I am very excited about my project, and feel that my training in my first year has been essential in equipping me with the knowledge and skills I need to get started.

**Julian Bartram (2011)**

The first three terms of the 4 year OXION DPhil programme consist of a comprehensive post-graduate training that includes lectures, seminars, demonstrations and two laboratory rotations. Many OXION students, including myself, have already obtained a Master’s degree prior to their DPhil training year, and are willing and able to make sensible choices for their own training schedule. For this reason, I was positively surprised when I learned that the OXION DPhil programme gives students a freedom in shaping their own training schedule, which I have not experienced to such an extent in my previous degrees. This is complemented by the impression that, for the first time, the purpose of the training courses and exercises is teaching and not assessing.

The two laboratory rotations in Hilary and Trinity terms are the most essential part of the training year. In my first mini-project with Dr Louise Upton and Dr Ed Mann, we investigated aspects of sensory-motor integration in the mouse. Using multielectrode linear arrays, we recorded *in vivo* from the somatosensory cortex, while stimulating motor cortex and generating whisker-evoked responses in the somatosensory cortex. I gained valuable training in *in vivo* electrophysiology and we acquired interesting results. Currently, I am working on my second project with Prof Angela Vincent, Dr Bethan Lang and Dr Louise Upton. Our aim is to establish a mouse model of autoimmune epilepsy. We are using a telemetric EEG/video system and injecting sera from patients with anti-NMDAR auto-antibodies into the mouse brain. These patients have NMDAR encephalitis and often show cognitive impairments and epileptic seizures. We hypothesise that there is a causal link between auto-antibody presence and occurrence of epileptic seizures and seek to confirm this with our EEG/video recordings. This project allowed me for the first time to work on a biomedical problem and further complemented my training in electrophysiology.

During my first year, I have been exposed to the entire spectrum of research OXION has to offer, which has now led to an informed decision regarding my choice of DPhil project.

**Hege Ekeberg Larsen (2011)**

As a first year OXION graduate student I have spent my first year doing two laboratory rotations. The first one was spent in Professor Paterson’s laboratory in the Department of Physiology, Anatomy and Genetics (DPAG), looking at the neuronal control of the heart in health and disease (hypertension being the first model of choice). Previous work in this field has produced conflicting results, in part due to technical limitations but also experimental design. We sought to overcome this by optimizing the experimental protocols and investing in an advanced optical mapping system that will allow us to, perhaps for the first time, fully understand the sensitive and reciprocal control of the heart during physiological and pathophysiological conditions. The second project was done in the lab of Professor Kieran Clarke, also in DPAG, looking at mouse models of heart failure. Ashrafian *et al.* 2012 created a cardiac specific fumarate hydratase knock out mouse and found that the elevated levels of fumarate that resulted was cardioprotective in 6 week old mice. However, as the mice grew to 3-4 months old, cardiac abnormalities were seen, suggesting a move towards heart failure. The project in the Clarke lab is designed at characterizing these abnormalities using cardiac MRI and MRS both in vivo and in vitro.

Being able to do rotations has been hugely beneficial, as it has exposed me to countless experimental techniques that I was previously unfamiliar with, and allowed me to explore different areas of science. Observing and being part of cutting edge research labs has given me valuable insight into life as an academic scientist and provided me with solid foundations on which I will base my main D.Phil project.

**Conor McClenaghan (2011)**

This first year of my OXION PhD has been an exciting and enjoyable time including a range of new experiences and training prospects. Early in the year we were given insights into a broad range of techniques including neurophysiology and anatomy, statistics, animal handling, proteomics, imaging and microarrays which gave me a basic understanding of these processes and the collaborative possibilities within OXION. Following this I undertook my first mini-project in Prof Fran Ashcroft’s lab. This project was an investigation into KATP channel structure and function using excised patch clamp electrophysiology of xenopus oocytes. I looked into the role of 2 amino acids (D322 and E323) postulated to be involved in the coupling of Kir6.2 and SUR1 subunits in the channel complex. This project allowed me to further develop electrophysiology skills in a new expression system as well as giving me a grounding in basic molecular biology techniques.

I then moved on to a second mini-project in the lab of Dr Stephen Tucker where I investigated the action of a family of naturally occurring plant-derived chemicals called akylamides on the TREK subfamily of Two-pore potassium channels. Using chimeric channels and 2 electrode voltage clamp I have been investigating the site of action of these modulators on TREK-1 and TRAAK.

I hope to continue working under both Dr Tucker and Prof Ashcroft as joint supervisors for my main project which will include a range of techniques in a multi-faceted approach to investigate potassium channel structure, function and pharmacology.

***What our past Training Fellows did next….***

**Dr. Nathan Absalom** (2004-2007) initially worked as a postdoctoral research worker with Professor Roger Cox at Harwell before returning to Australia to work in the Department of Pharmacy at the University of Sydney. He is currently studying the mechanism of action of gamma hydroxybutyrate, which is one of the most effective treatments for narcolepsy.

**Dr. Haris Alexopoulos** (2002-2005) now works on neurological disease in the Faculty of Medicine at the National and Kapodistran University of Athens.

**Dr. Timothy Craig** (2004-2007) went on to work for a couple of years with Professor Ashcroft. He now works in Professor Jeremy Henley’s group in the Department of Anatomy at the University of Bristol. His research focuses on neuronal SUMOylation in synaptic function.

**Dr. David Keays** (2005-2008) now runs his own research group at the Institute of Molecular Pathology in Vienna. He works on the molecular basis of magnetodetection in pigeons and also on new genes that cause neurodevelopmental disease.

**Dr. Jackie Kidd** (2003-2006) After a postdoctoral position at the University of Dundee, Jackie left scientific research to train as a pharmacist. She is now working as a pharmacist in Scotland.

**Dr. Heidi de-Wet** (2003-2006) is currently a postdoctoral research worker, working with Professor Ashcroft. She is engaged in functional and structural studies of the sulphonylurea receptor (the regulatory subunit of the KATP channel).

***What our past OXION graduate students did next…***

**Dr. Sian Alexander (2003)** completed her medical studies and then worked as a doctor on the Academic Foundation Programme at Addenbrooke’s Hospital in Cambridge. She has recently been awarded an Academic Clinical Fellowship in Neurology at Cambridge University Hospitals NHS Foundation Trust.

**Dr. Kasia Bera** **(2006)** is now studying medicine at the University of Oxford.

**Dr. Rebecca Clark (2006)** left academic research and became a science teacher. She is enjoying communicating her passion for science to young people, and frequently draws on experiences from her DPhil to inspire the next generation of scientists.

**Dr. Michael Craig (2006)**  took up a postdoctoral position at the National Institute of Child Health and Human Development in Bethesda, USA. He works in Dr. Chris Bain’s lab within the Laboratory of Cellular and Synaptic Neurophysiology on the role of interneurons in the development, generation and propagation of neuronal oscillations in the hippocampus.

**Dr. Tommas Ellender** **(2004)** took up a postdoctoral position in the Department of Pharmacology at Oxford. He is working with Dr. Colin Akerman on mouse cortical development.

**Dr. Amy Hoon (née Schou) (2006)**  is now a research study monitor for clinical trials at Queen Mary College London.

**Dr. Stephan Kaizik** **(2004)** is now working for Merck Millipore as a research support scientist.

**Dr. Michael Kohl** **(2005)** worked with Professor Ole Paulsen at the Department of Physiology, Development and Neuroscience. He also was awarded a Junior Research Fellowship at St. John’s College at the University of Cambridge. He has now moved to the University of California at Berkeley, to work with Drs. Hillel Adesnik and Ehud Isacoff on developing and using novel holographic two-photon stimulation modalities to study sensory processing in awake rodents.

**Dr. Brittany Zadek** **(2005)** went on to study medicine in the States. She will be specializing in radiology and has been accepted onto the radiology residency programme at Cornell University. She has retained an interest in research, working on breast imaging and is currently finishing up a retrospective study, looking at the benefits of screening mammography in women in their forties.

**\*Date in brackets is the start date of the 4 year DPhil-Vacation Studentships**

2005/6 Stefanie Schultrich

Project undertaken with Mathias Dreger: studied TRPV3

(member of the TRPV family of transient receptor potential

ion channels). **Follow up**: **Now a Helmholtz student studying for a PhD at the Max-Delbrueck Centre in Berlin in group of Michael Bader. Her project deals with the classical nuclear protein import pathway via importing alpha and importing beta. Due to finish end of 2011**.

.

2006 Laxmi Parajuli

Project undertaken with Stephen Tucker: prokaryotic K+

channel studies. **Follow up: Since studying for PhD in Japan, current**

**position unknown.**

2006 Helen Hager

Project undertaken with Angela Vincent: studied antibodies to

glycine receptor in acquired startle disease and stiff person’s

syndrome. **Follow up**: **completed Masters degree and is**

**now working for the consultancy firm McKinsey in Munich.**

2007 Mathilde Lafond

Project undertaken with Fran Ashcroft: studied techniques in

KATP channels. **Follow up: following a period working for the firm Biomérieux, now working for Professor Ashcroft as Research Technician.**

2007 Huza Zhang

Project undertaken with David Beeson: investigation of a region

of the muscle AChR that is involved in rapsyn-associated clustering.

**Follow up**: **currently a medical student but also doing a PhD as part of the MBPhD course at Imperial College, funded by the MRC.**

2007 Christopher Barkus

Project undertaken with Robert Deacon: studied cognitive

characterization of the NMDA/AMPA receptor KO mice to gain better

understanding of how to run object recognition tests in this species.

**Follow up**: **went on to do a PhD in Dept of Psychology, Oxford and now has a postdoctoral position in the Department of Psychiatry, Oxford.**

2007 Elizabeth Durkin

Project undertaken with Fran Ashcroft: worked on ATP-sensitive

potassium channels. **Follow up**: **is in final year as 4 Year PhD**

**student at UCL. Intends to stay as postdoc for one year at UCL to write up publications.**

2007 Ulrike Weirauch

Project undertaken with Mathias Dreger: Effect of oleic acid on protein

expression in β-cell mitochondria. **Follow-up**: **current position unknown.**

2008 Alexander Torrey Deng

Project undertaken with David Keays: study of molecular mechanism

underlying magnetoreception, using the honey bee Apis mellifera as

a model organism. **Follow up:** **is finishing clinical studies.**

2008 Anna Kaleva

Project undertaken with David Beeson: on epilepsy: ‘Screening for autoantibodies to GABAA in selected cohorts of patients with idiopathic epilepsies’

**Follow up:** **after continuing with her clinical studies, Anna has now**

**qualified as a foundation doctor and is about to start at Guy’s and**

**St. Thomas’s.**

2009 Chris Kneale

Project undertaken with David Sattelle: ‘Characterisation of proteins interacting with the human α7 nicotinic acetylcholine

receptor’. **Follow up: unknown.**

2009 Dhaneesha Senaratne

Project undertaken with David Beeson: ‘Molecular mechanisms

underlying the response of patients with *DOK7* mutations to

treatments with β2 adrenergic receptor agonists’. **Follow up:**

**continuing with clinical studies in Cambridge.**

2010 Katie Duffell

Project undertaken with Patrick Rorsman: ‘On regulation of glucagon

secretion’. **Follow up: has chosen to specialize in Chemistry and is staying on at University of Cambridge to do a Four Year Masters.**

2010 Hamish Jackson

Project undertaken with Kieran Clarke: ‘Use of cardiac magnetic resonance spectroscopy in the compilation of a detailed spectroscopic picture of cardiomyopathies drawing on all available data’. **Follow up: is studying graduate medicine at Imperial College and hopes eventually to do clinical science.**

2010 Qunxiang Ong

Project undertaken with Radu Aricescu: ‘Structure-based design of novel AMPAreceptor modulators’. **Follow up: currently undertaking a one-year research work at a chemical synthesis lab in Singapore and considering a PhD in either biochemical or chemical studies.**

2010 Karina Vanadzina

Project undertaken with Nick Rawlins: ‘Point mutation in NMDA receptor subunit NR2A impairs acquisition of spatial working memory but does not influence non-spatial long-term memory’. **Follow up: unknown.**

2011 Nathan Denton

Project undertaken with Fran Ashcroft: ‘Sulphonylurea sensitivity of mutant KATP channels’. **Follow up: Finished undergraduate degree at Brasenose, Oxford and is starting an MSc with Professor Fredrik Karpe on adipocyte development/epigenetics in type II diabetes at the Nuffield Dept. of Clinical Medicine. He hopes eventually to fulfill a physician-scientist role in the future.**

2011 Paul Giraud

Project undertaken with David Beeson: ‘New constructs to test for LRP4

antibodies in sera from ‘seronegative’ myasthenia gravis patients’. **Follow-up:**

**3rd year undergraduate at Paris-Ouest Medical School, France.**

2011 Suzanne Harrogate

Project undertaken with Gero Miesenböck: ‘Compensation of changes in

neuronal capacitance’. **Follow up**: **continuing with medical studies.**

2011 Maria Marin-Vilar

Project undertaken with Jonathan Flint: ‘Neurogenetics: genetic determinants of adult neurogenesis and brain anatomy in the mouse’ **Follow up:** **unknown.**

2012 Christine Böch (funded by University of Ulm)

Project undertaken with Ed Mann: ‘Functional characterization of ionotrop

glutamate receptors on parvalbumin-expressing interneurons in the medial

entorhinal cortex’

2012 Christian Hoffmann

Project undertaken with Phil Biggin: ‘Investigating the molecular gating

Mechanism of ionotropic glutamate receptors using computational approaches’

2012 Jun Siong Low

Project undertaken with Mike Hanna/Roope Mannikko: ‘Functional

characterization of *CLCN1* mutations causing Myotonia congenita’

2012 Katia Mattis

Project undertaken with Fran Ashcroft: ‘The heterologous expression system

of *Xenopus laevis* oocytes for the characterization of KATP channel mutants’

2012 Nipuna Senaratne

Project undertaken with David Beeson: ‘Investigation of how mutations in

Proteins involved in N-linked glycosylation affect the muscle acetylcholine

Transmission’

**OXION PUBLICATIONS (2011 – 2012)**

1. Allen K, **Rawlins JNP**, **Bannerman DM**, **Csicsvari J** (2012) Hippocampal place cells can encode multiple trial-dependent features through rate modulation. *Journal of Neuroscience* (in press).
2. Altun M, **Kramer HB**, Willems LI, McDermott JL, Leach CA, Goldenberg SJ, Kumar KG, Konietzny r, Fischer R, Kogan E, Mackeen M, McGouran J, Khorenenkova SV, Parsons JL, Dianov GL, Nicholson B, Kessler BM (2011) Activity-based chemical proteomics accelerates inhibitor development for deubiquitylating enzymes. *Chem Biol* **18(11)**:1401.
3. Andres-Enguix I, Shang L, Stansfeld PJ, Morahan JM, **Sansom MS**, Lafrenière RG, Roy B, Griffiths LP, Rouleau GA, Ebers GC, Cader ZM, **Tucker SJ** (2012) Functional analysis of missense variants in the TRESK (KCNK 18) K channel. *Science Rep* **2**:237.
4. **Ashcroft FM**, **Rorsman P** (2012) Diabetes Mellitus and the ß Cell: The Last Ten Years. *Cell* **148(6)**:1160-71.
5. Altun M, **Kramer HB**, Willems LI, McDermott JL, Leach CA, Goldenberg SJ, Kumar KG, Konietzyn R, Fischer R, Kogan E, Mackeen MM, McGouran J, Khoronenkova SV, Parsons JL, Dianov GL, Nicholson B, Kessler BM (2011) Activity-based chemical proteomics accelerates inhibitor development for deubiquitylating enzymes. *Chem Biol* **18(11):**1401-12.
6. **Bannerman DM**, Bus T, Taylor AM, Sanderson DJ, Schwarz I, Jensen V, Hvalby Ø, **Rawlins JNP**, Sprengerl R, Seeburg PH (2012) Dissecting spatial knowledge from spatial choice by hippocampal NMDA receptor deletion. *Nature Neuroscience* **15(8)**:1153-9.
7. **Barkus C**, Dawson LA, Sharp T, **Bannerman DM** (2012) GluN1 hypomorph mice exhibit wide-ranging behavioral alterations. *Genes Brain Behav* **11(3)**:342-51.
8. Bavro VN, De Zorzi R, Schmidt MR, Muniz JR, Zubcevic L, **Sansom MS**, **Vénien-Bryan C**, **Tucker SJ** (2012) Structure of a KirBac potassium channel with an open bundle crossing indicates a mechanism of channel gating. *Nat Struct Mol Biol* **19(2)**:158-63.
9. **Becker EB**, Zuliani L, Pettingill R, **Lang B**, Waters P, Dulneva A, **Sobott F**, Wardle M, Graus F, Bataller L, Robertson NP, **Vincent A** (2012) Contactin-associated protein-2 antibodies in non-paraneoplastic cerebellar ataxia. *J Neurol Neurosurg Psychiatry* **83(4)**:437-40.
10. **Belaya K**, Finlayson S, Slater CR, Cossins J, Liu WW, Maxwell S, McGowan SJ, Maslau S, Twigg SR, Walls TJ, Pascual SI, Palace J, **Beeson D** (2012) [Mutations in DPAGT1 Cause a Limb-Girdle Congenital Myasthenic Syndrome with Tubular Aggregates.](http://www.ncbi.nlm.nih.gov/pubmed/22742743) *Am J Hum Genet* **91(1)**:193-201.
11. Botcherby EJ, Smith CW, **Kohl MM**, Débarre D, Booth MJ, Juskaitis R, **Paulsen O**, Wilson T (2012) Aberration-free three-dimensional multiphoton imaging of neuronal activity at kHz rates. *Proc Natl Acad Sci USA*  **109(8)**:2919-24.
12. **Clark R**, Männiko R, Stuckey DJ, **Iberl M**, **Clarke K**, **Ashcroft FM**  (2012) Mice expressing a human KATP channel mutation have altered channel ATP sensitivity but no cardiac abnormalities. *Diabetologia* **55(4)**:1195-204.
13. Cossins J, Liu WW, **Belaya K**, Maxwell S, Oldridge M, Lester T, Robb S, **Beeson D** (2012) The spectrum of mutations that underlie the neuromuscular junction synaptopathy in DOK7 congenital myasthenic syndrome. *Hum Mol Genet* **21(17)**:3765-75.
14. **Deacon R** (2012) Assessing burrowing, nest construction and hoarding in mice. *J Vis Exp* Jan 5; (59). Pii:2607. Doi: 10.3791/2607.
15. **Deacon RMJ**, Dulu TD, Patel NB (2012) Naked mole-rats: behavioural phenotyping and comparison with C57BL/6 mice. *Behavioural Brain Research* **231**:193-200.

**De Wet H**, Shimomura K, Aittoniemi J, Ahmad N, Lafond M, **Sansom M, Ashcroft FM** (2012) A universally conserved residue in the SUR1 subunit of the KATP channel is essential for translating nucleotide binding at SUR1 into channel opening. *J Physiol* July 16 [Epub ahead of print].

1. Edwards A, **Treiber CD**, Breuss M, Pidsley R, Huang GJ, Cleak J, Oliver PL, **Flint J**, **Keays DA** (2011) Cytoarchitectural disruption of the superior colliculus and an enlarged acoustic startle response in the Tuba1 a mutant mouse. *Neuroscience* **195**:191-200.
2. **Galvanovskis J**, Braun M, **Rorsman P** (2012) Exocytosis from pancreatic ß-cells: mathematical modeling of the exit of low-molecular-weight granule content. *Interface Focus* **1(1)**:143-52.
3. Goodson M, Rust MB, Witke W, **Bannerman DM**, Mott R, Ponting CP, **Flint J** (2012) Cofilin-1: a modulator of anxiety in mice. *PLoS Genetics* (in press).
4. Guergueltcheva V, Müller JS, Dusl M, Senderek J, Oldfors A, Lindbergh C, Maxwell S, Colomer J, Mallebrera CJ, Nascimento A, Vilchez JJ, Muelas N, Kirschner J, Nafissi S, Kariminejad A, Nilipour Y, Bozorgmehr B, Najmabadi H, Rodolico C, Sieb JP, Schlotter B, Schoser B, Hermann R, Voit T, Steinlein OK, Najafi A, Urtizberea A, Soler DM, Muntoni F, **Hanna MG**, Chaouch A, Straub V, Bushby K, Palace J, **Beeson D**, Abicht A, Lochmüller H (2011) Congenital myasthenic syndrome with tubular aggregates caused by GFPT1 mutations. *J Neurol* Oct 6 [Epub ahead of print].
5. **Hanna MG**, **Kullmann DM** (2012) Chanelopathies. In: *Neurogenetics*  (Ed. NW Wood) Cambridge University Press.
6. **Harrison PJ**, Pritchett D, Stumpenhorst K, Betts JF, Nissen W, Schweimer J, Lane T, Burnet PW, Lamsa KP, Sharp T, **Bannerman DM**, Tunbridge EM (2012) Genetic mouse models relevant to schizophrenia: taking stock and looking forward. *Neuropharmacology* **62(3)**:1164-7.
7. Hoppa MB, Jones E, Karanauskaite J, Ramracheya R, Braun M, Collins SC, Zhang Q, Clark A, Eliasson L, Genoud C, Macdonald PE, Monteith AG, Barg S, **Galvanovskis J**, **Rorsman P** (2012) Multivesicular exocytosis in rat pancreatic beta cells. *Diabetologia* **55(4)**:1001-12.
8. Irani SR, **Alexander S**, **Vincent A** (2012) Case 34-2011: a man with memory loss and partial seizures. *N Engl J Med* **366(8)**:768-9; author reply 769.
9. Irani SR, Pettingill P, Kleopa KA, Schiza N, Waters P, Mazia C, Zuliani L, Watanabe O, **Lang B**, Buckley C, **Vincent A** (2012) Morvan syndrome: clinical and serological observations in 29 cases. *Ann Neurol* **72(2)**:241-55.
10. **Kohl MM**, **Shipton OA**, **Deacon RM**, **Rawlins JN**, Deisseroth K, **Paulsen O** (2011) Corrigendum: Hemisphere-specific optogenetic stimulation reveals left-right asymmetry of hippocampal plasticity. *Nat Neurosci* **14(12)**:1617.
11. Karunaratne A, Davis GR, Hiller J, Esapa CT, Terrill NJ, **Brown SD**, **Cox RD**, Thakker RV, Gupta HS (2012) Hypophosphatemic rickets is associated with disruption of mineral orientation at the nanoscale in the flat scapular bones of rachitic mice with development. *Bone* **51(3)**:553-62.
12. Karunaratne A, Esapa C, Hiller J, Boyde A, Head R, Bassett J, Terrill N, Williams G, Brown M, Croucher P, **Brown S**, **Cox R**, Barber A, Thakker R, Gupta H (2011)

Significant deterioration in nanomechanical quality occurs through incomplete extrafibrillar mineralization in rachitic bone: evidence from in-situ synchrotron X-ray scattering and backscattered electron imaging. *J Bone Miner* **27(4)**:876-90*.*

1. **Kramer HB**, Nicholson B, Kessler BM, Altun M (2012) Detection of ubiquitin-proteasome enzymatic activities in cells: application of activity-based probes to inhibitor development. *Biochim Biophys Acta* May 19 [Epub ahead of print].
2. Laatikainen LM, Sharp T, **Bannerman DM**, **Harrison PJ**, Tunbridge EM (2012) Modulation of hippocampal dopamine metabolism and hippocampal-dependent cognitive function by catechol-O-methyltransferase. *J Psychopharmacology* (in press).
3. Li J, Bravo DS, **Upton AL**, Gilmour G, Tricklebank MD, Fillenz M, Martin C, Lowry JP, **Bannerman DM**, McHugh SB (2011) Close temporal coupling of neuronal activity and tissue oxygen responses in rodent whisker barrel cortex. *Eur J Neuroscience* **34**:1983-1996.
4. Li D, Lee CW, Buckler K, **Parekh A**, Herring N, **Paterson DJ** (2012) Abnormal intracellular calcium homeostasis in sympathetic neurons from young prehypertensive rats. *Hypertension* **59(3)**:642-9.
5. Lizuka T, Leite MI, **Lang B**, Waters P, Urano Y, Miyakawa S, Hamada J, Sakai F, Mochizuki H, **Vincent A** (2012) Glycine receptor antibodies are detected in progressive encephalomyelitis with rigidity and myoclonus (PERM) but not in saccadic oscillations. *J Neurol* **259(8)**:1566-73.
6. McGouran JF, **Kramer HB**, Mackeen MM, di Gleria K, Altun M, Kessler BM (2012) Fluorescence-based active site probes for profiling deubiwuitinating enzymes. *Org Biomol Chem* **10(17)**:3379.
7. McTaggart JS, **Lee S**, **Iberl M**, Church C, **Cox RD**, **Ashcroft FM** (2011) FTO is expressed in neurons throughout the brain and its expression is unaltered by fasting. *PLoS One* **6(11)**:e27968.
8. Murray C, Sanderson DJ, **Barkus C**, **Deacon RM**, **Rawlins JN**, **Bannerman DM**, Cunningham C (2012) Systemic inflammation induces acute working memory deficits in the premed brain: relevance for delirium. *Neurobiol Aging* **33(3)**:603-616.e3.
9. Oeschger FM, Wang W-H, Lee S, García-Moreno F, Goffinet AM, Arbonés ML, Rakic S, and Molnár Z(2011) Gene Expression Analysis of the Embryonic Subplate *Cereb. Cortex* **22(6)**:1343-59.
10. Oliver PL, Finelli MJ, Edwards B, Bitoun E, Butts DL, **Becker EB**, Cheeseman MT, Davies B, **Davies KE** (2011) *PLoS Genet* **7(10)**:e1002338.
11. Oliver PL, Sobczyk MV, Maywood ES, Edwards B, **Lee S**, Livieratos A, Oster H, Butler R, Godinho SIH, Wulff K, Peirson SN, Fisher SP, Chesham JE, Smith JW, Hastings MH, **Davies KE** and Foster RG (2012) Disrupted circadian rhythms in a mouse model of schizophrenia. *Current Biology* **22(4)**:314-9.
12. Pritchett D, Wulff K, Oliver PL, **Bannerman DM**, **Davies KE**, **Harrison PJ**, Peirson SN, Foster RG (2012) Evaluating the links between schizophrenia and sleep and circadian rhythm disruption. *J Neural Transm* May 10 [Epub ahead of print]
13. Reeve JE, **Khol MM**, Rodriguez-Moreno A, **Paulsen O** and Anderson HL (2012) Addendum: caged intracellular NMDA receptor blockers for the study of subcellular ion channel function. *Commun Integrat Biol* **5**:3,1-3.
14. Sanderson DJ, **Rawlins JN**, **Deacon RM**, Cunningham C, **Barkus C**, **Bannerman DM** (2011) Hippocampal lesions can enhance discrimination learning despite normal sensitivity to interference from incidental information.  *Hippocampus* Dec 7. Doi: 10.1002/hipo.20995 [Epub ahead of print]
15. Schneider T, Skitt Z, Liu Y, **Deacon RM**, **Flint J**, Karmiloff-Smith A, **Rawlins NJ**, Tassabehji M (2012) Anxious, hypoactive phenotype combined with motor deficits in Gt2ird1 null mouse model relevant to Williams syndrome. *Behav Brain Res* **233(2)**:458-73.
16. Stuckey DJ, Carr CA, Camelliti P, Tyler DJ, **Davies KE**, **Clarke K** (2012) In vivo MRI characterization of progressive cardiac dysfunction in the mdx mouse model of muscular dystrophy. *PLoS One* **7(1)**:e28569.
17. Tan SC, Carr CA, Yeoh KK, Schofield CJ, **Davies KE**, **Clarke K** (2012) Identification of valid housekeeping genes for quantitative RT-PCR analysis of cardiosphere-derived cells preconditioned under hypoxia or with prolyl-4-hydroxylase inhibitors. *Mol Biol Rep* **39(4)**:4857-67.
18. Ternette N, Wright C, **Kramer HB**, Altun M, Kessler BM (2011) Label-free quantitative proteomics reveals regulation of interferon-induced protein with tetratricopeptide repeats 3 (IFIT3) and 5’-3’-exoribonuclease 2 (XRN2) during respiratory syncytial virus infection. *Virol J* **8(1)**:442.
19. Webster R, Maxwell S, Spearman H, Tai K, Beckstein O, **Sansom M**, **Beeson D** (2012) A novel congenital myasthenic syndrome due to decreased acetylcholine receptor ion-channel conductance. *Brain* **135(Pt 4**):1070-80.

**OXION SEMINARS: AUTUMN 2011 -2012**

**17th October**

**Professor Michisuke Yuzaki**, Keio University, Tokyo

‘Not an orphan anymore – a glutamate receptor GluD2 finds two partners, Cbln and D-Ser’

**16th November**

**Professor Bill Catterall**, University of Washington, USA

‘Sodium channels at atomic resolution: structure, function and disease’

**2nd December**

**Dr. Denis Burdakov**, Dept of Pharmacology, University of Cambridge

‘Brain circuits orchestrating sleep and energy balance’

**23rd March**

**Dr. Andreas Schaefer**, Max Planck Institute, University of Heidelberg

‘Inhibition and odour discrimination in mice’

**25th April**

**Professor Michael Hausser**, University College London

‘Dendritic computation’