

## EDITOR'S CHOICE

## EPILEPSY: Golden Oldies

There are few double-blind randomised clinical trials worthy of the name in epilepsy and those that do exist are fraught with difficulties including diagnostic heterogeneity. Childhood absence epilepsy is common and is easier to define than most types of epilepsy, although even this condition may be mistaken for other idiopathic epilepsies or occasionally for focal epilepsies. So it is a good condition to study and these authors have looked at very respectable numbers of patients (453 in total) and randomised them to receive ethosuximide, valproate or lamotrigine. They increased the dose incrementally until they achieved seizure-freedom. Bedside hyperventilation and one hour of video EEG were used to monitor whether seizure-freedom had been achieved at 16 weeks. This is an important measure as patients are not always aware of their own absences. Patients "failed" if they had ongoing absences, a single tonic clonic seizure, adverse effects or predefined changes in serum markers. 209 children had not failed treatment at 16 weeks (ethosuximide 53%, valproate 58% and lamotrigine 29%). Failures were due to lack of seizure control in 24% and adverse effects in 22% (valproate 12%, ethosuximide 14% and lamotrigine 47%). Adverse effects occurred in 24% of patients taking valproate and ethosuximide

and in 17% of patients taking lamotrigine. They also used something called the Connors' continuous performance test to assess cognitive function and quality of life in these children, whose activity had previously been interrupted by very regular absences. Valproate patients did significantly worse than the other two groups. So in conclusion, lamotrigine did not work well at controlling seizures whereas valproate and ethosuximide both worked well but ethosuximide had less neurocognitive adverse effects. Insofar as we understand the basis of absence epilepsy, there is an abnormality of thalamocortical function in which T-type calcium channels play a key part. Ethosuximide acts on T-type calcium channels so is the closest we have to a magic bullet in epilepsy. It is an endorsement of research into the understanding of basic biological mechanisms that this relatively purely targeted drug is more successful in this study than the other agents tested. Don't forget this drug in IGE, even in older patients. Moreover, it confirms that newer is not always better, but those of us who are longer in the tooth already know that.

– **Mark Manfred**

**Glauser T, Cnaan A, Shinnar S et al.**

**Ethosuximide, valproic acid and lamotrigine in childhood absence epilepsy. NEJM 2010;362:790-9.**

## 'And the deep lane insists on the direction'

That micro-electrodes can be inserted into distinct relevant parts of the brain of rat pups before their eyes have unsealed (~postnatal day 15, P15), and by recording, while they navigate for the very first time, reveal that representations of direction, and to a lesser degree spatial location, are relatively hard-wired, is staggering. These are two back-to-back papers from the teams of John O'Keefe at University College London ('Team Place') and Edvard and May-Britt Moser at the Kavli Institute, Trondheim, Norway ('Team Grid'). O'Keefe and colleagues are known for their identification in 1971 of cells in cornu ammonis 1 (CA1) of hippocampus that fire maximally if a rat is in a particular position in space: place cells. Taube identified head-direction responsive cells in 1990. The Mosers identified in 2005 cells in medial entorhinal cortex (MEC) that fire maximally when a rat passes a virtual point in space matching a vertex of a regular grid of equilateral triangles: grid cells. But how much of this tripartite hardware is generated from experience and learning, and how much is a pre-determined not-so-blank slate? Team Place recorded from P14 to P30 from rats foraging for food. Directional cells were present at P14, mostly in para-subiculum, place cells developing over P16 to P17, and grid cells at P20, rapidly developing to P22. Team Grid measured from P13, and also found directional cells first, P15, at the level found in adult rats. Place cells were seen at P16, with only a subsequent modest rise, and rudimentary grid cells were seen at P16 but only attained strict periodicity by P28. The groups disagree on the timing and development of nascent grid cells, and issues of cross-communication between cells are unresolved in these studies, but both groups show that experience (trial number) had a far weaker effect on cell number than age. Bartsch et al provide a nice human study, in the same issue of Science, of 14 patients with acute transient global amnesia, CA1 lesions and impaired place memory in a virtual Morris water maze.

– **Mike Zandi**

**Wills et al. (O'Keefe) Development of the Hippocampal Cognitive Map in Prewanling Rats. SCIENCE 2010;328:1573-6. Langston et al. (Moser) Development of the Spatial Representation System in the Rat. SCIENCE 2010;328:1576-80.**

**Palmer and Lynch. A Kantian View of Space. SCIENCE 2010;328:1487-8.**

**Bartsch T. et al. Focal Lesions of Human Hippocampal CA1 Neurons in Transient Global Amnesia Impair Place Memory. SCIENCE 2010;328:1412-15.**

**Eliot T.S. East Coker. London : Faber, 1940**

Immune cells and the brain  
new functions and interactions

There has always been a great deal of interest in the dialogue between the immune system and the brain, and we have regularly reviewed this area in the context of disease states such as MS and paraneoplastic or autoimmune conditions targeting receptors or ion channels. Of late though there have been a number of papers linking inflammation to neurodegenerative disorders and more recently epilepsy.

In this latter area, Maroso et al have shown in experimental work and human post mortem data that inflammation may be important in epilepsy and not just an incidental consequence of the pathological insult and/or ongoing ictal activity. These authors show that a variety of cells in the brain, such as microglia and possibly even astrocytes and neurons, secrete the well known intracellular chromatin associated protein; high-mobility group box 1 (HMGB 1)!! This protein seems to be released in some situations by these cells in epileptic tissue, and it then binds to the Toll-like receptor 4 (TLR4). This receptor is normally involved in the immune response of the organism to bacterial infections, and works through the release of inflammatory mediators such as interleukin 1 beta. Maroso et al have now shown that this receptor binds HMGB1 in animal models of epilepsy, which in turn causes a number of downstream effects in the different cell compartments, and that this may underlie the propagation of further ictal events such that blocking this system at an early stage may have significant anti-epileptic properties in terms of the severity and chronicity of epilepsy, a possibility reinforced in this paper by human post-mortem data on resected hippocampal tissue from patients with chronic temporal lobe epilepsy.

Another new area where the immune system may be having a role in CNS function is in the area of adult neurogenesis; a process by which new, mature, adult neurons are added to the hippocampus and olfactory bulb in the fully developed mammalian brain. In two recent papers, Hunt et al have shown that cyclosporin is good for this process, and Wang et al show that activated T-cells are bad for it!!

In the study of Wang et al, they show that activated T-cells can release granzyme B (GrB) and that this then binds to a G-protein associated receptor in the human neural precursor cells (NPCs) with a reduction in intracellular cAMP level. This process in turn causes an increase in the expression of the voltage dependent potassium channel, Kv1.3, in

these NPCs, which leads to an inhibition of NPC neurogenesis. The authors also show that these processes have some *in vivo* relevance and then try to link it to MS, by reporting that patients with MS have elevated levels of CSF GrB compared to controls. They do not, however, show that in MS, this process of altered neurogenesis is present nor relevant to disease course.

In the second study Hunt et al have shown that cyclosporin A (CyA) has a direct proliferative effect on NPCs *in vitro* and *in vivo*, and that this effect was not associated with any alteration in the differentiation profile of the cells derived from those NPCs. The exact mechanism by which CyA achieves this effect is not clear, but may relate to actions on cell adhesion within the NPC clones. Furthermore it is not clear exactly what this means *in vivo* given this study only looked at NPC turnover rather than differentiation, integration and function. Nevertheless this study adds further weight to the evidence that CyA has direct neurorestorative properties, independent of any effects it has on the T-cells, and that this may be of increased significance as we move towards the first clinical trials of allografted stem cells for patients with neurological disease.

– Roger Barker

Maroso M et al.

**Toll-like receptor 4 and high-mobility group box-1 are involved in iotogenesis and can be targeted to reduce seizures.**

NATURE MED 2010;16:413-19.

**Hunt Jet al. Cyclosporin A has direct effects on adult neural precursor cells.** J NEUROSCI 2010;30:2888-96.

**Wang T, et al. Activated T-cells inhibit neurogenesis by releasing Granzyme B: Rescue by Kv1.3 blockers.** J NEUROSCI 2010;30:5020-7.

## NMDA receptor-antibody mediated encephalitis?

The question of autoantibody pathogenicity in neurology has been investigated since the discovery of AChR antibodies in myasthenia gravis. In this disease, and subsequently in the peripheral nervous system diseases of Lambert-Eaton myasthenic syndrome and neuromyotonia, passive transfer of patient IgG to experimental animals mimicked some of the physiological and behavioural effects seen in the clinic (reviewed by Vincent 2006). Hughes et al examined the *in vitro* pathogenicity of N-methyl-D-aspartate receptor antibodies (NMDAR-Abs) in central nervous system neurons. NMDAR-Abs have recently been described in patients with an encephalitis characterised by psychosis, seizures, a characteristic movement disorder, dysautonomia and a reduction in consciousness (Dalmau et al 2007, 2008). Although initial reports suggested a very strong female predominance (around 8.5 females to 1.5 males) with the majority of patients having ovarian tumours, more recent studies have shown a predominance of non-paraneoplastic cases and upto 30% of cases being male (Irani et al 2010). Most patients respond to immunotherapies and/or oophorectomy and many regain independent function. Although NMDAR-Abs target the extracellular domain of the NR1 subunit of the excitatory NMDAR and NMDAR-Ab titres correlate well with outcomes in individual cases, the pathogenicity of these antibodies is yet to be formally, experimentally confirmed.

Hughes et al used various electrophysiological and immunofluorescent techniques to show that NMDAR-Abs were able to internalise NMDARs on the surface of hippocampal cultures and reduce surface NR1 subunit levels. In addition, and possibly more surprisingly, total cellular NR1 content was also reduced. Moreover, this downregulation did not affect the expression/localisation of the other receptors or postsynaptic proteins examined and did not alter the morphology or viability of the neurons. The reduction in NMDAR-density was also seen in hippocampi of mice infused with NMDAR-IgG for two weeks and humans who died of the condition. The magnitude of the effect was proportional to the concentration of the NMDAR-Abs. While this study strengthened the previous *in vitro* observations of Dalmau et al (2008), some important questions remain unanswered:

1. NMDAR-Abs are known to be predominantly of the complement fixing subtypes (IgG1-IgG3). No study has yet been presented to specifically examine the role of this potential cytotoxic or chemotactic mechanism.
2. If it is shown that NMDAR-Abs, when injected into experimental animals, can reproduce some of the clinical correlates of the human dis-

ease, this will have important therapeutic implications for patients, particularly those with emerging variants of the typical NMDAR-antibody encephalitis.

– Sarosh R Irani, Department of Clinical Neurology, Oxford.  
Hughes EG, et al. Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. J NEUROSCI. 2010;30:5866-75.  
and Vincent A. ACTA NEUROL SCAND 2006;Suppl;183:1-7.  
Dalmau et al. ANN NEUROL 2007;61:25-36.  
Dalmau et al. LANCET NEUROLOGY 2008;7:1091-8.  
Irani et al. BRAIN 2010;133(Pt 6):1655-67.

## On the bus-like arrival of ALS genes: eyeing up a new protein

For some 15 years there had been essentially only one model of ALS: mutant SOD1. Things changed in 2006 with the realization that ALS (except SOD1 ALS) is characterised by TDP-43 pathology, and with the subsequent discovery of ALS-linked mutations of *TDP-43* and *FUS*. This has led to intense investigation of the role of RNA-processing in neurodegeneration. Somewhat like the buses, in the past 12 months, four more genes have been linked with ALS. Homozygous *spatacsin* mutations cause a very slowly progressive autosomal recessive juvenile ALS (Orlacchio et al 2009). Heterozygous mutations in *FIG4* cause ALS, which in some cases has an upper motor neuron phenotype (Chow et al., 2009). A single family with typical ALS was recently linked to a dominant mutation in *D-amino acid oxidase (DAO)* (Mitchell et al 2010). A fourth gene, *OPTN* encoding optineurin, is perhaps the most intriguing.

*OPTN* mutations were found in a handful of Japanese ALS cases (Maruyama et al 2010). Two different homozygous mutations, both predicted to cause premature termination, were found in consanguineous families. A further missense mutation was found in autosomal dominant kindreds. The pathogenicity of the homozygous mutations is likely to be due to a loss of function as a result of nonsense-mediated decay of mRNA transcripts. The dominant missense mutation may act through an as yet unknown toxic gain of function. Interestingly, *OPTN* mutations are a cause of primary open angle glaucoma (Rezaie et al 2002).

Maruyama et al went on to conduct pathological studies demonstrating that a case with the missense *OPTN* mutation had typical TDP-43 inclusions as seen in ALS. Interestingly, these inclusions were also positive for optineurin. Furthermore, they also found optineurin to be a component of inclusions in sporadic ALS, suggesting that optineurin may have a broader role in ALS pathogenesis. Even more surprisingly it appeared that optineurin was also a component of inclusions in SOD1 linked ALS. Their pathological studies were conducted in a somewhat unorthodox manner as they retained pathological specimens rather than taking consecutive sections and staining separately for ubiquitin, TDP-43 and optineurin. Nevertheless, it is intriguing that while TDP-43 seemed to separate SOD1 ALS from sporadic ALS, optineurin appears to be a common denominator.

Although its functions remain unclear, optineurin is an ubiquitously expressed, cytoplasmic and perinuclear protein. It associates with the golgi apparatus and is implicated in vesicle transport, apoptosis and transcription, mechanisms that have previously been implicated by other ALS genes. ALS-linked optineurin mutants were found by Maruyama et al to lack the ability to inhibit activation of the transcription factor NF- $\kappa$ B, while the mutation commonly associated with glaucoma retained this property. Further genetic and pathological studies are needed to determine the wider role of optineurin in ALS pathogenesis. It will also be interesting to elucidate how *OPTN* mutations can cause neurodegeneration in such seemingly disparate locations as the retina and the motor pathways.

– Jemeen Sreedharan, Guy's and St Thomas' NHS Trust, London.

**Maruyama H et al. Mutations of optineurin in amyotrophic lateral sclerosis.** NATURE 2010;465:223-6.

**and Orlacchio A, et al. SPATACSIN mutations cause autosomal recessive juvenile amyotrophic lateral sclerosis.** BRAIN 2010;133:591-8.

**Chow CY, et al. Deleterious variants of FIG4, a phosphoinositide phosphatase, in patients with ALS.** AM J HUM GENET 2009;84:85-8.

**Mitchell J et al. Familial amyotrophic lateral sclerosis is associated with a mutation in D-amino acid oxidase.** PROC NATL ACAD SCI USA 2010;107:7556-61.

**Rezaie T, et al. Adult-onset primary open-angle glaucoma caused by mutations in optineurin.** SCIENCE 2002;295:1077-9.

## Torsins and LAP-1: explaining neuronal vulnerability in DYT1 dystonia

A curious feature of many genetic conditions is that mutations in widely expressed genes cause tissue-specific illness. One example is DYT1 dystonia, a dominantly inherited form of primary dystonia which causes patients to suffer involuntary movements or postures due to co-contraction of antagonistic muscles. DYT1 dystonia is caused by a 3 base-pair deletion in the *TOR1A* gene which results in removal of a glutamic acid from the protein torsinA. This protein normally resides in the endoplasmic reticulum/nuclear envelope endomembrane system, but nobody really knows what it does. In animal models of DYT1 dystonia, neuronal nuclear membranes in all brain regions show characteristic abnormalities on electron microscopy (outpouchings originating from the inner nuclear membrane or blebs), whereas non-neuronal cells look normal. Is torsin A doing something different in neurones, or are neurones more vulnerable to this condition (and if so, why)?

Kim and colleagues began by focussing on one protein known to interact with torsinA – lamina-associated polypeptide 1 (LAP1). They disrupted the gene which encodes LAP1 and showed that the neurones of homozygous mutant mice exhibited the same nuclear membrane abnormalities as *TOR1A* mutant mice. They also showed that eliminating torsinA function in LAP1 heterozygotes caused the same blebs in neural and non-neural cells, supporting the functional relationship between torsinA and LAP1 and indicating that they are operating together in all tissues.

TorsinA is a member of a family of proteins which includes torsinB, torsin2 and torsin3. The authors postulated that these torsins may share redundant cellular functions and that differing expression patterns of family members may account for the neuronal vulnerability seen in DYT1 dystonia. They began by showing that torsinB protein levels were significantly higher in non-neural tissues than in neural tissue. They then showed that by selectively reducing torsinB levels (using lentivirus-mediated RNA interference), there was a dramatic increase in the frequency of nuclear membrane blebs in mutant torsinA cortical neurones and non-neuronal cells (fibroblasts in this case).

The paper argues that expression of torsinB in non-neuronal cells is largely responsible for protecting them from the disease mutation underlying human DYT1 dystonia. Other proteins also interact with torsinA (such as 'printror' and 'nesprin-3 $\alpha$ ') and they too might contribute to the cell-specific phenotype caused by torsinA dysfunction. The authors make the interesting point that polymorphisms in LAP1 and torsinB may affect penetrance of the *DYT1* mutation (which is incomplete) or alter the severity of symptoms in patients with DYT1 dystonia.

– David P Breen, Cambridge Centre for Brain Repair, University of Cambridge.

Kim CE, et al. A molecular mechanism underlying the neural-specific defect in torsinA mutant mice. *PNAS* 2010;107:9861-66.

## Occult PML

When faced with a patient with sub-acute cognitive decline and characteristic white matter changes on MRI, progressive multifocal leukoencephalopathy (PML) tends only to come into the differential diagnosis if the patient is immunosuppressed, whether from HIV, haematological malignancy or drugs. Ghuens et al describe five cases of CSF PCR+ or histologically-confirmed PML with minimal/occult immunosuppression and a literature review of similar cases. They found an additional 33 cases and of the total, 7 had hepatic cirrhosis, 5 renal failure, 2 were pregnant, 2 had dementia, 1 dermatomyositis, and 22 no diagnosis (of these, 5 had low CD4 counts). 71% of all cases were fatal within 120 months (median 8 months) and interestingly some patients stabilised or improved (after treatment with mirtazepine or interferon alpha, follow up only to 6-9 months).

There are problems with this study, acknowledged by the authors; a relatively large group of 19 patients had not been tested for HIV. The authors (although I'm not sure I agree!) felt the patients were very unlikely to have had HIV because 12 cases were before the AIDS epidemic, 16 had no histological features of HIV at autopsy and 14 had no haematological features of HIV. Furthermore, not all had had a thorough work up for immunosuppression since PML was unsuspected in life and

found only at autopsy (19 cases from table 1 had PML diagnosed on autopsy with no CSF PCR data so it could be assumed the cases without 'thorough work up' are these, although this is not elaborated upon in the paper).

It is clear those patients with, for example hepatic and renal failure have changes to their cellular immune system, and one could speculate that these changes were enough to predispose to PML. The 22 cases with no identified disease (assuming nothing was missed on pre-mortem testing) are also very interesting - the authors mention 5 cases with idiopathic low CD4 counts and mention that CD4 counts can fluctuate, thus speculating that the other cases may also have had changes in T cells normalised by the time their blood was tested. Speculatively, these cases may have had signalling defects downstream of lymphocyte numbers.

That 3 cases stabilised or improved (albeit with a short follow up time) is interesting and raises the possibility that cases with occult/ minimal immunosuppression do better and respond to treatment. It highlights also the importance of making an early diagnosis: although PML is untreatable, some agents hold promise (mirtazepine, mefloquine, IL2) and reversal of immunosuppression may improve outcome (as we have seen from the HAART era). If there is clinical suspicion for PML even in apparently immunocompetent patients, the CSF should be tested for JCV. And, as in many cases of rapidly progressive dementia with no clear cause and MRI changes, a brain biopsy should be considered and may test positive even if CSF JCV is negative (particularly given that post HAART, the sensitivity of PCR in the CSF decreases, and it could be speculated that the sensitivity of CSF PCR may be lower in patients with minimal immunosuppression).

– Wendy Phillips, Neurology Unit, Addenbrooke's Hospital, Cambridge, UK. Ghuens S, et al. Progressive multifocal leukoencephalopathy in individuals with minimal or occult immunosuppression. *J NEUROL NEUROSURG PSYCHIATRY* 2010;81(3):247-54.

## Rehabilitation: are variations in pulse after brain injury due to deconditioning or uncoupling?

Autonomic instability is a well-documented feature of the early stages of recovery following a severe brain injury. This may manifest with any of the signs or symptoms of sympathetic over-activity and has a putative neuroanatomical basis in lesions of the frontal lobe. Over time, unexpected variations in heart rate and blood pressure gradually resolve, and cardiovascular instability is not usually considered in the longer term amongst the survivors of acquired brain injury. This study looks at heart rate at rest and under conditions of submaximal exercise in a group of young males (age 7 – 13) surviving a severe brain injury. Given that ambulation was an inclusion factor, the group had all made a reasonable physical recovery. The control group was made up of age and BMI-matched subjects without health problems. Both groups had their heart rates measured at rest and on mobilisation at their "comfortable walking velocity". The brain-injured group had significantly higher resting and walking heart rates. Given that exercise should, at least in theory, form part of the longer term rehabilitation strategy for survivors of acquired brain injury, the potential for autonomic dysfunction perhaps needs to be considered. It would be enlightening to have a sense of the longer term cardiac morbidity and mortality in this patient group. While this is an important issue, given the increasing long-term survival rates following severe brain injury, there are a number of important questions that this study fails to address. What is the contribution of deconditioning? The study group had all sustained their brain injuries at different (undefined) points in the past and presumably were in hospital for different lengths of time. Adequate analysis of these variables or a more appropriate control group may have gone some way to addressing this fundamental issue.

– Lloyd Bradley, Western Sussex Hospitals Trust Katz-Leurer M et al. Heart rate and heart rate variability at rest and during exercise in boys who suffered a severe traumatic brain injury and typically-developed controls. *BRAIN INJURY* 2010;24(2):110-14.