NEUROPHYSIOLOGY

Cause for excitement

Axo-axonic cells (AACs) are interneurons that constitute the sole inputs to the axon initial segment of pyramidal cells and do not innervate other cell types. They are traditionally thought to provide only inhibitory input that is mediated by GABA (γ-aminobutyric acid). However, writing in *Science*, Szabadics and co-workers report a surprising role for these cells: they can trigger GABA-mediated excitatory as well as inhibitory postsynaptic responses in adult pyramidal cells.

> These results highlight for the first time a dual role for AACs in triggering excitatory as well as inhibitory responses in cortical microcircuitry

As action potential initiation is most likely to occur in axons, these synapses are in a prime position for influencing neuronal output. In the mature cortex, the GABA-mediated inhibitory effects of AACs on the postsynaptic membrane depend on the potassium chloride co-transporter 2 (KCC2), which promotes a net outflow of Cl^-, thereby reducing the intracellular concentration of Cl^-. Under normal circumstances, activation of ionotropic $GABA_A$ (GABA type A) receptors increases the membrane permeability to Cl^-, which causes a net flow of Cl^- into the cell, resulting in hyperpolarizing responses.

To study the function of AACs in the mature cortex, Szabadics and colleagues used high-resolution immunolocalization to reveal the distribution of KCC2 and its influence on the polarity of the postsynaptic response in rat and human layer 2/3 pyramidal cells. In both the rat and human neocortex, there was a substantially lower distribution of KCC2 on the axonal initial segments compared with the somatic and dendritic membranes, and the cytoplasm of pyramidal cells. The higher intracellular Cl^- concentrations associated with reduced KCC2 expression is likely to support $GABA_A$-mediated outflow of Cl^-, thereby promoting depolarizing responses.

To test this possibility, these researchers compared the effects of GABA-mediated AAC input to axon initial segments, and basket cell input to perisomatic regions of pyramidal cells in layer 2/3 rat somatosensory cortex. Basket cell input resulted excusively in hyperpolarizing responses that led to inhibitory postsynaptic potentials. By contrast, AACs gave rise to a relatively depolarized response, thereby increasing the likelihood of generating action potentials. Indeed, GABA-mediated AAC input triggered postsynaptic action potentials in the axon initial segments of two pyramidal cells that could not be achieved with basket cell input at any of their target sites.

These findings were confirmed in rat and human supragranular cortical layers in which single spikes generated a series of inhibitory postsynaptic potentials that in some cases were followed by longer lasting disynaptic excitatory postsynaptic potentials.

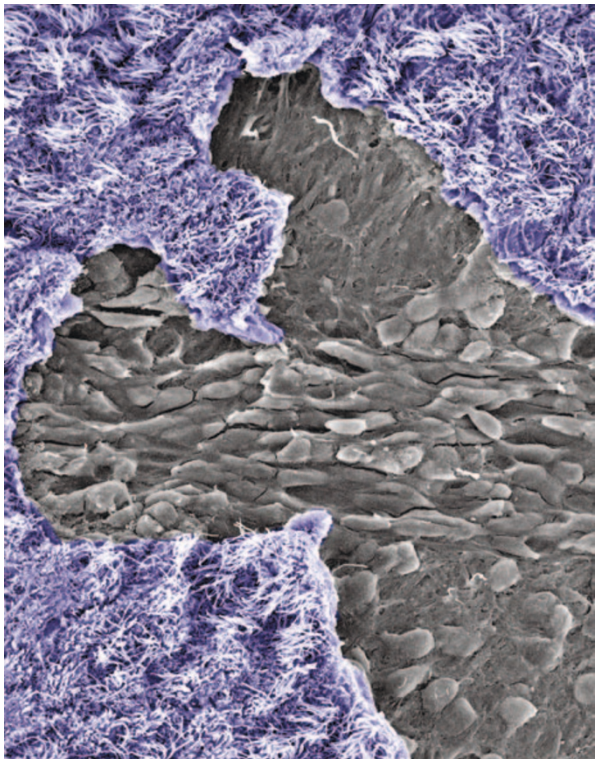
These results highlight for the first time a dual role for AACs in triggering excitatory as well as inhibitory responses in cortical microcircuitry, and pin-point a mechanism by which GABA-mediated input can have an excitatory effect on postsynaptic cells in the adult brain. It is hoped that future work will shed light on the function of this excitatory drive for pyramidal cells.

Alison Rowan

ORIGINAL RESEARCH PAPER Szabadics, J. & Varga, C. *et al.* Excitatory effect of GABAergic axo-axonic cells in cortical microcircuits. *Science* **311**, 233–235 (2006)

NEURONAL MIGRATION

Marching of the neurons



Scanning electron microscopy image of the ventricular wall with part of the ependymal surface (blue) peeled off to show the chains of migrating cells oriented similarly to the bending of ependymal cilia. Image courtesy of A. Alvarez-Buylla, University of California at San Francisco, USA.

In the adult brain, neuroblasts that are born in the subventricular zone (SVZ) have to travel a long way to their final destination in the olfactory bulb. How do they orient over such a distance and through complex territories? Writing in *Science*, Sawamoto and colleagues show that these newborn neurons are guided by signalling molecules streaming in specific directions with the flow of cerebrospinal fluid (CSF) in the brain ventricles.

It has been proposed that the flow of CSF might be crucial for neural function, but researchers do not know how the direction of CSF movement in the ventricles is established, or its associated functional consequences. Sawamoto *et al.* found that the flow of CSF correlated with the planar polarity of the ependymal cells that line the ventricles. When Indian ink was deposited onto the exposed surfaces of dissected walls of lateral ventricles, the pattern of ink flow, which was generated by the beating ependymal cilia, paralleled that of CSF flow observed *in vivo*. So, ependymal ciliary beating and the planar polarity of ependymal cells are important for establishing CSF flow in the brain.

The researchers then traced migrating neuroblasts at different locations in the SVZ and determined the direction of migration by inferring the average orientation of the leading processes. Interestingly, the orientation of neuroblast migration correlates with the direction of CSF flow rather than with the relative position of the olfactory bulb. In $Tg737^{orpk}$ mutant mice, which have severe defects in ciliary motility, the flow of CSF is aberrant and only ~9.3% of the neuroblasts generated in the SVZ reach the olfactory bulb.

CSF is mainly secreted by the choroid plexus, which is located in caudal regions of the lateral ventricles. The choroid plexus is also a source of chemorepulsive factors,

SYNAPTIC PHYSIOLOGY

More than a flicker

A new study from Richard Tsien's laboratory provides compelling evidence that 'kiss-and-run' is the main type of vesicle fusion at hippocampal synapses. The kinetics of kiss-and-run and vesicle re-use vary strikingly with stimulus frequency, which might allow small nerve terminals to conserve limited vesicular resources in the face of a demanding array of input patterns.

Of the many vesicles present at small central synapses, only ~30 are thought to be actively involved in transmitter release. Classically, fusion with the plasma membrane leads to the full collapse of each vesicle, involving complete aqueous continuity between the vesicle lumen and the extracellular solution. Retrieving, refilling and repriming a collapsed vesicle before the next release event could take several tens of seconds — an interval that would restrict the speed of synaptic information transfer.

Evidence gathered during the past few years for a second, non-classical mode of fusion, known as kiss-and-run or 'flicker', seemed to provide a solution to the problem. In the case of kiss-and-run, vesicles fuse transiently with the plasma membrane to release their contents, but remain intact for refilling and further transmitter release (re-use). However, because vesicle fusion at small terminals is particularly difficult to study, the defining features of kiss-and-run have remained unclear, and its existence has even been called into question.

> “Tsien and colleagues have provided a powerful technique to answer the many questions that remain about the physiological role of kiss-and-run fusion.”

Tsien and colleagues developed a novel fluorescence quenching technique to overcome some of the limitations of methods used previously to record or visualize kiss-and-run fusion events. They used a small hydrophilic quencher (bromophenol blue) that could rapidly enter vesicles from the extracellular medium during brief fusion events to quench FM dye or enhanced green fluorescent protein (EGFP) that was tagged to the lumenal domain of the vesicular protein VAMP2 (synaptobrevin 2).

They confirmed that during first fusion, FM1-43 was partially trapped in vesicles loaded with the dye — firm evidence for rapid vesicle retrieval characteristic of kiss-and-run, rather than classical collapse — and that the prevalence of kiss-and-run was strongly frequency dependent, increasing at lower frequencies to ~80%. What's more, the researchers were able to probe the kinetics of first fusion and the rate of vesicle re-use,

including members of the SLIT family. The researchers found that SLIT was distributed in a gradient along the dorsal SVZ, with the highest concentration in the caudal region, which declined rostrally. This gradient corresponds to the direction of CSF flow and neuronal migration. These results suggest that the flow of CSF generates the chemorepulsive gradient in the SVZ, which might help guide neuroblasts along the treacherous journey towards the olfactory bulb.

It has been reported that nodal cilia are important for the determination of left–right symmetry. Therefore, it might be a general theme that polarized ciliated cells provide important vectorial information for body-plan development.

Jane Qiu

ORIGINAL RESEARCH PAPER Sawamoto, K. *et al.* New neurons follow the flow of cerebrospinal fluid in the adult brain. *Science* 12 January 2006 (doi:10.1126/science.1119133)
FURTHER READING Okada, Y. *et al.* Mechanism of nodal flow: a conserved symmetry breaking event in left–right axis determination. *Cell* **121**, 633–644 (2005)
WEB SITE
Alvarez-Buylla's laboratory: http://neurosurgery.medschool.ucsf.edu/faculty_staff/department_faculty/alvarez_buylla.html

and to show that these measures were also influenced by stimulation frequency.

Tsien and colleagues have added considerably to our understanding of the characteristics of kiss-and-run, and have provided a powerful technique to answer the many questions that remain about the physiological role of this mode of fusion.

Rebecca Craven

ORIGINAL RESEARCH PAPER Harata, N. C. *et al.* Frequency-dependent kinetics and prevalence of kiss-and-run and reuse at hippocampal synapses studied with novel quenching methods. *Neuron* **49**, 243–256 (2006)
FURTHER READING Harata, N. *et al.* Limited numbers of recycling vesicles in small CNS nerve terminals: implications for neural signaling and vesicular cycling. *Trends Neurosci.* **24**, 637–643 (2001) | Rizzoli, S. O. & Betz, W. J. Synaptic vesicle pools. *Nature Rev. Neurosci.* **6**, 57–69 (2005)
WEB SITE
Tsien's laboratory: http://www.stanford.edu/group/Tsienlab/

NEUROLOGICAL DISORDERS

Making steps in stroke therapy

Agents currently used in the treatment of stroke have a narrow window of time for therapeutic application and dose-limiting adverse effects. The development of new therapies has been hampered by a lack of adequate models of blood vessel dysfunction that mimic the vascular disruptions that occur in human stroke. Two new potential therapeutic strategies for the treatment of stroke and three new models of stroke have recently been reported.

Rupture or blockage of a blood vessel in the brain causes rapid cell death in the core of the injured region, and triggers mechanisms in the surrounding area — the penumbra — that lead, for example, to increases in the concentrations of intracellular Ca^{2+} and reactive oxygen species (ROS), which initiate cell death. Targeting these mechanisms is a promising route for the development of therapies for the treatment of stroke.

One such strategy, reported by Jiang and colleagues, involved using the metal chelator PAN-811 (also known as Triapine) to 'mop up' intracellular Ca^{2+}. PAN-811 was also shown to scavenge ROS, and so protects neurons from the degenerative effects of both chemical species. In a rat model of ischaemic stroke, intracerebroventricular administration of PAN-811 one hour after occlusion of the middle cerebral artery (MCA) led to a 59% reduction in infarction volume. In addition, PAN-811 has been shown to have a favourable safety and pharmacodynamic profile in the range required for neuroprotection.

In a different approach, Kawano and colleagues focused on enzymes implicated in the neurotoxicity that occurs after stroke, specifically the cascade involving cyclooxygenase 2 (COX2). Long-term treatment with COX2 inhibitors is thought to increase the risk of cardiovascular complications, so Kawano and colleagues sought to identify molecules downstream of COX2 that are associated with COX2 neurotoxicity. One such receptor in this pathway is the prostaglandin E_2 receptor EP1. In mice, administration of the EP1 receptor inhibitor SC51089 six hours after occlusion of the MCA reduced brain injury. This receptor could, therefore, be an alternative target to COX2 in stroke.

Current models of stroke involve invasive intravascular injections, and the location and size of the occlusion produced can vary. Nishimura and colleagues have found a way to occlude a single microvessel deep below the surface of the brain. After selecting a vessel using two-photon microscopy, ultrashort laser pulses were applied to disrupt the vessel. By varying the energy of the pulse, the authors were able to produce three models of stroke: haemorrhagic (high energies); extravasations with continued blood flow (low energies); and clots with full vessel occlusion (multiple irradiation with increasing energy). These models and the strategies described above will be important in the continued progress in the development of new therapies for stroke.

Samantha Barton

ORIGINAL RESEARCH PAPERS Jiang, Z.-G. *et al.* A multifunctional cytoprotective agent that reduces neurodegeneration after ischemia. *Proc. Natl Acad. Sci. USA* **103**, 1581–1586 (2006) | Kawano, T. *et al.* Prostaglandin E_2 EP1 receptors: downstream effectors of COX-2 neurotoxicity. *Nature Med.* **12**, 1–5 (2006) | Nishimura, N. *et al.* Targeted insult to subsurface cortical blood vessels using ultrashort laser pulses: three models of stroke. *Nature Methods* **3**, 99–108 (2006)

Image courtesy of L. Keogh.

PSYCHIATRIC DISORDERS

Another role for leptin?

Depression and obesity are both serious and expensive health problems worldwide. Now, imbalances in one hormone — leptin — have been implicated in both. Leptin's effect on body weight regulation has already been well documented; recent research from The University of Texas, USA, has shown that it can also attenuate depression-like behaviour in rats.

Lu and colleagues found significant reductions in plasma leptin levels in rats that had undergone chronic unpredictable stress or chronic social defeat. Rats in the former group also showed a concomitant increase in corticosterone, the human equivalent of cortisol, elevations of which are often seen in human patients with depression. When leptin was administered to the chronically stressed rats, it attenuated the hedonic deficit that is associated with chronic stress. However, no effect was seen in non-stressed rats.

The authors then used the forced swim test, which measures 'despair' behaviour — characterized by cessation of escape behaviours such as climbing and swimming — and is commonly used to investigate the efficacy of antidepressant drugs. They found that, in rats subjected to this test, systemic administration of leptin diminished immobility in a dose-dependent manner and increased swimming times — in short, it decreased despair behaviour. The positive control, desipramine (a tricyclic antidepressant that selectively inhibits the reuptake of noradrenaline), also reduced rats' immobility in this test, but resulted in increased climbing rather than swimming, indicating that leptin has a different mode of action from the noradrenaline reuptake inhibitors.

Lu and co-workers went on to use *in situ* hybridization to investigate whether the altered behaviour seen in the forced swim test after leptin administration was accompanied by changes in brain activation. Measuring c-*fos* mRNA expression, they found increased activity in areas of the hippocampus that included CA1, CA3 and the dentate gyrus, and in the amygdala — most notably in the basolateral nucleus. This suggests that particular limbic structures could be responsible for leptin's effects in this test. Intrahippocampal infusion of leptin during forced swimming produced similar results to systemic administration, indicating that the hippocampus is crucial in meditating leptin's antidepressant-like effects. Meanwhile, intrahypothalamic leptin infusion did not affect behaviour in the forced swim test, indicating that its antidepressant effect is not connected to its role in energy homeostasis.

At present, whether leptin is involved in human depression, and the validity of animal models of depression are both controversial. However, these findings are encouraging, and might pave the way for new antidepressant treatments.

Sarah Archibald

ORIGINAL RESEARCH PAPER Lu, X.-Y. *et al.* Leptin: a potential novel antidepressant. *Proc. Natl Acad. Sci. USA* **103**, 1593–1598 (2006)
FURTHER READING Berton, O. & Nestler, E. J. New approaches to antidepressant drug discovery: beyond monoamines. *Nature Rev. Neurosci.* **7**, 137–151 (2006)

SYNAPTIC PHYSIOLOGY

Message in the binding

The translation of neurotransmission into appropriate postsynaptic signals is achieved through the clustering of neurotransmitter receptors and their associated signalling molecules in the postsynaptic density (PSD). This clustering is mediated by ligand-recruiting scaffold proteins such as PSD-95, but the mechanisms involved are unclear. Writing in the *Journal of Neuroscience*, Nonaka and colleagues report that it is the ligand-binding ability of PSD-95 that is important for its clustering and synaptic localization, as well as its association with the PSD.

The amino terminus of PSD-95 contains three tandem, ligand-binding PDZ domains, at least one of which is required for the clustering and synaptic targeting of PSD-95. What is the role of these tandem PDZ domains and their ligand interactions in the clustering and localization of PSD-95?

To address this question, Nonaka and co-workers constructed a series of full-length PSD-95 mutants lacking ligand-binding ability individually in each of PDZ1, PDZ2 and PDZ3, in both PDZ1 and PDZ2, or in all three. They showed that decreased ligand-binding affinity resulted in decreased clustering of PSD-95, and, furthermore, revealed an independent and approximately additive contribution of PDZ domains to this process. In addition, they found that ligand-binding deficiency caused

DEVELOPMENT

Eye catching

For visual information to be accurately relayed from the eye to the brain precise topographic connections between retinal cells and visual centre neurons are essential. New work by Tabata and colleagues reveals that, during development of the *Drosophila* visual system, Hedgehog (HH) signalling from growing retinal axons (R axons) to neurons of the lamina optic ganglion is required for the two to catch hold and establish connections. The group also identify the transcription factor, Single-minded (SIM), as a lamina neuron target of HH, and show that this, too, is required for R axon–lamina connections.

The *Drosophila* compound eye is composed of 750 visual units, each of which contains eight photoreceptor (retinal) cells. During development of the visual system, the first six of these (R1–6) extend axons (R axons) to the lamina optical ganglion. Immature precursor neurons, residing in the pre-assembling region of the lamina, receive HH signals from arriving R axons and are induced to differentiate. Subsequent associations between these differentiating neurons and R axons form preliminary structures called lamina columns. These arise in the assembling region and later consolidate synaptic partnerships into final lamina cartridges.

To investigate the precise role of HH in the formation of lamina columns and cartridges, Tabata *et al.* generated flies containing both wild type and *smo*$^{-/-}$ lamina neurons — Smoothened (SMO) being an essential component of the HH receptor. They found that *smo*$^{-/-}$ lamina neurons, unlike their wild type counterparts, were never observed in the assembling region (supporting a similar finding by Huang and Kunes) but were, instead, restricted to the pre-assembling region, suggesting that *smo*$^{-/-}$ cells were defective in their ability to interact with R axons and form lamina columns.

As the expression of SIM protein is restricted to lamina neurons and is abolished in cells that lack SMO, the group asked whether SIM might

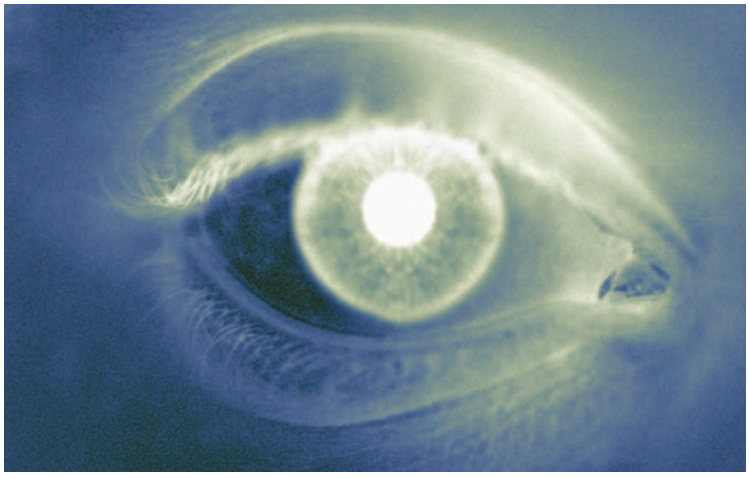
the association of PSD-95 with the PSD to be destabilized, which suggests that ligand interactions have a role in anchoring PSD-95 to the PSD.

Surprisingly, the decreased ligand-binding affinity of mutant PSD-95 also resulted in aberrant spine morphology. Whereas mature PSD-95 clusters are normally localized in spines close to the dendritic shaft, a significant number of the surviving clusters formed by mutant PSD-95 were located away from this site, on the tips of elongated, seemingly immature spines. Moreover, there was an inverse correlation between the cluster–shaft distance and the amount of clustering. Combined with the finding that ligand-binding affinity contributes additively to clustering, this correlation implies that PDZ domains also make an additive contribution to spine maturation.

These findings suggest two separate functions for ligand-binding events at the PDZ domains of PSD-95: regulation of spine maturation, and recruitment of ligands into the PSD. The implication of multivalent PDZ binding ability is that the synaptic clustering of PSD-95, and its association with the PSD, can be dynamically modulated, potentially in response to local synaptic activity. Studies that disrupt the PDZ-binding ability of PSD-95 ligands should reveal the relative contribution of their interactions to synapse development and function.

Daniel McGowan

ORIGINAL RESEARCH PAPER Nonaka, M. *et al.* Essential contribution of the ligand-binding βB/βC loop of PDZ1 and PDZ2 in the regulation of postsynaptic clustering, scaffolding, and localization of post-synaptic density-95. *J. Neurosci.* **26**, 763–774 (2006)



also be involved in lamina–R axon connections. They found that, like *smo*$^{-/-}$ neurons, *sim*$^{-/-}$ neurons were restricted to the pre-assembling region. In addition, ablating SIM function in lamina neurons by overexpressing a dominant-negative mutant of dARNT protein (a transcription factor partner of SIM) also prevented these cells from entering the assembling region to form lamina columns. Therefore, knocking out SIM reproduced the phenotype of knocking out HH signalling.

Although it is yet to be determined whether SIM overexpression can recover the *smo*$^{-/-}$ phenotype, it seems likely that the two factors act in the same pathway. And, as both HH and SIM have conserved mammalian homologues, such studies into the fly's visual system might well provide insight into the pathways responsible for topographic network formation in mammals.

Ruth Williams

ORIGINAL RESEARCH PAPER Umetsu, D. *et al.* The highly ordered assembly of retinal axons and their synaptic partners is regulated by Hedgehog/Single-minded in the *Drosophila* visual system. *Development* 26 Jan 2006 (doi:10.1242/dev.02253)
FURTHER READING Huang, Z. & Kunes, S. Signals transmitted along retinal axons in *Drosophila*: Hedgehog signal reception and the cell circuitry of lamina cartridge assembly. *Development* **125**, 3753–3764 (1998)

IN BRIEF

DEVELOPMENT

Neurogenin 2 is required for the development of ventral midbrain dopaminergic neurons.

Kele, J. *et al. Development* **133**, 495–505 (2006)

Proneural proteins, including MASH1, and neurogenin 1 and 2 (NGN1/2), are essential for producing multipotent neural precursor cells, and for driving these cells to assorted neuronal fates. Now Kele *et al.* have found that mice with mutations in *Ngn2*, but not *Ngn1* or *Mash1*, have an almost complete loss of dopaminergic neurons from the ventral midbrain region. As loss of dopaminergic neurons is a key feature of Parkinson's disease, this finding could have exciting implications for neurogenesis-based cell-replacement therapies.

NEUROLOGICAL DISORDERS

Trak1 mutation disrupts $GABA_A$ receptor homeostasis in hypertonic mice.

Gilbert, S. L. *et al. Nature Genet.* **38**, 245–250 (2006)

Hypertonia, which is observed in conditions such as cerebral palsy, stroke and spastic paraplegia is marked by abnormal increase in muscle tension that causes rigidity, dystonia and uncontrollable spasms. In some conditions, hypertonicity is thought to arise from defects in GABA (γ-aminobutyric acid)-mediated motor neuron inhibition. Gilbert *et al.* confirmed that in HYRT mice, which have motor defects consistent with hypertonia, symptoms can be relieved by treatment with $GABA_A$ receptor-potentiating drugs, and went on to clone *Trak1*, a $GABA_A$ receptor-interacting factor, as the gene responsible for the HYRT phenotype.

SENSORY SYSTEMS

Imaging hair cell transduction at the speed of sound: dynamic behaviour of mammalian stereocilia.

Fridberger, A. *et al. Proc. Natl Acad. Sci. USA* **103**, 1918–1923 (2006)

Sound perception requires stereocilia of the inner ear to convert nanometre displacements into receptor transmissions along the auditory nerve. Previous methods of studying the relationship between sound, stereocilia deflection and receptor transmission involved either manual stimulation of stereocilia (limited to artificial deflection data) or sound stimulation (which provides little or no information about stereocilia deflection). Fridberger and colleagues have now overcome these limitations, devising a new method of rapid confocal imaging, which allows visualization of stereocilia deflection by direct sound stimulation.

NEUROIMMUNOLOGY

Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood.

Ziv, Y. *et al. Nature Neurosci.* **9**, 268–275 (2006)

Boosting the immune system might boost brain power. Autoimmune T cells are known to promote neuronal survival and renewal following CNS injury, and Ziv *et al.* propose that this might reflect a normal, homeostatic role for T cells in adult neurogenesis (and therefore learning and memory). After 6 weeks, rats housed in enriched environments showed greater neurogenesis than rats housed in standard cages, but this increase in neurogenesis by environmental stimulation did not occur in immunodeficient mice. Furthermore, mice with excess autoimmune T cells showed both increased neurogenesis and improved learning and memory in water maze tests.

In the news

TIMID AS A MOUSE

Social phobias, depression and post-traumatic stress disorder all have crippling effects on a person's quality of life. Although current antidepressants can be used to alleviate feelings of withdrawal and fear, for many they have no effect. Now, scientists at the University of Texas Southwestern Medical Center, Dallas, USA, have shown that brain-derived neurotrophic factor (BDNF) is involved in the negative responses seen in such disorders.

Eric Nestler and Olivier Berton and their colleagues were interested in BDNF because it is part of the brain's reward circuits — typically associated with drug addiction. To induce symptoms of withdrawal and fear, every day for 10 days a mouse of one strain was exposed to a different, larger mouse from a strain Nestler refers to as "… naturally mean mice" (*News@Nature.com*, 9 February 2006). Bullied mice became withdrawn and fearful and, even after their tormentors were removed, remained wary, hiding in the corners of their cages when introduced to new mice. These symptoms persisted for 4 weeks after the bullying stopped.

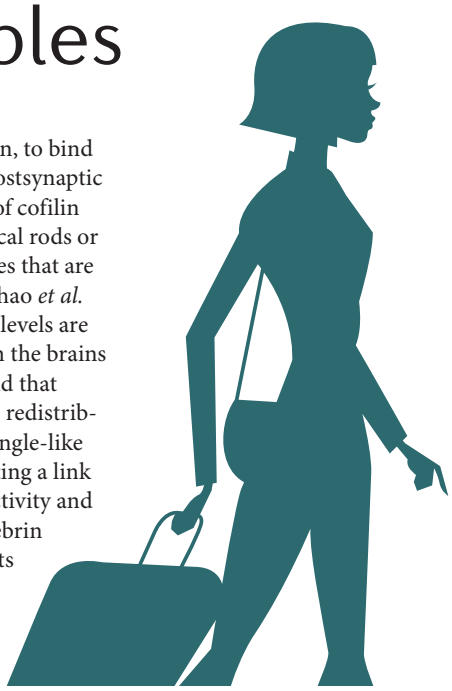
Bullied mice had an altered mesolimbic dopamine system — the brain circuit that is linked to addictive behaviour — with BDNF production induced in the reward circuit. Blocking BDNF activity stopped the smaller mice from being afraid of the bigger, more aggressive mice. This effect mimics that of chronic antidepressant treatment.

BDNF is important in several areas of the brain, and so targeting it directly might lead to problematic side effects. However, identifying particular pathways in which BDNF acts, as Berton comments, provides "… a way to develop antidepressants that work faster and in more people" (*Reuters*, 9 February 2006). So, manipulating the molecules that interact with BDNF could help to alleviate the symptoms of social phobias.

Samantha Barton

NEURODEGENERATIVE DISEASES

PAK up your troubles



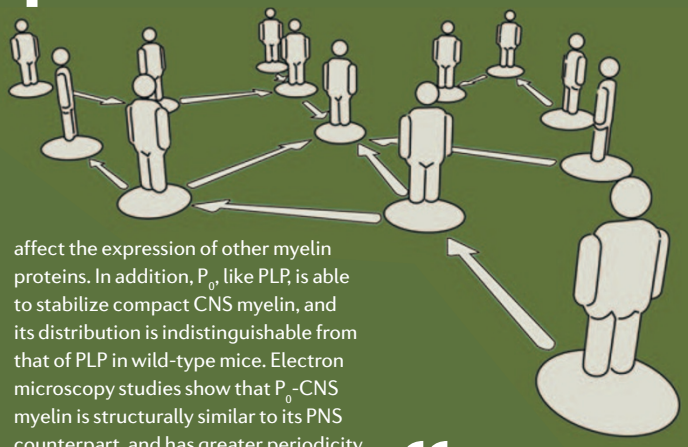
PAKs (p21-activated kinases) are key regulators of the actin cytoskeleton that, in neurons, have a role in dendritic spine morphogenesis. In mice, mutation of *Pak3* causes X-linked, non-specific mental retardation, and perturbation of the PAK pathway leads to cognitive deficits and dendritic spine defects. Now, in *Nature Neuroscience*, Zhao *et al.* report a loss of PAK activity in the brains of patients with Alzheimer's disease (AD) and suggest that this might cause the dendritic spine and cognitive defects observed in AD.

PAK signalling inactivates cofilin, which destabilizes interactions between actin subunits, causing it to detach from actin. This enables another protein, drebrin, to bind and regulate actin in postsynaptic spines. In the absence of cofilin inactivation, pathological rods or inclusions form, features that are characteristic of AD. Zhao *et al.* show that soluble PAK levels are significantly reduced in the brains of patients with AD, and that phosphorylated PAK is redistributed to granular and tangle-like accumulations, suggesting a link between loss of PAK activity and cofilin aggregation, drebrin loss and synaptic defects observed in AD.

These same features were observed in an Alzheimer's model

GLIA

Changing places



Although myelin serves similar functions in the PNS and CNS, its main protein constituent differs: the type I integral membrane protein P_0 (protein zero) is present in PNS myelin, whereas the tetraspan membrane protein PLP (proteolipid protein) resides in the CNS. It is thought that P_0 was initially the primary structural protein of both PNS and CNS myelin — which first appeared ~440 million years ago in cartilaginous fishes — but was replaced by PLP in the CNS after the divergence of the bony fishes ~400 million years ago. What could be the benefits of the P_0 to PLP conversion during evolution?

To address this issue, Yin and colleagues reversed the evolutionary step by generating transgenic mice that expressed P_0, rather than PLP, in the CNS. In these P_0-CNS animals, the level of P_0 expression in the CNS is similar to that of PLP in normal mice, and replacing PLP with this ancestral protein in the CNS does not affect the expression of other myelin proteins. In addition, P_0, like PLP, is able to stabilize compact CNS myelin, and its distribution is indistinguishable from that of PLP in wild-type mice. Electron microscopy studies show that P_0-CNS myelin is structurally similar to its PNS counterpart, and has greater periodicity (membrane spacing) than that of wild-type CNS myelin.

Next, the researchers studied the effect of replacing PLP with P_0 by measuring the animals' motor function. In P_0-CNS mice, motor performance was normal at 6 months of age, but declined significantly (by 90%) by 1 year — by which time 50% of the animals had died. These observations are consistent with the precocious accumulation of

> "What could be the benefits of the P_0 to PLP conversion during evolution?"

mouse engineered to produce high levels of amyloid-β (Aβ). Reducing the Aβ burden of these mice, by passive immunization with an anti-Aβ antibody, led to an increase in PAK and drebrin levels. Furthermore, the addition of Aβ oligomers to cultured primary hippocampal neurons induced rapid and persistant reductions in PAK and drebrin, which supports a role for Aβ in inducing PAK signalling defects. Expressing wild-type PAK1 in these neurons limited drebrin loss; conversely, pharmacological inhibition of PAK in adult mice recapitulated many of the features of AD.

These findings suggest that PAK and its downstream effectors represent potential therapeutic targets for AD.

Daniel McGowan

ORIGINAL RESEARCH PAPER Zhao, L. *et al.* Role of p21-activated kinase pathway defects in the cognitive deficits of Alzheimer disease. *Nature Neurosci.* **9**, 234–242 (2006)

the amyloid precursor protein — a reliable indicator of axonal pathology in primary myelin disease affecting PLP-deficient mice — in the brains of P_0-CNS mice at a young age. So, PLP, but not P_0, might provide trophic support for axons in the CNS, thereby delaying the onset of neurodegene ration.

This elegant study indicates that the shift from P_0 to PLP during CNS myelin evolution was associated with an important neuroprotective function of myelin-forming glia. This finding may further our understanding of human myelin diseases, in which a spectrum of neurological disabilities is associated with null mutations, deletions and point mutations in the *PLP* gene.

Jane Qiu

ORIGINAL RESEARCH PAPER Yin, X. *et al.* Evolution of a neuroprotective function of central nervous system myelin. *J. Cell Biol.* 30 January 2006 (doi:10.1083/jcb.200509174)
FURTHER READING Yoshida, M. & Colman, D. Parallel evolution and coexpression of the proteolipid proteins and protein zero in vertebrate myelin. *Neuron* **16**, 1115–1126 (1986)
WEB SITE
Trapp's laboratory: http://www.lerner.ccf.org/neurosci/trapp/

DEVELOPMENT

MicroRNAs mediate synapse development

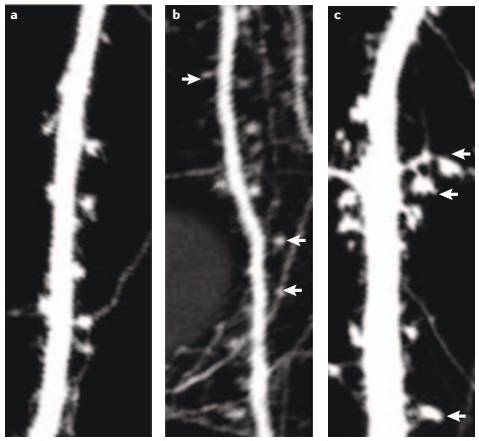
MicroRNAs (miRNAs) are small non-coding RNAs that have a key role in development in a wide variety of organisms. A team led by Michael Greenberg has now identified, for the first time, a particular miRNA — miR-134 — that is crucial for the formation of synapses.

miRNAs can regulate gene expression by attaching to mRNAs and inhibiting their translation. mRNA translation is an important aspect of synaptic development and plasticity, so it is possible that miRNAs are involved in these processes.

Schratt and co-workers investigated the expression and localization of candidate miRNAs in rat hippocampal neurons and found that miR-134 expression is confined to the brain. Intriguingly, this miRNA was more highly expressed in the hippocampus during development, and levels peaked at the time when synapses became mature. *In situ* hybridization and immunostaining revealed that miR-134 was specifically located close to synaptic sites in dendrites, implicating it in synaptic functions.

These researchers then studied the nature of miR-134 involvement in synaptic functions. They found that overexpression of miR-134 led to a marked decrease in the volume of mature spines that was attributed to a decrease in spine width, whereas inhibition of miR-134 resulted in increased volume and width of spines. These data indicate that miR-134 negatively regulates dendritic spine volume.

The next step was to understand how miR-134 influences spine volume. Schratt and colleagues isolated several mRNA targets for miR-134, and focused their explorations on *Limk1* (Lim-domain-containing protein kinase 1) as this is known to be involved in spine development. miR-134 and *Limk1* were co-localized in dendrites both *in vitro* and *in vivo*. In line with the known inhibitory effect of miRNAs on mRNAs, overexpression of miR-134 blocked translation of *Limk1* mRNA, whereas preventing miR-134 activity increased *Limk1* expression, effects that were specific to dendritic compartments. Moreover, co-expression of miR-134 with a mutant form of *Limk1* mRNA that could not bind with miR-134 rescued the decrease in spine volume, whereas the same construct with wild-type *Limk1* did not.



Dendritic spines in rat hippocampal neurons that express a control construct (**a**), increased levels of miR-134 (**b**) or a miRNA antisense inhibitor (**c**). Arrowheads point to examples of spines that illustrate the reduced spine size in (**b**) and the increased spine size in (**c**).

These findings suggest that miR-134 mediates its effects on spine volume by disrupting *Limk1* mRNA translation in dendrites.

The translation of dendritic mRNAs at synaptic sites might not be activated until synaptic stimulation triggers the release of extracellular factors such as brain-derived neurotrophic factor (BDNF). Interestingly, Schratt and colleagues found that exposure to BDNF resulted in increased *Limk1* mRNA translation, which suggests that BDNF stimulation can release *Limk1* mRNA translation from inhibition by miR-134.

This comprehensive study not only pin-points a specific miRNA that is important for synaptic development, but also sheds light on its mechanism of action. The development of dendritic spines seems to depend on a fine balance between the inhibitory effect of miR-134 on Limk1 mRNA translation and, following synaptic activity, the release of this repression by BDNF to allow development to progress. It remains to be seen whether other miRNAs and their target mRNA species are involved in the development of synapses.

Alison Rowan

ORIGINAL RESEARCH PAPER Schratt, G. M. *et al.* A brain-specific microRNA regulates dendritic spine development. *Nature* **439**, 283–289 (2006)
FURTHER READING He, L. & Hannon, G. J. MicroRNAs: small RNAs with a big role in gene regulation. *Nature Rev. Genet.* **5**, 522–531 (2004)