C01

The role of fascin and drebrin in neuroblast migration

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Adult neurogenesis persists in the subventricular zone (SVZ), a crucial brain area that can respond to insults by producing neuronal progenitor cells able to migrate to sites affected by injury and neurodegeneration. Understanding the property of these cells is essential to fully elucidate their potential in neuroregenerative strategies. In the past few years several factors regulating neuronal migration have been identified, however still very little is known about the exact molecular mechanisms controlling neural progenitor motility. In this study we investigate the function of two proteins, fascin and drebrin, which are highly expressed in the postnatal brain by SVZ-derived migrating neuroblasts. Fascin is an actin-bundling protein that contributes to the architecture and function of cell protrusions in cell adhesion, interaction and motility. Drebrin is expressed by migrating neuroblasts; interestingly, cessation of migration in these cells coincides with the disappearance of the protein. We show here that RNAi-mediated depletion of fascin significantly impairs neuroblast migration in vitro. Phosphorylation of fascin on Ser39 is important for its actin-bundling activity and is regulated by protein kinase C (PKC). We demonstrate that Ser39 phosphorylation plays a fundamental role in neuroblast migration. Indeed, the migration of neuroblasts is strongly impaired when these cells are nucleofected either with a phosphomimetic (S39D) or a non-phosphorylatable (S39A) fascin variant, pointing to a crucial role for PKC in the regulation of neuroblast motility. Importantly, these cells still retain the ability to extend a protrusion in the direction of migration. Similarly, drebrin knockdown also substantially impairs neural progenitor migration in vitro and leads to a highly branched, unpolarised neuroblast morphology. We are currently validating our findings in vivo by performing postnatal electroporation of shRNA-expressing plasmids and time-lapse imaging of cultured brain slices from CD1 lactating P3 mouse pups. Our preliminary observations strongly suggest that drebrin depletion may severely affect neuroblast morphology also in vivo. In summary, both fascin and drebrin appear to play a fundamental function by controlling neural progenitor motility and morphology. Our future studies will aim at elucidating the signalling pathways involving both proteins during neural progenitor migration.

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

C02

From stem cells to inborn behaviour: clonal origin and genetic specification of a motor control centre in Drosophila

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Inborn behaviours rely on neural circuits that are genetically determined during development. However, it is currently unknown how such circuits are formed. Here we show that stem cell-derived clonal units in the Drosophila brain generate neural circuit elements underlying inborn walking behaviour. Tracing the progeny of embryonic neuroblasts ppd5/8, we found that these neural stem cells give rise to large-field ring neurons of all subtypes, R1-R4, that constitute an entire set of circuit elements intrinsic to the ellipsoid body of the central complex in the adult brain. During development, initial formation of these neural lineages depends on Engrailed function and their maintenance requires FGF8 signalling. Prospero activity limits the number of neuronal progeny and Dscam is involved in ring neuron synaptogenesis, both of which are necessary for correct formation of the ellipsoid body neuropil. A GABAergic subset of these clonally-related cells, comprising R2 and R4 neurons, are required for specific aspects of inborn walking behaviour. Our findings suggest that an inherited genetic program specifies stem cell-derived ontogenetic clones that form functional units of neural circuitry underlying inborn animal behaviour.

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C03

Blockade of ADAM10/17 impairs sphere growth and promotes neuronal differentiation from brain tumour stem cells

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High grade primary glial tumours are characterised by rapid proliferation and diffuse infiltration, contributing to a dismal prognosis. The recent concept of the tumour stem cell, able to proliferate indefinitely as well as generate progeny comprising the tumour bulk, leads us to the investigation of the niche within which these cells exist. Niche components include the ADAMs, cell surface proteins which contribute to cell-cell adhesion and have enzyme activity central to signalling, growth and invasion by tumours. We have derived sphere cultures derived from

high grade tumour stem cells, and maintained these through multiple passages to amplify the stem cell component. Using RT-qPCR, we have established the expression profile of 10 ADAM molecules across 38 primary tumours and in sphere cultures developed from some of these. We have shown that compared to control adult and foetal neural tissue, ADAMs 10, 12, and 17 are over-expressed in low grade tumours, more so in high grade tumours, and to a greater degree still in the stem-cell-enriched sphere cultures. We have used immunohistochemistry to localise them within tissues and spheres. These tumours are known to be heterogeneous, and the pattern of ADAM overexpression differs from one sample to another, with a degree of overlap in function between ADAM family members. We have investigated the inhibition of ADAM function using pharmacological inhibitors, demonstrating an impact on sphere formation which correlates with the degree of overexpression in a given stem cell line. We have confirmed the specificity of the pharmacological inhibition with blocking antibodies. As well as impairing growth, we have observed marked effects on differentiation and resulting cell morphology, with potential implications for the pattern of invasion.ADAMs represent a promising therapeutic target in a group of tumours notoriously refractory to existing treatments.

Funding from the Royal Society and the Southampton University Hospital Trust

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C04

Semaphorins and Plexins signal via alpha2-chimaerin in the normal development of the oculomotor projection and Duane's Retraction Syndrome

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Eye movements in vertebrates depend on the function of six extraocular muscles, which are innervated by three cranial nerves. In humans, incorrect development of this pattern of innervation leads to eye movements disorders such as strabismus, which affects 1% of the population, and may result in amblyopia or partial blindness (1). However the etiology of these syndromes, and the guidance cues and molecular mechanisms involved in the precise axon projections of the ocular motor system are largely unknown. We have recently identified mutations in the α2-chimaerin (α2-chn) [an isoform of the chimaerin-1 (CHN1) gene] which have been shown to be responsible for a congenital form of strabismus, Duane's Retraction Syndrome (DRS) (2). Expression of α2-chn forms harbouring identified human mutations in the oculomotor nerves of chick embryos suggest a role in axon pathfinding (2), placing α2-chn downstream of yet unknown guidance cues.
Supported by previous evidence, we hypothesize herein that repellent guidance cues such as Semaphorins might orchestrate topographic axon branching of the

oculomotor nerve to its particular extraocular muscle targets. We sought to investigate and dissect the molecular pathways that guide the OMN to its target muscles, as well as their implications in DRS. We used the chick embryo as model, and the main techniques involved in this work include: (i) in situ hybridization from embryos at stages 28 to 31 (5 to 6 days old), (ii) in ovo electroporation of the ventral midbrain at stage 11-12 (2 days of development), and then allowing them to grow to stage 30 (6 days of development), and finally (iii) OMN primary cultures from 5-day-old embryos. We demonstrate that Sema3A/C and Plexin receptors expression patterns are consistent with their possible role in oculomotor axon guidance. We further show that in vivo knock-down of Sema3A/C-Plexin signalling or of α2-chimaerin function by means of shRNA electroporations into the oculomotor neurons of embryos lead to characteristic axon guidance phenotypes, reminiscent of DRS. In primary cultures of OMN neurons, Sema3A induces growth cone collapse, which is blocked or potentiated by the expression of either α2-CHN shRNA or gain-of-function constructs, respectively. Finally, in vivo expression of a combination of plexin loss-of-function and α2-chn gain-of-function constructs rescue the Plexin loss of function phenotype to generate an essentially normal axon projection pattern. This work provides both in vitro and in vivo evidence that α2-chn is critical in the pathfinding of oculomotor neurons as a downstream component of the Sema3A/C-Plexin signalling pathway.

Engle, E.C. Human genetic disorders of axon guidance. Cold Spring Harbor perspectives in biology. 2010 Mar; 2(3).

Miyake, N., et al. Human CHN1 mutations hyperactivate alpha2-chimaerin and cause Duane's retraction syndrome. Science. 2008 Aug 8;321(5890):839-43.

Wellcome Trust & MRC funding.

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C05

NO signalling potentiates neuronal $Ca_V2.1$ and $Ca_V2.2$ in the mouse Medial Nucleus of the Trapezoid Body (MNTB) by different mechanisms

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Nitric Oxide (NO) is a diffusible second messenger that modulates ion channels and contributes to synaptic plasticity. In light of its known modulation of high voltage-activated (HVA) potassium channels in the postsynaptic principal neurons of the medial nucleus of the trapezoid body (MNTB) (Steinert et al., 2008), we have investigated its modulation of native voltage-gated calcium channels (Ca_V). Using barium as the charge carrier, whole-cell patch recordings were made from *in vitro* brain slices from P13-15 CBA mice. Slices were incubated with nNOS inhibitor 7-nitroindazole (7-NI, 10μM) and treated with pharmacological channel blockers to isolate

identified Ca^{2+} currents. Unpaired observations in the presence and absence of the NO donor sodium nitroprusside (SNP, 100μM) were made to elucidate NO-dependent modulation of the expressed Ca_V subtypes. Results showed that there was a differential effect of NO on the channel subtypes: the Ca_V1 (L-type) conductance was unaffected by NO, whereas $Ca_V2.1$ and $Ca_V2.2$ (P/Q and N-type) conductances were potentiated. P-type current was increased from 0.53±0.04nA (n=9, mean±SEM) to 0.82±0.04nA (n=6, mean±SEM) and N-type from 0.24±0.02nA (n=4, mean±SEM) to 0.42±0.07nA (n=9, mean±SEM). Our results also showed that $Ca_V2.1$ channels were potentiated via the canonical NO-cGMP pathway, as SNP induced potentiation was abolished in the presence of the guanylyl cyclase blocker 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 1μM). In contrast, NO-dependent potentiation of N-type channels was not removed by inhibition of guanylyl cyclase activity, and this suggests that this channel subtype is modulated via a different pathway, i.e. by nitrosylation. This shows for the first time that NO-dependent modulation of a host of differentially expressed postsynaptic Ca_V channels can occur and our findings provide insights in to the mechanisms of cGMP-dependent and -independent regulation of voltage-gated Ca^{2+} entry by NO.

Steinert JR, Kopp-Scheinpflug C, Baker C, Challiss RA, Mistry R, Haustein MD, Griffin SJ, Tong H, Graham BP & Forsythe ID. (2008). Nitric oxide is a volume transmitter regulating postsynaptic excitability at a glutamatergic synapse. Neuron 60, 642-656.

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

C06

Aged-related changes to hippocampal intrinsic excitability and Na^+ channel gating

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Neurological function declines during the normal ageing process resulting in cognitive impairment in the elderly. The cellular mechanisms underlying age-related deficits in cognitive function are not well understood, although changes to hippocampal intrinsic excitability may play a role (Matthews *et al.* 2009). To explore the effects of ageing on neuronal excitability, we made whole-cell recordings from CA1 hippocampal neurones in slices prepared from 1-2 month (young: Y), 7-9 month (middle aged: M) and 22-24 (aged: A) month old C57/BL6 mice. Current clamp recordings revealed no difference in the resting membrane potential (Y: -77.6 ± 0.5 mV, n=20; M: -76.0 ± 0.4 mV, n=33; A: -76.8 ± 1.0 mV, n=17; P=0.25 1-way ANOVA), input resistance (Y: 131 ± 8 MΩ; M: 123 ± 6 MΩ; A: 123 ± 10 MΩ; n as above, P = 0.74) or sag (Y: 27 ± 1 %; M: 26 ± 2 %; A: 29 ± 1 %; n as above, P = 0.29). In response to a 500 ms, 300 pA current injection all of the recorded neurones fired a series of action potentials (APs). A waveform analysis of the first AP induced by this stimu-

lus revealed that whilst the peak and maximum rate of rise were not affected by age there was a significant difference in AP threshold. Thus, at 7-9 months of age the AP threshold was significantly more hyperpolarised that at the other age points (Table 1). A change in AP threshold is suggestive of a change in the gating properties of the Na^+ channels which underlie the AP. To explore this possibility, nucleated macropatches were excised from the soma of CA1 pyramidal neurones and inward, TTX-sensitive Na^+ currents (I_{Na}) were recorded under voltage clamp, using a Cs-based internal solution. No difference was observed in the absolute conductance or the steady state inactivation properties of I_{Na}, however, the $V_{½}$ of activation was shifted in a hyperpolarised direction at 7-9 months of age, mirroring the shift in AP threshold (Table 1). Thus, CA1 pyramidal neurones exhibit an age-related form of intrinsic plasticity in which hyperpolarising shifts in the activation properties of I_{Na} and AP threshold arise during adulthood (from young to middle aged). This may relate to previously reported forms of activity-dependent changes to AP threshold (Xu *et al.*, 2005). The greater excitability observed in mid-life was reversed as ageing continued, such that old animals possess reduced intrinsic excitability, which likely contributes to age-related cognitive impairments.

		1-2 month			7-9 month			22-24 month			P (ANOVA)
		Mean	S.E.M.	n	Mean	S.E.M.	n	Mean	S.E.M.	n	
AP properties	AP Peak (mV)	38.7	1.1	20	39.3	0.8	33	37.1	1.1	17	0.23
	Max dV/dt (V/s)	592.4	17.0	20	575.0	15.6	33	588.5	17.3	17	0.82
	AP thres (mV)	-58.6	0.6	20	**-61.9**	0.8	33	-58.7	0.7	17	**0.0002**
I_{Na} properties	G_{max} (nS/pF)	6.6	1.0	16	5.9	0.7	15	4.9	0.6	18	0.3
	$V_{½}$ act (mV)	-30.7	0.5	16	**-33.2**	0.7	15	-30.9	0.9	18	**0.036**
	$V_{½}$ inact (mV)	-72.4	0.9	16	-74.7	0.7	15	-74.9	0.9	18	0.1

Matthews EA *et al.* (2009) *J Neurosci* **29**: 4750-5

Xu J *et al.* (2005) *J Neurosci* **25**: 1750-1760

This work was supported by Pfizer Global R&D.

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C07

Adaptation reduces sensitivity to save energy without information loss in the fly visual system

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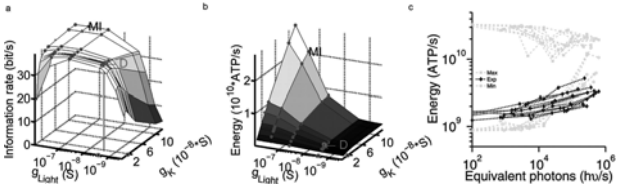
Signal processing in neurons is constrained by internal noise and energy consumption. Neural systems often invest energy in amplifying signals to protect them against internal noise. There is no general expression for the trade-off between energy and information, because it is strongly dependent on the properties of signal, noise and underlying mechanism. We present the first analysis of this energy-information trade-off in a specific system, the R1-6 photoreceptors of the blowfly,

Calliphora vicina. These photoreceptors adapt to light level by adjusting their transduction gain (number of light-gated channels opened per photon) and potassium conductance. By combining experimental measures of photoreceptor impedances, transfer functions and signal quality in a basic membrane model we discovered that photoreceptors do not adapt to maximise their sensitivity. Instead, they use a lower sensitivity at which they capture ~90% of the information from naturalistic stimuli. This strategy is efficient because it avoids the wasteful amplification of noisy inputs and reduces energy consumption. The membrane model is a typical RC-circuit in which two opposing conductances (light-gated and potassium) determine the response amplitude and time constant. The light-gated conductance is driven by a Poisson process whose rate varies according to mean luminance and the contrast power spectrum of moving natural scenes viewed through blowfly optics (van Hateren, 1992)1.

Figure 1. (a-b.) Theoretical information and energy surface as a function of light-gated (gLight) and potassium conductance (gK). MI corresponds to the maximum information and D to the theoretical value at the conductances measured in an experiment with white noise presentation. One can see that the experimental information is located at the edge, where still 99% of the maximum information is available while the energy is drastically reduced. The blue dots on the surfaces correspond to combination of conductances that achieve 99% of the maximum information. The blue dots on the energy surface span a variety of different energy consumptions, while all leading to roughly same information rate. (c.) The theoretical maximum and minimum energy consumption that achieves at least 99% of the maximum information are shown in grey lines at increasing intensities. The black lines are the experimental energy consumption.



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BBSRC and Royal Society

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C08

A model for the electrical responses of human rod photoreceptors to the onset of steady backgrounds

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The activation phase of a photoreceptor's electrical response to light flashes has been modelled using biochemical analysis of phototransduction (Lamb & Pugh, 1992). This "LP model" has been successfully applied to a range of recordings from single cells (Lamb & Pugh, 1992) and living subjects (Cideciyan & Jacobson 1996). Here we investigated whether a simple modification of the equation would predict responses to the onset of steady backgrounds and tested this against electroretinogram recordings. Scotopic electroretinograms were recorded from three normal human subjects, using conductive fibre electrodes, in response to both light flashes (white or blue, ranging in intensity from 0.13 to 740 scotopic cd m^{-2} s) and steady backgrounds (white, ranging in intensity from 33 to 1900 scotopic cd m^{-2}). The rod-system response was isolated by subtracting responses recorded in the presence of a blue rod-saturating background (38 scotopic cd m^{-2}) from responses to identical stimuli recorded in the dark. Subjects' pupils were dilated pharmacologically using 1% tropicamide. The standard "flash response" form of the LP model was applied to flash responses, as in previous studies, to obtain values for the three parameters: maximal response amplitude a_{max}, amplification constant A, and delay time t_d. A new "step response" equation was derived using Eqn 6.6 of Lamb & Pugh (1992), as

$1 - \exp\{ -I A (t - t_d)^3 / 6 \}$

where I is the step intensity. This equation provided a good fit to rod responses to steps of light using the same parameter-values as for the flash responses. The results support the applicability of the LP model to ERG responses, and we have derived a new expression to fit responses to onset of backgrounds. Diseases affecting photoreceptors have been shown to alter the parameters obtainable using the LP model, and this work will support future attempts to analyse the effects of such abnormalities more quantitatively in living subjects.

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Thanks to David Cunningham, Sara Haenzi, Rebecca Rewbury, Oshini Shivakumar and Mathew Vithayathil for assistance with experiments.

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C09

TDP-43 causes defects in motor axon projections

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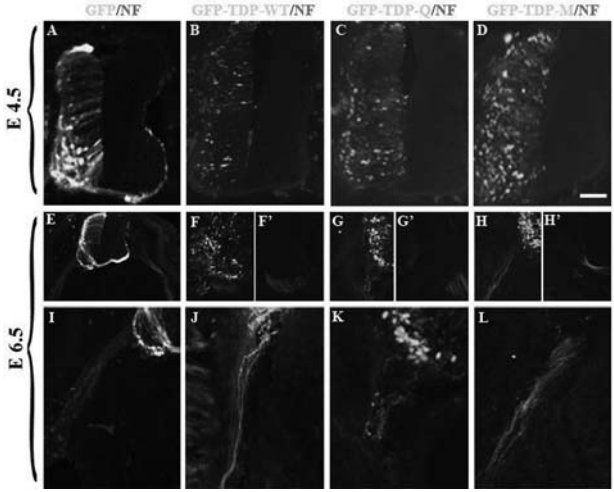
Progress in the MND field has been fuelled by the discoveries that mutations in RNA-processing proteins are causative in ALS. We and others have identified mutations in the genes encoding TDP43 in patients with ALS and FTLD-U. TDP43 is a RNA-processing protein, with DNA and RNA binding properties and multiple roles in neuronal survival and function. In this study, we have analysed the role of TDP43 in an embryonic in vivo system by testing the effects of over-expression of wild-type and mutant TDP43 on motor neuron viability, axon outgrowth, branching and cytoskeletal integrity. By understanding the relative impact of TDP43 mutant and wild-type forms on cytoskeletal integrity, we demonstrate a link between TDP43 proteinopathy and axon cytoskeleton. We have used the chick embryo as an acute model for TDP43 function.

We electroporated GFP labelled TDP43 wild-type and mutant constructs in the chick embryo spinal cord (GFP-TDP-43WT, GFP-TDP-43Q331K and GFP-TDP-43M337V). The GFP fluorescence was widespread in the spinal cord demonstrating that almost 90-95% of NPCs were transfected. TUNEL staining showed a statistically significant increase in the number of apoptotic nuclei in embryos electroporated with TDP-43WT (p-value 0.0337), TDP-43Q331K (p-value 0.0026) and TDP-43M337V (p-value 0.0045) as compared to those expressing pEGFPC1.

We next tested if there was any mis-localisation of TDP-43 proteins in our in vivo chick spinal cord model system. At E3.5 wild-type TDP-43, myc/HA and GFP tagged, protein was predominantly nuclear. However, the mutant forms of TDP 43 (particularly those tagged with GFP) showed more frequent cytoplasmic localisation aggregates while retaining the nuclear expression. We also demonstrate that at E6.5 all forms were detected cytoplasmically and in axons, suggesting an enhanced cytoplasmic localisation over time. We have demonstrated that at E6.5, TDP43 wild type and mutant forms lead to down-regulation of neurofilament associated protein. Careful confocal analysis demonstrated that the mis-localisation of TDP43 protein to the axon cytoplasm is accompanied by premature truncation of the axon projections and premature de-fasciculation of the axon bundles. In order to achieve statistical values for the effect of TDP43 on the length of axon projections, we carried out transfection of TDP43 in primary chick spinal motor neurons. Our in vitro data suggests a decrease in the total process length which is actually due the decrease in length of the longest process.

The chick model offers a relatively quick and cost-effective means of validating genetic discoveries and will provide valuable insights into disease mechanisms.



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I would like to thank the Motor Neuron Disease Association for funding.

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C10

Dynamic association of tau with neuronal membranes is regulated by phosphorylation and the tyrosine kinase fyn

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Tau is an abundant cytosolic protein which regulates cytoskeletal stability by interacting with microtubules in a phosphorylation-dependent manner. In Alzheimer's disease, tau becomes hyperphosphorylated and forms paired helical filament (PHF) aggregates and intracellular tangles. Although tau is largely cytosolic, approximately 20% of tau is associated with the plasma membrane, at least in rat pheochromocytoma cells. Membrane-association of tau may be regulated by its phosphorylation state. We therefore determined whether tau is associated with the neuronal membrane, and investigated the mechanisms regulating this association. We performed cellular fractionation by differential centrifugation in cultured rat cortical neurons and analyzed tau in cytosolic and membrane fractions using western blotting. Data were analyzed by two-way ANOVA followed by the post-hoc Dun-

nett's multiple comparison test. Significance level was set to $P < 0.05$. Fractionation of cortical neurons revealed that approximately 10% of neuronal tau is membrane-associated. This tau was only weakly recognised by PHF-1, a phospho-dependent tau antibody, but was strongly detected by tau-1, an antibody against tau dephosphorylated at Ser199/202. Isolation of plasma membrane-associated proteins by biotinylation demonstrated the association of tau with the plasma membrane in neurons. Pre-treatment of neurons with tau kinase inhibitors revealed that inhibition of either glycogen synthase kinase-3 (GSK-3) by lithium, or casein kinase 1 (CK1) by IC261, strongly dephosphorylated tau and increased the amount of tau in the membrane relative to the cytosolic fraction. The effect of IC261 on membrane-association of tau was reversible and four hours after treatment, tau localization in treated cells was similar to that in control cells. The effect of IC261 was prevented by pre-treatment with okadaic acid to inhibit phosphatase activity. Mutation of serine/threonine residues in the N-terminal half of tau to glutamate, to mimic a permanent state of phosphorylation, prevented tau localisation to membranes in transfected CHO cells. In contrast, intracellular localisation of tau was unaffected by mutation of serine/threonine residues to glutamate in the C-terminal half of the protein. Inhibiting CK1 in neurons lacking the tyrosine kinase fyn induced tau dephosphorylation but did not increase its membrane association. Furthermore, inhibition of CK1 increased binding of neuronal tau to the fyn-SH3 domain. We conclude that dephosphorylation of tau, especially at CK1 and GSK-3 sites, promotes trafficking of tau from the cytosolic compartment to the neuronal membrane and requires the presence of fyn.

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C11

Polyglutamine Atrophin provokes autophagic neurodegeneration by repressing fat

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Polyglutamine pathologies are neurodegenerative diseases that manifest both general polyglutamine toxicity and mutant protein specific effects. Dentatorubropallidoluysian Atrophy (DRPLA) is one of these disorders caused by mutations in the Atrophin-1 protein. We have generated several models for DRPLA in Drosophila and have analysed the mechanisms of cellular and organism toxicity. Our genetic and ultrastructural analysis of neurodegeneration suggests may play a role in cellular degeneration when polyQ proteins are overexpressed in neuronal and glial cells. Clearance of autophagic organelles is blocked at the lysosomal level after correct fusion between autophagosomes and lysosomes. This leads to accumulation of autofluorecent pigments and of proteinaceous residues usually degraded by the

autophagy-lysosome system. In these circumstances, further pharmacological and genetic induction of autophagy does not rescue neurodegeneration by polyQ Atrophins, in contrast to many other neurodegenerative conditions. Large alterations in transcription also accompany neurodegeneration in polyglutamine diseases. We report that the fat tumour suppressor gene mediates neurodegeneration induced by the polyglutamine protein Atrophin. We have monitored early transcriptional alterations in a Drosophila model of Dentatorubral-pallidoluysian Atrophy and found that polyglutamine Atrophins downregulate fat. Fat protects from neurodegeneration and Atrophin toxicity through the Hippo kinase cascade. The Fat/Hippo signalling does not provoke neurodegeneration by stimulating overgrowth, rather it alters the autophagic flux in photoreceptor neurons, thereby affecting cell homeostasis. Our data thus provide a crucial insight into the specific mechanism of a polyglutamine disease and reveal an unexpected neuroprotective role of the Fat/Hippo pathway.

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F.Napoletano, S.Occhi, P.Calamita, V.Volpi, E.Blanc, B.Charroux, J.Royet and M.Fanto (2011). Polyglutamine Atrophin provokes neurodegeneration by repressing fat. The EMBO Journal doi:10.1038/emboj.2011.1 (Advance Online Publication).

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C12

Reprogramming of Tau isoforms by RNA trans-splicing: a potential gene therapy approach for tauopathies

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Tau (microtubule-associated protein Tau, MAPT) is a protein predominantly expressed in neuronal axons, which promotes microtubule polymerisation and stabilisation. The exon 10 (E10) of the MAPT gene encodes the second of four imperfect microtubule-binding repeats in the Tau protein. Exclusion or inclusion of E10 by alternative splicing gives rise to Tau isoforms with three (3R) or four (4R) microtubule-binding repeats, respectively. In the normal adult human brain the ratio 4R/3R is 1. Tauopathies are a group of neurodegenerative diseases characterized by the intracellular accumulation of Tau. Recent evidence suggests that some of these conditions are associated with abnormal alternative splicing at the level of E10, which affects the normal ratio between 4R/3R Tau isoforms.
The aim of this project is to modulate the ratio between 4R/3R Tau isoforms by reprogramming the inclusion of Tau E10. The strategy used is the spliceosome-mediated RNA trans-splicing (SMaRT). SMaRT creates a chimaeric mRNA through

a trans-splicing reaction between the 5' splice site of an endogenous target pre-mRNA and the 3' splice site of an exogenously delivered RNA molecule. To test this strategy in a prototypic model of Tau pathology, we used a mouse transgenic model that carries the human Tau gene in the mouse Tau knock out background (hTau mice).
Trans-splicing molecules (PTMs) were delivered by lentiviral vectors into differentiated primary neurons of hTau mice. Different lentiviral concentrations (multiplicity of infection, moi) were used to achieve the best transduction efficiency. At moi=10 (i.e. 10 viral particles per neuron) PTMs produced efficient trans-splicing with the Tau RNA. The trans-spliced product was detected at the RNA level by RT-PCR and at the protein level by immunoprecipitation and western blot. The efficiency of Tau isoform conversion was on average 35 %. These results provide promising perspectives of a plausible gene therapy approach to correct aberrant RNA splicing in tauopathies.

This work is supported by Wellcome Trust and the Medical Research Council.

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C13

PACAP induces neuroprotection through the induction of activity-dependent signalling through the CREB coactivator CRTC1

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Pituitary adenylate cyclase-activating peptide (PACAP) is a known neuroprotective peptide widely assumed to exert its effect directly through the cAMP-protein kinase A (PKA_ pathway. Contrary to this, we find that PACAP-induced PKA signalling in cortical neurons is a poor direct activator of neuroprotective pathways, and actually exerts its effects indirectly through inducing synchronized action potential firing. To investigate the actions of PACAP, dissociated cortical cultures were prepared from E17 Sprague-Dawley rat embryos. Using Fluo-3 calcium imaging, PACAP was shown to induce a PKA-dependent increase in action potential (AP) firing and associated calcium transients (n = 6). These were shown to be essential for the anti-apoptotic actions of PACAP, as tetrodotoxin (TTX) blocked PACAP mediated protection against trophic deprivation (n = 3) and staurosporine (n = 4). Furthermore PACAP stimulation induced neuroprotective ERK 1/2 phosphorylation in a TTX sensitive manner (n = 4), and ERK pathway inhibition by PD98059 blocked PACAP induced protection. Through direct activation by PKA, PACAP caused CREB phosphorylation at serine-133, in the presence of TTX. Moreover, using a CRE-Firefly Luciferase-reporter showed that PACAP could induce some CRE-dependent gene expression in the absence of APs (n = 7), though this was significantly reduced. In the presence of TTX PACAP is unable to induce the activation and nuclear import

of CREB co-activator CRTC1, which is dependent on firing activity-dependent calcineurin signalling (n = 6). Over-expression of CRTC1 is sufficient to rescue PACAP-induced CRE-mediated gene expression in the face of activity-blockade (n = 4). A Dominant-Negative form of CRTC1, and the inhibitory CREB isoform ICER both blocked long-term PACAP neuroprotection (n = 4). Thus, the enhancement of AP firing may play a significant role in the neuroprotective actions of PACAP and other adenylate cyclase-coupled ligands.

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C14

In vivo imaging of long-term reactions of glia and neurones after lesions of the spinal cord in triple-transgenic mice with fluorescent proteins

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Neuronal regeneration after spinal cord injury may be impeded by a glial scar, but its character and temporal development is still vague. Triple-transgenic mice with labeled projection neurons and glial cells with different fluorescent proteins (axons/YFP, microglia [MG]/GFP, astrocytes/CFP) were imaged with two photon microscopy before and after laser-induced spinal cord lesions (20-40 μm diameter at L4). The lesions were regularly re-inspected for up to one year. The initial laminectomy at spinal cord L4 and re-opening for re-inspections were performed under full volatile anaesthesia (1:1 N2O:O2 and isoflurane initially 5%, then 2%). The days after operation, the mice received buprenorphin (0.1 mg/kg per day i.p.). Within minutes after the lesion, MG sent their processes towards the lesion. During the next days, nearby MG cells migrated toward the lesion accumulating and staying there for about a week, then slowly diminishing during the next five months. Monocytes (also GFP-labeled) crossing the blood-brain barrier during the acute reaction and invading the lesion site were not found. Astrocytes were slowly activated and started to extend processes to the lesion after two days. An astroglial reaction surrounding the lesion site was fully developed after a week and subsequently decreased within five months. Within hours after the lesion, dissected axons formed bulbous debris which was partly engulfed by MG processes directed to the lesion. Other axonal bulbs remained at their place caudal to the lesion for weeks. Occasionally, axonal sprouting was detected about three months after injury. Initially, neuronal sprouts were not always straightly directed towards the lesion but later they could cross the site of injury, when the glial accumulation had almost vanished. Mechanically induced injuries evoked similar spatiotemporal cellular reactions but did not allow such a systematic investigation since they were less well

defined. The combination of multi-cellular labeling with long-term imaging allowed an exploration of the time course of complex cellular responses after spinal cord injury. Detailed knowledge of the temporal behavior of the main cell types involved in the reaction to spinal cord injury may provide valuable hints for a therapeutical approach to improve axonal survival and regeneration.

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C15

Endogenous stem cells in the postnatal mouse cortex following Traumatic Brain Injury

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Interest to promote regeneration of the injured nervous system has recently turned towards the use of endogenous stem cells. Following a robust stab injury, mature astrocytes in the adult mouse cortex proliferate and contribute to gliogenesis in the mouse cortex in vivo, but also neurogenesis in sphere cultures in vitro (Buffo 2008). This raises two questions critical for efforts to harness endogenous stem cells for repair. Firstly, what cues are involved in driving these precursor cells out of quiescence following injury? Secondly, what are the environmental signals that promote gliogenesis rather than neurogenesis in the injured cortex? We investigate whether a source of potential endogenous stem cells that resides in the cortex is activated following Traumatic Brain Injury (TBI), and correlate signaling pathways with this activity.

Using a validated organotypic stretch injury model (Morrison 2006), cultured postnatal mouse cortico-hippocampal slices were stretched to induce an injury equivalent to a severe TBI. In uninjured cortex, proliferating cells cultured in vitro (number of neurospheres per well) are virtually absent in older mice (equivalent postnatal day 15 compared to postnatal day 8). However, following a severe stretch injury, this neurosphere forming capacity is increased in the hippocampus, and significantly, is also restored in the cortex (3.0 +/- 0.3 spheres per well in control and 6.0 +/- 0.5 in the injured hippocampus after 11 days in vitro and 0.5 +/- 0.1 in control and 7.1 +/- 1.3 in injured cortex; $p<0.01$ 2-way Anova with Bonferroni post-hoc). Moreover, these proliferating cells derived from the injured cortex are multipotent. To determine the endogenous source of these cells following TBI, we examined cells from the injured cortex that express the protein Glial Fibrillary Acidic Protein (GFAP). Using cortical cells that express GFP in GFAP cells (Nolte 2001), flow cytometry was used to separate injured cells into GFP positive and negative populations. Positive cells accounted for the majority of proliferating neurospheres formed (3.4 +/- 1.0 in the positive population compared to 0.7 +/- 0.2; $p<0.001$ student t-test). The Sonic Hedgehog (Shh) signaling pathway is activated following a corti-

cal freeze injury (Amankulor 2009) and contributes towards cellular proliferation. In our TBI model, there is a transient upregulation of the Shh receptor, Patched 1, 6 hours post-injury, which may contribute to the proliferative effect observed after TBI.

Our results indicate that a source of endogenous stem cells residing in the post-natal mouse cortex proliferate in vitro only following Traumatic Brain Injury (TBI). Moreover, these proliferating cells are multipotent and are derived mostly from GFAP expressing cells. This raises the possibility of utilising an endogenous source of stem cells for repair following TBI.

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Funded by WNCT, WMR and RCSEng.

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C16

Endocannabinoids regulate the migration of subventricular zone-derived neuroblasts in the post-natal brain

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In the adult brain, neural stem cells proliferate within the subventricular zone before differentiating into migratory neuroblasts that travel along the rostral migratory stream (RMS) to populate the olfactory bulb with new neurons. Because neuroblasts have been shown to migrate to areas of brain injury, understanding the cues regulating this migration could be important for brain repair. Recent studies have highlighted an important role for endocannabinoid (eCB) signaling in the proliferation of the stem cell population, however it remained to be determined if this pathway also played a role in cell migration. We now show that mouse migratory neuroblasts express cannabinoid receptors, diacylglycerol lipase alpha (DAGLα), the enzyme which synthesizes the endocannabinoid 2-arachidonoylglycerol (2-AG), and monoacylglycerol lipase (MAGL), the enzyme responsible for its degradation. Using a scratch wound assay for a neural stem cell line and RMS explant cultures, we show that inhibition of DAGL activity or CB1/CB2 receptors substantially decreases migration. In contrast, direct activation of cannabinoid receptors or preventing the breakdown of 2-AG increases migration. Detailed analysis of primary neuroblast migration by time-lapse imaging reveals that nucleokinesis, as well as the length and branching of the migratory processes are under dynamic control of the eCB system. Finally, similar effects are observed in vivo by analyzing

the morphology of GFP-labeled neuroblasts in brain slices from mice treated with CB1 or CB2 antagonists. These results describe a novel role for the endocannabinoid system in neuroblast migration in vivo, highlighting its importance in regulating an additional essential step in adult neurogenesis.

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C17

A cannabinoid-RalA signalling pathway controlling neural precursor migration

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In the mammalian brain, neural precursors derived from neural stem cells residing in the subventricular zone migrate towards the olfactory bulb following a very well defined path, the rostral migratory stream (RMS). Importantly, these precursors have the capacity to migrate away from their native route to areas of pathological damage in the adult brain (1). While our understanding of neural precursor migration has increased over the years, the exact molecular mechanisms remain to be fully elucidated. Understanding the migratory properties of these cells is essential to fully exploit their potential in neuroregenerative strategies. The endocannabinoid system has been previously shown to play an important role in the regulation of neural stem cell proliferation. We have recently shown that it is also involved in controlling the migration of RMS precursors both in vitro and in vivo (2). Indeed, agonists of the G protein-coupled cannabinoid receptors CB1 and CB2 markedly increase neural precursor migration, while CB receptor antagonists significantly impair it. In an effort to analyse the CB-dependent signalling pathways regulating neural precursor motility, we find that stimulation of CB1/CB2 receptors leads to a significant activation of RalA, a Ras-like GTPase previously shown to be involved in the control of neuronal morphology and polarity (3,4). RalA appears to be abundantly expressed in a vesicular pattern in migrating neural precursors. Using time-lapse imaging of RMS explants, we show that siRNA-mediated knockdown of RalA abolishes cannabinoid-stimulated motility and strongly impairs nucleokinesis, a crucial step for efficient migration. Current work is aimed at dissecting the molecular components of this cannabinoid-RalA signalling pathway, including other small GTPases and regulators of nuclear movement. In addition, our preliminary data suggest that other factors known to stimulate neural precursor migration can also activate RalA. We are currently examining the effect of RalA knockdown on neural precursor migration in vivo using electroporation of shRNA-expressing plasmids in the postnatal mouse forebrain together with time-lapse imaging of fluorescently labelled precursors in cultured brain slices.

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Sangeetha Gajendra is supported by a BBSRC CASE studentship. This work is supported by a Wellcome Trust Project grant awarded to Patrick Doherty and Giovanna Lalli.

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C18

Stress and inflammation reduce BDNF expression in first-episode psychosis: a pathway to smaller hippocampal volume

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Background: Reduced brain-derived neurotrophic factor (BDNF) levels have been reported in the serum and plasma of patients with psychosis. The aim of this study was to investigate potential causes and consequences of reduced BDNF expression in these patients, by examining the association between BDNF levels and measures of stress, inflammation and hippocampal volume in first-episode psychosis.
Methods: BDNF, interleukin (IL)-6, and tumour-necrosis-factor (TNF) alpha mRNA levels were measured in leukocytes of 49 first-episode psychosis patients (DSM-IV criteria) and 30 healthy controls (recruited between January 2006 and December 2008). In the same subjects, we measured salivary cortisol levels, and collected information about psychosocial stressors (number of childhood trauma, number of recent stressors, and perceived stress). Finally, hippocampal volume was measured, using brain MRI, in a subsample of 19 patients.
Results: Patients had reduced BDNF (effect size d=1.3, p<0.001) and increased IL-6 (effect size d=1.1, p<0.001) and TNF-alpha (effect size d=1.7, p<0.001) gene expression levels, when compared with controls, as well as higher levels of psychosocial stressors. A linear regression analysis in patients showed that a history of childhood trauma and high levels of recent stressors predicted lower BDNF expression through an inflammation-mediated pathway (adjusted R square=0.23, p=0.009). In turn, lower BDNF expression, increased IL-6 expression, and increased cortisol levels, all significantly and independently predicted a smaller left hippocampal volume (adjusted R square=0.71, p<0.001).
Conclusions: Biological changes activated by stress represent a significant factor influencing brain structure and function in first-episode psychosis, through an effect on BDNF.

This research has been supported by: a King's College Development Trust (UK) Studentship, a NARSAD Young Investigator Award, and a grant from the University of London Central Research Fund to Valeria Mondelli; a KCL Translational Research Grant to Paola Dazzan; the South London and Maudsley NHS Foundation Trust & Institute of Psychiatry NIHR Biomedical Research Centre for Mental Health; the BIAL Foundation; the British Academy; the UK Medical Research Council; the Commission of European Communities 7th Framework Programme Collaborative Project Grant Agreement n°22963 (Mood Inflame); and the Wellcome Trust (Grant Number: WT087417).

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

C19

Molecular mechanisms mediating dietary modulation of adult hippocampal neurogenesis and associated behaviour

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It is now well established that during adulthood, new neurons are generated from adult neural stem cells residing in the dentate gyrus of the hippocampus, a region important for memory and learning function as well as mood in rodents and humans (1). In the rodent, an increase of neurogenesis in the hippocampus is associated with improved learning/memory abilities (2), whereas a decrease is associated with symptoms of depression (3). The level of adult hippocampal neurogenesis can be regulated by factors such as enriched environment, physical activity, aging and stress but also by diet (4).

We first present dietary parameters responsible for adult hippocampal neurogenesis and memory and learning as well as mood regulation. We show, in the mouse animal model (mus musculus, strain C57BL/6) that meal frequency, independently of calorie intake, affects adult neurogenesis, learning/memory and mood.

We next identified possible molecular mechanisms mediating the effects of diet on adult hippocampal neurogenesis. We show that the gene Klotho is highly expressed in the hippocampus and its expression is up-regulated by 2 fold upon intermittent fasting-induced increased adult hippocampal neurogenesis ($n=3$, $p<0.05$). Klotho is also known as the 'ageing suppressor gene', due to the symptoms of a knockout mouse resembling human aging. In turn in a mouse model over-expressing Klotho lifespan is extended up to 30% (5). Up to date, the role of Klotho in the central nervous system has not been investigated. To further examine the role of Klotho on cellular and molecular mechanisms underlying the influence of food intake on hippocampal neurogenesis, we used a human embryonic hippocampal progenitor cell line.

Threshold analysis in the dentate gyrus of mice maintained on an intermittent fasting diet (fed ad libitum every other day) for 3 months shows an increase of Klotho

protein compared to ad libitum fed mice ($p<0.0297$, n=5) but no increase in the number of Klotho positive cells.
Immunocytochemical analysis of the cells upon Klotho knockdown showed a decrease in proliferation, differentiation, gliogenesis while apoptosis is increased. Markers used were BrdU and Ki67 for proliferation, Dcx and MAP2 for differentiation, s100beta ($p=0.0363$) for glial cells and cleaved Caspase 3 ($p=0.0290$) for apoptosis.
Our data suggest that diet modulates adult hippocampal neurogenesis through Klotho regulation, and underline a central role for Klotho in regulating hippocampal neurogenesis.
Data are presented as mean ± SEM. All statistical analyses were performed with GraphPad Prism5 on independent biological replicates (indicated as n=3). One-Way ANOVA with Newman-Keuls post hoc test was used for multiple comparisons among treatment groups. Student's t-test was used to compare means of two independent treatment groups. P-values <0.05 were considered significant.

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C20

Elucidating the functions of the schizophrenia susceptibility gene ZNF804A using human neural cells

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Genome-wide association studies have identified susceptibility genes for common psychiatric disorders with unprecedented confidence. The first genome-wide significant finding for the broad phenotype of psychosis (encompassing both schizophrenia and bipolar disorder) was with a variant within the ZNF804A gene (O'Donovan et al, 2008). Although the associated gene variant appears to impact on brain structure and activity (Esslinger et al, 2009), ZNF804A has, to date, been a gene of unknown function. In order to shed light on the functions of ZNF804A in an unbiased manner, we manipulated its expression in human neural progenitor cells using

RNA interference and assessed effects on the cellular transcriptome using whole genome microarrays. Transcriptional consequences of the two non-overlapping siRNA targetting ZNF804A were compared with a control siRNA (n=4 for each of the 3 conditions). There were 153 nominally significant (t-test $P < 0.05$) probe expression differences between ZNF804A siRNA and control conditions that were shared by the two ZNF804A siRNA conditions. Gene ontology analysis using DAVID bioinformatics resources (http://david.abcc.ncifcrf.gov) showed a significant ($P = 0.002$) enrichment of genes involved in 'biological adhesion'. We sought to confirm the most significant gene expression changes shared by the two ZNF804A siRNA conditions in repeat experiments using qPCR. Two of the 3 genes showing consistent gene expression changes across experiments are involved in neurite outgrowth; the other is of unknown function. These data demonstrate how transcriptional profiling in appropriate cell systems can be used to generate hypotheses as to the physiological role of genes of unknown function.

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C21

Astrocytic Nrf2 is a mediator of ischemic preconditioning

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The transcription factor Nrf2 regulates the Phase II antioxidant response, enhancing resistance to oxidative insult. Whether Nrf2 forms part of an endogenous protective response in the brain however, is not clear. Nrf2 has been reported to be activated following mild oxidative stress in non-neural tissues, we therefore sought to determine whether the same is the case in neural tissue. To achieve this, Nrf2 target gene expression was quantified by qrtPCR in DIV 9-11 primary mouse cortical neuron/astrocyte cultures (90%NeuN+/10%GFAP+), which were exposed to either peroxide (0, 25, 50uM) or oxygen glucose deprivation (OGD, 3 hour, treatments same throughout). Nrf2 target genes sulfiredoxin (*Srxn1*) and heme-oxygenase (*Hmox1*) were both significantly induced by peroxide (n=4, $p<0.05$ student t-test throughout unless otherwise specified) and OGD (n=5, $p<0.05$). To confirm Nrf2-specific induction, experiments were repeated in Nrf2 -/- neurons, revealing no induction post-OGD or post-peroxide (n=4-8, $p>0.05$), demonstrating Nrf2-dependent gene induction. Astrocytes appear to be the sole locus for Nrf2 activa-

tion as peroxide or OGD exposure fail to induce gene induction in pure neuronal cultures (>98%NeuN+, n=4-5, p>0.05), but significantly increase both *Srxn1* and *Hmox1* expression in pure astrocytic cultures (>96%GFAP+, n=3, p<0.05). Moreover, Hmox1 protein induction in mixed cultures is localized to astrocytes post-insult. To determine whether astrocytic Nrf2 activation could protect against a subsequent insult a preconditioning protocol was established. Exposure to 1.5 hour OGD significantly protected cells from a subsequent 3 hour insult 24 hours later (cell death assessed by DAPI-assisted quantification, n=5, p<0.05, ANOVA + post-hoc). Both *Srxn1* and *Hmox1* are upregulated following 1.5 hour OGD (n=4, p<0.05), indicating that the preconditioning stimulus is sufficient to recruit Nrf2. Applying the preconditioning protocol to both Nrf2 wild type and -/- neurons (n=5,11 respectively), revealed a significant decrease in protection in the absence of Nrf2 (p<0.05, ANOVA + post-hoc), highlighting the central role of astrocytic Nrf2 in mediating an acquired neuroprotective response. Nrf2 may also have a role in acquired neuroprotection *in vivo* as transient occlusion of the middle cerebral artery in adult mice, following anesthesia induction and maintenance with isoflurane (2%) in a mixture of 30% O_2 and 70% N_2O by face mask, for a duration known to trigger preconditioning, led to a significant increase in *Srxn1* and *HO-1* gene expression in the preconditioned hemisphere (n=6, p<0.01, paired t-test). The observed Nrf2-dependent recruitment of distinct and complimentary antioxidant pathways following transient episodes of oxidative stress both *in vitro* and *in vivo*[1] suggests the presence of an endogenous protective response and highlights the importance of Nrf2 as a therapeutic target.

Bell KF et al. (2011). Proc Natl Acad Sci U S A 108:E1-2; author reply E3-4.

Work was supported by the Medical Research Council, the Royal Society, and the Wellcome Trust. KB is the recipient of a CIHR Fellowship.

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

C22

MicroArray analysis of transduced cell lines to study glycyl tRNA synthetase (GARS) related neurodegeneration

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Background:GARS gene encodes the critical protein, Glycyl-tRNA Synthetase, which is an enzyme linking tRNA to glycine. Mutation in GARS have been shown to cause distal spinal muscular atrophy (dSMA) and Charcot-Marie-Tooth disease type 2D (CMT2D). In both conditions, the prominent early feature is wasting and weakness of the muscle of the hands and feet caused by degeneration of peripheral nerves (1). Two start codons in the Gars gene generate a short and a long version of the protein, which localize mainly to mitochondria and cytoplasm respectively. How-

ever, the function and relative role of the two isoforms in triggering neurodegeneration is unknown. The aim of this study is to identify the correlated genes differently expressed between wild type and mutant of the short and long Gars isoforms using a stable neuronal cell line model. Material and method:Lenti-XTM Tet-On® advanced inducible expression system was used to generate 4 types of mouse stable cell lines, with mouse cDNA: wildtype Gars and mutant_P278KY (2) in short or long Gars isoforms. 4 types of cDNA were tagged with an OneStrep tag and were cloned into pTightPuro vector. These were packaged into lentivirual particles and cotransduced with the regulator TetOn virus into target NSC-34 cells. We thereby obtained a system with Doxycycloine regulated expression of the gene of interest. Cells were tested with immunofluorescence and western blotting to confirm the expression of the transduced gene. RNAs were extracted from the expressed cell lines and were then carried out Affymetrix microarray study. Positive targets were validated using quantitative real-time PCR.Result: Immunofluorescence and western blot showed that NSC-34 cells were successfully transduced as indicated by the expression of the Strep tag. Using polyclonal cell lines, microarray data showed a few genes expressed differently between wildtype and mutant type with significant fold changes.

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The project is funded by MRC.

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

PC01

Skin stretch at the human wrist reduces the accuracy of tactile distance discrimination

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Stretching the skin around the human wrist, e.g. by altering flexion-extension angle, has been shown to impair tactile localization (Cody et al., 2010). This effect is probably due to stretch-induced enlargement of the receptive fields (RFs) of local touch units. To test whether a related tactile discrimination task is similarly affected by skin stretching, we have compared the precision of tactile distance discrimination at two wrist angles.

Twenty young adults (16 female, 4 male) were recruited. A 7-point linear stimulus array (0, 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0mm) was marked in ink along the longitudinal axis of the shaved dorsal surface of the wrist of the non-dominant (test) side whilst the relaxed joint was supported at a neutral angle. A single pair of stimulus points (corresponding to loci 0 and 7.5mm) was marked on the contralateral, dominant (reference) wrist.

In each trial, two brief, sequential tactile stimuli were applied, respectively, to the 0 and 7.5mm loci of the reference wrist using a von Frey hair (rating 70mN). Shortly after, a pair of sequential stimuli was applied to two loci of the 7-point array on the test side. The separations of these test stimulus pairs, in the neutral posture, ranged from 0mm (both stimuli at 0mm) to 15mm (stimuli at 0 and 15mm). The subject, eyes closed, was asked to state whether the separation distance (variable) between the two stimuli on the test side was “longer” or “shorter” than the separation distance (constant 7.5mm) on the reference side. Each test separation, ordered randomly, was repeated 10 times and the probability of the response “longer” (relative to reference separation distance 7.5mm) was calculated. The interval of uncertainty (IU, a measure of discriminatory threshold for separation distance) was estimated from standard psychophysical functions (probability of judgement “longer” versus test separation distance). Discriminatory acuity for separation distance was determined in this way under (1) baseline conditions (test wrist supported at neutral angle, no applied skin stretch) and (2) skin-stretched conditions (test wrist supported at 70deg. flexion).

Statistical analysis indicated that IU for discrimination of separation distance was significantly greater (less accurate) under skin-stretched than baseline (neutral) conditions (Wilcoxon 2-tailed test, $p = 0.048$). We interpret this finding as suggesting that tactile distance discrimination is strongly dependent upon the RF dimensions of regional touch units in an analogous manner to that previously demonstrated for localization of stimuli.

Cody FWJ et al. (2010) Neurosci Lett 484, 71-75

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PC02

NG2-glia use the retinoic acid signalling pathway to create a permissive environment for neurite outgrowth

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Inhibitory chondroitin sulphate proteoglycans (CSPGs), such as NG2, are highly up-regulated following central nervous system (CNS) injury and removal of these CSPGs promotes functional recovery. The NG2 CSPG is also expressed by a population of glial cells known as NG2-glia that are present throughout the developing and adult CNS, and these NG2-glia have been shown to support regenerating axons. Interestingly, following injury NG2-glia also express retinaldehyde dehydrogenase (RALDH) 2, the key retinoic acid (RA) synthesising enzyme. Retinoic acid stimulates neurite outgrowth via activation of the retinoic acid receptor (RAR) β. We therefore hypothesised that activation of the RA signalling pathway in NG2-glia would create a permissive environment for axonal regeneration. Using a co-culture system comprising adult dissociated dorsal root ganglia (DRG) sensory neurons with NG2-glia, we have investigated the effects of NG2-glia and RA on neurite outgrowth. Briefly, optic nerves from postnatal day (P) 7 mice were used to culture NG2-glia which were then co-cultured with dissociated DRG neurons. After 24 hours co-cultures were fixed in 2% PFA and immunolabelled for NG2 to identify NG2-glia, β-III tubulin to identify neurons and RARβ. Cells were then imaged using a Zeis LSM 700 confocal microscope and the percentage of neurite bearing neurons was calculated. Our results show that NG2-glia, as a unique glial cell type, are permissive to neurite outgrowth from DRG, and specifically in presence of the RARβ agonist, CD2019 (400nM), axonal outgrowth from DRG neurons is significantly enhanced ($p<0.05$, Two way ANOVA with Bonferroni post-hoc test). Furthermore, DRG neurites are closely associated with NG2-glia. Digestion of the NG2 CSPG from NG2-glia, via incubation with the enzyme chondroitinase ABC (ChABC), also significantly enhances neurite outgrowth ($p<0.001$, Student's T-test) and increases neurite branching from DRG neurons in DRG-NG2 co-cultures ($p<0.01$, Student's T-test). This effect can be blocked and then rescued by the addition of the RALDH2 inhibitor disulphiram (10μM), followed by all-trans RA (100nM). Pharmacological intervention with the pan RAR antagonist (10μM) also demonstrates that NG2-glia constitutively secrete RA, and this significantly enhances neurite outgrowth ($p<0.05$, One way ANOVA followed by Bonferroni post hoc test). Together, these data demonstrate that despite the presence of the NG2 CSPG itself, NG2-glia offer a favourable environment for neurite outgrowth, which is mediated via the RA signalling pathway.

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

PC03

Effects of vitamin-E and L-arginine on experimental diabetic neuropathy in rats

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Diabetic polyneuropathy is a common chronic complication of diabetes. Oxidative stress is increased in both human and experimental diabetes and has been related to the development of diabetic neuropathy. Vascular factors also have been implicated in the pathogenesis of experimental diabetic peripheral neuropathy (EDPN). The aim of this study was to evaluate the possible contribution of the two pathways, vascular or metabolic, to the development of such neural complication.
Ninety adult male Wistar rats (200-250 g) were assigned into 9 groups (n = 10 for each). One group served as a control (vehicle solution, 40 mg.kg-1, I.P.), and 8 experimental groups included streptozotocin-induced (40 mg.kg-1, I.P.) diabetic rats. One diabetic group did not receive any further treatment. Four diabetic groups were treated with a single treatment of the following: insulin (1 IU, S.C., daily), vitamin-E (300 mg.kg-1, I.M., 3 times a week), vitamin-E (600 mg.kg-1, I.M., 3 times a week), L-arginine (50 mg.kg-1, intragastric, daily). Three diabetic groups were treated with 2 treatments (insulin plus one of the 3 other treatments mentioned above). All experimental treatment regimes continued for 4 weeks, after which, under ether (10% for induction, and 5% for maintenance) anaesthesia, nerve conduction velocity (NCV) and amplitude of muscle contraction (AMC) studies were performed, and retro-orbital fasting blood samples were taken for measuring of serum glucose levels. The rats were then humanely killed, under terminal anaesthesia by cervical dislocation, and sciatic nerves were dissected for measuring of malondialdehyde (MDA), glutathione peroxidase (GPx), endothelial nitric oxide synthase (eNOS). Data are expressed as mean±S.D. and Significance ($P<0.05$) tested with ANOVA.
Diabetic rats had significantly higher serum glucose levels (382.5%), increased oxidative stress (MDA; 261.6%), lower eNOS (74.8%), delayed NCV (63.6%), and lower AMC (36.4%) as compared with the control group. Solitary insulin treatment (but not vitamin-E or L-arginine) corrected serum glucose to control values. However, treating diabetic rats with vitamin-E significantly reduced oxidative stress (using vitamine-E at the dose of 600 mg.kg-1, MDA was decreased by 50%, with increased GPx activity from 0.91±0.21 to 2.79±0.42 nmol.mg-1), and corrected NCV (reducing the latency by 72.03%) and improved AMC by 228.87%. On the other hand, L-arginine treatment had no effect on the oxidative stress markers, but significantly improved NCV (reducing the latency by 79.72%) and increased AMC (by 256.60%). This study supported the notion that EDPN is a multifactorial complication, caused by hyperglycemia, oxidative stress and vascular impairment. It is concluded that

conjugate treatment with vitamin-E, especially in higher doses, with insulin could be of great value. Moreover correction of impaired nerve blood flow by drugs that induce nitric oxide has proved to be efficient in the protection against, and correction of EDPN.

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PC04

The MRC London neurodegenerative diseases brain bank: a resource for neuroscience research

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Clinically and neuropathologically well-characterised human brain tissue is one of the most important resources for neuroscience research and is essential in the battle to develop new strategies and treatments for neurodegeneration. There has been significant research progress in recent decades and post-mortem tissue has played a major role in enabling advances in diagnosis, characterisation of pathological features, molecular genetics and bioinformatics.

The MRC London Neurodegenerative Diseases Brain Bank (LNDBB) is one of the largest brain banks in the UK. Since its establishment in 1989 it has collected over 2000 cases (formalin fixed and frozen samples). We focus our banking on neurodegenerative diseases, including Alzheimer's disease, Dementia with Lewy Bodies, Motor Neurone Disease and Frontotemporal dementia and age-matched controls but also house smaller collections, such as psychosis and paediatric disorders, in order to enhance research in these areas. The LNDBB operates a transparent and open-door policy for provision of central nervous system tissue to researchers. So far we have completed over 1270 requests and provided over 10,000 samples to national and international institutions. We are part of the MRC UK Brain Banks and Brains for Dementia Research networks which aim to encourage and facilitate both tissue donation and accessibility and use by researchers.

The brain bank also carries out studies into the best methods of preservation of tissue and the research potential of archival fixed tissue. We are constantly updating our procedures to ensure tissue is of the best quality for use in current research techniques.

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PC05

Effect of Hydrogen Peroxide on the Delayed Rectifier Potassium Current in Dissociated Hippocampal CA1 Neurons

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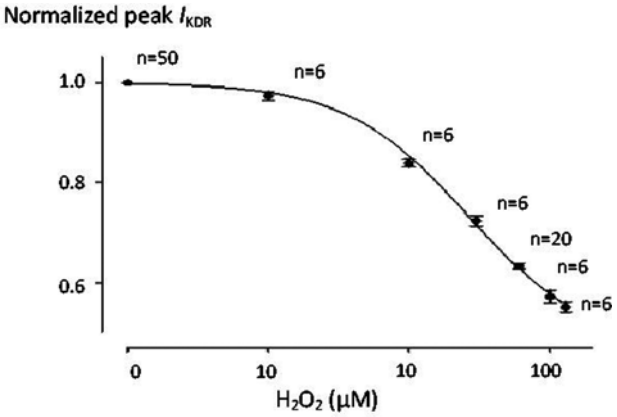
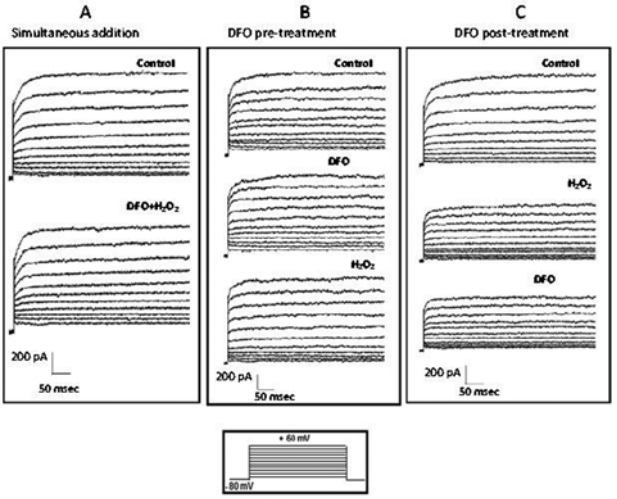
Introduction: Hydrogen peroxide (H_2O_2) is a reactive oxygen species that is generated as part of normal cellular metabolism. When in excess, H_2O_2 is known to cause damage to various biomolecules. Application of H_2O_2 has been frequently used in previous studies to induce oxidative stress conditions. The present study examined the effect of H_2O_2 on the delayed rectifier potassium current (I_{KDR}), a voltage – dependent current of paramount importance for neuronal excitability. Methods: Young adult Sprague Dawley rats were housed in the Animal Resources Centre at the Faculty of Medicine, Kuwait University; animals were humanely treated in accordance with the rules of animal care in this institution. Rats were sacrificed by terminal anaesthesia with ether and hippocampal CA1 neurons were isolated as explained earlier [1]. Whole cell voltage - clamp experiments were performed on freshly dissociated neurons before and after treatment with H_2O_2 using the method explained earlier [2]; in some cases different antioxidants and reagents were used in order to reveal the mechanism behind the H_2O_2 induced changes in I_{KDR}. Data were acquired using the p-CLAMP10 software (Axon Instruments, USA) and analyzed using the GraphPad PRISM 5 software (GraphPad Sowtware Inc., USA). The data were compared using a two-tailed unpaired Student's t-test and a difference between groups (n=5-6 per group) was considered statistically significant if $p<0.05$. Results: The external application of H_2O_2 inhibited I_{KDR} in hippocampal CA1 neurons in a concentration dependent manner (Figure 1). H_2O_2 reduced I_{KDR}'s amplitude and voltage dependence. Desferoxamine (DFO), an iron - chelator that prevents hydroxyl radical generation, prevented H_2O_2 - induced reduction in I_{KDR} (Figure 2). Application of the cysteine-SH oxidizing agent, 5,5 –dithio-bis-nitrobenzoic acid (DTNB) mimicked the effect of H_2O_2, whereas SH - reducing agents dithiothreitol (DTT) and glutathione (GSH) reversed and prevented the inhibition in I_{KDR} respectively. Addition of reducing agents DTT and GSH alone did not affect I_{KDR}. Membrane impermeable oxidative and reducing agents had effects only when added intracellularly. Conclusions: H_2O_2 reduced I_{KDR}, and this reduction was prevented by DFO thus identifying hydroxyl radicals as the intermediate oxidants responsible for the decrease in current amplitude. The reversal of H_2O_2 - induced reduction of I_{KDR} by SH - reducing agents identified SH groups as oxidative targets. Thus, the oxidative modulation of I_{KDR} by H_2O_2 was probably *via* hydroxyl radicals which targeted free SH groups of cysteine residues found in the intracellular aspect of the delayed rectifier potassium channel protein.



Concentration response curve for H_2O_2 inhibition of I_{KDR}. CA1 cells were treated with increasing H_2O_2 concentrations (10 – 1300 μM) for 6 minutes. Points represent mean of normalized current amplitude measured at 460 msec at + 60 mV. Values are mean ± S.EM, n indicated in the figure.



The effect of DFO on the H_2O_2 – induced decrease in I_{KDR}. A: Addition of DFO simultaneously with H_2O_2 showed no decrease in I_{KDR} currents. B: Addition of DFO alone for up to 5 minutes showed no change in current. Addition of H_2O_2 for 6 minutes showed no change in I_{KDR}. C. H_2O_2 addition for 6 minutes caused a decrease in I_{KDR} that was not reversed by DFO. Currents were elicited during eleven 500 msec clamp potentials between - 40 mV (bottom trace) and + 60 mV (top trace) in steps of 10 mV. Holding potential was - 80 mV.

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Authors acknowledge financial support from the College of Graduate Studies, Kuwait University.

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PC06

Generation and characterisation of mouse embryonic stem cell lines for the study of Huntington's disease

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Huntington's disease (HD) is caused by a CAG repeat expansion in the IT15 gene, which results in a long stretch of polyglutamines close to the amino-terminus of the HD protein, huntingtin (htt). HD is characterised neurologically by motor dysfunction, cognitive decline and psychiatric disturbances, which lead to progressive dementia, severe weight loss and death approximately 15–20 years after disease onset. Mutant htt forms intracellular aggregates, and causes selective neurodegeneration of the γ-aminobutyric acid-releasing spiny-projection neurons of the striatum although loss of neurons in many other brain regions has also been reported.

We have established mouse embryonic stem cell (ESC) lines from the R6/2 fragment model for HD. Currently we are investigating several protocols for the directed differentiation of the cell lines into neural stem cells (NSC) and neurones. We will evaluate the suitability of the cell lines as in vitro models of HD by determining if they display HD-related phenotypes. Such studies will include the investigation of ESC, NSC and neurones for htt expression, localisation and aggregation, accelerated cell death, transcriptional dysregulation and alterations in neurite outgrowth. Furthermore, ESC derived neurones will be used in our lab in the search for potential therapeutics.

Support: CHDI Foundation

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PC07

Electrophysiological characterization of glutamic acid decarboxylase 65-GFP neurons in the lateral hypothalamic area

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Gamma-aminobutyric acid (GABA) producing neurons of the lateral hypothalamus (LH) are functionally important in regulation of feeding (Leinninger et al., 2009). Some LH GABA neurons are also sleep-active (Hassani et al., 2010). The electrophysiological phenotypes of GABAergic neurons show considerable diversity all over the mammalian brain but their electrophysiology has not been studied in the LH before. Therefore in this study we characterised these neurons according to categories previously applied to cortical GABAergic neurons (Young and Sun, 2009). We used whole cell patch clamp recordings of glutamic acid decarboxylase 65 (GAD65) expressing neurons in acute slices prepared from transgenic mice expressing GFP under the GAD65 promoter (Erdélyi et al., 2002). Statistical testing was done using Student's t-test and data are reported as mean ± sem.
The majority of the cells (79/87) were spontaneously active with a mean firing rate of 11.6 ± 0.8 Hz (n = 79) at rest in slices prepared during the light period. Spontaneous firing was not significantly different in slices prepared during the dark period (firing at rest at 12.3 ± 1.6 Hz, n = 7/7, P = 0.77) whereas 7/7 GFP expressing cells in layer 1/2 of somatosensory cortex did not spike at rest and had resting membrane potential of -64.5 ± 0.9 mV (n = 7). By following the categorisation scheme of Young and Sun (2009), we were able to identify 11.5% of LH GAD65-GFP cells as low threshold spiking (LTS), 16.1% as late spiking (LS), 66.7% as regular spiking (RSNP) and 5.7% as fast spiking (FS) cells. 7/10 LTS cells expressed a type T calcium current whereas the other types of cells rarely showed this. LS cells expressed a significantly larger A-current than other GFP+ cells (1242 ± 146 pA in LS cells, n = 10; 316 ± 44 pA in others, n = 32, P < 0.0001). The FS cells had high maximum firing frequencies (> 250 Hz) and narrow action potentials (< 0.5 ms half-width) but, in contrast to published data on cortical FS neurons, showed considerable spike frequency adaptation (adaptation ratio was 0.44 ± 0.05, n = 5).
In this poster we show that GABAergic cells in LH slices prepared during both night and day are spontaneously firing and can be divided into 4 subclasses, and we summarise relevant electrophysiological parameters for each class.

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This work was funded primarily by the European Research

Council (FP7 Grant to DB). MK was also supported by the

Osk. Huttunen Foundation (PhD studentship).

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PC08

Methylomic Profiling Across Multiple human Brain Regions and Blood to Detect Differentially Methylated Regions (DMRs) and Allele-Specific DNA Methylation (ASM)

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There is mounting evidence that epigenetic factors play an important role in neurodegenerative diseases such as Alzheimer's disease. To date, little is known about normal patterns of DNA methylation across different regions of the human brain, and how these compare to those observed in peripheral tissues such as blood. Creating a reference epigenomic map across brain regions and peripheral tissues represents an important first step in disease-focused studies.
We carried out methylated DNA immunoprecipitation combined with next-generation sequencing (MeDIP-seq) using peripheral blood and post-mortem tissue representing eight brain regions from the same three individuals. Using the same samples, we also undertook a genome-wide screen for allele-specific DNA methylation (ASM) across brain regions and peripheral blood.
We were able to create a comprehensive map of the brain methylome and identify differentially-methylated regions (DMRs) across tissues. Moreover our ASM investigation revealed numerous examples where allelic patterns of DNA methylation are tissue-specific.
Overall, patterns of DNA methylation differ across brain regions, with cortical regions being distinct from cerebellum and blood. Levels of DNA methylation at promoter regions tend to be more strongly correlated across tissues, adding to the growing body of evidence that many tissue-specific DMRs are located outside of classic CpG islands. In addition we also found numerous examples of tissue-specific ASM. These data provide a valuable reference for future epigenetic and genetic studies of dementia and neuropsychiatric disease. Data is available for the research community as part of the NIH Epigenome Atlas.

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PC09

Expression of N-arachidonoylethanolamine (anandamide)-synthetizing enzymes in rat primary sensory neurons

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The endovanilloid/endocannabinoid systems control the activity and excitability of a major sub-population of nociceptive primary sensory neurons (PSN) (1), through the signalling molecule anandamide (AEA) and its main targets, the excitatory transient receptor potential vanilloid type 1 ion channel (TRPV1) and the inhibitory cannabinoid 1 (CB1) receptor (2; 3). AEA that intriguingly is also produced by PSN expressing TRPV1 and CB1 receptors, is thought to be synthesized by multiple enzymatic pathways (4). At present, the enzymatic pathway(s) involved in AEA synthesis in PSN is not known. Here, we aimed to find the expression pattern of enzymes, which might participate in AEA production in PSN.

Dorsal root ganglia (DRG) were collected from male Sprague Dawley rats (80-100g). Total RNA and protein were isolated both from intact DRG and 2 days old cultures of PSN prepared from the DRG. Half of the cultures grew in the presence of capsaicin (10 μM) overnight to induce degeneration of the great majority of TRPV1-expressing PSN (5). Reverse-transcriptase polymerase chain reaction (RT-PCR), quantitative RT-PCR (RT-qPCR) and western immunoblotting were used for studying enzyme expression. All data are expressed as mean ± SEM. Differences are regarded significant at $p<0.05$ calculated by Student's t-test.

RT-PCR and RT-qPCR revealed that mRNA of all enzymes are expressed in intact DRG, though inositol 5' phosphatase (SHIP-1) and protein tyrosine phosphatase non-receptor type 22 (PTPn22) mRNA levels were low. Culturing caused a significant down-regulation in the expression of alpha/beta-hydrolase 4 (ABHD4) and group 1B soluble phospholipase (sPLA2G1B) transcripts (0.18±0.01, $p<0.01$ and 0.8±0.03, $p<0.05$, respectively; n=4), whereas it elevated the expression of protein tyrosine phosphatase, non-receptor type 22 (PTPn22) transcript (2.75 ± 0.18 $p<0.01$) when compared to that of intact DRG. Capsaicin treatment resulted in significant increase in ABHD4 transcription (1.87 ± 0.25 $p<0.01$; n=4) in comparison to that of untreated cultures.

Western-blotting revealed that N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD), glycerophosphodiester phosphodiesterase 1 (GDE-1) and PTPn22 proteins are present in intact DRG. Culturing increased the level of these enzymes. In addition, sPLA2G1B became also detectable in cultures. Capsaicin treatment diminished the expression of NAPE-PLD and GDE-1 proteins (0.74±0.02 $p<0.05$ and 0.62±0.06 $p<0.05$, respectively; n=3).

Our data indicate that several putative AEA-synthesising pathways could indeed be involved in AEA production in PSN. Among these pathways, NAPE-PLD and/or GDE-1 might produce AEA in TRPV1-expressing PSN.

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Angelika Varga was supported by The European Union (Marie Curie Intra-European Fellowship; 254661) and Agnes Jenes by BJA/RCoA Project Grant.

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PC10

The neuronal actions of anti-Müllerian hormone appear to be independent of amyloid precursor like protein 2

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Anti-Müllerian Hormone (AMH) is a regulator of neurons with multiple unrelated functions. In embryos it is a testicular hormone, which contributes to the male bias in the brain and behaviour (1). In mature animals, AMH is produced by neurons of both sexes where it may serve as an anterograde regulator of neural networks (2,3). AMH is a member of the transforming growth factor β superfamily of proteins that are synthesised as large precursor proteins and then cleaved to give an N-terminal peptide and a C-terminal signalling peptide. The C-terminal of AMH signals through an AMH-specific type II receptor and a type I receptor that is shared with the bone morphogenetic proteins. In sperm and transfected CHO cells, AMH has non-classical signalling, involving the N-terminal fragment binding to amyloid precursor like protein 2 (APLP2), leading to activation of the extracellular regulated kinases 1/2 (ERK1/2) via a G_0 protein mechanism (4). APLP2 and amyloid precursor protein are redundant regulators, without which synapses degrade (5). Furthermore, the ERK pathways mediate part of the neurotrophin signalling. We have therefore examined the possibility that AMH activates ERK1/2 in neurons through APLP2.
Hippocampal neurons from 16-day-old mouse embryos were cultured for 4 days, and were then treated with either an AMH-related peptide (an N-terminal AMH peptide, the C-terminal fragment or the full-length AMH), brain-derived neurotrophic factor (BDNF) as a positive control or vehicle. The dose of the AMH peptides ranged from 0 to 75 nM, with both their acute (0-30 min) and chronic (0-6 days) effects on the phosphorylation of ERK1 and ERK2 being measured. For each condition, 3 independent primary cultures with 2 replicates were used. The phosphorylation of ERK1/2 was detected by Western blot using anti-ERK and anti-phospho-ERK antibodies and IRDye-conjugated secondary antibodies.

The presence of APLP2 in the cultures was verified by Western blots and immunohistochemistry. All three isoforms (a-c) of APLP2 were detected in the cultures and the brain, whereas only the b and c isoforms were present in the testes. The positive control, BDNF, increased the ratio of phosphorylated-ERK1/2 to ERK1/2 by over 3 fold (6.00±0.21, vehicle 2.66±0.46, n=2). In contrast, none of the AMH variants altered the phosphorylation of ERK1 or ERK2. For example, the p-ERK1/ERK1 ratio with 75 nM of the N-terminal AMH was 0.23±0.11 compared to a control of 0.20±0.11(n=6, P>0.05, Student T). The cultured neurons secreted AMH but this was not responsible for the low level of basal phosphorylation of ERK, as it persisted when *Amh*$^{-/-}$ neurons were cultured (0.27±0.05). In conclusion, the reported data is inconsistent with AMH regulating neurons via ERK1/2, suggesting that AMH regulation of the brain is solely through the classical pathway.

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The Marsden Fund (Royal Society NZ)

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PC11

Short term, low dose fluoxetine treatment increases brain allopregnanolone concentrations in female rats and abolishes estrous cycle-related stress-induced hyperalgesia

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Fluctuations in brain concentrations of female gonadal hormones during the ovarian cycle can impact significantly on behaviour. During late diestrus (LD) in rats, a sharp fall in the concentration of allopregnanolone (ALLO), a neuroactive metabolite of progesterone (PROG) triggers a withdrawal-like response, leading to increased responsiveness to anxiogenic stress (1). We investigated whether an acute replacement of ALLO with the synthetic analogue ganaxolone or an enhancement of ALLO production by fluoxetine (FLX) treatment can prevent the stress-induced hyperalgesia of LD. Female Wistar rats were confined in a Perspex tube for measurement of tail flick latencies (TFLs) in response to noxious radiant heat applied to the tail. Nociceptive thresholds were similar at all stages of the estrous cycle. Exposure to anxiogenic vibration stress (4 Hz for 5 min) during LD, but not at other stages,

evoked hyperalgesia (21±3.4% average decrease in TFL n=16, mean±SEM, P<0.01, one-way ANOVA). Pre-treatment with FLX (1.75mg Kg-1 i.p. at 16:30h on the day of early diestrus and again on the morning of LD 1h prior to behavioural testing) or the synthetic analogue of ALLO, ganaxolone (GNX, 7mg Kg-1, same dosing protocol as FLX) prevented development of stress-induced hyperalgesia (-4±5.8%, n=10 and +5.4±3.6%, n=7 change in TFL, respectively following stress). FLX or GNX had no effect when administered at other stages of the cycle. Since the dose of FLX used is reported to be subthreshold for effects on 5-HT systems (2) we investigated whether its effect was due to central steroidogenic activity (2). Rats were dosed with FLX or vehicle (n=5-6) as described above but instead of undergoing behavioural testing, they were killed by decapitation and the brain, minus olfactory bulbs, snap frozen at -80oC. Free steroids were extracted and fractionated from individual whole brain samples and derivatised for identification and assay by gas capillary chromatography-electron impact mass spectrometry (3). FLX induced a significant (P<0.05, Student's t-test) increase in whole brain concentrations of ALLO (1.4±0.2 v. 0.7±0.2ng/g) and its reduced metabolites allopregnanediol (2.7±0.4 v.1.2±0.4ng/g) and 5α-pregnan-3α,17-diol-20-one (1.0±0.2 v. 0.4±0.1ng/g). Concentrations of pregnenolone, the metabolic precursor of PROG decreased (3.0±0.5 v. 1.5±0.2ng/g) but there was no change in PROG or 5α-dihydroprogesterone. These measurements suggest an enhancement by FLX of the reducing activity of the 3α-hydroxysteroid dehydrogenase enzyme which catalyses ALLO production. Our results show that short term replacement of ALLO by ganaxolone, or the enhancement of the production of this steroid by FLX treatment prevents development of the stress-induced hyperalgesia normally associated with the fall in brain ALLO at LD.

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PC12

The effects of streptozotocin-induced diabetes on tight junction protein expression in the choroid plexus epithelium and on the physical integrity of the blood-cerebrospinal fluid barrier in rat

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It has been shown that streptozotocin (STZ) induced diabetes in rats altered expression of the BBB tight junction (TJ) proteins [1]. This study investigated effects of the STZ-induced diabetes in rat on the expression of the TJ proteins occludin, claudin

1 and 2 in the choroid plexus epithelium (CPE) and on the physical integrity of blood-cerebrospinal fluid barrier (BCSFB).
Methods. Diabetes was induced in Sprague-Dawley rats by i.p. injection of STZ, 55 mg/kg; control group (CG) animals received a vehicle. Only animals with blood glucose >17 mM were included in the diabetic group (DG). Animals from the DG groups and CG were used for the study at either 7 or 28 days after the STZ/vehicle injection. At the day of experiment rats were anesthetized with urethane (1.2–1.5 g/kg, i.p.), CSF samples were collected from *Cistern Magna* and choroid plexuses were collected. The expression of TJ proteins was explored at the transcript level by real-time PCR using hydrolysis probes, with 18S mRNA as an internal control and the CGs as calibrators. The fold change in mRNA expression was calculated as described earlier [2]. The expression of TJ proteins at the protein level was explored by immunoblotting; rat β-actin was used as internal control. The physical integrity of the BCSFB was explored by estimating the CSF/perfusate ratio for [^{14}C]sucrose after 10 min of *in situ* brain perfusion and the *in vivo* CSF/serum ratio for albumin. Statistical analysis was done using Student's t -test.
Results. The amount of mRNA for occludin in the DG after 7 days decreased to 64% of the amount in the CG; no significant change in the amount of mRNA for claudins were observed. After 28 days, amount of mRNA for all 3 TJ proteins decreased to <50% of the amount in the CG, (Table 1). No significant change in TJ proteins expression at the protein level, relative to expression of rat β-actin, was detected in DGs. Neither the CSF/perfusate ratio for [^{14}C] sucrose nor the *in vivo* albumin CSF/serum ratio differed between the DGs and CGs at 7 and 28 days.
Conclusion. STZ-induced diabetes has caused a decrease in the amount of occludin mRNA after 7 days and a decrease in the amount of mRNA for all three TJ proteinsa after 28 days. The relative expression of those proteins and the physical integrity of the BCSFB did not change.

The mRNA fold change ($2^{-\Delta\Delta Ct}$) in diabetic groups compared to the control groups as calibrators.

	7 days treatment		28 days treatment	
Gene of interest	Normalized expression ($2^{-\Delta\Delta Ct}$)	Range [($2^{-\Delta\Delta Ct+SD}$) - ($2^{-\Delta\Delta Ct-SD}$)]	Normalized expression ($2^{-\Delta\Delta Ct}$)	Range [($2^{-\Delta\Delta Ct+SD}$) - ($2^{-\Delta\Delta Ct-SD}$)]
Occludin	0.64	0.53 - 0.76	0.43	0.26 - 0.70
Claudin 1	0.79	0.39 - 1.58	0.47	0.27 - 0.83
Claudin 2	1.17	0.42 - 3.32	0.14	0.05 - 0.45

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This study was supported by the Kuwait University Research Administration grant No MY 04/07.

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PC13

Autophagy and the ubiquitin-proteasome system in the degradation of tau

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The microtubule-associated protein tau is mainly expressed in neurons and is essential for microtubule polymerisation and neurite outgrowth. Tau binding to microtubules is dependent on the phosphorylation state of the protein. In several neurodegenerative diseases, including Alzheimer's disease, in which tau deposition is apparent, tau is found hyperphosphorylated and aggregated into neurofibrillary tangles. Dysfunction of autophagy has been linked to a number of neurodegenerative disorders that are associated with an accumulation of misfolded protein aggregates. Several studies appear to implicate the autophagic process in tau degradation. The aim of this study was to examine the effects of permanent mimics of tau phosphorylation on its degradation via the autophagic route in primary cortical neurons and mouse embryonic fibroblasts (MEFs). Plasmids expressing EGFP fused to the longest human CNS tau isoform (2N4R), two phosphomimic mutants E18tau and E27tau each containing 18 or 27 serine/threonine sites mutated to glutamate and an additional A18tau mutant in which 18 serine/threonine sites were mutated to alanine to mimic dephosphorylation were expressed in MEFs, either wild-type (Atg5+/+) or autophagy-deficient (Atg5-/-). Cells transfected with tau were treated with 10 mM 3-methyladenine, to inhibit autophagy, 1 μM MG132, to inhibit the proteasome, or 100 μM cycloheximide to inhibit protein synthesis. We found that neither wild-type nor phosphorylation mutant forms of full-length tau protein form aggregates in Atg5+/+ or autophagy deficient Atg5-/- MEFs. In primary cortical neurons, inhibition of autophagy results in the accumulation of all of the tau constructs. Inhibition of the proteasome also results in accumulation of exogenous tau. Our results suggest that in primary embryonic neurons both autophagic and proteasomal routes may be important for the degradation of exogenously expressed tau. In the absence of autophagy, the proteasome and/or calpain systems are able to degrade tau in MEFs. We conclude that the phosphorylation state of the tau protein may be important for its degradation.

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PC14

Chronic inflammation increases hyperpolarization-activated current in C-, but not Aδ-, fibre nociceptive neurons in rats *in vivo*

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Hypersensitivity to painful stimuli (hyperalgesia) and/or normally non-painful stimuli (allodynia) is a hallmark of inflammation. This inflammatory pain hypersensitivity results partly from increased excitability of nociceptive dorsal root ganglion (DRG) neurons innervating inflamed tissue. These sensitized neurons exhibit increased spontaneous activity (SA) and decreased activation threshold (Woolf & Ma, 2007). The underlying molecular and ionic mechanisms of SA and hyperexcitability in these neurons are poorly understood. Hyperpolarization-activated cyclic nucleotide-gated channels (HCN) are key modulators of neuronal activity by producing the depolarizing Ih current (Biel et al. 2009) which has been implicated in nerve injury-induced hyperexcitability in DRG neurons (Chaplan et al. 2003). Here we examined whether, after tissue inflammation, expression and/or activation properties of Ih change in nociceptive DRG neurons. Hindlimb inflammation was induced by a 150μl intradermal injection of complete Freund's adjuvant (CFA) into the plantar surface of the left hindpaws of Wistar rats. Discontinuous single electrode voltage clamp (dSEVC) was performed in untreated (control) and CFA treated rats deeply anaesthetised with sodium pentobarbitone (60 mg/kg, i.p.). C-and Aδ-fibre nociceptors were identified on the basis of their dorsal root conduction velocities and their responses to noxious mechanical and thermal stimuli. Ih was assessed with 1 sec hyperpolarizing voltage steps from resting potential to -130 mV and was identified in vivo on the basis of its activation properties, time-dependant rectification, reversal potential, and blockade with Ih antagonist ZD7288 in some neurons. Ih was considered to be present if it was >50 pA. Interestingly 5-7 days after CFA induced inflammation a significantly higher proportion of C-nociceptors in CFA rats expressed Ih compared to control (76% (38/50) vs 48% (21/43), $p<0.01$, Fisher's exact test). Furthermore, CFA inflammation induced significant increases in the median Ih amplitude in these neurons (0.21 nA (CFA, n=50) vs 0.03 nA (normal, n=43), $P<0.0001$ Mann-Whitney U test), the median Ih density (2.91 pA/pF (CFA) vs 0.68 pA/pF (normal), $p<0.01$) and the rate, but not voltage dependence, of Ih activation. In contrast, there was no change in Ih expression or function in Aδ-fibre nociceptors. These results suggest that Ih/HCN channels are involved in inflammation-induced hyperexcitability of C-nociceptive DRG neurons.

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Supported by MRC grant to LD.

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PC15

Cornichon proteins modify functional properties of recombinant and native AMPA receptors

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Fast excitatory synaptic transmission in the CNS is mediated mainly by AMPA-type glutamate receptors (AMPARs). These ligand-gated ion channels are formed from hetero- or homomeric combinations of four pore-forming subunits (GluA1-4). Functional diversity among AMPARs is determined by their subunit composition, and by associated proteins that act as trafficking molecules and auxiliary subunits. A family of transmembrane AMPAR regulatory proteins (TARPs) regulate various key AMPAR properties (Nicoll et al, 2006). These include agonist affinity, single-channel conductance, desensitization and deactivation kinetics and, for calcium-permeable (CP-) GluA2-lacking AMPARs, block by intracellular polyamines and relative calcium permeability (Soto et al, 2007). Recently, another family of transmembrane proteins capable of interacting with and modifying AMPARs was identified, when proteomic analysis revealed cornichon (CNIH) -2 and -3 as partners of GluA2 in brain (Schwenk et al, 2009).

We found that co-expression in tsA-201 cells of CNIH-2 or -3 with GluA1 or GluA2 subunits affected many of the same channel properties that are modified by TARPs. Thus, desensitization (τ_{des}) of homomeric GluA1 receptors was slowed by CNIH-2 (from 2.9 ± 0.2 to 5.9 ± 1.0 ms; n = 8 and 11). The mean channel conductance, measured using non-stationary fluctuation analysis, was also increased (from 20.0 ± 2.5 to 30.9 ± 3.6 pS; n = 6 and 7). TARPs greatly decrease the block of CP-AMPARs by intracellular polyamines, reducing their characteristic inward rectification. CNIH-2 similarly caused a rightward shift in the conductance-voltage plot for GluA1 receptors (depolarization in $V_{1/2}$ of Boltzmann function from –67.3 ± 3.5 to –55.2 ± 3.0 mV; n = 7 and 6).

There is no consensus as to the role of cornichons in neurons (Shi et al, 2010; Kato et al, 2010). This issue has not been addressed in non-neuronal cells. In the original study (Schwenk et al, 2009), CNIH-2/3 immunoreactivity was observed in various glial cells, but functional effects were not investigated. To address this, we examined oligodendrocyte precursor cells (OPCs) – immature glia that give rise to oligodendrocytes responsible for myelination. Non-permeabilized OPCs cultured from rat optic nerve showed intense labelling with a CNIH-2/3 antibody. Over-expression of CNIH 3 in OPCs slowed the τ_{des} of glutamate-evoked current (from 6.1 ± 0.8 to 10.0 ± 0.8 ms; n = 10 and 12), indicating incorporation of CNIH-3 in functional AMPARs. Together, our data demonstrate that cornichons assemble with

both CP- and calcium-impermeable AMPARs in expression systems, altering their properties. The presence of CNIH-2/3 at the surface of OPCs and the effects of CNIH over-expression suggest that these effects may influence AMPAR signalling in vivo.

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Supported by the Wellcome Trust (SGC-C and MF). MZ was in receipt of an MRC (LMCB, UCL) studentship.

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PC16

Investigation of the effects of ApoE genotype on astrocytic protein secretion

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The most significant genetic factor in Alzheimer's disease (AD) is the increased risk of developing this disease that is conferred by possession of one or two alleles of apolipoprotein E4 (ApoE4). However, despite much research, the mechanism by which AD pathogenesis is modulated by the different ApoE isoforms (ApoE2, ApoE3 and ApoE4) remains obscure. The consequences of ApoE isoforms conferring differential susceptibility to AD include differences in (1) the ability of the various isoforms to produce or remove neurotoxic forms of amyloid beta-peptide, a constituent protein of the plaques deposited outside neurons in AD brain, and (2) sensitivity to amyloid neurotoxicity. Several studies have shown that over-expression of mutant forms of amyloid precursor protein (APP) leads to the widespread appearance of amyloid-containing deposits in the brains of transgenic mice. However, mice lacking ApoE are reported to be relatively resistant to the neurotoxic effects of amyloid beta-peptide when crossed with mice expressing mutant APP. Importantly, replacing mouse ApoE with human ApoE3 in mutant APP over-expressing mice delays the development of amyloid deposits compared to replacement with human ApoE4. Furthermore, over-expression of ApoE4 appears to exacerbate amyloid deposition in APP mutant mice. These findings indicate that ApoE may be involved in the clearance of amyloid and that ApoE4 may be less able to provide a functional compensation compared to that of ApoE3. ApoE functions as

a cholesterol acceptor in the brain and is also involved in cholesterol trafficking, a function dependent on the state of ApoE lipidation. Astrocytes and microglia are the primary sources of ApoE in the brain, with little ApoE being expressed by neurons. Here we are investigating astrocytes from ApoE knockout and wild-type mice to enable a comparison of (1) the secreted lipoproteins and (2) the release of pro-inflammatory cytokines. Differences are apparent in the protein composition of lipoprotein particles secreted in the absence of ApoE, and in the presence of different ApoE isoforms, compared to those produced by wild-type astrocytes. Furthermore, we anticipate that astrocytes from ApoE knockout mice will exhibit differential responses of cytokine release, compared to wild-type astrocytes, in response to stress. The results will elucidate mechanisms underlying the altered risk of developing AD that is caused by harbouring different ApoE alleles.

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PC17

Cannabinoid CB1 receptor-mediated signalling in the ducky2J model of cerebellar ataxia

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The ducky2J (du2J) mutation causes expression of a truncated, non-functional alpha2delta-2 Ca2+ channel subunit to provide a model of cerebellar ataxia and absence seizures. Du2J mutants have been shown to exhibit altered spontaneous Purkinje cell activity (Donato et al., 2006). It is also known that activation of cannabinoid CB1 receptors (CB1Rs) can induce cerebellar dysfunction, causing severe motor incoordination, including forms of ataxia (Patel & Hillard, 2001). Therefore, we have investigated CB1R-mediated modulation of cerebellar neuronal activity in the ducky2J model. Electrophysiological data were acquired via multi-electrode array (MEA) and whole cell patch clamp in acute cerebellar slices from wild type (+/+) and heterozygous (+/du2J) mice (male; 3-5 weeks old). Data are expressed as % of control; statistical significance was determined by Friedman tests followed by Dunn's tests unless stated. In +/+ mice, MEA recordings from the Purkinje cell layer (PCL) showed that the CBR agonist WIN55,212-2 (WIN55; 5μM) significantly increased firing rate (130 ± 8%; $P<0.05$); subsequent addition of AM251 (2μM) decreased PC firing rate (98 ± 7%; $P<0.001$ vs WIN55). In +/+ mice, the firing rate of granule cell layer (GCL) neurones were unaffected by 5μM WIN55 and 2μM AM251 (both $P>0.05$). These data are consistent with CB1R regulation of presynaptic inputs onto Purkinje cells, but not granule cells, in wild-type mouse cerebellum. Whole cell patch clamp recording from PCs in +/+ mice confirmed CB1R ligand effects in +/+ mice, whereby AM251 increased inhibitory postsynaptic (IPSC) frequency ($P<0.01$; Repeated one-way ANOVA followed by Tukey test); these data are also consistent with a localization of CB1R to inhibitory presynaptic terminals onto PCs (Ma et al.,

2008; Wang et al., 2011). In +/du2J mice, WIN55 (5μM) and AM251 (2μM) had no effect on in PCL firing rate; these CB1R ligands also had no effect on GCL firing rate. These data point to differential CB1R regulation of presynaptic inputs onto Purkinje cells between wild-type and +/du2J mutant mouse cerebellum. In whole cell patch clamp recording from PCs in +/du2J mice, WIN55 (5μM) and AM251 (2μM) had no effect on IPSC frequency. Taken together, these results suggest that CB1 receptor mediated signalling is compromised in du2J mutant mice. It will be of interest to determine if aberrant endocannabinoid signalling underlies an ataxic phenotype.

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PC18

Trafficking of Roundabout by Commissureless in the Drosophila Central Nervous System

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Both longitudinal and commissural neurons in the Drosophila central nervous system are guided by a number of repellent and attractant cues. One of these is Slit, which is produced by cells at the midline. Slit signals through the Roundabout (Robo) receptor and acts as a negative signal to steer axons away from the midline.

Commissural neurons express Robo but are insensitive to Slit prior to crossing the midline and become responsive to Slit after crossing. This differential sensitivity occurs through Commissureless (Comm), which regulates Robo protein distribution in the axon. Comm acts as an intracellular sorting protein that is able to sequester Robo within intracellular vesicles in commissural axons prior to crossing (Araújo and Tear 2003). To dissect the intracellular mechanism by which Robo is trafficked by Comm we have investigated the role of the Rab family of GTPases and additional components that regulate membrane protein transport. METHODS: Mutated forms of Rab proteins, acting as dominant negatives, were expressed in the Drosophila melanogaster CNS or cultured cells using the Gal4/UAS system (Brand and Perrimon 1993). Immunofluorescence techniques were used to analyze possible morphological changes in the CNS due to the overexpression of these proteins during embryonic development. RESULTS: Surprisingly, the growth of neither ipsi- nor contralateral projecting neurons was affected when dominant negative forms of Rabs were expressed in the CNS during development. We were able to

show the efficacy of these dominant negative constructs, however, in a different developmental setting. The same dominant negative constructs expressed in a Drosophila cell line did show a role for one specific Rab protein in the trafficking of Robo alone (in the absence of Comm), while none had any effect on the trafficking of the Comm-Robo complex. To characterise the sorting process in more detail we are now studying the dynamics of both Robo and Comm in axons in vivo. Preliminary results show the process to be highly dynamic in living embryos. With the results of these ongoing studies we hope to gain insight into the precise regulatory mechanisms that direct the distribution of Robo and Comm within the extending commissural axons.

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We are grateful for the fly community and the Bloomington Stock Center for sending us fly stocks. This work was supported by grants from the BBSRC and MRC.

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PC19

The role of the orphan nuclear receptor TLX in IL-1β-induced changes in proliferation of adult rat hippocampal neural precursor cells *in vitro*

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The orphan nuclear receptor TLX is a key regulator of neurogenesis, which occurs throughout the embryonic brain, and in the dentate gyrus of the adult hippocampus. It is required to maintain neural precursor cells (NPCs) in an undifferentiated state (Shi et al, 2003). Neurogenesis is impaired in Alzheimer's disease, major depression and bipolar disorder. Inflammation is also implicated in these disorders due to increased levels of the pro-inflammatory cytokine interleukin-1β (IL-1β) in the hippocampus. IL-1β has also been shown to negatively influence neurogenesis. Although data is emerging on the role of TLX in neural development, the effect of inflammatory modulators on TLX has not yet been explored. The aims of this study were to 1) assess the effect of IL-1β on the expression of TLX within proliferating NPCs prepared from adult rat dentate gyrus, and 2) determine if pharmacological inhibition of the IL-1 type 1 receptor (IL-1R1) prevents IL-1β-induced changes in TLX expression within proliferating cells. Neurosphere cultures were established from dentate gyri pooled from two 2-3 month old male Sprague Dawley rats and were allowed to proliferate for 7 days *in vitro* (DIV) as previously described (Nolan et al., 2010). Cells were treated with IL-1β (10ng/ml or 100ng/ml) and/or IL-1 receptor antagonist (IL-1RA) (1μg/ml) for the final 24h of culture. Cells were also treated during this period with 5-bromo-2-deoxyuridine (BrdU) (0.2μM) for detection of proliferating cells. Dissociated neurosphere cells were immunocytochemically

stained for IL-1R1 and co-stained for TLX and BrdU. The intensity of TLX expression in proliferating cells was measured by densitometry. IL-1β did not affect the percentage of $BrdU^+$ cells within the culture. Analysis of TLX expression by densitometry showed that IL-1β (100ng/ml) significantly decreased TLX expression in proliferating cells compared to control (10.4±0.4 vs. 7.9±0.3; $p<0.001$; ANOVA; n=3), while no change was observed in TLX expression within proliferating cells after treatment with IL-1β (10ng/ml). Co-treatment of NPCs with IL-1RA and IL-1β (100ng/ml) blocked the IL-1β-induced decrease in the intensity of TLX within proliferating cells (9.5±0.3 vs 7.9±0.3; $p<0.05$; ANOVA; n=3). Treatment with IL-1RA alone resulted in a significant increase in the fluorescence intensity of TLX within proliferating cells (11.8±0.3 vs 10.4±0.4; $p<0.05$; ANOVA; n=3). An IL-1β-induced decrease in expression of TLX in adult hippocampal NPCs suggests that IL-1β prevents these cells from remaining in an undifferentiated state. These results indicate that TLX is susceptible to inflammatory insult and may act as a regulator of adult hippocampal NPC fate in an inflammatory environment.

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This material is based upon works supported by the Science Foundation Ireland under Grant No. SFI/RFP/NSC1298

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PC20

A bioinformatic and *in situ* screen for novel axon guidance molecules

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Sophisticated genetic and biochemical screens for axon guidance molecules have identified a number of ligands and their axonal receptors. Many of these fall into a few major classes that share a limited number of structural motifs which are conserved from invertebrates to vertebrates. Although it has been speculated that the majority of axon guidance molecules have been discovered it is unlikely that sufficient molecules have been identified to encode the complete wiring of the embryonic nervous system.

In order to identify further axon guidance molecules we have taken a systematic bioinformatic approach to identify novel transmembrane proteins that contain any of a number of conserved extracellular protein domains found in known axon guidance molecules. This screen has identified 158 genes in Drosophila that fulfil these

criteria and their expression patterns were subsequently determined by in situ hybridization. This study yielded 46 candidates that show neural expression in the embryo during the period of axon extension. These include 8 genes that have orthologues in vertebrates including the CG32635/Neto and Ten/Odz families, Gogo and CG8403/Pikachurin.
Pikachurin is a type II transmembrane receptor-like protein with predicted EGF and laminin domains. It has been identified to interact with dystroglycan and have a role in positioning the bipolar cell dendrites in the mouse photoreceptor ribbon synapse (Sato et al 2008). Drosophila Pikachurin is widely expressed in the embryonic nervous system. A small deletion in pikachurin created by imprecise excision of an existing P-element reveals a likely role in the targeting of the ISNb motor neurons.

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PC21

An *in vivo* model to investigate tau hyperphosphorylation in Alzheimer's disease

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Hyperphosphorylation of tau plays a critical role in Alzheimer's disease (AD) pathology. Many kinases phosphorylate tau *in vitro*, but it is not certain which ones are responsible for the neurodegenerative process in AD. *Drosophila* provides a model system for studying tau toxicity *in vivo*. Expression of human tau in *Drosophila* photoreceptor neurons results in neurodegeneration that bears many of the hallmarks of AD. We are utilising *Drosophila* to identify the human kinases and their phosphorylation sites responsible for the generation of toxic forms of tau *in vivo*.
Expression of human tau in the photoreceptors of several transgenic *Drosophila* lines revealed varying levels of degeneration by scanning electron microscopy (SEM). We investigated the transcript levels of human tau expressed by qPCR. The levels of Sarcosyl-soluble and insoluble tau produced were quantified by western blot (WB). Data were analyzed by t-test or one-way ANOVA followed by the post-hoc Tukey's multiple comparison test. Significance level was set to $P < 0.05$. We found that the level of tau transcript and insoluble but not soluble human tau expression correlates with the degenerative phenotype in the eye.
Human kinases were expressed together with human tau in the fly eye to see whether the tau toxicity was enhanced. Glycogen synthase 3β (GSK3β) is one of the major kinases involved in tau hyperphosphorylation in AD brains. When human GSK3β was co-expressed with human tau the eye degenerative phenotype increased compared to that seen with tau alone. Western blot analysis showed that the degenerative enhancement is coincident with specific phosphorylation of human tau by

GSK3β on sites recognised by PHF-1, a phospho-dependent tau antibody for Ser396/404. This demonstrates the phosphorylation sites on tau responsible for increased toxicity *in vivo* can be identified using *Drosophila*. In the same way we are investigating additional kinases potentially responsible for tau hyperphosphorylation in AD including the dual-specificity tyrosine-phosphorylation regulated kinase 1A (DYRK1A). DYRK1A expression also enhances the human tau phenotype in the fly eye. We are currently mapping the phosphorylation sites responsible by western blot.
We find increased photoreceptor degeneration when expressing both human tau and human kinases in the Drosophila eye. This allows us to evaluate the contribution made by individual human kinases and specific sites to generate toxic forms of tau *in vivo*.

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PC22

Effect of transthyretin on thyroxine and β-amyloid removal from brain and cerebrospinal fluid in mice

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Transthyretin (TTR) is a serum and cerebrospinal fluid (CSF) chaperone protein for the thyroid hormones, retinol and β-amyloid peptide. TTR is synthesized systemically in the liver, and centrally by the choroid plexus (CP) and ependymal cells lining the cerebral ventricles. The secretion of TTR by the CP into CSF has been suggested to play a key role in the transfer of T4 from the blood to the CSF, as a unidirectional secretion of TTR to the CSF may drive T4 across the blood CSF barrier. On the other hand, T4 transport from CSF into blood is significantly reduced by TTR in a dose-dependent manner. TTR also binds β-amyloid peptide to attenuate amyloid plaque development, a hallmark of Alzheimer's disease. It is however, unclear whether TTR affects the clearance of β-amyloid from the brain. The aim of this study was to investigate the role of TTR in β-amyloid and T4 efflux from the brain.
Eight week old male 129sv mice were anaesthetized i.p. with 0.1 ml ketamine (100mg.ml-1) and 0.2 ml medetomidine (1mg.ml-1). The mice were placed in a stereotaxic frame and a cannula inserted into one lateral cerebral ventricle. A total 1μl artificial CSF was infused containing 125I-Aβ40 and 3H-inulin, or 125I-T4 and 3H-mannitol and the brain removed 2, 4, 8, 16 or 32 minutes after infusion. Brain samples were dissolved in Solvable and scintillation fluid added before counting. All procedures were within the guidelines of the Animals (Scientific procedures) Act, UK, 1986.

At baseline, levels of both 125I-T4 and 125I-Aβ40 in the brain were significantly higher than the extracellular markers and the net uptake of 125I-T4 into the brain was significantly greater than that for 125I-Aβ40. With increased time post infusion, levels of 125I-T4, 125I-Aβ40 and extracellular makers declined in brain. These generated linear relation curves, by which the half time for efflux was calculated. For 125I-T4, the half time for efflux was the fastest, 5.14 min, and faster than the marker mannitol (7.6 min), suggesting removal was via a transport system and not just bulk drainage of CSF and interstitial fluid. The half time for 125I-Aβ40 efflux was slower than T4 (17.5 min) but still faster than its extracellular marker, 3H-inulin (23.7 min). The introduction of TTR resulted in a significant increase in whole brain uptake and retention of 125I-T4 doubling the half time for efflux whilst extracellular markers remained similar to control. TTR increased 125I-Aβ40 accumulation sigmificantly in the CPs which may act as a route for delayed Aβ40 removal.
This study indicates that TTR acts differentially on T4 and Aβ40 efflux from brain. TTR prevents loss thyroxine from the brain but increases the accumulation of Aβ40 only in the CP which may enhance later CNS removal.

We are grateful for the support Dr Nouhad Kassem and funding by the BBSRC.

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PC23

Chronic hindlimb inflammation increases excitability of C-, but not Aδ-, fibre nociceptive neurons in rats *in vivo*

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Hypersensitivity to painful stimuli (hyperalgesia) and/or normally non-painful stimuli (allodynia) is a hallmark of inflammation. This inflammatory pain hypersensitivity is partly due to sensitization of primary afferent dorsal root ganglion (DRG) neurons. These sensitized neurons exhibit increased spontaneous activity (SA) and decreased activation threshold (Woolf & Ma, 2007). Previous studies have shown increased proportions of both C-and Aδ-fibre nociceptors with SA following acute inflammation (Koltzenburg et al., 1999, Xu et al., 2000). More recently we have shown that the proportion of Aδ-fibre nociceptors showing SA was greater than that of C-fibre nociccetors at both 1 and 4 days after CFA (Complete Freund's Adjuvant)-induced hindlimb inflammation (Djouhri et al., 2006). Here we examined the impact of CFA-induced chronic inflammation (5-7 days post CFA) on excitability of C-and Aδ-fibre nociceptors innervating inflamed tissue. Hindlimb inflammation was induced by two intradermal injections of 150μl of CFA into the left hindlimb of Wistar rats (one into the plantar surface of teh hindpaw and the other into the knee region). This was to induce inflammation in the whole hindlimb as described previously (Djouhri et al., 2006). Intracellular recordings of somatic action potentials

evoked by dorsal root electical stimulation were made from L4/L5 DRG neurons in untreated (control) and CFA treated rats deeply anaesthetised with sodium pentobarbitone (60 mg/kg, i.p). C-and Aδ-fibre nociceptors were identified on the basis of their dorsal root conduction velocities and their responses to noxious mechanical and thermal stimuli. We found that long term inflammation (5-7 days post CFA) increased excitability of C-, but not Aδ-fibre nociceptors as indicated by an afterdischarge response (response that outlasts the stimulus duration) to a brief noxious stimulus (pinch with toothed forceps), increased incidence of SA, and decreased electrical (dorsal root) threshold. Indeed compared to controls, C-fibre nociceptors of CFA-treated rats exhibited a highly significant decrease in electrical threshold ($P<0.001$, Mann- Whitney U test) and a significant increase in the incidence of SA ($P<0.05$ Fisher's exact test). The percentage of SA increase was from 5% (2/43) to 24% (12/50%). Furthermore, 28% of C-nociceptors in CFA rats exhibited afterdischarge responses to a brief noxious pinch. In contrast, Aδ-nociceptors showed no afterdischarge responses, no significant change in the electrical threshold and no SA (0% in control (n=15) vs 0% in CFA, n=12). These results suggest that C-nociceptors contribute more to chronic inflammatory hypersensitivity that Aδ-fibre nociceptors.

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Supported by MRC grant to LD.

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

PC24

Mitochondrial dysfunction causes age-related and cell type-specific neurodegeneration

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Mitochondrial dysfunction has been associated with several major neurodegenerative disorders, including Parkinson's disease (PD). Among the pathological changes observed in PD is the progressive loss of dopaminergic (DA) neurons in the Substantia Nigra of the ventral midbrain. Ageing individuals with PD have high levels of mitochondrial DNA (mtDNA) alterations, and mutations in PD-related genes PINK1 and parkin have been associated with reduced mtDNA copy numbers and decreased mitochondrial respiratory activity. However, it is not clear whether mtDNA alterations and mitochondrial dysfunction are causally related to DA neurodegeneration. In order to address this question in vivo, we knocked down the sole mito-

chondrial DNA polymerase, POLGα, in a cell-type specific manner in Drosophila. POLGα knockdown dramatically decreased the level of mtDNA. POLGα-related mitochondrial dysfunction in dopaminergic, but not in cholinergic or in serotonergic neurons, resulted in adult-onset, age-related motor deficits and DA-specific neurodegeneration. Lethality caused by POLGα knockdown could be rescued, at least to some extent, by targeted genetic manipulation of either PINK1/parkin signalling or by Drp1, both of which have been implicated in mitochondrial dynamics. Full rescue of lethality was achieved by genetically bypassing respiratory deficiencies affecting complexes 1, 3 and 4. In addition, enhancement of the electron transport chain by nicotinamide, ameliorated locomotor defects and prevented DA neuron loss. Our results demonstrate that mtDNA alterations lead to respiratory chain deficiencies that cause age-related DA neurodegeneration underlying PD.

Supported by the Medical Research Council and Parkinson's UK.

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PC25

Expression of N-acyl phosphotidylethanolamine phospholipase D in rat dorsal root ganglion neurons

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The enzyme N-acyl phosphotidylethanolamine phospholipase D (NAPE-PLD) is involved in Ca2+-dependent synthesis of anandamide (1) that is an endogenous activator of the transient receptor potential vanilloid type 1 ion channel (TRPV1) (2) and the cannabinoid 1 (CB1) receptor (3). TRPV1 and the CB1 receptor are involved in the regulation of the activity and excitability of a major sub-population of nociceptive primary sensory neurons (PSN) which play a pivotal role in the initiation and maintenance of acute pain as well as pain associated with pathological condition, such as inflammation (4). We have shown previously that TRPV1-expressing cells produce anandamide in a Ca2+-dependent manner. Further we have shown recently that NAPE-PLD mRNA is expressed in a sub-population of PSN and that most of the NAPE-PLD-expressing cells express TRPV1 (5). Here, we assessed the neurochemical properties of NAPE-PLD-expressing PSN.
Multiple immunofluorescent staining was used on the L4 dorsal root ganglia (DRG) of naive, adult Wistar rats (n=3) or rats injected intraplantarly, under isoflurane anaesthesia (5% for induction and 3% for maintainance), with compelet Freund's Adjuvant (CFA, n=3) or incomplete Freund's Adjuvant (IFA; n=3) 3 days prior to tis-

sue harvesting. We used anti- NAPE-PLD-, anti-neurofilament 200 (NG200)-; anti-calcitonin gene-related peptide (CGRP)- anti-CB1 receptor-, anti-TRPV1-antibodies and biotinylated Griffonia simplicifolia lectin (IB4).
Immunoreactivity for NAPE-PLD was detected in ~40% of the cells. Most of them were immunonegative for NF200 that identifies cells with myelinated axons. Triple staining revealed that NAPE-PLD immunopositivity was present in cells expressing the nociceptive PSN markers, CGRP or IB4 binding sites. About 1/3 of the NAPE-PLD immunopositive cells expressed both CGRP immunopisitivity and IB4 binding sites. Further staining revealed that NAPE-PLD immunopositivity is highly co-expressed with immunopositivity for both of the anandamide-responding receptors, TRPV1 (~2/3), and the CB1 receptor (~90%). CFA but not IFA injection produced an overall increase in the number of NAPE-PLD immunopositive neurons ($p<0.05$, ANOVA). The increase was particularly apparent in large sized cells.
Our results confirm recent data that NAPE-PLD is expressed predominantly in nociceptive cells. The high degree of co-expression between NAPE-PLD, TRPV1 and the CB1 receptor suggests that NAPE-PLD through synthesizing anandamide might play an important role in regulating the activity and excitability of nociceptive DRG neurons. The increase in NAPE-PLD expression following CFA injection in PSN neurons further suggests that that regulatory role could be enhanced in inflammatory conditions.

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This work has been supported by FCT, Portugal.

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PC26

Defining the spinal cord connectome: axon projection and dendritic fields in the developing *Xenopus* spinal cord

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How nervous systems develop with the remarkable specificity required for complex functions is a question that developmental neuroscientists have been chasing for many years. Are sophisticated recognition mechanisms required during early stages of vertebrate development, when simpler circuits assemble in the axial nervous system to generate first movements?

In hatchling *Xenopus laevis* tadpoles, previous work has elucidated morphology, physiology and synaptic connections of neuron subclasses forming the spinal motor circuit that generates swimming. This puts us in a position to ask how these circuits self-assemble. Unexpectedly, paired neuron recordings showed at least some synapses between all neuron types raising the possibility of low specificity and that axons potentially form synapses with any dendrite they contact (Li et al 2007). Are connections in early circuits determined primarily by geographical location of axons and dendrites of different neuron types?

To record 3D information on location of axons and dendrites in the tadpole CNS, we devised a measuring microscope. We obtain data on morphology of individual neurobiotin-filled neurons from electophysiological experiments where tadpoles were anaesthetised with 0.1% MS222 and then immobilised with alpha bungarotoxin. We define their relative position in 3D space in the nervous system. Neurons were viewed in whole mounts of the tadpole CNS under a Nikon Optiphot microscope and DeltaPix camera. Specimen position was controlled by a Scientifica PatchStar micromanipulator and LinLab software which allowed 3D co-ordinates describing neuronal morphology and relative location to be recorded.

Excitatory descending interneurons (dINs) drive other swim circuit neurons during swimming (Dale & Roberts, 1985, Soffe et al 2009). Paired whole-cell recordings have shown that they synapse with each other (Li et al 2006). 3D co-ordinates of dIN axon and dendrite trajectories were recorded within the spinal cord and hindbrain, which is ~4mm rostro-caudally and ~100μm dorso-ventrally. We defined the rostral edge of the hindbrain, the midbrain border (MBB), as x=0 and the ventral edge of the spinal cord as y=0. Preliminary assessment of a dIN sample (n=17) whose somata ranged from 830-2278μm from MBB showed axon trajectories extending to 3340μm from MBB. The sample axon dorso-ventral occupation ranged from 1-71μm from the ventral edge; closely matched by dendritic arborisation dorso-ventral occupation, which ranged from 1-65μm from the ventral edge.

These initial data show that the geography of dIN axons would allow contact with dIN dendrites to form synaptic connections. More detailed inspection may reveal possible relationships within dINs that may influence connectivity, e.g. do more rostral dINs have more ventral axons? Data on dINs provides an example of the method which we hope will predict the global network connectivity of the identified and recorded neuron types within the spinal cord.

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PC27

The effects of oxytocin on hippocampal neurogenesis

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The neurohypophysial hormone oxytocin, best known for its role in lactation and parturition, has been shown to play an important role in the central nervous system, modulating stress responses, pain perception, learning and different aspects of social behaviour, from trust to maternal care (Neumann, 2008). Animal and human studies showed that administration of oxytocin produces anxiolytic effects and decreases the release of stress hormones (Yoshida et al., 2009). The majority of studies of oxytocin levels in depression showed its negative correlation with depressive symptoms.

It has been found that human hippocampal neurogenesis is involved in the pathogenesis of depression. It has been shown that depressed patients exhibit reduced levels of neurogenesis in hippocampal dentate gyrus, and antidepressants counteract this effect (Boldrini et al., 2009). Specifically, our laboratory has recently demonstrated that antidepressants exert their effect through the glucocorticoid receptor (Anacker et al., 2011). It has been shown that oxytocin *in vivo* has a suppressive effect on glucocorticoid receptor expression in the hippocampus (Petersson and Uvnäs-Moberg, 2003). Considering that, we hypothesised that oxytocin may have an antidepressant-like effect on hippocampal neurogenesis, which would strengthen the notion of its therapeutic potential for stress-related disorders, including depression.

We used a human neural stem cell line HPC03A/07 (provided by ReNeuron Ltd., Surrey, UK) as an *in vitro* model of hippocampal neurogenesis. We assessed the effect of oxytocin treatment on cell proliferation by BrdU immunocytochemistry. The results showed that oxytocin has a stimulating effect on the proliferation of neural progenitors (20.13% increase upon 1μM oxytocin, $p<0.05$, t-test, n=3, and 20,51% increase upon 100nM oxytocin, $p<0.05$,t-test, n=3).

These findings show that oxytocin has a positive effect on adult hippocampal neurogenesis, a process which is altered in depressed patients and proposed to be an alternative target of antidepressant treatment, therefore confirming the therapeutic potential of neurohormone oxytocin for depressive disorders.

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PC28

Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor

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Antidepressants increase adult hippocampal neurogenesis in animal models, and indeed, this increase in neurogenesis has been shown to be required for antidepressants to alleviate depressive-like behaviour in rodents (1). However, the molecular mechanisms underlying the effects of antidepressants on neurogenesis are unknown. Studies by us and others have shown that antidepressants regulate the function of the glucocorticoid receptor (GR) in animals and in cellular models (2). Here we wanted to test specifically whether the GR may be involved in the effects of antidepressants on human neurogenesis. We therefore used a human hippocampal progenitor cell line (HPC03A/07, from ReNeuron, UK) to investigate the molecular pathways involved in the antidepressant-induced regulation of neurogenesis.

We treated cells with the antidepressant, sertraline (1μM), for 10 days and investigated neuronal differentiation by doublecortin (Dcx) and by microtubulin-associated protein-2 (MAP2) immunocytochemistry. Cell proliferation was assessed by 5'-bromodeoxyuridine (BrdU, 10μM) incorporation and immunocytochemistry after 3 days of treatment. Gene expression and GR-phosphorylation upon antidepressant treatment was investigated by quantitative Real-Time PCR and by Western Blot, respectively.

Our data show that sertraline increases both immature, Dcx-positive neuroblasts (by 16±2%, $p<0.001$, n=3), and mature, MAP2-positive neurons (by 26±4%, $p<0.01$, n=3). This effect was abolished by the GR-antagonist, RU486 (at 50nM). Interestingly, progenitor cell proliferation was only increased when cells were co-treated with sertraline and the GR-agonist, dexamethasone (1μM) (by 14±3%, $p<0.05$, n=6), an effect which was also abolished by RU486. Moreover, sertraline increased GR phosphorylation at its serine residue S203 (by 1.6±0.2 fold, $p<0.05$, n=4), increased GR transactivation (by 20±3%, $p<0.05$, n=3), and increased expression of the GR-

regulated cyclin dependent kinase-2 (CDK2) inhibitors, p27Kip1 (by 1.9±0.2 fold, $p<0.05$, $n=3$) and p57Kip2 (by 1.8±0.2 fold, $p<0.05$, $n=3$).
In conclusion, our data demonstrate that the antidepressant, sertraline, increases human hippocampal neurogenesis via a GR-dependent mechanism that requires GR phosphorylation and activation of a specific set of genes, including p27Kip1 and p57Kip2. Our data point towards an important role for the GR in the antidepressant-induced modulation of neurogenesis in humans and may provide a future drug target to overcome neurogenesis-related disturbances in depression.

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Funded by a studentship to C. Anacker from the NIHR "Biomedical Research Centre for Mental Health"

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PC29

Characterising functional, anatomical and electrophysiological changes from acute to chronic stages of spinal contusion injury

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Traumatic spinal cord injury (SCI) generally results in severe motor, sensory, and autonomic deficits below the level of the injury. As contusion injuries are the most common form of SCI in humans, an animal model of spinal contusion injury provides a clinically relevant tool for studying pathological changes that occur following SCI and to assess the efficacy of potential therapeutic interventions. We have performed a detailed characterisation of some of the physiological changes that occur from acute to chronic stages post injury in this model, using a novel electrophysiological technique to assess axonal conduction through the lesion over time. Anaesthetised adult rats (using a mixture of ketamine (60 mg/kg) and medetomidine (0.25 mg/kg), administered i.p.) received a 150kD (Infinite Horizons) contusion injury. Saline (3–5 ml) and baytril (5 mg/kg) were given subcutaneously twice daily for 3 and 7 days, respectively, post-injury. Electrophysiological recordings were performed at a number of time points (1, 7, 14, 28 and 84 days) post-injury. Animals were deeply anaesthetised with urethane (1.25 g/kg, i.p.), and depth of anaesthesia was regularly assessed by monitoring pedal withdrawal reflexes and respiratory rate. Acutely (1 day) post-injury there was a complete absence of conduction across the contusion site. This increased slightly in the sub-acute stage (1 week), with the

percentage of axons conducting across the injury gradually increasing as the injury progressed to chronic stages (4 – 12 weeks). The behavioural assessments (BBB locomotor scale and ladder walking) exhibited a similar pattern to the electrophysiological data at earlier time points, highlighting an initial severe functional deficit, with gradual improvement over the sub-acute stage. There were however, no further improvements in performance beyond 4 weeks using behavioural assessments. Anatomical characterisation was also performed at the different injury time points, to assess the degree of tissue loss, cell death and glial scarring. Furthermore, demyelination and remyelination was assessed following contusion injury at the electron microscopic level. Thus, we have provided a detailed characterisation of changes over time in a clinically relevant spinal injury model. Such thorough assessments, which combine electrophysiological and behavioural function with anatomical measures, could prove invaluable to furthering our understanding of mechanisms underlying the pathological events that occur following spinal cord injury, and for assessing potential therapies aimed at promoting repair.

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PC30

Assessing the therapeutic potential of novel lentiviral vector delivery of chondroitinase ABC in adult rats with a spinal contusion injury

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The bacterial enzyme chondroitinase ABC (ChABC) is a promising treatment option for spinal cord injury (SCI), degrading chondroitin sulphate proteoglycans (CSPGs) which are one of the key molecules inhibitory to repair. However, in vivo treatments to date have been suboptimal based on drawbacks such as enzyme stability and invasiveness of previous delivery strategies. Recently, a bacterial chondroitinase cDNA has been engineered that allows the expression and secretion of active chondroitinase enzyme by mammalian cells (Muir et al. 2009). Gene delivery of ChABC may have a number of advantages compared to previous treatment paradigms, including sustained CSPG degradation as well as reduced invasiveness and risk of infection. We have evaluated the effectiveness of lentiviral vector delivery of ChABC in an animal model of spinal contusion injury, which represents the most common form of SCI in humans and, therefore, provides a clinically relevant tool for assessing the efficacy of potential therapeutic interventions. Anaesthetised adult rats (using a mixture of ketamine (60 mg/kg) and medetomidine (0.25 mg/kg), administered i.p.) received a 150kD (Infinite Horizons) contusion injury and lentiviral vector incorporating the ChABC gene or a control GFP was immediately injected rostral and caudal to the injury site. Saline (3–5 ml) and baytril (5 mg/kg) were given subcutaneously twice daily for 3 and 7 days, respectively, post-injury. We have

shown prolonged and widespread CSPG degradation with ChABC lentiviral vectors. Additionally, using a number of behavioural, electrophysiological and anatomical outcome measures, we have demonstrated improved function in animals treated with lentiviral ChABC and changes in lesion pathology. We demonstrate the potential advantages of lentiviral vector delivery of ChABC, such as sustained and widespread CSPG degradation that is associated with improved anatomical and functional outcomes.

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PC31

PGC-1α regulates NMDA and AMPA-type glutamate receptor expression in rat cultured cortical neurones

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There is strong evidence that disruption of mitochondrial biogenesis and function play a causal role in neuronal vulnerability in neurodegeneration[1]. Indeed selective loss of highly metabolic regions of the brain in Huntington's disease (HD) suggest a link between mitochondrial dysfunction and oxidative stress in disease pathogenesis. The transcriptional coactivator PPARgamma coactviator 1alpha (PGC-1α) is a regulator of mitochondrial biogenesis and function[2,3] and is decreased in the striatum of patients with HD. Furthermore PGC-1α undergoes targeted downregulation by mutant huntingtin protein (mHtt)[2] and PGC-1α knockout mice have striatal lesions similar to mHtt mice[4]. In addition, PGC-1α partially reverses the toxic effects of mutant huntingtin in cultured striatal neurones[2] while *in vivo* administration of PGC-1α to the striatum in a mouse model of HD reduces neuronal volume loss[2]. Synaptic NMDAR-activity can drive the expression of PGC-1α which is neuroprotective against oxidative and excitotoxic stress *in vitro* (Soriano *et al*. (2011) Antioxidants & Redox Signaling in press), whereas extrasynaptic NMDAR expression is increased in HD[5]. Excessive NMDAR activity leads to excitoxic death in neurones and its regulation has been targeted in the search for therapeutic interventions for multiple neurological disorders. This study proposes a novel mechanism of neuroprotection provided by PGC-1α via the regulation of glutamate receptor expression.

Electrophysiological whole-cell patch-clamp recordings from rat primary cortical neuronal cultures (days *in vitro* 9-11) indicated that over expression of PGC-1α caused a 29.5 ± 6.0% decrease (N=6, n=24, t-test $p < 0.05$) in whole-cell NMDAR-mediated currents in response to bath application of NMDA (100 μM). Conversely, siRNA knock-down of PGC-1α caused a 48.7 ± 13.4% (N=6, n=26, t-test $p < 0.05$) increase in whole-cell NMDAR-mediated currents. Increased expression of PGC-1α

also caused a significant decrease (26.3 ± 5.8%, N=3, n=12, t-test $p < 0.05$) in whole-cell AMPAR-mediated currents together with a reduction in the gene promoter activity of GluA1 and GluA2 AMPAR subunits (36.1 ± 3.6% and 18.0 ± 5%, respectively, n=5, t-test $p<0.05$). In conclusion our data suggests PGC-1α can regulate the functional expression of both NMDA and AMPA ionotropic glutamate receptor subtypes. Such regulation many contribute to the control of neuronal survival.

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This work has been supported by the Medical Research Council. CAP is in receipt of a BBSRC PhD studentship.

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PC32

Characterisation of Late Infantile Neuronal Ceroid Lipofuscinosis gene CLN7 using Drosophila as a model organism

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Neuronal ceroid lipofusciosis (NCL) disorders are a collection of childhood neurodegenerative diseases that fall into the larger category of lysosomal storage disorders. The different variants of the disease are defined by their age of onset with the most aggressive affecting children in their first year of life. The genes affected in ten of these disorders have been identified, the most recent of which is CLN7. Mutations in CLN7 cause late infantile NCL which follows the classical pathology of the other NCLs and exhibits the hallmark accumulation of autofluorescent pigment in all tissues, although as is seen in all the variant forms, only neuronal loss is observed. Very little is understood of the normal function of CLN7 nor how its mutation leads to disease. Drosophila has an orthologue of CLN7 and we hope to gain clues to the human disease by utilising the advantages of Drosophila to study the gene's function. CLN7 is a 12 pass transmembrane protein. We have shown both human and fly CLN7 resides within the membrane of acidic vesicles when expressed in Drosophila derived BG3 cell lines, we also show that when Drosophila CLN7 is driven in primary cultured neuronal cells it co-localises with lysotracker in acidic vesicles. We have conducted a large-scale p-element excision screen to create a gene deficiency. From 750 excision lines we have 3 imprecise excision lines which carry varying sizes of deletion in the CLN7 locus but retain the surrounding genetic material. All 3 lines are homozygous viable. Our examination of the cln7-MB3 allele

shows significantly decreased number of boutons at the neuromuscular junction (n=16, mean 14) compared with wild type (n=13, mean 19) using Mann-Whitney test. Combined with the localisation data, the results at the neuromuscular junction appear to suggest CLN7 plays a role in synaptic trafficking controlling bouton growth regulation.

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PC33

The effect of glioblastoma cells on the function of efflux transporters in the blood-brain barrier

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Background and Aim: Efflux transporters such as P-glycoprotein (Pgp), breast cancer resistance protein (BCRP), and multi-drug resistance associated proteins (MRPs), are expressed on the blood- facing surface of blood-brain barrier (BBB) endothelia, and help restrict the movement of compounds from blood to brain. This has been a major problem for delivery of therapeutic drugs to the brain and particularly in treatment of brain tumours because chemotherapeutics are substrates for efflux transporters. It is unclear however why brain tumours are so resistant to chemotherapy and whether BBB efflux transporters play a role. Here we investigate whether BBB efflux transporters are affected during tumour progression in the brain using an in vitro assay.
Methods: An efflux activity assay was developed, where 3H-MPP+ (a substrate of Pgp, BCRP and MRPs) was loaded into primary porcine brain endothelial cells (PBECs), and 3H-MPP+ efflux into an extracellular buffer was then measured in the presence and absence of Pgp, BCRP and MRPs inhibitors (verapamil 50μM, haloperidol 60μM, prazosin 35μM Ko143 0.2μM MK571 10μM). Efflux studies were conducted on PBECs in mono-culture or in non-contact co-culture with either primary rat astrocytes or C6 rat glioblastoma cells on suspended Transwell® filters.
Results and Conclusions: The presence of inhibitors decreased apical efflux of 3H-MPP+ from PBECs co-cultured with primary astrocytes, demonstrating functionally active Pgp, BCRP and MRPs at the apical (blood face) of the endothelia. The % of apical efflux due to activity of each of the transporters was Pgp 29.4±1.8%, BCRP 20.0±0.7%, MRPs 14.9±0.8% (n=3-8) demonstrating the dominance of Pgp in these cells. When BBB endothelial cells are exposed to C6 glioma cells, Pgp and MRP activity was attenuated, but in contrast BCRP activity was up-regulated. MPP+ efflux due to each transporter was; Pgp 17.8±0.9%, BCRP 29.6±1.5%, MRPs 9.2±0.4%. Down-regulation of Pgp and MRPs would be beneficial for delivery of some chemotherapeutics which are substrates for these efflux transporters, however, up-regulation of BCRP may partially compensate for the decrease in other transporter activity. It

may, however, be beneficial for treatment strategies to avoid chemotherapeutics that are substrates for BCRP, such as the anthracyclines (doxorubicin, mitoxantrone) used in the treatment of gliomas.

Abbott NJ et al. Neurobiol Dis. 2010 37(1):13-25

We are grateful for the support of BBSRC CASE award in partnership with AstraZeneca.

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

PC34

Chronic inflammation increases expression of hyperpolarization-activated and cyclic nucleotide-gated channel subunit 2 in small dorsal root ganglion neurons in the rat

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Chronic inflammatory pain is characterised by hypersensitivity to painful stimuli (hyperalgesia) and/or normally non-painful stimuli (allodynia). This hypersensitivity is partly due to sensitization of primary afferent dorsal root ganglion (DRG) neurons innervating inflamed tissue. These sensitised neurons exhibit increased excitability (Woolf & Ma, 2007). Hyperpolarization-activated and cyclic nucleotide-gated cation channels, which consist of four subunits (HCN1-4), are key modulators of neuronal activity (Biel et al. 2009). The aim of the present study was to examine whether chronic inflammation alters HCN1-3 channel protein expression in DRG neurons. We focused on these subunits because they are clearly expressed in rat DRG neurons, whereas expression of HCN4 is uncertain (Chaplan et al. 2003). Inflammation was induced by two injections of 100μl of Complete Freund's adjuvant (CFA) into the left hindpaw and left knee region of female Wistar rats (150-175g) under 3% isoflurane anaesthesia. Seven says post CFA, we performed immunohistochemistry in L4/L5 DRGs and spinal cord, from control and CFA treated rats, using well characterised anti-HCN1-3 antibodies (Alomone Laboratories). We also examined both the control and CFA tissues for co-localization of HCN1-3 with isolectin IB4, a neuronal marker of a subpopulation of C-fibre neurons. The somatic sizes of HCN1-3 positive neurons were measured and divided into 3 groups: small diameter (≤30 μm), medium-diameter (~31-40 μm) and large diameter (>40 μm) neurons.Seven days after CFA-induced inflammation, there was a significant increase ($P < 0.01$, Mann-Whitney U test) in HCN2-immunoreactivity in small neurons (putative nociceptors) determined by densitometric comparison of DRG sections from control and CFA rats and in the percentage of small and medium DRG neurons-expressing HCN2 ($P<0.01$, Fisher's exact test). Increased HCN2-immunoreactivity was also seen in axons (possibly both the peripheral and central processes) of these small neurons-expressing HCN2, as well as the superficial dorsal horn of the spinal

cord, where the central terminals of C- and Aδ- nociceptive neurons terminate. In contrast, there was no change in HCN1- or HCN3- immunoreactivity in any cell-type of DRG neurons or spinal cord following CFA-induced inflammation. These results are consistent with recent findings (Papp et al. 2010) that show increased HCN2-immunoreactivity in the axon terminals of C- and Aδ-fibre neurons in the superficial dorsal of the spinal cord, 3 days after CFA-induced inflammation. Taken altogether, the data suggest that HCN2 channels contribute to the hyperexcitability of DRG neurons and hypersensitivity associated with tissue inflammation.

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Supported by MRC grant to LD.

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

PC35

Retinal ganglion cell axons need microRNA function for correct pathfinding during mouse visual system development

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The visual system has been largely investigated to identify the molecular mechanisms controlling the formation of neuronal connectivity during development. In animals with binocular vision, the optic chiasm is a major decision point where retinal ganglion cell (RGC) axons arising from the eyes sort into ipsi- and contralateral projections. In mice, about 97% of the axons cross to the contralateral side, whereas the rest stays ipsilaterally, projecting towards the lateral geniculate nucleus and then into the superior colliculus. Here, RGC axons form connections that obey the rules of a defined topographic map.

In the last years intensive studies in the field highlighted the roles of several polypeptide-encoding genes controlling the different morphogenetic subroutines, which ensure the correct wiring of the visual system. Recently, through the use of conditional inactivation of RNase III enzyme Dicer, we and others demonstrated that microRNAs (miRNAs), a class of small non-coding RNAs, are fundamental regulators of retinal histogenesis and axon guidance decisions at the optic chiasm.

Our results show that mouse embryos with an early loss of Dicer –from E7.5- in the retina and in the ventral diencephalon, exhibit a microphthalmia phenotype associated to a high rate of apoptosis during neurogenesis. In these mutant embryos we also noticed a significant increase of ipsilateral projections and defasciculated axons at the optic chiasm and in the retina. Moreover, a considerable number of

RGC axons aberrantly project from one eye into the other or enter the diencephalon ectopically. Spatiotemporal transcripts regulation of genes involved in patterning and axon guidance due to the direct or indirect action of miRNAs, both in the optic chiasm and in the retina, may lead to the detected phenotypes in mice conditionally Dicer deleted. Nevertheless, using in situ hybridization and immunohistochemistry we find that Dicer mutant retinae and optic chiasm are patterned normally along both axes without showing any changes of guidance molecule expression pattern. Similarly, the number and location of Zic2-expressing RGCs is unchanged in Dicer mutant mice, compared to wildtype, suggesting that the specification of the ipsilateral retinal domain is not controlled by miRNAs.
At the moment, in order to define a possible cell-autonomous way-of–action of miRNAs during axon outgrowth, we are analyzing mouse embryos with Dicer deletion only in subset cells of retina from E10.5. This will help to clarify the molecular pathways involved in RGC axon extension, pathfinding and synapse formation. Our work presents a role for miRNAs as a linchpin for the establishment of the visual circuitry during development.
Support: MRC G0601182 and Wellcome Trust 087883/08

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PC36

Early motor development, activity-dependent plasticity, dystonia & deep brain stimulation (DBS)

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Dystonia is thought to arise from excessive cerebral plasticity & reduced inhibition. Dystonia seems activity-dependent. Dominant genes predispose to dystonias & Brain Derived Nerve Growth Factor (BDNF) single nucleotide polymorphisms may determine manifest cariers. Rapid repetitive pre-synaptic stimulation produces release of BDNF from electrically active neurones & enlargement of dendritic spines leading to Long Term Potentiation (LTP). LTP is enhanced in immature compared to adult brain. By contrast, inactivity, immobility, slowly repetitive pre-synaptic stimulation may lead to reduction in AMPA receptors in post-synaptic membranes stimulating type I metabotropic glutamate receptors that activate phosphoinosotide turnover in dendritic spines leading to Long Term Depression (LTD) & reduced BDNF production. Primary dystonias utilize fundamental mechanisms for neuronal sensori-motor organization, though the balance between LTP & LDP may underpin many other functions including memory. Repetitive transcranial magnetic or direct current stimulation may reduce cortical plasticity & increase inhibition, altering LTP-LTD neuronal balance in focal dystonias, offering benefits lasting weeks. Deep brain stimulation (DBS) may continuously maintain a functional neuronal LTP-LDP

balance over many years. Potent environmental inputs may initiate a subtle reorganization of the sensori-motor plasticity essential for normal human function & development.
Dystonic disordered motor control resembles that of developing babies & infants adapted by a long underwater evolutionary process. In water, slow movements are economical but rapid movements inhibited by viscous drag: the occasional flick is possible to dart away but cannot be sustained. Water exerts a constant pressure on all surfaces of the body. Gravity is partly counter-balanced by buoyancy. In air, buoyancy & viscous drag are lost, gravity bears down. The movements of the newborn are slow & ponderous, but within months become frankly fast, rhythmic or fragmented, though undirected. In infancy (1-12 months) bizarre limb postures & attitudes flow in a constant rehearsal of future purposeful movements. These infantile motor patterns appear dystonic, exhibiting abundant plasticity & little inhibition. Early excessive movements offer the immature motor system opportunities to explore the physical environment. By contrast the immobile embryo, newborn or infant has a bleak neurodevelopmental prognosis without exploratory motor behaviours to gain rewards for their actions. Certain forms of cerebral palsy may represent excessive cerebral plasticity.

This work has been supported by Guy's & St Thomas' Charity

Project Code G060708

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PC37

Mapping the dynamics of oculomotor nerve projections to the extraocular muscles in the zebrafish and the role of α2-chimaerin

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In vertebrates, eye movements are controlled by six extraocular muscles, innervated by three cranial nerves – the oculomotor, the trochlear and the abducens. Studies in the chick embryo have shown that the oculomotor nerve (OMN) undergoes a stereotyped pattern of outgrowth and branching to its four muscle targets(1). Perturbations of this wiring pattern in humans give rise to congenital eye movement disorders such as Duane's Retraction Syndrome (DRS), which can arise due to mutations in the RacGAP signalling molecule α2-chimaerin(2). However, the dynamics of axon behaviour which govern topographic axon projections in the ocular motor system have not been characterised, nor has the role of α2-chimaerin been elucidated. We have therefore used the zebrafish model system to study the developmental dynamics of axon guidance to the extraocular muscles, and the role of α2-chimaerin in this process.

We have used two-photon time-lapse imaging of the Isl1-GFP transgenic zebrafish line, between 24 and 96 hours post fertilisation, to map the normal development of the OMN and to describe its dynamics at key time-points, e.g. branching decisions. Here we show that the OMN first projects filopodia over a wide area, before restricting protrusions to particular areas of the environment corresponding with muscle anlage. Together with immunostaining of embryos at fixed time points, these movies have also revealed a hierarchical order of appearance of oculomotor axon segments. Mosaic expression techniques to image single GFP-expressing neurons have revealed that axons project from individual OMN subnuclei to muscle targets. This suggests that neuromuscular connectivity is generated by direct axon projections from subnuclei to muscles, rather than axon branching to multiple muscles and subsequent pruning. We have also found that single OMN neurons which express α2-chimaerin harbouring human mutations display extensive filopodial extension and exploratory behaviour as for wild-type axons. However, axons expressing mutant α2-chimaerin do not restrict their protrusions to select a particular muscle target, resulting in a cell-autonomous stalling phenotype. We are currently creating stable transgenic zebrafish lines expressing mutant forms of α2-chimaerin. This will allow us to use time-lapse imaging to model the human DRS phenotype and to investigate the effects of α2-chimaerin mutations on the entirety of cranial nerve projections to the extraocular muscles.

Chilton, J. and Guthrie, S. (2004). J. Comp. Neurol. 472, 308-317.

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Funded by Fight For Sight.

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

SA01

Live imaging reveals novel aspects of asymmetric division and neurogenesis

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During embryonic neurogenesis many ventricular zone progenitors divide to generate one daughter that retains progenitor potential and replenishes the ventricular progenitor pool while the other daughter becomes a neuron and migrates away to the developing grey matter. We have used the superior optics of the zebrafish brain and live imaging techniques to examine how this asymmetry in daughter fates is achieved and observe an unexpected twist to the origin of the neuron. Previous hypotheses had suggested that the daughter that inherits the apical domain is most likely to retain progenitor characteristics while the more basal daughter becomes the differentiated cell. However our time-lapse analyses shows that the cell destined to become a neuron is derived from the more apical of the two daughters, while the more basal daughter replenishes the apical progenitor pool. In addition we observe novel cell behaviours during the very early stages of neuronal differentiation that lead us to propose a new mechanism for generating neuronal spacing patterns in the spinal cord.

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SA02

Functional characterization of motion-sensitive inputs to the zebrafish optic tectum

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In the visual system different classes of retinal ganglion cell (RGC) respond to and convey qualitatively distinct information about the visual environment to the brain. From these parallel inputs the brain builds a complete and coherent representation of the visual scene and then generates appropriate behavioural responses. We are using the optic tectum of larval zebrafish as a model system to ask how the brain performs these functions and how the underlying circuitry develops. A prerequisite to understanding how visual information is processed by the tectum is a detailed description of the nature and organisation of visual inputs to the tectum. To describe both the functional properties and laminar organization of different types of RGC input to the tectum we have fused the synaptic vesicle protein, synaptophysin to the genetically encoded calcium sensor GCaMP3 (SyGCaMP3). We have generated a stable transgenic line of zebrafish that express SyGCaMP3 specifically

in RGCs. By combining in vivo imaging of SyGCaMP3 responses with patterned visual stimulation we can record stimulus-evoked calcium influx at identified presynaptic terminals in the brain. We are also able to assign RGC terminals with defined response properties to defined locations within the tectum. Using this approach we are building a map of motion-sensitive inputs to the tectum. By performing these experiments at different developmental stages we can ask how this map develops and how the functional properties of RGCs themselves emerge. We hope that this information will serve as a useful point of reference for understanding how visual information is processed within the tectum.

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SA03

Development of respiratory rhythm generating circuits in the mouse embryo hindbrain

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We breathe roughly half a billion times in a lifetime, generally in an effortless and even unconscious manner owing to activity of a respiratory central pattern generator (CPG) located in the hindbrain. The respiratory CPG relies on the coupling of two prominent rhythmogenic sites located in the medulla, the pre-Bötzinger Complex (preBötC) and the para-Facial Respiratory Group (pFRG). Working in the mouse embryo, we have identified the emergence of forerunning versions of these two oscillators using developmental genetics tools, electrophysiological and optical recordings. We have defined molecular and functional signatures for cells destined to form each oscillator. More precisely, we have shown the independent development of (i) an Egr2- (also known as Krox20-) derived, Phox2b/Lbx1/Atoh1-expressing embryonic parafacial oscillator and (ii) a Dbx1-derived population of glutamatergic interneurons required for both preBötC rhythm generation and bilateral synchrony. These results indicate that each oscillator is not assembled from cells of disparate origins. Rather, each oscillator is made of cells featuring selective built-in functional properties that derive from a discrete transcriptionally defined domain of the neuroepithelium. Hence, the dual organisation of the respiratory CPG seems to reflect the modular origin of its composing cells.

Work supported by CNRS, INSERM, ANR-07-Neuro-007-01 grant to GF

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SA04

Building thalamocortical connections

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The neocortex, which is hallmark of mammals, controls essential brain functions via a remarkable architecture of its internal microcircuits as well as via an extensive wiring with the rest of the brain. In particular, topographically organized thalamocortical connections convey sensory and motor input from the periphery to distinct areas of the neocortex. During embryonic development, thalamocortical projections establish a blueprint of the neocortical extrinsic connectivity, which can be remodeled postnatally. Understanding how these projections are formed is essential not only to progress in our comprehension of neural wiring and neocortical functioning, but also to unravel the mechanisms underlying the "high-jacking" of major functions by the neocortex during the evolutionary emergence of the mammalian brain.
We previously showed that mouse thalamocortical connections are guided internally towards the neocortex by the tangential migration of guidepost "corridor" neurons in an intermediate target. Here, we show that corridor neurons act as a hub to orient the topographic positioning of thalamocortical projections towards distinct cortical areas, by expressing combination of guidance cues that elicit expected and paradoxical responses in thalamic axons. These results reveal a novel function of cell migration in the internal pathfinding and topographic orientation of thalamocortical projections and raise the question of how this process emerged during evolution. Using comparative studies in mammals, reptiles & birds, as well as functional experiments, we found that species-specific differences in the migration of conserved corridor neurons regulate the opening of a mammalian neocortical route for thalamic axons. We further show that the midline repellent Slit2 orients the migration of corridor neurons and thereby switches the position of thalamic axons to a mammalian-specific path. Our study reveals that subtle differences in the migration of conserved intermediate neurons trigger large-scale changes in thalamic connectivity, and opens novel perspectives on the development and evolution of brain wiring.

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SA05

Sensory-driven plasticity in developing visual circuits

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The development of neural circuits involves a diverse array of endogenous and exogenous signals, including both spontaneous and environmentally driven neural activity. Early seminal experiments illustrated the remarkable sensory-driven plasticity of the developing visual system, and later studies have established that activity is not just permissive but plays an instructive role in the formation of visual circuits. Evidence suggests that neurons can learn about the spatiotemporal properties of the visual environment by utilising temporally asymmetric Hebbian learning algorithms, such as spike-timing dependent plasticity. However, it is not known how developing systems control the statistical properties of their activity in order to ensure that features in the environment are translated into functional properties of their circuits. I will describe results from a series of experiments using in vivo recordings in the optic tectum of Xenopus laevis embryos during early stages of development. These show that the receptive field of tectal neurons can be "trained" by repeatedly presenting a visual stimulus and that the resulting changes reflect the spatiotemporal properties of the training stimulus. At these stages of development, tectal neurons are establishing their synaptic connections with presynaptic glutamatergic and GABAergic neurons. I will present evidence that local GABAergic circuits are critical for sensory-driven plasticity. When GABAergic transmission in the tectum is disrupted, the instructive effect of the visual input upon receptive fields is eliminated. This elimination of instructive learning is linked to changes in spike-timing patterns because when GABAergic inputs are blocked, there is a substantial increase in the spike-timing correlations between tectal cells and greater potential for tectal-tectal synaptic plasticity. In support of this, instructive learning is eliminated when spike-time correlations between tectal neurons are artificially increased by electrical stimulation and the relative timing of synaptic inputs to tectal cells is consistent with this role for GABAergic signaling. Rather than decreasing the variance in spike-timing, as they do in many adults systems, early GABAergic circuits in the optic tectum enhance spatiotemporal differences in spiking patterns and minimise correlations that may be introduced via recurrent excitation. This may provide a mechanism to ensure that receptive field changes are primarily instructed by the statistics of the visual environment.

This work was supported by the Biotechnology and Biological Sciences Research Council (BB/E0154761), the Medical Research Council (G0601503) and the European Research Council under the European Community's Seventh Framework Programme (243273).

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SA06

The embryonic origin of GABAergic hub neurons in the developing hippocampus

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Connectivity in the developing hippocampus displays a functional organization particularly effective in supporting network synchronization, as it includes superconnected hub neurons. We have previously shown that hub network function is carried out by a subpopulation of GABAergic interneurons that display dense and widespread axonal arborisations (Bonifazi et al. Science 2009). However the fate of hub neurons remains unknown. Specifically it is unclear whether these hub cells are only transiently present or later develop into distinctive subclasses of interneurons. These questions are difficult to assess given the complexity of the GABAergic neurons and the poor expression of interneuron markers at early developmental stages. To circumvent this conundrum we used "genetic fate mapping" that allows for the selective labelling of interneurons based on their place and time of origin. Following theoretical predictions, we tested the hypothesis that pioneer cells could develop into hub neurons.

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SA07

Disentangling electrical and chemical synaptic signalling in a network of inhibitory interneurons

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Relatively little is known about how electrically coupled interneuron networks respond to excitatory synaptic input. Cerebellar Golgi cells are an attractive system to study electrical signalling because they do not form reciprocal chemical synaptic connections. Using paired whole-cell recordings from acute slices of mouse cerebellum, we show that gap junction-mediated potentials have a largely inhibitory effect on neighbouring Golgi cells. This 'electrical inhibition' is due to the propagation of afterhyperpolarising potentials through connexion-36 gap junctions located on dendrites. Neighbouring Golgi cells tended to synchronize their activity under quiescent conditions. However, stimulation of excitatory mossy fibre input triggered different behaviours in directly innervated Golgi cells and those that did not receive an input. Moreover, when the excitatory input occurred out-of-phase

with the intrinsic firing, antiphase firing was triggered. Biologically detailed network models closely reproduced the experimental results and predict that sparse synaptic excitation causes a transient desynchronization of spiking across the network. Our results suggest that several features of sensory-evoked behaviour in the cerebellar granule cell layer could arise from synaptic excitation of the electrically coupled Golgi cell network.

Funded by the MRC, BBSRC and Wellcome Trust.

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SA09

RNA Binding proteins in Neurodegeneration

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TAR DNA binding protein (TDP-43) and Fused in Sarcoma (FUS) are predominantly nuclear proteins that regulate gene transcription, splicing and transport. They are also involved in micro-RNA biogenesis. TDP-43 is the major protein component of cytoplasmic inclusions in fronto-temporal lobar dementia (FTLD-TDP-43) and amyotrophic lateral sclerosis (ALS) while FUS is deposited in a small number of MAP tau and TDP-43 negative FTLD cases. Mutations in the genes encoding TARDBP and FUS are detected in 1-4% of familial ALS cases and are also seen in ~1% of sporadic ALS cases. TDP-43 and FUS mutant proteins show reduced nuclear import and are neurotoxic strongly implicating mislocalisation in the pathogenesis of ALS and FTD. Mutations are not detected in the vast majority of ALS and FTLD cases in which these proteins are deposited. Thus the mechanisms involved in regulating the nuclear import or cytoplasmic degradation may be important in the pathogenesis of these disorders.

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

SA10

An unusual mouse modeling aspects of Down syndrome

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Down syndrome in humans is caused by trisomy of chromosome 21 (Hsa21). It is a relatively common, complex disorder that arises from a defect in gene dosage.

If we knew which of the ~300 genes on Hsa21 were involved in which aspects of the syndrome, such as the leukemia, early onset dementia, etc., then it would help us understand which pathways were involved and help in designing treatments not only for the Down syndrome population but also for the euploid population which is affected by the same disorders. A handful of mouse models exist that result in trisomy for portions of the regions homologous to human chromosome 21. We have taken models these a step further by placing human chromosome 21 into a mouse and so creating a strain that is aneuploid, and carries a freely segregating human chromosome. We have conducted a phenotypic analysis of this strain and find that it does indeed model human Down syndrome. This model plus other new mouse strains will help us dissect the link between genotype and phenotype in this disorder.

We thank the Wellcome Trust, the UK Medical Research Council, the Brain Research Trust, and the AnEUploidy grant from Framework Programme 6 from the European Union Commission for funding. We thank Ray Young for graphics.

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SA11

Loss of BDNF in Huntington's Disease: from molecular mechanisms to therapeutic strategies

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Changes in the level and activity of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), have been described in a number of neurodegenerative disorders, including Huntington's disease, Alzheimer's disease and Parkinson's disease. It is only in Huntington's disease, however, that gain-of-function and loss-of-function experiments have linked BDNF mechanistically with the underlying genetic defect. Altogether, these studies have led to the development of experimental strategies aimed at increasing BDNF levels in the brains of animals that have been genetically altered to mimic the aforementioned human diseases, with a view to ultimately influencing the clinical treatment of these conditions. In this presentation, I will focus on available data concerning changes in BDNF levels in HD cells, mice and human postmortem samples, describe the molecular evidence underlying this alteration, and review the data concerning the impact of the experimental manipulation of BDNF levels on HD progression. Finally, I will describe how the targeting of a specific mechanism that is responsible for the BDNF dysfunction could be a valid option to increase BDNF in HD.

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

SA12

Mediation of synaptic dysfunction by tau mislocalisation to dendritic spines

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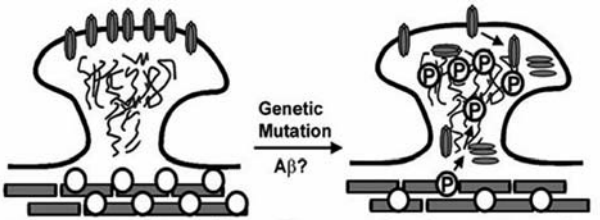
Alzheimer's disease (AD) is a progressive neurodegenerative disease that exhibits gradual memory loss. The two histopathological hallmarks of AD are beta-amyloid plaques containing Amyloid beta proteins (Abeta) and neurofibrillary tangles containing phosphorylated tau proteins. Although Abeta and tau proteins have been proposed to be the main contributors to memory loss in AD patients, their roles in different stages of pathological development are still unclear. Recent advances in AD research indicate that brain dysfunction precedes neurodegeneration. Here, we show that early tau-related deficits develop not from the loss of synapses or neurons, but rather as a result of synaptic abnormalities caused by the accumulation of hyperphosphorylated tau within intact dendritic spines, where it disrupts synaptic function by impairing glutamate receptor trafficking or synaptic anchoring. Mutagenesis of 14 disease-associated serine and threonine amino acid residues to create pseudohyperphosphorylated tau caused tau mislocalization while creation of phosphorylation-deficient tau blocked the mis-targeting of tau to dendritic spines. Thus, tau phosphorylation plays a critical role in mediating tau mislocalization and subsequent synaptic impairment. Interestingly, our recent experimental results show that Swedish mutation in Abeta can also aberrantly drive tau proteins to dendritic spines. These data establish that Abeta- and tau-mediated pathologies are likely to converge on dendritic spines.



Tau proteins (circles) are driven to dendritic spines after phosphorylation (circles with "p"), causing synaptic dysfunction

Sources of funding for this study include B. Grossman and her family, the American Health Assistance Foundation (D.L.) and the National Institutes of Health (NIH) of the United States (R01-DA020582, K02-DA025048 to D.L.; R01-NS049178 to L.M.L.; T32-DA007234 to R.D.P.; R01-NS049129 to L.L.Y.; T32 DA022616-02 to M.N.R.; R01-AG026252, R01-NS063214 to K.H.A.).

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SA13

Molecular approaches to investigate the PTEN tumour suppressor in the mouse CNS

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Loss of the PTEN tumour suppressor has recently been identified as a persuasive target for increasing regenerative capacities of neurons affected in degenerative conditions or following injury to the nervous system. In mice, for example, loss of PTEN expression has been shown to promote axonal elongation and increases survival in cell bodies and axon terminals of degenerating motor neurons, as well as promoting regenerative growth of axonal processes following injury to the CNS. Here, we present a technique to specifically tune PTEN function in mammalian tissue with high specificity and temporal control. In this technique, destabilising domains derived from *E. coli* dihydrofolate reductase (DHFR*) or from the FK506-binding protein 12 (FKBP*) are fused to proteins of interests, causing their efficient degradation. Presence of DHFR* or FKBP* specific ligands - Trimethoprim (TMP) and Shield, respectively - stabilise tagged proteins and confer biological activity. We combined in utero electroporation (under isoflurane in O_2 anaesthesia) of DHFR* or FKBP* tagged proteins into the cortex of mouse embryos with subsequent stabilisation by systemic application of TMP or Shield. We established methods to deliver the synthetic ligands, analysing their abilities to cross the blood-brain barrier and tested the functional efficacies of stabilized proteins.
We successfully generated active Cre-DHFR* and FKBP*-PTEN protein expression systems, which, in combination with floxed-PTEN mouse alleles, enables precise control of PTEN-loss and re-installing PTEN. In this way, PTEN levels and activity can be managed precisely within specific experimental paradigms. We believe such information will prove essential for understanding the degree in which transient PTEN inhibition can indeed be seen as an amenable target for reinstalling growth in degenerative conditions or following injury to the CNS.

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

SA14

Increasing the regenerative ability of axons

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Most CNS axons have a low intrinsic ability to regenerate, the reasons for which remain to be solved. Three possible reasons are:
Local mRNA translation. Axons in the peripheral nervous system contain many mRNAs, and the machinery to translate these into proteins. This ability is important for axon regeneration, because blocking local translation inhibits axon regeneration. Comparing the mRNAs from embryonic and adult PNS axons there are many changes, including the absence of kinesin mRNAs in adult axons. We find that one of these, kif3C, plays a key role in growth cone regeneration.
Integrins and axon regeneration. In order to grow through the extracellular matrix axons must express appropriate integrins. The main matrix glycoprotein in the damaged CNS is tenascin-C, but tenascin-C binding integrins are lacking. We have transfected alpha9 integrin into neurons, giving them the ability to grow long axons on tenascin in vitro. Transduction of DRG neurons in vivo enhances their ability to regenerate their axons, but only modestly. One problem is that integrin transport into axons is blocked at the axon initial segment. Integrin trafficking relies on Rab11 and Rab coupling protein. Another problem is that CNS inhibitory molecules inactivate integrins.
Gangliosides and axon regeneration. Axons contain the membrane enzyme PMGS, which desialyates GD1a ganglioside to produce GM1. We find that axotomy in halothane-anaesthetised rats activates PMGS, converting axonal ganglioside to GM1, and that blocking the enzyme inhibits axon regeneration. Retinal axons from the CNS do not convert their surface gangliosides to GM1 after axotomy, but application of an external sialidase causes both ganglioside conversion and promotes axon regeneration. The activation pathway involves Ca2+ and P38.

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

SA15

Promoting axonal regeneration within an inhibitory environment

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Large spinal cord or brain injuries lead to life-long, often major functional deficits. In contrast, smaller lesions of the CNS often have a good prognosis with extensive functional recovery; the underlying mechanisms are not well understood, however. In adult rats, spinal cord injury transecting the hindlimb corticospinal (CST) axons or stroke-mediated destruction of parts of the motor cortex induce spontaneous sprouting of spared fibers in the upper spinal cord and brain stem. Rehabilitative training enhances both the functional recovery of precision movements and the anatomically demonstrated fiber growth. Following hindlimb CST axotomy, forelimb sensory connections expand to the former hindlimb motor cortex that now is re-connected to the forelimb spinal cord, demonstrating a major map shift of the sensory and motor somatotopic representations. In all these cases, however, extent and length of fiber growth was limited to about 0.2 - 2 mm.

Twenty years ago, our group has discovered the presence of specific neurite growth inhibitiory factors in myelin of the CNS, among which the membrane protein Nogo-A, currently the most potent known neurite growth inhibitor. Function blocking antibodies against Nogo-A have been generated and applied to rats and macaque monkeys with spinal cord injuries as well as animals with strokes in the sensory-motor cortex. Biochemical readouts showed an up-regulation of growth specific proteins. On the anatomical level, injured fibers showed enhanced regenerative sprouting as well as long-distance regeneration with formation of large terminal arbors. Simultaneously, spared fiber tracts showed enhanced compensatory sprouting, often covering relatively long distances. In animals with cortical strokes, fibers from the intact corticobulbar or corticospinal system crossed the midline, supplying innervation to the denervated brain stem or spinal cord under the influence of anti- Nogo-A antibodies. Behavioural experiments for locomotion, grid and beam walk, swimming, as well as skilled forelimb reaching showed marked improvements of functional recovery in the Nogo-A antibody treated injured animals. These results show that the spontaneously occurring repair processes after CNS injury can be potentiated by the functional suppression of the endogenous neurite growth inhibitory protein Nogo-A. In collaboration with Novartis, a clinical trial is correntlly conducted in acutely spinal cord injured patients.

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SA18

Epigenetic reprogramming of brain cancer stem cells

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Cancer cells are driven by both genetic and epigenetic changes, but their relative contribution in driving the malignant phenotype remains unclear. We have used induced pluripotent stem (iPS) methodology to demonstrate that highly malignant and aneuploid human glioblastoma cells can be epigenetically reprogrammed. Glioblastoma-iPS cells (GiPSCs) activate expression of early embryonic markers such as NANOG, and display widespread reconfiguration of DNA methylation patterns including reactivation of aberrantly silenced tumour suppressor. Removal of epigenetic restrictions enables these GiPSCs to enter alternative differentiation programs in vitro and in vivo.

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SA19

From pluripotent stem cells to cortical circuits

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The cerebral cortex consists of several hundreds of different types of neurons, organized into specific cortical layers and areas, that display specific profiles of gene expression, morphology, excitability and connectivity.
The identification and characterization of factors capable of (re)specifying the identity of cortical neurons has important implications regarding our understanding of neurodevelopmental diseases and in the context of therapies for neurological disorders.
Embryonic stem (ES) and other pluripotent stem cells constitute a promising tool for the modelling and treatment of human neural diseases.
Here we describe a novel pathway by which pluripotent stem cells, whether of mouse or human origin, recapitulate in vitro the major milestones of cortical development, leading to the sequential generation of a diverse repertoire of neurons that display most salient features of genuine cortical neurons. When grafted into the cerebral cortex of newborn, or lesioned cortex of adult anaesthetised mice, these neurons develop specific patterns of axonal projections corresponding to endogenous cortical projections in vivo.
Intrinsic corticogenesis sheds new light on the mechanisms of neuronal specification, and constitutes an innovative tool to model normal and pathological cortical

development, including in the human species. In the long run, cortical neurons generated in vitro could be used also in the perspective of brain repair, for several diseases striking cortical neurons.

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SA21

Integrating epigenetic factors into studies of neuropsychiatric disease

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Given the high heritability estimates for most complex neurobiological disorders, current approaches to understanding etiology have primarily focussed on uncovering a genetic contribution to disease-onset. Whilst a number of novel susceptibility loci for disorders including Alzheimer's disease, schizophrenia and autism have been uncovered using recent genome-wide association approaches, these loci account for only a small proportion of attributable risk and the mechanism behind their action remains unknown. There is growing recognition that epigenetic mechanisms are important in the etiology of complex disease, acting at the interface between the genome and the environment. Recent technological advances mean that it is now feasible to study epigenetic marks such as DNA methylation at base-pair resolution across the genome. In this talk I will present data from our group showing how dynamic epigenetic processes are involved in neuropsychiatric phenotypes, and can be influenced by environmental, genetic and stochastic factors. We propose a novel etiological approach to complex disease based on the integration of genetic and epigenetic information.

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SA22

Differential expression and functions of protein-coding and noncoding genes across mouse neocortical layers

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In the mammalian cortex, neurons and glia form a patterned structure across six layers whose complex cytoarchitectonic arrangement has likely contributions to cognition. The cerebral cortex has a uniform laminar structure that historically has been divided into six layers. Sub-classes of pyramidal neurons and interneurons populate specific layers, each characterized by a different depth in the cortex with specific pattern of dendritic and axonal connectivity. However, analyzing these laminar differences is difficult and often suffers from subjectivity. We sequenced transcriptomes from layers 1-6b of the adult (P56) mouse primary somatosensory cortex to understand the transcriptional levels and functional repertoires of coding and noncoding loci for cells constituting these layers. 5,835 protein-coding genes and 66 noncoding RNA loci were found to be differentially expressed ('patterned') across the layers, based on a machine-learning model (naïve Bayes) approach. Layers 2-6b are each associated with specific functional and disease annotations that provide insights into their biological roles. This new resource greatly extends currently available resources, such as the Allen Mouse Brain Atlas and microarray data sets, by providing quantitative expression levels, by being genome-wide, by including novel loci, and by identifying candidate alternatively spliced transcripts that are differentially expressed across layers.

Funding has been provided by the Marshall Scholarship, New College Oxford, the NIH-Oxford-Cambridge Scholars Program, a Marie Curie Fellowship, the Intramural Research Program of the National Human Genome Research Institute, St. John's College, Oxford, and the UK Medical Research Council. We thank A. Hoerder-Suabedissen for sectioning; R.A. Chodroff, E.D. Green, A. Hoerder-Suabedissen, A. Heger, L. Goodstadt, M. Goodson, C. Webber and J. Becker for helpful discussions.

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SA23

Histone acetylation, memory and mental retardation

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Epigenetic changes of the chromatin, such as histone acetylation, represent an attractive molecular substrate for long-term memory and other forms of adaptation to the environment. We will discuss here the role of CBP, a histone acetyltransferase involved in mental retardation, in neuronal viability and plasticity using different strains of genetically modified mice. Our studies in forebrain-restricted CBP mutants show that the loss of this protein in forebrain principal neurons causes modest memory and transcriptional defects and a dramatic reduction of histone acetylation, but does not affect cell viability. In another line of research, morphological and behavioral analyses on cbp+/- mice demonstrate that environmental enrichment (EE) can ameliorate some deficits associated to CBP-deficiency, but these mice still show a strong defect in environment-induced neurogenesis that correlates with attenuation of the transcriptional program associated with this process and with impaired EE-enhanced spatial navigation. Overall, our experiments provide novel insights into the etiology of Rubinstein-Taybi mental retardation, clarify some of the standing questions concerning the role of CBP and histone acetylation in activity-driven gene expression, memory formation and neurodegeneration, and identify CBP-dependent transcriptional neuroadaptation as an important mediator of EE-induced benefits, a finding with important implications for mental retardation therapeutics.

Research at Barco's lab is supported by the grants from the Spanish Ministry of Science and Innovation BFU2008-00611, CSD2007-00023 and SAF2008-03194-E (part of the coordinated ERA-Net NEURON project Epitherapy).

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