Previously, we have shown that 1 μM capsaicin and 50mM KCL elicit comparable rises in $[Ca^{2+}]_i$ in TRPV1 positive neurones and that in both cases this leads to a desensitisation of TRPA1. We now further report that TRPA1 responsiveness to cinnamaldehyde (200 μM) remains intact following activation of TRPV1 by capsaicin (1 μM) in reduced $[Ca^{2+}]_i$ conditions. In nominally calcium-free extracellular solution, the response to cinnamaldehyde was reduced (by 30 %), but not abolished, by preceding application of capsaicin.

We conclude that calcium is a crucial second messenger involved in TRPV1-mediated inhibition of TRPA1. We propose that pharmacological targeting of second messengers could be a potential avenue for blunting TRPA1-mediated nociception.

We wish to thank the Health Research Board, Ireland for funding this work.

*Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.*

---

PC38

**The contribution of melanopsin-driven photoreception to light-evoked activity within the mouse olivary pretectal nucleus**

A.E. Allen, T.M. Brown, J. Gigg and R.J. Lucas

*Faculty of Life Sciences, University of Manchester, Manchester, UK*

In addition to rod and cone photoreceptors, the retina contains a subset of retinal ganglion cells that are rendered intrinsically photosensitive due to the expression of the photopigment melanopsin. These cells, termed mRGCs (melanopsin-expressing retinal ganglion cells), innervate several central targets, notably those associated with non-image forming responses to light. One such area is the olivary pretectal nucleus (OPN), a relay-station for the pupillary light reflex.

We aimed to characterise the contribution of melanopsin-photoreception to light-evoked activity within the OPN *in vivo*. To achieve this, changes in spike-firing rate were assessed via multi-electrode recordings in the pretectum of mice anaesthetised with urethane (1.5g/kg). Responses to increasing intensities of blue (460nm) and red (640nm) light were measured, for both short (2s) and long (30s) duration stimuli. To isolate rod/cone vs. mRGC-driven activity, we compared responses in wildtype mice with those of *rodless+coneless* mice, and melanopsin knockout (*Opn4*$^{-/-}$) mice.

Light responses within the wildtype OPN were characterised by strong transient increases in firing rate at lights ON and OFF, and sustained elevations in firing rate for the duration of stimulus presentation. Responses showed irradiance-dependent increases in amplitude and response speed at intensities ranging from 9.8 to 15.8 $\log_{10}$ photons/cm$^2$/sec. In *rodless+coneless* mice, transient increases in firing rate were entirely absent. Instead, neurons typically showed sluggish increases in firing rate that gradually increased to a peak around 10s after stimulus onset, and persisted for around 20s after offset. The *rodless+coneless* OPN also was much less sensitive, with a threshold for detectable responses around 13.8 $\log_{10}$ photons/cm$^2$/sec. Conversely, *Opn4*$^{-/-}$ mice retained fast ON and OFF transient responses as seen in wildtypes, but showed relatively little sustained responses throughout light stimuli. The sensitivity of *Opn4*$^{-/-}$ mice was similar to that of wildtype mice, with irradiance-dependent responses occurring in the range of 9.8 to 15.8 $\log_{10}$ photons/cm$^2$/sec.

These data reveal that while rod and cone photoreceptors drive the fast transient responses to light onset and offset, melanopsin contributes to sustained elements of light-evoked activity within the OPN, especially at higher light intensities. They further suggest that melanopsin makes a unique contribution to the sensory capabilities of the mammalian visual system.

This work was supported by the BBSRC and the University of Manchester Alumni Fund.

*Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.*

---

PC39

**Thalamic extrasynaptic GABAA receptors are required for typical absence seizures**

G. Di Giovanni, G. Orban, S.J. Fyson, V. Crunelli and D.W. Cope

*School of Bioscience, Cardiff University, Cardiff, UK*

Aberrant GABAergic inhibition in thalamo-cortical networks has been identified as a potential mechanism for spike-and-wave discharge (SWD) generation. Thalamocortical (TC) neurons in the ventrobasal (VB) thalamus receive both 'phasic' and 'tonic' GABAA receptor mediated inhibition, generated by synaptic and extrasynaptic delta-subunit containing receptors, respectively [Cope et al., 2005; J. Neurosci. 25: 11553]. We have shown a selective increase of tonic GABAA receptor-mediated inhibition in pharmacological as well as in polygenic and monogenic rat and mouse models of absence epilepsy due to an impairment of GABA transporter-1 (GAT-1) activity [Society for Neuroscience 2007, 142.7, 142.8, 142.9]. Therefore, we suggested that extrasynaptic GABAA receptor gain-of-function in VB TC neurons is a necessary requirement for the appearance of SWDs.

To directly test this hypothesis, we have now pharmacologically and genetically targeted extrasynaptic GABAA receptors in VB and monitored EEG and behavioural correlates of absence epilepsy in normal Wistar rats, Genetic Absence Epilepsy Rats from Strasbourg (GAERS) and GABAA receptor delta-subunit knockout mice. All experiments were conducted in accordance with the UK Animal Scientific Procedure Act. Reverse microdialysis of the selective extrasynaptic GABAA agonist THIP (70 and 100 μM, both n=5) and the selective GAT-1 inhibitor NO-711 (200 μM, n=5) into the VB induced SWDs and behavioural correlates of absence seizures in normal Wistar rats. THIP- and NO-711-induced SWDs were suppressed by systemic administration of the anti-absence drug ethosuximide (ETX, 100 mg/kg, n=5). In addition, we "knocked-down" extrasynaptic GABAA receptors in TC cells of GAERS by directly infusing a δ subunit specific antisense oligodeoxynucleotide (ODN, 1 and 2 nMol/μl, n=5 and 6, respectively) into the VB. The antisense ODN produced a marked reduction (~70% at 2 nMol) of the total time spent

in seizures and the number of SWDs, whilst infusion of a missense ODN (1-2 nMol/μl, n=5) was ineffective. Lastly, systemic administration of 50 mg/kg of gamma-butyrolactone induced absence seizures in wild type mice, which were significantly reduced in the delta-subunit knockout mice (84% decrease in the total time spent in seizures and 53% reduction in number of SWDs).
Our data demonstrate that extrasynaptic GABAA receptor gain-of-function in VB TC neurons is a necessary and sufficient requirement for the appearance of a pure absence epilepsy phenotype, and that GAT-1 critically controls SWD genesis.

This work was supported by the Wellcome Trust. D.W. Cope is an Epilepsy Research UK Fellow.

*Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.*

---

PC40

**Effects of TTA-P2, a novel potent and selective T-type calcium channel blocker on thalamic cell excitability**

N. Leresche[2], F.M. Dreyfus[2], A. Tscherter[2], A.C. Errington[1], V. Crunelli[1] and R.C. Lambert[2]

*[1]School of Bioscience, Cardiff University, Cardiff, UK and [2]UMR7102 UPMC-CNRS, Paris 75005, France*

Although it is well established that low-voltage activated T-type Ca2+ channels play a key role in many neurophysiological functions and pathological states, the lack of selective and potent antagonists has so far hampered a detailed analysis of the full impact that these channels might have on single cell and neuronal network excitability as well as on calcium homeostasis.
Using thalamic slices (prepared from fully anaesthetized Wistar rats or transgenic mice, in accordance with the UK Scietific Procedure Act), we now show that the novel piperidine-based molecule TTA-P2 exerts a specific, potent (IC50: 22nM) and reversible inhibition of T-type Ca2+ currents (IT) in both thalamocortical (TC) and reticular thalamic nucleus (NRT) neurons without any action on HVA Ca2+ currents. Under current clamp conditions, 1μM TTA-P2, a concentration that fully blocks IT (96±1%, n=7), has no effect on tonic firing and action potential characteristics (threshold, half-width, amplitude, afterhyperpolarization), but abolishes the low threshold Ca2+ potential (LTCP)-dependent high frequency burst firing of thalamic neurons. In addition, when TC and NRT neurons are held at -60mV, application of 1μM TTA-P2 produces a tonic hyperpolarization of 3.1±0.5mV (n=11) and 5±2.2mV (n=6), respectively. Such hyperpolarization is not observed when TC neurons are held at -70mV or in TC neurons from Cav3.1 KO mice that are recorded at -60mV (n=6). These data demonstrate that the TTA-P2 induced hyperpolarization is due to the block of the window component of IT and that this current contributes to the resting membrane potential of thalamic neurons. In addition, we could show that application of 1μM TTA-P2 blocks membrane potential bistability of TC neurons in slices that are perfused with the h-channel blocker ZD 7288 (100μM) (n=4). Thus, the use of TTA-P2 has allowed to consolidate and enlarge our current understanding of the contribution of IT to single TC neuron excitability, and to provide the first direct demonstration that the window component of IT underlies the intrinsically generated slow (<1Hz) sleep oscillation of thalamic neurons.

*Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.*

---

PC41

**Integrative *in vivo* approaches to studying anaesthetic mechanisms**

D.R. Carr

*Department of Biophysics, Imperial College, London, UK*

Although modern general anaesthesia has been used for over 100 years in medical and veterinary practice, the mechanisms of how such a wide variety of pharmacological agents induce reversible loss of consciousness are not completely known (Franks, 2008). Given that loss of consciousness can only be measured in live animals, in vivo experiments therefore provide the most optimal setting for the elucidation of such unknowns.
Electroencephalographic (EEG) methods have been widely used in both research and clinical settings to monitor anaesthetic depth for surgical and non-surgical procedures in humans and animals. The technique relies on recording network activity of synchronously oscillating neurons in the mammalian brain. The utility of the technique is that it can provide a real-time measure of brain activity on millisecond timescales.
We recorded the EEG activity of C57BL/6 mice chronically implanted (under ketamine (80 mg/kg) and xylazine (15 mg/kg) mix anaesthesia) with gold-plated electrodes in response to increasing doses of the volatile anaesthetic halothane. We recapitulated experiments that show a prominent theta oscillation (centred at 5 Hz) in the EEG and found this effect to be sensitive to muscarinic antagonism with atropine (50 mg/kg i.p. or 50 μg bolus in the medial septum) (Pang et al., 2009).
There is evidence that this theta oscillation results from the concerted action of cholinergic and GABAergic neurotransmitter systems in the medial septum that form projections to the hippocampus (Yoder & Pang, 2005). So for our further investigations we injected various pharmacological agents directly into the medial septum thus allowing a more specific neurophysiological analysis.

Franks, NP (2008) Nat Rev Neurosci 9(5): 370-86.

Pang, DS et al. (2009) Proc Natl Acad Sci, 106, 17546-51.

Yoder, RM & Pang, KC (2005) Hippocampus 15(3): 381-92.

Funded by BBSRC and CIMPP.

*Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.*