PHENOTYPE

Issue 28 | Michaelmas Term 2017

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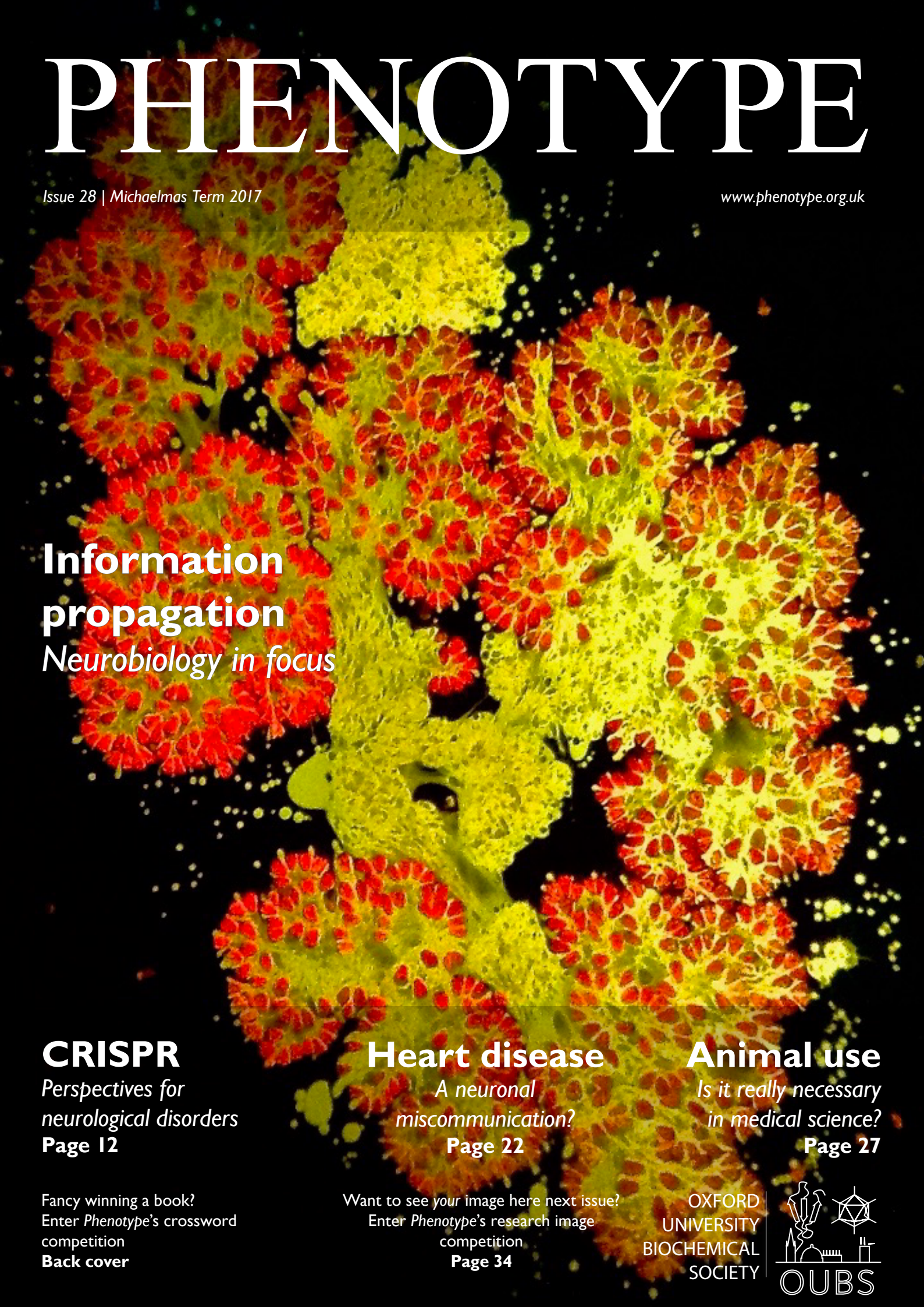

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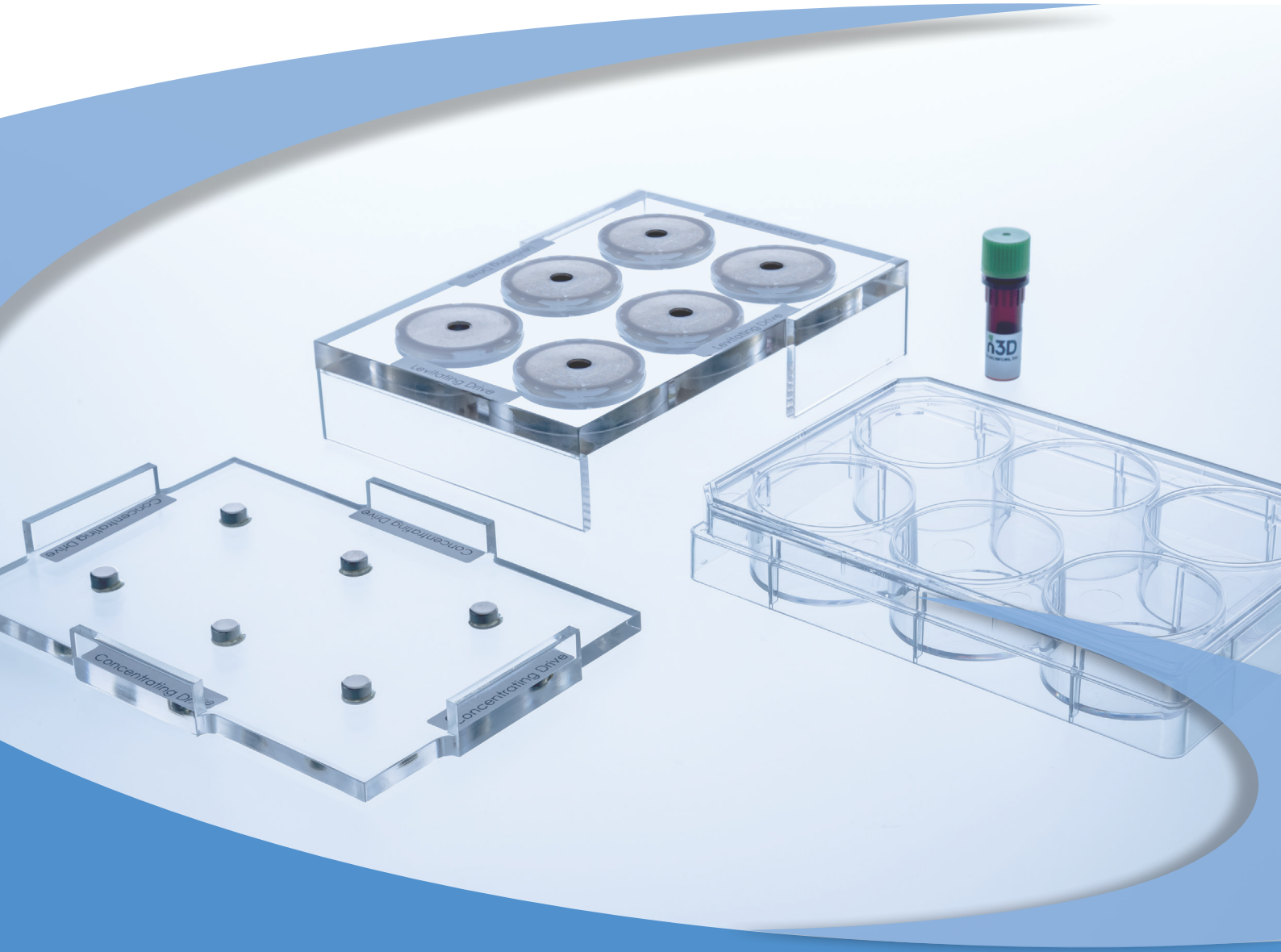

3D Cell Culture

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EDITORIAL

Welcome to the 28th issue of Phenotype! After many months of preparation and amazing work by our authors and editorial team, we are excited to share this edition with you.

This term we have focused on neuroscience. We present articles on many aspects of the nervous system, from basic functionality to effective treatments. For a brief overview of the field, take a look at the excellent infographic by Katharina Schleicher on ***page 6.***

Following Katharina's cartoon figure of axons, action potentials and communication between neurons, we turn to ***page 22*** *where Emma Bardsley describes the role of neuronal miscommunication in cardiovascular diseases, and to* ***page 8*** *where Ian Robertson explains how nerve cells respond to their physical environment. Ian underlines the potential of developing new drugs that change how nerve cells 'sense' their surroundings.*

Talking about nerve cells & brains… don't forget that we're not the only animals with brains! The worm Caenorhabditis elegans has actually been used as a model organism for Alzheimer's disease, as highlighted by Josh Newman on ***page 10****.*

Delving into the biochemistry and molecular networks of the nervous system, on ***page 20*** *Claire Hill tells us about the biochemistry of Fragile X Syndrome, a form of inherited intellectual disability. On* ***page 16****, Professor Russell Foster and his team help us identify the genes that generate and regulate circadian rhythms.*

Turn to ***page 14*** *for an insightful article into the promises and pitfalls of induced pluripotent stem cell-based models of neurodegenerative diseases. In this article, Lauren Watson explains that disease-relevant patient cells allow the investigation of disease progression and the development of novel therapeutic approaches. In fact, a promising tool for studying both the genetic and the epigenetic causes of various neurological disorders is the CRISPR-Cas9 system, as Veronika Hartleb discusses on* ***page 12****.*

Besides Neuroscience, this issue also features an article about antibiotics by Henry Stennett on ***page 24*** *that highlights the urgency of finding new antibiotics to fight bacterial infections.*

Apart from our Features articles, check out our Regulars articles and the Science & Society section, where you can learn about Science Activism on ***page 29****.*

Don't forget to enter our prize competitions! Read about the winner of our Snapshot Image competition on ***page 34*** *and enter this term's competition for a chance to get your research image on the front cover of Phenotype, and to win a £50 voucher from Oxford University Press. On the back cover of this issue you will find our neuroscience-themed crossword. Send your answers for a chance to win one of the Wiley-Blackwell textbooks reviewed on* ***pages 32-33****.*

I hope you enjoy reading this issue and your Michaelmas term! If you are interested in getting involved with Phenotype, please contact me at ***stefania.kapsetaki@new.ox.ac.uk****. We are always happy to see enthusiastic new faces joining our team!*

Stephanie Kapsetaki
Editor-in-Chief

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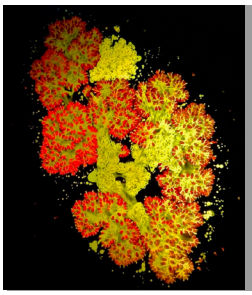


On the cover

Botryococcus braunii, a lipid-rich freshwater green alga.

Read more on page 34!

EDITORIAL TEAM

RESEARCH HIGHLIGHTS

by
Oleg Sitsel

Wright R *et al.* (2017) *J Neurosci* 37(22):5447-5462.

Neuronal chloride regulation via KCC2 is modulated through a $GABA_B$ receptor protein complex

$GABA_B$ receptors are G-protein coupled receptors (GPCRs) that form part of an inhibitory synaptic signal transmission cascade. In contrast to $GABA_A$ receptors, which use chloride ions to quickly relay inhibitory signals, $GABA_B$ receptors utilise G-protein signalling in order to generate a slower inhibitory signal. These proteins have complex regulatory mechanisms that include usage of various subunits and post-translational modifications. In this study, Wright *et al.* discovered another example of $GABA_B$ receptor versatility.

Using mass spectrometry, co-immunoprecipitation, and immunofluorescence, $GABA_B$ receptors in rat hippocampal neurons were reproducibly found to associate with the potassium-chloride cotransporter KCC2. This association was also confirmed to take place in KCC2-transfected CHO cells expressing the receptor subtypes $GABA_B$R1b and $GABA_B$R2, even when KCC2's N- or C-terminal domains were deleted. Subsequent experiments explored how associations with $GABA_B$ receptors affect KCC2's chloride transport. The application of SKF97541, a specific $GABA_B$ receptor agonist, resulted in an increase in intracellular chloride concentration, leading to the conclusion that activating $GABA_B$ receptors decreases the chloride transport of associated KCC2. Several control experiments were then performed, which confirmed that modulation of KCC2 was responsible for the observed changes in intracellular chloride concentration. The authors also discovered that, following SKF97541 treatment, the amount of membrane-bound KCC2 decreased significantly, yet no degradation occurred. By using a clathrin-mediated endocytotic pathway inhibitor, the study demonstrated that this pathway is responsible for KCC2 removal from the membrane. Finally, electrophysiological recordings made in GABAergic interneurons and CA3 pyramidal neurons showed that activated $GABA_B$ receptors use KCC2 as a middleman to affect $GABA_A$ receptor activity *in vivo*.

In conclusion, this study identifies and characterizes a novel mechanism of communication between different GABA receptor types, shedding more light on the complex world of inhibitory neurotransmission.

Wei WC *et al.* (2017) *J Physiol* [Epub ahead of print].

Functional expression of calcium-permeable canonical transient receptor potential 4-containing channels promotes migration of medulloblastoma cells

Ca^{2+} signalling is crucial to a host of intra- and intercellular processes, therefore errors in signalling can have dire consequences. In cancerous tissue, this includes enhanced motility of malignant cells and acceleration of metastatic spread. Medulloblastoma (MB) is the most common type of brain cancer found in children, and it originates from precursor cells of the cerebellum. Previous research by Maike Glitsch's group on an MB cell line, DAOY, showed that this type of tumour senses and responds to acidification of interstitial fluid (a consequence of malignant cell metabolism) via stimulation of the proton-sensing GPCR, OGR1, leading to downstream Ca^{2+} release from intracellular stores. In this study, Wei *et al.* investigated whether there is a link between OGR1 signalling and expression of the Ca^{2+}-permeable channel TRPC4 in normal and cancerous cells, and how it might contribute to the transformation from one to the other.

Markedly reduced mRNA levels of TRPC4 and TRPC5 were detected in primary granule cell cultures derived from *OGR1*-knockout mouse cerebella compared to wild-type, with similar observations at the protein level for TRPC4. Using the TRPC4/5 activator (–)-Englerin A to investigate the function of channel expression in primary granule cells, the group observed that activation resulted in small increases in intracellular Ca^{2+} concentration, without any noticeable effect on the proliferation and migration of primary granule cells.

In DAOY cells, application of (–)-Englerin A triggered Ca^{2+} influx, without any effect on cell survival. However, cell migration rates were noticeably increased and could be returned to basal levels by TRPC4/5 inhibitors and decreased by *TRPC4* knockdown. This establishes a crucial role for TRPC4 in migration of cancerous granule cells. When both *OGR1* and *TRPC4* were found to be strongly expressed in nine separate human MB tissue samples, the possibility of OGR1 activation by acidic pH leading to increased TRPC4 Ca^{2+} influx was tested and confirmed.

Overall, this study provides an initial understanding into the role of OGR1 and TRPC4 in MB, and a foundation for further investigations of the topic.

Infographic: NEUROSCIENCE

by Katharina Schleicher

Notable disorders of the nervous system

Alzheimer's Disease
Degenerative disease of the brain. Characterised by dementia, always fatal.

Cerebral Palsy
Motor disorder. Caused by damage to the cerebrum at birth.

Depression
Serious mood disorder. Characterised by insomnia, loss of appetite, prolonged unhappiness.

Epilepsy
Condition characterised by periodic perturbation of brain electrical activity. Can lead to seizures, loss of consciousness, sensory disturbances.

Huntington's Disease
Inherited disorder resulting in the death of brain cells. Can cause psychiatric problems, abnormal movements, difficulty with feeding or communication.

Multiple Sclerosis
Progressive disease affecting nerve conduction. Characterised by episodes of weakness, lack of coordination, speech disturbance.

Parkinson's Disease
Progressive disease of the brain. Leads to difficulty initiating voluntary movement.

Schizophrenia
Severe psychotic illness. Characterised by delusions, hallucinations, unusual behaviour.

Spinal Paralysis
Loss of feeling and movement caused by traumatic damage to the spinal cord.

Stroke
Loss of brain function caused by disruption of blood supply to the brain. Usually leads to permanent sensory, motor, or cognitive deficit.

Methods in neuroscience

Since 1801: Transcranial direct current stimulation (tDCS)
Local stimulation of the brain by a small electrical current applied to the scalp.

Since 1875: Electroencephalography (EEG)
Recording regions of neural activity with electrodes placed on the scalp.

Since 1949: Intracellular recording
Measuring the potential difference across a membrane using an intracellular microelectrode filled with a concentrated salt solution, and an extracellular electrode.

Since 1971: Magnetic resonance imaging (MRI)
Constructing a map of the brain by using a magnetic field to acquire a picture of all the hydrogen atoms in the head.

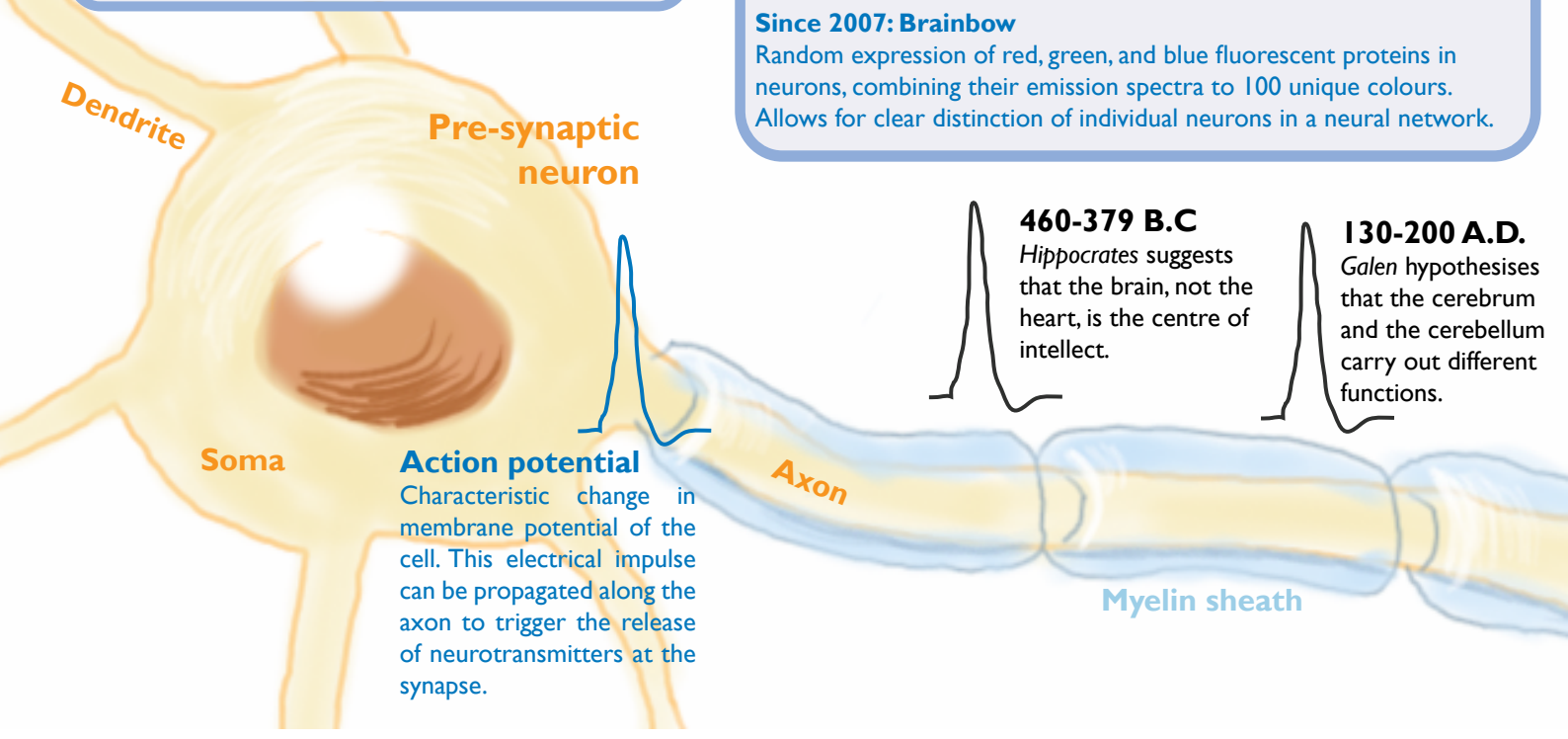
Since 1975: Positron emission tomography (PET)
Determining metabolic activity in the brain by measuring consumption of a positron-emitting glucose derivative by brain cells.

Since 1976: Patch clamp
High resolution recordings of currents passing through single ion channels isolated in a tight membrane seal using a glass pipette.

Since 1985: Transcranial magnetic stimulation (TMS)
Manipulating the brain by repeated pulses of a magnetic field.

Since 2005: Optogenetics
Using genetic tools to activate and inhibit mammalian neurons with light.

Since 2007: Brainbow
Random expression of red, green, and blue fluorescent proteins in neurons, combining their emission spectra to 100 unique colours. Allows for clear distinction of individual neurons in a neural network.

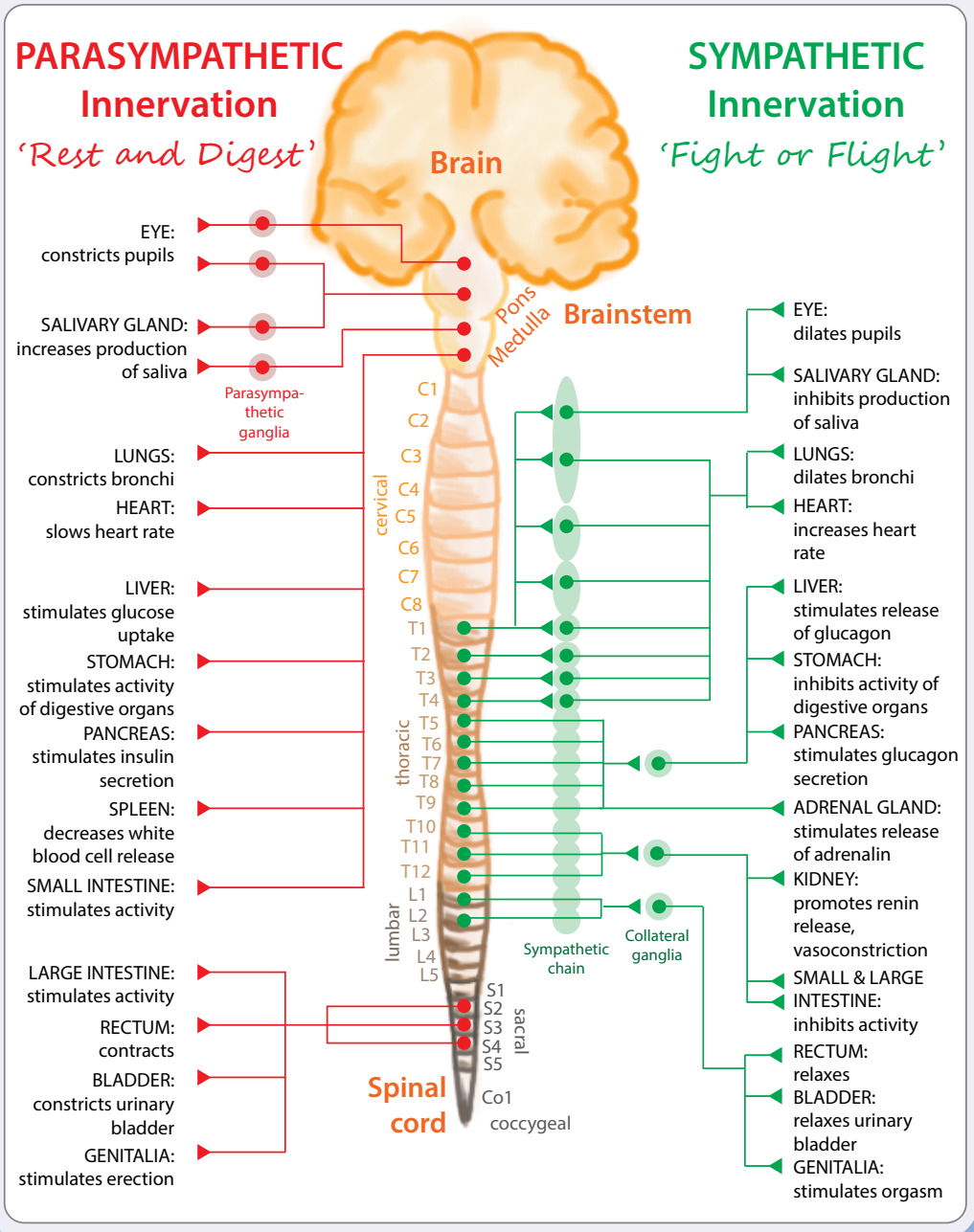


460-379 B.C
Hippocrates suggests that the brain, not the heart, is the centre of intellect.

130-200 A.D.
Galen hypothesises that the cerebrum and the cerebellum carry out different functions.

The human autonomic nervous system

What your central nervous system does for you without you even knowing



Post-synaptic neuron

Synapse
Communication site between two neurons. Here, the electrical signal of the action potential is converted into a chemical signal by the presynaptic cell. This chemical can diffuse to the post-synaptic cell, which detects the signal.

Neuro-transmitter

1921
Otto Loewi confirms that neurons can communicate by releasing chemicals.

1888
Santiago Ramón y Cajal develops the idea that neurons communicate by contact.

1873
Camillo Golgi discovers a method for staining brain neurons in their entirety.

1861
Paul Broca concludes that a particular brain region is specifically responsible for the production of speech.

1810
Charles Bell attributes motor function to ventral root ganglia.
François Magendie shows that dorsal root ganglia carry sensory information.

ca. 1800
The nervous system has been completely dissected, anatomically.

Oligodendroglial cell

Feeling their way in the dark: neurons respond to their physical environment

by Ian Robertson

How our brains develop, from a simple neural tube to a structure capable of thinking and feeling, an organised network of 86 billion neurons, has to be one of the greatest wonders of human development. Through this marvel, evolution has ultimately built a device capable of understanding the very mechanisms of its own creation. However, while our combined minds have achieved a great deal in the search for detailed self-awareness, there are many mysteries that still remain.

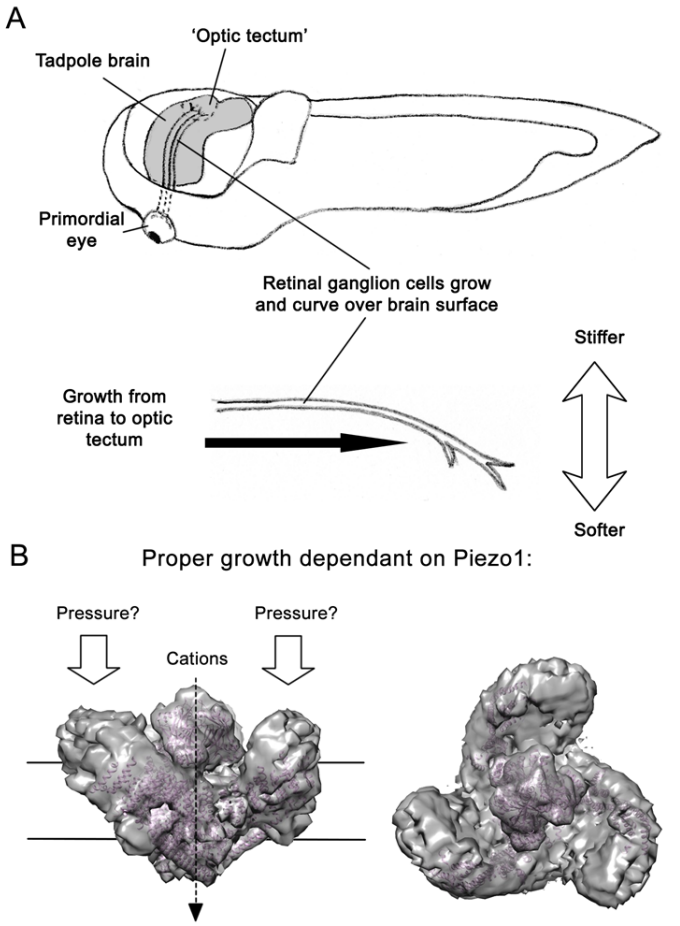


Figure 1. (A) An artist's impression of a developing tadpole with the brain exposed. (B) Structural model of the Piezo1 cation channel (2).

One such mystery being how our billions of neurons know which way to grow in order to connect the correct sensory signals with the right processing areas of the brain. Originally it was thought that they achieve this through a combination of their innate genetic programme and by interpreting signalling molecules from surrounding cells, which can attract or repel them. Recently, a third mechanism to help guide developing neurons has been proposed: the mechanical stiffness of their extracellular environment, also called the extracellular matrix (ECM).

Studies of neurons cultured from the optic nerve of Xenopus tadpoles showed that these neurons grew much further when cultured on stiff gel substrates as opposed to soft gels (1). The authors went on to show that this was relevant in the developing Xenopus brain by following the growth of retinal ganglion cell axons. During the normal process of development, these cells send their axons out of the retina and let them grow along the brain surface in order to connect the tadpole's eyes to the optic tectum (the region of the brain that processes visual signals in Xenopus). Normally these neurons curve as they grow across the brain surface in order to reach the correct brain area, a process which is directed by chemical signals such as slit1/2 and semaphorin. Atomic force microscopy measurements also showed stiffness gradients in developing tadpole brains that could help direct axon growth. Disrupting ECM stiffness, either by applying chondroitin sulfate to dissected brains, or by compressing the brain region with force from a cantilever, inhibited the elongation and curving of the axons, preventing proper optic nerve development.

The detailed mechanisms of how these neurons sense the biomechanics of their surroundings are not completely clear, but they seem to be dependent on 'Piezo1' mechanosensitive cation channels. These channels can be blocked by a peptide toxin derived from tarantula venom, called GsmTx4, such that when tadpoles are exposed to this toxin, their growing neurons stop responding to substrate stiffness. Furthermore, morpholino knockdown of piezo1 in Xenopus tadpoles also disrupted the ability of retinal ganglion cells to grow and curve properly in their brains.

Aside from being a cation channel, the exact mechanism by which Piezo1 functions is still not fully understood. Recent cryo-electron microscopy and crystallography studies have shown that Piezo1 forms a large trimer with a central pore around which three large 'paddles' project into the extracellular space (2). These paddles might act as 'push buttons' to open and close the central pore, allowing cations to rush into the cell, which can both trigger a neural impulse and also affect internal cell signalling. Piezo1 may not only be responding to stiffness though. Another study suggested its involvement in detecting the 'nano-roughness' of a cell's environment (3), where neurons growing on rougher surfaces produce fewer, but longer, neurite extensions. The authors speculate that this nano-roughness may be important in pathologies such as Alzheimer's disease, where extracellularly aggregated amyloid plaques in the brain can alter the topography of a cell's environment.

Whatever mechanisms neurons use to 'feel' their surroundings, it is now clear that the nature of their

physical environment should also be considered when trying to understand their behaviour. In the future, developing drugs that modulate how neurons sense the biomechanics of their surroundings may provide valuable new weapons in the fight against neurodegenerative disorders.

Dr Ian Robertson is a postdoctoral researcher in Prof Penny Handford's research group in the Department of Biochemistry.

References

1. Koser DE, *et al.* (2016) Mechanosensing is critical for axon growth in the developing brain. *Nat Neurosci* 19(12):1592–1598.
2. Ge J, *et al.* (2015) Architecture of the mammalian mechanosensitive Piezo1 channel. *Nature* 527(7576):64–69.
3. Blumenthal NR, *et al.* (2014) Stochastic nanoroughness modulates neuron-astrocyte interactions and function via mechanosensing cation channels. *Proc Natl Acad Sci USA* 111(45):16124–16129.

Can worms get Alzheimer's?

by Josh Newman

Way back in 1902, Auguste Deter found herself in a mental institution in Frankfurt, Germany. Not that she really understood where she was, why she was there, or what she was doing. She had lost touch with the world, her surroundings, the people she knew and loved, and famously uttered the words "*Ich hab mich verloren*" – I have lost myself. But what had this woman lost herself to? The answer became clearer after she was visited by a man whose name would go on to identify her malady – that man was Dr Alois Alzheimer.

Fast forward over 100 years and, whilst we understand a huge amount more about this type of dementia, we are yet to truly understand the underlying molecular mechanisms that initiate and spread the disease around the central nervous system. The pathology is defined by two different types of inclusion that form together in the brain; extracellular amyloid beta plaques, and intracellular neurofibrillary tau tangles. Whilst the field is nicely divided between 'tauists' and 'baptists', there is clear evidence of an interplay between the two proteins, the importance of which, however, is not fully understood or appreciated.

Amyloid precursor protein (APP), as the name would suggest, is the membrane protein precursor to amyloid beta – under normal circumstances, this protein is cleaved by alpha-secretases in a pathway that is considered non-amyloidogenic. Conversely, APP can also be cleaved by beta- and gamma-secretases to produce the amyloidogenic Aβ peptide (1), which is capable of aggregating and forming plaques extracellularly. Within a neuron, however, the tau protein can also go awry and wreak havoc. Normally this protein functions to stabilise microtubules but, when hyperphosphorylated, can form inclusions known as tangles. To this day, we are uncertain as to which of these proteins is the primary culprit in this disease, nor are we sure as to why this happens in the first place, or how it causes cell death. It is information like this that is key to working out an effective treatment.

"By trying to understand these mysterious processes of self-templating, aggregation, and propagation, we will hopefully be able to find new ways to treat these diseases."

The focus of my PhD is the latter of the two – tau; this protein is particularly interesting as it has been implicated in a number of other neurodegenerative diseases, such as progressive supranuclear palsy (PSP), Pick's disease, and corticobasal degeneration (CBD), yet we still do not really understand its role in such disorders. The differences between these tauopathies is reflected in the composition of their tangles, and the regions these tangles appear to form. The core hydrophobic region of this protein, the microtubule binding domain, has a number of repeats that can be alternatively spliced from the nascent *MAPT* mRNA transcript – this results in three repeat (3R) or four repeat (4R) isoforms. In PSP and CBD, inclusions are comprised of 4R tau; in Alzheimer's disease, a mixture of 3R and 4R; and in Pick's disease, purely 3R tau (2). Is this an important part of the pathology? That is somewhat unclear. However, there is evidence that there is a seeding barrier between these isoforms, preventing the spread of misfolding between 3R and 4R (3).

Seeding and spreading are difficult concepts to define in these diseases without a back story, and for that we must cast our minds back to before Auguste Deter was born, and think about an organism we probably overlook in science more often than not; the sheep. In the 1700s, sheep farming was a major industry across Europe and the trading of certain breeds was a hard deal to strike. In fact, the Merino sheep of Spain were so successful as a breed that the Spanish outlawed their trading, with the punishment of death coming to those who defied that order (4). This sheep-based protectionist economy soon fell on its own sword however as, over time, generations of in-breeding gave rise to a dramatic narrowing of the gene pool, and with it came an increased genetic susceptibility to a disease with devastating degenerative consequences. These once-prized sheep would experience changes in their posture, poor balance, convulsions and eventually death. During the course of the disease, they would obsessively rub themselves so vigorously against anything they could find that they would scrape the wool from their skin, leaving gruesome bleeding lesions in its place instead; this led to the disease becoming known as Scrapie. Scrapie is characterised by extensive neurodegeneration that appears as large holes of cell death in the brain, resulting in this family of diseases becoming known as transmissible spongiform encephalopathies, as these holes resemble those in a sponge. The transmissible nature of this disease, and similar diseases in other mammals, including mad cow disease in cattle and Creutzfeldt-Jakob disease in humans, led many to believe that some kind of viral or bacterial agent was to blame. Alas, this was not to be the case, and in the 1980s, Stanley Prusiner proposed the controversial theory that this disease was actually caused by a proteinaceous infectious particle, coining the term prion (5).

The protein-only hypothesis was highly contested – after all, how can an infectious agent propagate without nucleic acids? However, Prusiner went on to win the

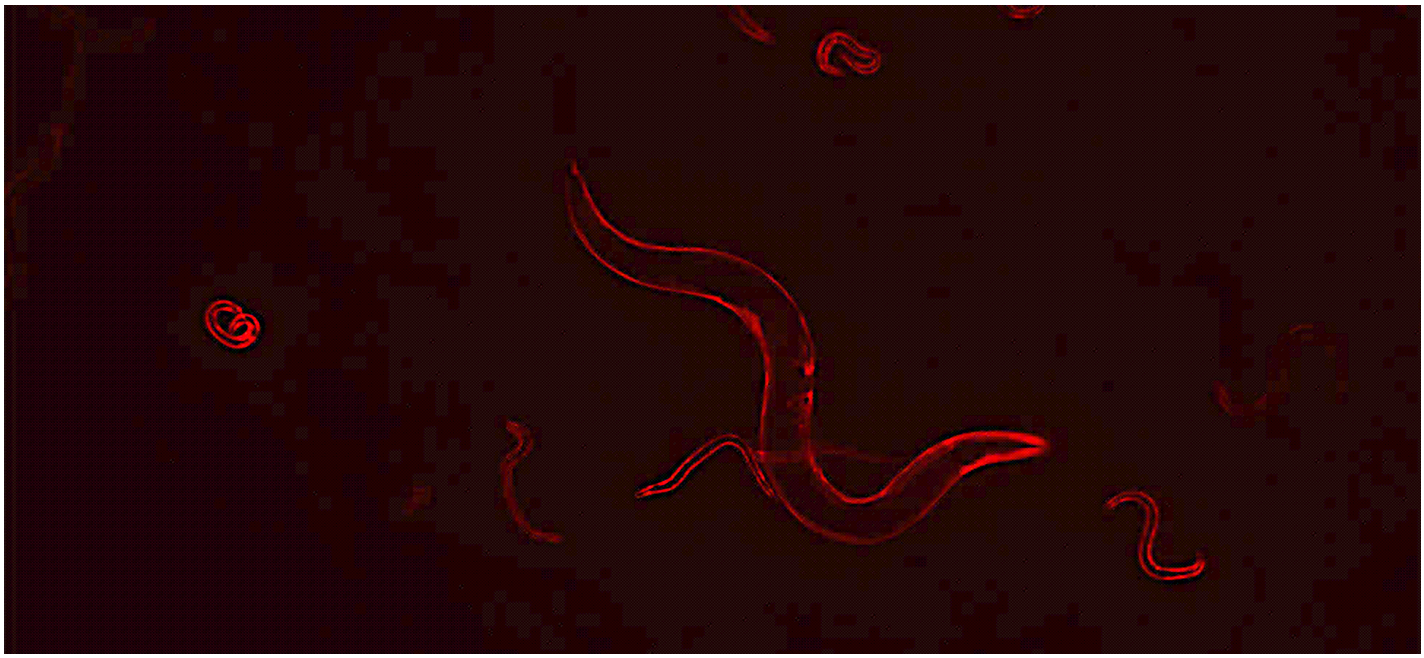


Figure 1. Muscle-specific expression of fluorescently tagged tau in *C. elegans*.

Nobel Prize for this discovery and prion disease is now well-characterised. But how does this relate to Alzheimer's disease? It all comes down to the ability of the pathological prion protein, PrP^{Sc} - an aberrantly folded conformation of the PrP^{C} protein - to induce misfolding events in its correctly folded counterparts, which can then form aggregates. These misfolding events are able to propagate between cells and spread throughout the entire brain. This is similar to what is seen in a number of neurodegenerative diseases, many of which exhibit these prion-like behaviours. This is thought to be the case for tau, with evidence showing pathology spreading to synaptically connected regions and inducing misfolding events in other cells (6). By trying to understand these mysterious processes of self-templating, aggregation, and propagation, we will hopefully be able to find new ways to treat these diseases, rather than just giving drugs to mask the symptoms, which is all we are able do at the moment.

So that's a brief introduction to the background of Alzheimer's disease. But how do I actually study it? Well, I spend my days with a microscopic, transparent nematode known as *Caenorhabditis elegans*. One of the first questions I often get after explaining that I look at Alzheimer's disease in worms is… "But how can you tell if a worm has Alzheimer's? Do they start forgetting things?". The answer to that is, unfortunately, no, memory deficits are not something I study. Instead, I look at how this spread occurs and how we can influence it. As these worms are transparent, fusing the tau protein with a fluorescent marker allows us to observe it within their cells (Figure 1). This is incredibly useful as, unlike previous *in vivo* models of tauopathy, we can obtain real-time visualisation of the spread of pathology and how this changes when we manipulate the system.

But worms don't get these diseases naturally – they don't express the same set of proteins that higher organisms such as you and I do. Instead, we must induce these pathologies by genetically engineering them to express human forms of these proteins – by doing this, we can give worms human diseases and try to figure out what conserved cellular mechanisms might be in place to help or hinder their progression. In particular, we are interested in the effects of non-pathological proteins that may influence the seeding and propagation of these pathological protein aggregates. By assessing these influences, we hope that we may identify future targets that can help us to treat these conditions.

That's the beauty of using a model organism with a very basic and well understood biology – it simplifies the science and hopefully points us in directions that may be evolutionarily conserved in humans. So, from a German woman with an undiagnosed case of early onset Alzheimer's disease in the 1900s to worms on an agar plate today, we certainly have come a long way. But we still have much further to go and many more exciting discoveries to make.

References

1. Zhang Y, *et al.* (2011) APP processing in Alzheimer's disease. *Mol Brain* 4(1):3.
2. Spillantini MG & Goedert M (2013) Tau pathology and neurodegeneration. *Lancet Neurol* 12(6):609–622.
3. Dinkel PD, *et al.* (2011) Variations in filament conformation dictate seeding barrier between three- and four-repeat tau. *Biochemistry* 50(20):4330–4336.
4. Schneider K, *et al.* (2008) The early history of the transmissible spongiform encephalopathies exemplified by scrapie. *Brain Res Bull* 77(6):343–355.
5. Prusiner SB (1982) Novel proteinaceous infectious particles cause scrapie. *Science* 216(4542):136–144.
6. Liu L, *et al.* (2012) Trans-synaptic spread of tau pathology in vivo. *PLoS One* 7(2):e31302.

Josh Newman is a PhD Student at the MRC Laboratory of Molecular Biology (University of Cambridge).

Applications for CRISPR-Cas9 genome editing in neurological disorders

by Veronika Hartleb

There are numerous tools currently available for editing the human genome, one of which is the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-Cas9 system. CRISPR-Cas9 relies on a mechanism whereby the Cas9 enzyme is directed by a single-guide RNA (sgRNA) to a desired location in the genome, where it causes double-strand breaks in the DNA. These breaks can subsequently be repaired by either of two possible pathways. One is an error-prone process called 'non-homologous end-joining' (NHEJ), which often generates insertions or deletions that lead to frameshift mutations or gene knockouts. The other pathway is the more precise 'homology-directed repair' (HDR), which can introduce or correct specific mutations by adding an exogenous DNA sequence. Once corrected, these cells should demonstrate increased functional protein expression (1).

The advantages of CRISPR-Cas9 genome editing

CRISPR-Cas9 can easily be designed to target a desired location in a gene, and can even permit the simultaneous targeting of multiple loci by using numerous sgRNAs. This process is called 'multiplexing', which can be useful for modelling multigenic diseases and generating large-scale libraries of gene knockouts in order to identify potential causes of disease. It can also be modified to study the transcriptional control and epigenetic modulation of genes, which can have a lasting effect on neural function (2). Importantly, it has been shown that CRISPR-Cas9 can effectively target somatic tissues, e.g. neurons in the brain (3). Additionally, animal models using the CRISPR-Cas9 system are useful for studying not only the complex synaptic and circuit functions but also neuronal development and its links to neurological diseases (1).

Animal models developed using CRISPR-Cas9

Parkinson's disease is known to affect the motor system. It is caused by the death of neurons within the substantia nigra portion of the brain, resulting in a loss of dopamine. Although the pathophysiology is not fully understood, mutations in genes PARK2, DJ-1, PINK1, and especially LRRK2 and SCNA, are involved. However, the degree and extent of their involvement remains unclear, since knocking out these genes individually in rodent and pig models does not fully mirror the pathophysiology of the disease. CRISPR-Cas9 genome editing could help create animal models with various gene knockout combinations that would ultimately reveal how these genes interact, as well as aid in the discovery of druggable targets (1).

Many different genetic mutations are known to cause Alzheimer's disease (AD), one of which is the gene encoding for the amyloid precursor protein (APP) involved in early-onset AD. CRISPR-Cas9 has already been successfully used to correct a mutant APP in human fibroblasts and might also be employed in the brain of animal models, using HDR to decrease the amyloid plaque formation observed in Alzheimer's (1).

CRISPR-Cas9 as a clinical therapeutic

CRISPR-Cas9 genome editing also holds great promise for treating human genetic disorders by directly correcting pathogenic mutations in affected cells and tissues. Indeed, human embryos have already been successfully edited, either to cure monogenic diseases like beta thalassaemia by correcting common HBB gene mutations (4) or to make them resistant to HIV infections by inserting a CCR5Delta32 mutation (5). Recently, a group has employed HDR to successfully correct the MYBPC3 mutation that causes cardiac myopathy (6). A number of clinical trials are currently underway using genome editing for cancer.

Neurological disorders, however, are difficult to treat because they have a complex pathophysiology and are either multigenic or multifactorial, and therefore involve complex gene-to-gene and gene-environmental interactions. Furthermore, the brain contains hundreds of neuronal subtypes, each with its own biochemical and physiological functions, and malfunctions of a given cell type in different regions of the brain contribute to correspondingly different neuropsychiatric phenotypes. It is therefore crucial to improve the method for targeting a specific subtype of cells in any given region of the brain (7). Moreover, neurons are generally post-mitotic (they do not divide), which makes them more difficult to edit. Nevertheless, a recent study has shown successful in vivo genome editing of neurons in different areas of the mouse brain, including the hippocampus, striatum and cortex (3). Most of the breaks caused by CRISPR-Cas9 in targeted neurons are repaired by NHEJ. The more precise HDR, which allows desirable, specific DNA corrections, is less likely to work in the post-mitotic neurons (8). Hence, a mechanism to overcome this needs to be identified. CRISPR-Cas9 genome editing aimed at transcriptional regulation might be a feasible option. Since it is independent of DNA repair pathways, it could be useful for diseases like Angelman syndrome, which is characterised by a loss of the *UBE3A* gene encoding for a transcriptional co-activator (9). The faulty genes in neurological disorders generally need to be modified using CRISPR-Cas9 directly in vivo, which makes the procedure more challenging than for other diseases, such as haematological disorders. For example, in sickle cell anaemia, the target cell population can be retrieved from the body and after editing, transplanted back into the patient. In vivo editing is particularly difficult as it mainly relies on the delivery of Cas9 via a viral vector,

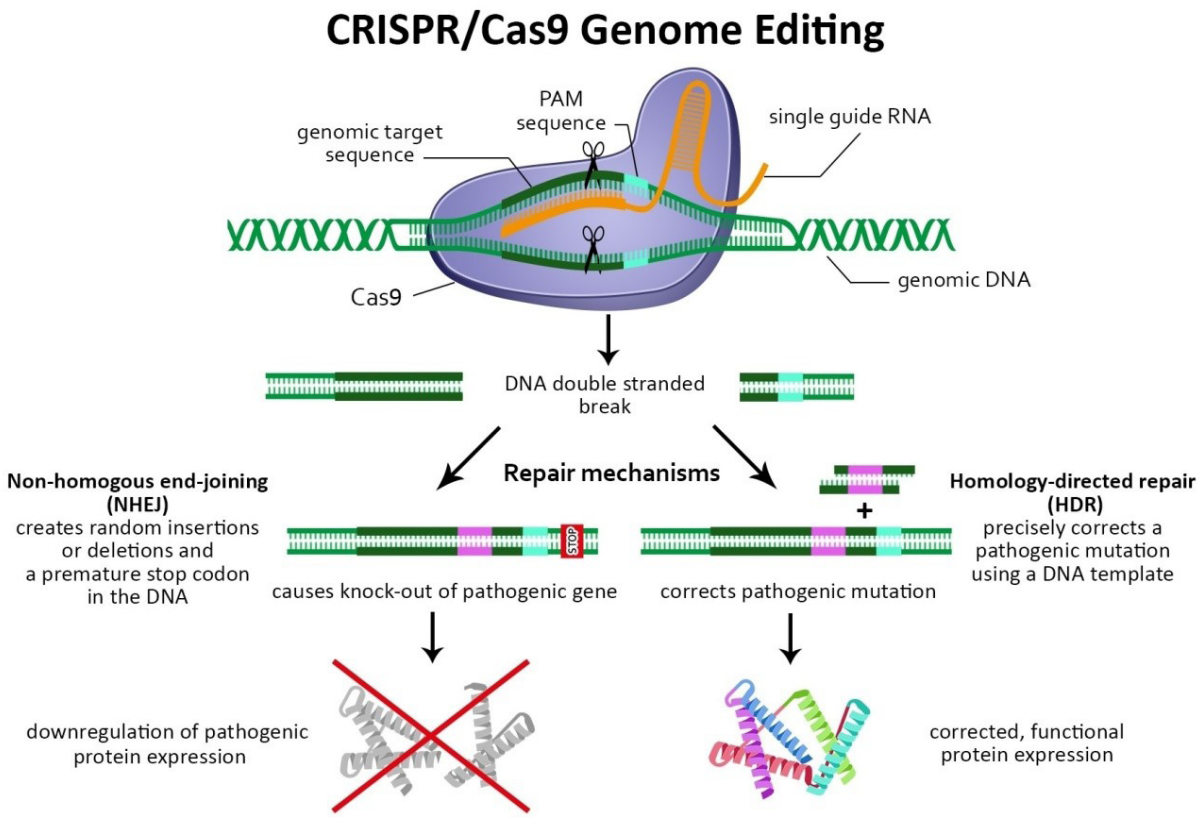


Figure 1. A schematic depicting the concept of CRISPR-Cas9 genome-editing. The Cas9 nuclease is directed by a single guide RNA to cause double-strand breaks in the DNA in close proximity to the PAM sequence. These double-strand breaks are subsequently repaired either via non-homologous end-joining (NHEJ), causing the downregulation of pathogenic protein expression, or via homology-directed repair (HDR), leading to corrected, functional protein expression.

such as a recombinant adeno-associated viral vector (rAAV). This poses further challenges, e.g. the limited packaging capacity of the rAAV, which usually requires an orthologue of the most commonly used *Streptococcus pyogenes* Cas9 (spCas9) – the Staphylococcus aureus-derived saCas9. Still, even spCas9 has been used to edit a mouse brain (10).

+

Safety concerns

The CRISPR-Cas9 system is based on the sgRNAs, which are approximately 20–23 base pairs long. As such, there is a high probability that the sgRNA sequence exists elsewhere in the genome apart from the desired target region and could therefore cause other, unwanted breaks, known as 'off-target effects'. A sgRNA used in CRISPR-Cas9 genome editing could encounter a binding sequence in the genome every 4^{12} (4 trillion) bases when using spCas9 (11). Many groups are now working to minimise these undesirable off-target effects, and the ultimate goal for clinical applications is to abolish them altogether.

In summary, CRISPR-Cas9 is a useful tool for studying both the genetic and the epigenetic causes of various neurological disorders. Although CRISPR-Cas9 could potentially be employed to treat neurological diseases, methods for delivering Cas9 and sgRNAs to the brain and the mechanisms for targeting post-mitotic neurons need to be optimised. Safety concerns must also be carefully addressed to reduce off-target effects. The advent of CRISPR-Cas9 has not only helped to identify and characterise disease-causing genes but will ultimately assist in the drug discovery process.

References

1. Kolli N, *et al.* (2017) Application of the gene editing tool, CRISPR-Cas9, for treating neurodegenerative diseases. *Neurochem Int.*
2. Sweatt J (2013) The emerging field of neuroepigenetics. *Neuron* 80(3):624–632.
3. Staahl B, *et al.* (2017) Efficient genome editing in the mouse brain by local delivery of engineered Cas9 ribonucleoprotein complexes. *Nat Biotechnol* 35(5):431-434.
4. Liang P, *et al.* (2015) CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. *Protein Cell* 6(5):363–372.
5. Kang X, *et al.* (2016) Introducing precise genetic modifications into human 3PN embryos by CRISPR/Cas-mediated genome editing. *J Assist Reprod Genet* 33(5):581–588.
6. Ma H, *et al.* (2017) Correction of a pathogenic gene mutation in human embryos. *Nature* 548(7668):413–419.
7. Nelson S, *et al.* (2006) The problem of neuronal cell types: a physiological genomics approach. *Trends Neurosci* 29(6):339–345.
8. Heidenreich M & Zhang F (2016) Applications of CRISPR-Cas systems in neuroscience. *Nat Rev Neurosci* 17(1):36–44.
9. Jamal I, *et al.* (2017) Rescue of altered HDAC activity recovers behavioural abnormalities in a mouse model of Angelman syndrome. *Neurobiol Dis* 105:99–108.
10. Ran F, *et al.* (2016) In vivo genome editing using Staphylococcus aureus Cas9. *Nature* 520(7546):186–191.
11. Lee H, *et al.* (2016) Genome Engineering with TALE and CRISPR Systems in Neuroscience. *Front Genet* 7:47.

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iPSC-based models of neurodegenerative diseases: promises and pitfalls

by Lauren Watson

As the global burden of neurodegenerative diseases continues to rise, the identification of suitable models in which to study these conditions is becoming a pressing concern. Patients' post-mortem brain tissue, commercially-available cell lines and animal models have all provided valuable insights into disease pathology. However, none of these are able to fully recapitulate human disease progression. Recent developments in the field of induced pluripotent stem cell (iPSC) technology offer a unique opportunity to generate disease-relevant neuronal cells from human patients, capable of overcoming many of the limitations of traditional models, and with the potential to serve as screening tools for therapeutic development.

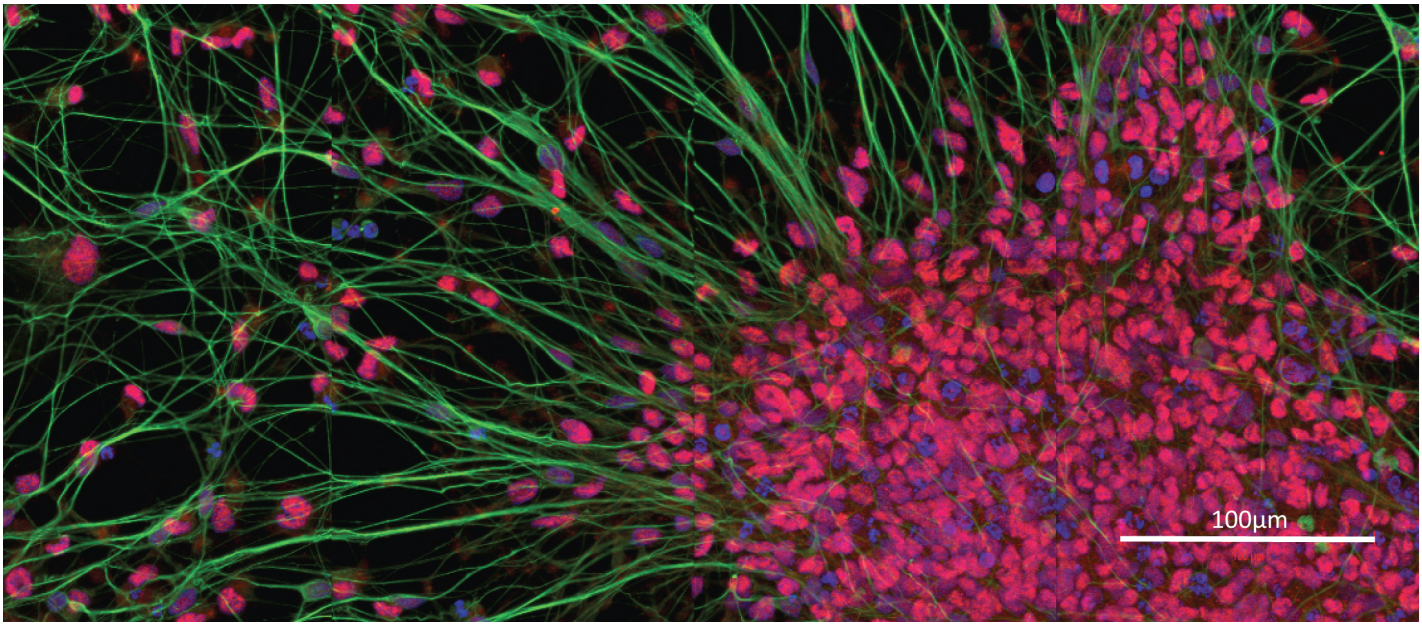

Figure 1. iPSC-derived neurons, generated from a patient with the neurodegenerative disease spinocerebellar ataxia type 7, express the disease-associated protein, Ataxin-7 (red) as well as the early neuronal marker, βIII-tubulin (green). Nuclei are stained with DAPI (blue). Image taken by Lauren Watson and used courtesy of the Confocal and Light Microscope Imaging Facility of the University of Cape Town.

Despite this immense promise, iPSC-based models still face a number of technical challenges, which must be overcome before their application to neurodegenerative disease research becomes more widely applicable.

The inaccessibility of central nervous system (CNS) tissue from living patients presents a particular challenge for studies of neurological disease. Post-mortem human brain tissue typically represents an advanced stage of pathology, often compounded by the effects of ageing, and thus offers only limited insights into how the disease progresses. Classical approaches to circumvent this challenge include the use of cell and animal models, generated via the overexpression of exogenous copies of the disease-associated gene of interest. Although such approaches have yielded significant breakthroughs, they face a number of limitations, including lineage-specific and species-specific differences in gene expression and regulation, as well as experimental artefacts induced by the high levels of transgene expression required to elicit a phenotype.

Attention has therefore turned in recent years to the development of disease-relevant human cell models, which express the pathogenic protein at physiological levels, while still permitting experimental manipulations. Advances in the field of iPSC technology have enabled the robust reprogramming of patient somatic cells into pluripotent stem cells, via the transient expression of embryonic genes (1). These stem cells can then be differentiated into any cell of the body, thereby presenting the ideal solution: CNS cells which not only carry the patient genomic context, but which can also be assayed over time, in theory allowing for the study of neurodegeneration throughout the lifetime of a neuron. Indeed, in the decade since iPSCs were first described, considerable advances have been made in understanding the aetiology and progression of a diverse array of neurodegenerative conditions, including amyotrophic lateral sclerosis (ALS), spinal muscular atrophy, Parkinson's disease, Alzheimer's disease (AD), Huntington's disease and the spinocerebellar ataxias (SCAs) (2). Well-established protocols now exist for the differentiation of human iPSCs into cells with

phenotypes resembling glutamatergic, GABAergic, dopaminergic and motor neurons, as well as medium spiny neurons of the striatum, and glial progenitors (2). These cells have been used extensively to identify novel disease phenotypes, as well as to screen potential therapeutic approaches *in vitro*. Perhaps most excitingly, iPSC-based models have also provided the opportunity to study cells from patients with sporadic neurodegenerative conditions without prior knowledge of the causative genetic defects, enabling, for example, the identification of novel disease-associated gene regulation networks in sporadic AD (3).

However, despite these significant breakthroughs, a number of challenges remain to be addressed. Chief among these is the need for a precise understanding of the complex molecular events underpinning the development of each neuronal subtype, and an accurate set of criteria for the evaluation and characterisation of the generated cells. This has proven a particular obstacle for the generation of iPSC-based models of the cerebellum, whose complex development and highly specialised cellular structures have been extremely difficult to reproduce *in vitro* (4). Even in cases where developmental pathways are well established, protocols remain complex, requiring lengthy periods of differentiation and expensive reagents, and generating heterogeneous cell populations due to variable differentiation efficiencies, which may confound results. More recent protocols have therefore sought to move away from the step-wise delivery of multiple growth factors and small molecules towards a more refined approach, using combinations of fewer factors which trigger differentiation through the induction of endogenous signals (5).

In addition, while the two-dimensional neuronal cultures generated by many protocols facilitate close monitoring of the development of individual cells, such approaches may be limited in terms of long-term maturation and survival, due to a lack of structural and trophic support. Certain phenotypes arising from non-cell-autonomous mechanisms or cell-environment interactions, including those affecting neuronal migration and synapse formation, may also be lost in these cultures. To overcome this, several research groups have pioneered the development of three-dimensional cultures or "organoids", which deliberately include multiple cell types, and mimic the *in vivo* architecture of a particular brain region (reviewed in (6)). Although well-established for the cerebral cortex, organoid models present several limitations of their own, including the need for specialised equipment (such as bioreactors) to maintain long-term viability through the facilitation of nutrient and gaseous exchange (6).
Concerns also remain regarding the correlation between iPSC-derived disease models and neurons in the human brain, particularly relating to age and disease stage. Despite expression of mature neuronal markers in some models, transcriptomic analyses suggest that iPSC-derived neurons are generally still in an embryonic state (7). These cells may be useful for the study of neurodevelopmental defects and early stages of disease progression. However, it is likely that functionally mature cells, and/or the use of exogenous stressors will be required in order to fully recapitulate the phenotypes of late-onset neurodegenerative disease.
iPSC-based models nevertheless offer distinct advantages in the study of neurodegeneration, allowing for the investigation of disease progression and the development of novel therapeutic approaches, using disease-relevant patient cells. In combination with cutting-edge molecular biology techniques such as CRISPR/Cas9 genome editing and single-cell sequencing, these models are poised to deliver invaluable insights into CNS development and degeneration.

"iPSC-based models offer distinct advantages in the study of neurodegeneration, allowing for the investigation of disease progression and the development of novel therapeutic approaches, using disease-relevant patient cells."

References

1. Takahashi K & Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126(4):663–676.
2. Ross CA & Akimov SS (2014) Human-induced pluripotent stem cells: potential for neurodegenerative diseases. *Hum Mol Genet* 23(R1):R17-26.
3. Hossini AM, *et al.* (2015) Induced pluripotent stem cell-derived neuronal cells from a sporadic Alzheimer's disease donor as a model for investigating AD-associated gene regulatory networks. *BMC Genomics* 16(1):84.
4. Watson LM, *et al.* (2015) Induced pluripotent stem cell technology for modelling and therapy of cerebellar ataxia. *Open Biol* 5(7):150056.
5. Muguruma K, *et al.* (2015) Self-organization of polarized cerebellar tissue in 3D culture of human pluripotent stem cells. *Cell Rep* 10(4):537-550.
6. Kelava I & Lancaster MA (2016) Dishing out mini-brains: Current progress and future prospects in brain organoid research. *Dev Biol* 420(2):199–209.
7. Ardhanareeswaran K, *et al.* (2017) Human induced pluripotent stem cells for modelling neurodevelopmental disorders. *Nat Rev Neurol* 13(5):265–278.

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Identifying the genes that generate and regulate circadian rhythms

by
Aarti Jagannath,
Stuart Peirson,
Russell Foster

Life has evolved under a 24h rhythm where environmental factors such as temperature and light fluctuate with a daily predictable sequence. As a consequence, most organisms have evolved circadian clocks that anticipate these regular environmental changes and establish endogenous 24h rhythms to gate the correct physiology and behaviour to the appropriate time window each day (1). The mechanisms underlying circadian regulation are cell autonomous transcription-translation feedback loops (TTFLs): in mammals, the transcription factors CLOCK and BMAL1 drive the expression of *Period* (*Per1/2*) and *Cryptochrome* (*Cry1/2*), whose products are proteins that in turn inhibit CLOCK and BMAL1 (Figure 1).

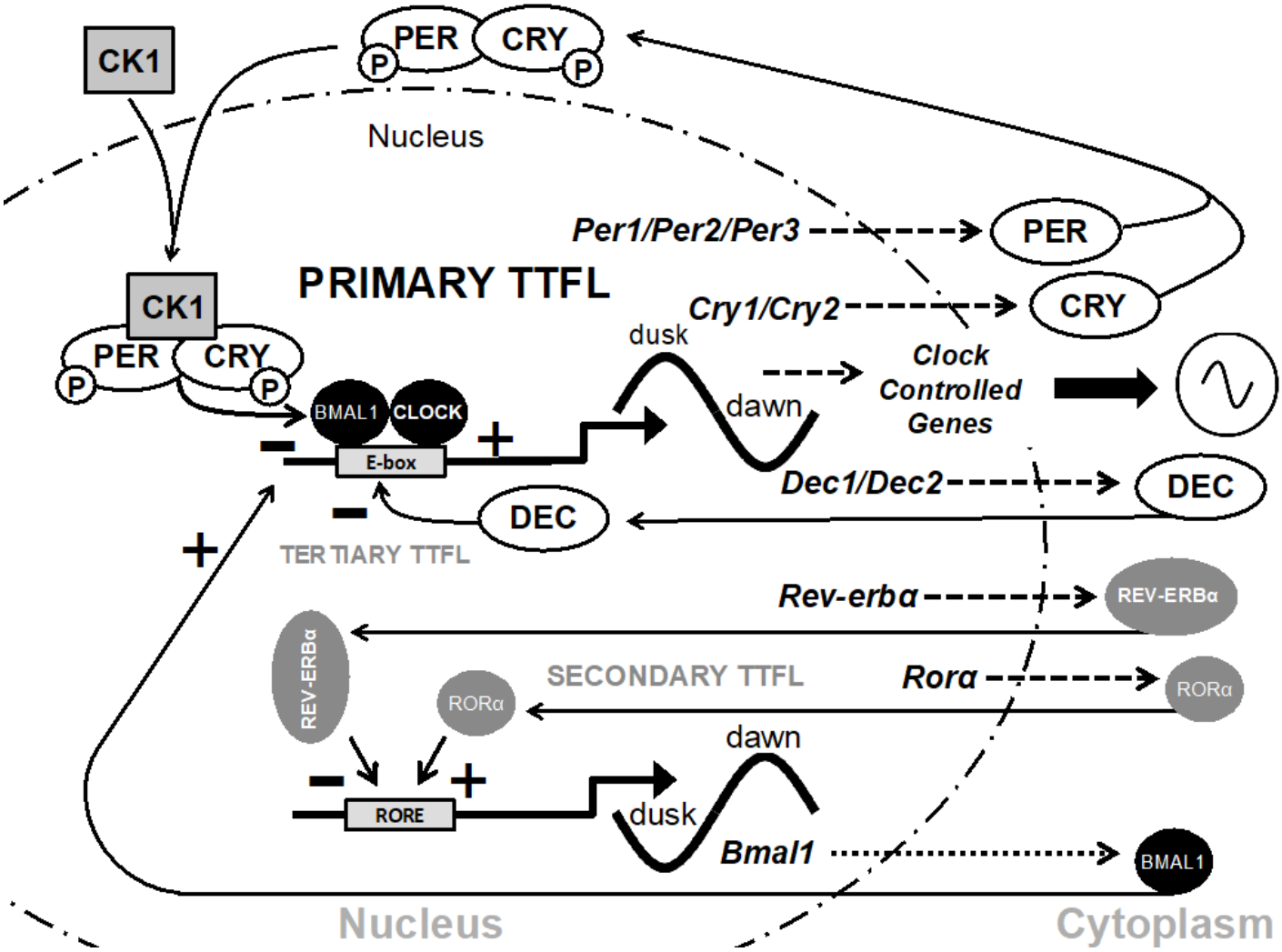

Figure 1. The mammalian molecular clock is highly complex, but the primary driving force is transcription controlled by two proteins, the constitutively expressed "Circadian Locomotor Output Cycles Kaput" (CLOCK) and the rhythmically produced "Brain muscle arnt-like 1" (BMAL1). The heterodimer CLOCK-BMAL1 complex binds to E-box promoters, driving rhythmic transcription of multiple genes, including those encoding PER and CRY proteins. Phosphorylation-regulated PER-CRY-casein kinase 1 (CK1) complexes form, enter the nucleus, and inhibit CLOCK-BMAL1-mediated transcription, forming a negative transcriptional/translational feedback loop (TTFL). Separate to this, light upregulates *Per1/2* transcription, and CRY-PER-CK1 complex expression levels peak at dusk, before declining as PER and CRY are degraded, and reaching the lowest level just before dawn. This light-sensitive PER expression entrains the molecular clockwork to the dawn/dusk cycle. Further levels to the TTFL are achieved through the *BMAL1* promoter elements, *RORα*, which drives *BMAL1* expression, and *REV-ERBα*, which represses *BMAL1* expression. Both these proteins have E-box promoters in their encoding genes, but different transcription/translation rates for the two proteins mean that BMAL1 levels cycle, in antiphase to PER/CRY levels. Additional regulation of the cycle is accomplished by the products of other downstream CLOCK target genes, such as *DEC1* and *DEC2*, both inhibitors of *CLOCK-BMAL1* transcription.

Downstream of these four factors lie thousands of clock-controlled genes that orchestrate the oscillation of tissue-specific metabolic and physiological functions. Most cells in the body possess a molecular clock and are maintained in synchrony by a master pacemaker located in the suprachiasmatic nuclei (SCN) of the hypothalamus.

Light regulation of the circadian network

In order for the circadian network to have adaptive value, it must receive and respond to signals called zeitgebers that provide temporal cues. Zeitgebers modulate the temporal expression patterns of clock genes, such as *Per1/2*, to set the phase, amplitude and period of the molecular clockwork. Light, which signals the dawn-dusk cycle, is the most well characterised zeitgeber (1). In mammals, dawn-dusk photoentrainment is mediated by photoreceptors within the eye, but the classical photoreceptors of the eye play only a secondary role. The eye is the best understood part of the central nervous system. Decades of research have explained how we see: photons are detected by the rods and cones and their graded potentials are assembled into an 'image' by inner retinal neurones, followed by advanced visual processing in the brain. The eye and the brain are connected via the retinal ganglion cells (RGCs), whose topographically mapped axons form the optic nerve. This representation of the eye left no room for an additional class of ocular photoreceptors.

However, studies in the late 1990s showed that mice lacking all their rod and cone photoreceptors could still entrain their circadian rhythms to light perfectly normally. When the eyes were covered, photoentrainment was lost, so another photoreceptor within the eye had to be involved. When this theory was originally proposed, there was considerable hostility to the idea. Vision researchers argued that the eye had been the focus of intensive study for over 150 years, and

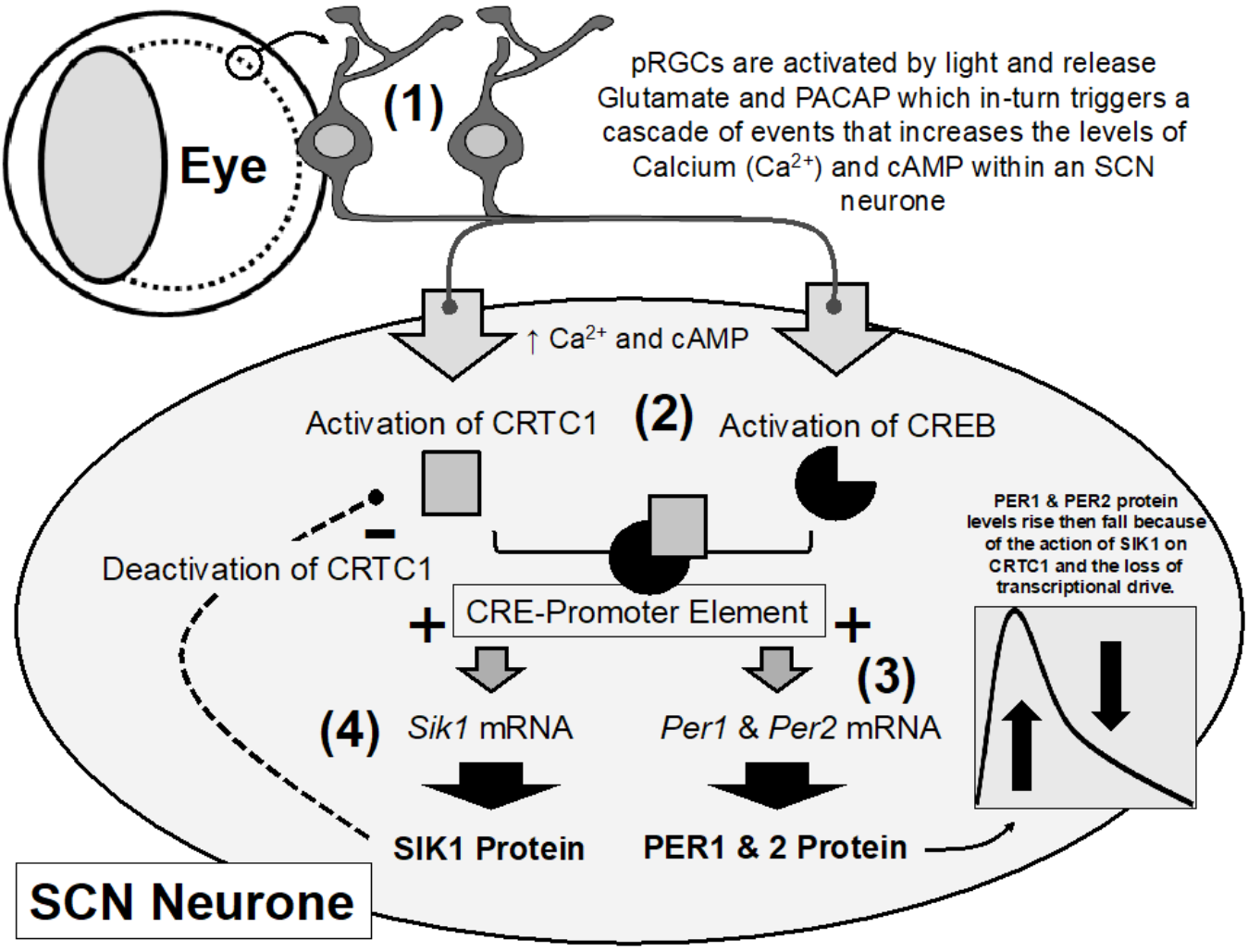

Figure 2. Light regulation of the molecular clockwork in mammals. (1) Melanopsin-expressing photosensitive retinal ganglion cells (pRGCs) detect light, releasing neurotransmitters at pRGC terminals synapsing onto neurons in the ventral SCN. These neurotransmitters trigger signalling cascades that increase levels of calcium and 3'5'-cyclic adenosine monophosphate (cAMP) within SCN neurons. (2) These increased calcium/cAMP levels activate cAMP response element-binding protein (CREB) via phosphorylation by Protein Kinase A, and CREB-regulated transcription coactivator 1 (CRTC1) via dephosphorylation. CREB and CRTC1 together bind to the promoter for the *Per1*, *Per2*, and *Sik1* genes. (3) CRE-driven PER1/PER2 expression shifts the molecular clockwork, advancing and delaying the clock at dawn and dusk, respectively. (4) However, SIK1 phosphorylates and deactivates CRTC1, stopping transcription of *Per1*/2 and limiting the effects of light on the clock and allowing the system to re-set for future light detection.

how could an entire class of photoreceptors have been overlooked? Nevertheless, it was demonstrated first in fish (2), then in mammals (3), that the retina contains a small population of photosensitive retinal ganglion cells, or pRGCs, which comprise approximately 1-2% of all retinal ganglion cells (Figure 2) (4).

The pRGCs utilise a blue light sensitive photopigment termed "melanopsin" or OPN4. Genetic ablation of the rods, cones and melanopsin-pRGCs in mice eliminates circadian responses to light, demonstrating that there are no additional photoreceptors either within the eye, or indeed elsewhere in the body, that regulate the circadian system. Although the rods and cones are not required for circadian entrainment, they are now known to contribute to the light responses of the melanopsin pRGCs under certain circumstances. Genetic silencing of OPN4 in the pRGCs does not block photoentrainment in mice. Mice can still entrain, but with reduced sensitivities (5). Rods and cones send indirect projections via the inner retinal neurones (bipolar and amacrine cells) to the pRGCs, and it seems that in the absence of OPN4, the rods and cones can partially compensate for its loss. A complex pattern is emerging of how the different photoreceptor populations interact.

Just like mice, humans who have lost all of their rods and cones as a result of a genetic disease show normal circadian entrainment and pRGC responses that peak in the blue part of the spectrum (6). This finding is having a major impact in the clinic. Ophthalmologists now appreciate that eye loss deprives us of both vision and a proper sense of time. Furthermore, genetic diseases that result in the loss of the rods and cones, and cause visual blindness, often spare the pRGCs. Under these circumstances, individuals who possess eyes, are visually blind, yet possess functional pRGCS, should be advised to seek out sufficient light to entrain their circadian system. This realisation that the eye provides us with both our sense of space and our sense of time has redefined the diagnosis, treatment and appreciation of human blindness.

The pRGCs project directly to the ventral SCN through synaptic connections, where glutamate signalling then drives cAMP response element binding factor (CREB-CRTC)-mediated transcription of *Per* genes in the SCN (Figure 2) (7). Peripheral circadian clocks throughout the body receive inputs from the SCN and numerous additional signals, including feeding, glucocorticoids, temperature, and indicators of physiological conditions such as metabolic state and sleep history. The mechanisms by which many of these zeitgebers interact with the molecular clockwork of the peripheral clocks remain unclear. Significantly, circadian clock outputs have a profound impact upon the biology of a cell, with anywhere between 2 and 30% of each tissue's transcriptome displaying a circadian rhythm. Interaction between clock transcription factors and tissue-specific transcription factors overlay a circadian

Physical	Mental Health	Behavioural	Performance
Risk of cancer	Risk of depression	Risk of sleepiness	Impaired attention and concentration
Cardiovascular disease and stroke	Psychiatric relapse	Road traffic accidents	Decreased memory
Disorders of the HPA	Mood fluctuation	Falls and fractures	Reduced multi-tasking
Metabolic abnormalities	Delirium	Repeat prescribing	Impaired decision-making
Weight gain and obesity	Impulsivity	Alcohol and drug dependency	Reduced creativity
Reduced immunity	Anger and frustration	Increased sedative and stimulant use	Reduced communication
Bodily sensations of pain	Higher risk of suicide	Less likely to attend appointments	Reduced socialisation
Thermoregulatory problems	Anxiety and hyperarousal	Longer stay in hospital	Less likely to be employed
Vulnerable seizure threshold	Chronic fatigue	Earlier admission to long-term care	More likely to be on benefits

Table 1. Sleep and circadian rhythm disruption (SCRD) can have both short-term and longer-term impacts upon health and well-being. Physical pathologies such as the increased risk of cancer, obesity and cardiovascular disease develop after years of SCRD. Furthermore, short-term SCRD of even a few days can result in a notable drop in normal behaviour and performance (1). Individuals with a risk of mental health problems are more likely to experience an exacerbation of symptoms after SCRD.

rhythm onto tissue-specific gene expression patterns, resulting in the appropriate circadian transcriptome and, in turn, appropriately timed physiology and behaviour.

Sleep and Circadian Rhythm Disruption (SCRD)

In view of the ubiquity and importance of circadian rhythms it is perhaps no great surprise that sleep and circadian rhythm disruption (SCRD) is a common feature shared by some of the most challenging diseases of our time. Sufferers of mental illnesses such as schizophrenia, bipolar disorder and depression; neurological conditions like Alzheimer's, stroke and multiple sclerosis; and serious disorders of the eye, all exhibit SCRD. Various forms of SCRD are also widespread across the population, as seen in developmental disorders such as autism and Smith-Magenis Syndrome, during teenage development, in those who do work shifts, and indeed everyone affected by the demands of today's 24/7 society; and not least in the ageing population (1). Despite the prevalence of SCRD, its origins remain a mystery, its detection is frequently overlooked, it is rarely treated, and treatment options are severely limited (see below). Yet the health consequences of SCRD are profound. It is important to stress that SCRD is far more than feeling sleepy at an inappropriate time. It promotes multiple poor health conditions physically, mentally and behaviourally, as summarised below (Table 1).

New treatments for sleep and circadian rhythm disruption (SCRD)?

Despite our growing knowledge of the molecular mechanisms underlying the 24h circadian clock and its role in the development of chronic and debilitating diseases, there are limited therapeutic options available for the treatment of sleep and circadian rhythm disruption (SCRD) (8). As light is the primary zeitgeber for the SCN clock, bright light therapies and cognitive behavioural therapies that strengthen natural zeitgebers, such as scheduled outdoor exercise, have been shown to have some success (1). However, potent pharmacological interventions are still lacking. Melatonin has long been characterised as an output of the circadian clock and can be used to modify the phase of the clock, presumably acting via melatonin receptors expressed in the neurones of the SCN and multiple other cell populations across the body.

Melatonin has therefore been studied as a possible chronotherapeutic drug and seems promising in certain circadian-related conditions. Prolonged release of melatonin (tradename *Circadin*) is used to treat primary insomnia in the elderly, whilst the agonist *Agomelatine* is used in the treatment of major depressive disorders. Most recently, *Tasimelteon* was approved in the United States in an orphan circadian disorder, non-24h sleep-wake disorder in the totally blind. Targeting the melatonin system, however, has limited efficacy. For example, *Tasimelteon* showed a beneficial effect on stabilising sleep-wake in only 20% of patients after one month of treatment (1). Consequently, recent efforts have focused on developing alternative therapies, mainly targeting the core clock. Solt *et al.* reported that a novel REV-ERBa receptor agonist was effective at regulating both sleep and metabolism in mice, whilst Hirota *et al.* have developed a small molecule Cryptochrome activator. An alternative strategy now being developed in Oxford is the identification of molecules that act on the light input pathway to the clock, providing a pharmacological replacement for light in the treatment of SCRD.

References

1. Foster RG & Kreitzman L (2017) *Circadian Rhythms: A very short introduction.* Oxford University Press, Oxford.
2. Soni BG, *et al.* (1998) Novel retinal photoreceptors. *Nature* 394(6688):27–28.
3. Freedman MS, *et al.* (1999) Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. *Science* 284(5413):502–504.
4. Sekaran S, *et al.* (2003) Calcium imaging reveals a network of intrinsically light-sensitive inner-retinal neurons. *Curr Biol* 13(15):1290–1298.
5. Hattar S, *et al.* (2003) Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. *Nature* 424(6944):76–81.
6. Zaidi FH, *et al.* (2007) Short-wavelength light sensitivity of circadian, pupillary, and visual awareness in humans lacking an outer retina. *Curr Biol* 17(24):2122–2128.
7. Slanger TE, *et al.* (2016) Person-directed, non-pharmacological interventions for sleepiness at work and sleep disturbances caused by shift work. *The Cochrane Library* (8):CD010641.
8. Hughes S, *et al.* (2015) Chapter six: photic regulation of clock systems. *Method Enzymol* 552:125–143.

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The biochemistry of Fragile X Syndrome (FXS)

by Claire Hill

Fragile X Syndrome (FXS) was one of the first genetic diseases in which the link between RNA regulation and cognitive function began to emerge. It is a form of inherited intellectual disability, with a prevalence of approximately 1 in 4000 males (1) and 1 in 8000 females (2). FXS is caused by the mutation of a single gene, fragile X mental retardation 1 (*FMR1*), which encodes a heterogeneous group of fragile X mental retardation protein (FMRP) isoforms.

Most commonly, FXS occurs due to massive expansion of the number of CGG repeats in the 5' untranslated region (UTR) of the *FMR1* gene (increasing from 5 – 44 repeats to over 200), causing a fragile site on the X chromosome (Figure 1). The CGG repeats subsequently become hyper-methylated, which leads to transcriptional silencing of the *FMR1* gene. In some cases, FXS patients may have a normal number of CGG repeats, but instead carry a missense mutation in *FMR1*. This may lead to a change from arginine to glutamine (R138Q) in the nuclear localisation sequence (NLS) (3) or an isoleucine to asparagine change (I304N) in the second K homology (KH) RNA-binding domain (KH2) (4). As FXS is an X-linked disease, affected males exhibit a stronger phenotype than females. The most significant clinical characteristics of FXS include developmental delay and inherited intellectual disability. The question that remains, however, is how does a loss of FMRP lead to these observed clinical phenotypes? FMRP is a known RNA-binding protein, but the molecular basis of its involvement in memory and learning during development is unclear.The *FMR1* gene is widely expressed in human and mouse tissues, particularly within the organs most affected by FXS, such as the adult brain and testes. FMRP contains clusters of arginine and glycine residues (an RGG box) and two ribonucleoprotein (RNP) KH domains, indicating that it is an RNA-binding protein (5). Importantly, the I304N mutation is located within the second KH domain of FMRP, which suggests that the ability of FMRP to associate with RNA is essential for its normal function. RNA sequence specificity is likely achieved by the first 400 bases, whilst the C-terminal region, which contains the RGG box, provides a non-specific RNA-binding surface.

Immunohistochemistry studies have shown that FMRP (also referred to as FMR-1) is a cytoplasmic protein and is abundantly present in neurons and found at very low levels in glial cells (6). Research carried out by Feng et al. (1997) further characterised FMRP localisation, finding that it co-fractionated with polysomes and rough endoplasmic reticulum in human cell lines and mouse brain. Co-fractionation of FMRP with ribosomes in synaptosomal preparations also confirmed the presence of FMRP in dendrites, which suggests that it may be involved in translation of proteins that are important for dendritic structure or function. Immunogold studies have suggested that nucleo-cytoplasmic shuttling of FMRP occurs. Dendritic localisation, which was confirmed by co-fractionation of FMRP with ribosomes in synaptosomal preparations, also suggests that FMRP might be involved in the translation of proteins important for dendritic structure or function. Studies carried out in mouse NIH 3T3 cells and human HeLa cells have also shown that FMRP may be an mRNA chaperone protein, interacting with messenger ribonucleoprotein (mRNP) particles within the translational apparatus (8). In addition, a study carried out in cultured hippocampal neurons documented the presence, motility and activity-dependent regulation of FMRP granule trafficking in dendrites and neuronal spines (9). Again, FMRP granules were shown to co-localise with ribosomes, as well as ribosomal RNA and MAP1B mRNA, the latter of which encodes a protein important for microtubule and actin stabilisation.

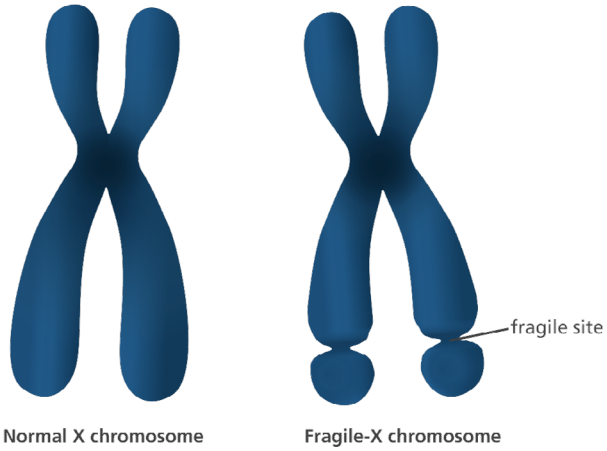

Figure 1. Schematic diagram representing the appearance of normal and Fragile-X chromosomes (13).

Finally, FMRP has also been shown to associate with Dicer and the RNA-induced silencing complex (RISC) (10). As such, it has been proposed that FMRP guides microRNA produced by Dicer into the RISC complex, resulting in gene silencing. In addition, FMRP has been shown to associate with two miRNAs in the mouse brain, miR-125b and miR-132, which are known to be involved in controlling synaptic plasticity and dendritic spine morphology (11). Although the exact role of FMRP remains largely unknown, these data suggest that one of the roles of FMRP in neurons is to regulate synaptic plasticity via the transport and translation of specific mRNAs at dendritic spines, and through the miRNA pathway. FMRP also appears to play a role in

the general regulatory system of microtubule formation, particularly during brain development.

The study of FMRP is a diverse and often conflicted research area; however, new techniques such as high-throughput sequencing cross-linking immunoprecipitation (HITS-CLIP) are providing a snapshot of where FMRP binds RNA across the entire transcriptome in living cells. This research has generated a list of potential FMRP targets, enabling the design of functional assays that will help to define the molecular role of FMRP in controlling translation (12). This work is also shedding light on the nature and function of FMRP, and its role in neuronal function and cognitive development, ultimately paving the way towards the development of potential therapeutics.

References

1. Turner G, *et al.* (1996) Prevalence of fragile X syndrome. *Am J Med Genet* 64(1):196–197.
2. Saul RA & Tarleton JC (1993) FMR1-Related Disorders (University of Washington, Seattle) Available at http://www.ncbi.nlm.nih.gov/pubmed/20301558 [Accessed 10th March 2017].
3. Collins SC, *et al.* (2010) Identification of novel FMR1 variants by massively parallel sequencing in developmentally delayed males. *Am J Med Genet Part A* 152(10):2512–2520.
4. Feng Y, *et al.* (1997) FMRP associates with polyribosomes as an mRNP, and the I304N mutation of severe fragile X syndrome abolishes this association. *Mol Cell* 1(1):109–118.
5. Siomi H, *et al.* (1993) The protein product of the fragile X gene, FMR1, has characteristics of an RNA-binding protein. *Cell* 74(2):291–298.
6. Devys D, *et al.* (1993) The FMR–1 protein is cytoplasmic, most abundant in neurons and appears normal in carriers of a fragile X premutation. *Nat Genet* 4(4):335–340.
7. Feng Y, *et al.* (1997) Fragile X mental retardation protein: nucleocytoplasmic shuttling and association with somatodendritic ribosomes. *J Neurosci* 17(5):1539–1547.
8. Corbin F, *et al.* (1997) The fragile X mental retardation protein is associated with poly(A)+ mRNA in actively translating polyribosomes. *Hum Mol Genet* 6(9):1465–1472.
9. Antar LN, *et al.* (2005) Localization of FMRP-associated mRNA granules and requirement of microtubules for activity-dependent trafficking in hippocampal neurons. *Genes, Brain Behav* 4(6):350–359.
10. Siomi H, *et al.* (2004) RNA interference: A new mechanism by which FMRP acts in the normal brain? What can Drosophila teach us? *Ment Retard Dev Disabil Res Rev* 10(1):68–74.
11. Edbauer D, *et al.* (2010) Regulation of Synaptic Structure and Function by FMRP-Associated MicroRNAs miR-125b and miR-132. *Neuron* 65(3):373–384.
12. Darnell JC, *et al.* (2011) FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. *Cell* 146(2):247–261.
13, What is Fragile-X Syndrome? (2016) Image credit: Genome Research Limited. Available at http://www.yourgenome.org/facts/what-is-fragile-x-syndrome [Accessed 31st August 2017].

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Cardiovascular disease: a neuronal miscommunication?

by Emma N. Bardsley

The expansion of the aging population has led to a concomitant rise in age-related illnesses. Amongst these, cardiovascular diseases are the most prevalent accounting for 15 million deaths worldwide every year. Hypertension is central in determining cardiovascular risk and is a potent indicator of morbidity and mortality; however, there remains an unmet clinical need for disease-modifying and prophylactic interventions. The Paterson research group addresses this need by investigating the processes that modulate sympathetic activity, a major determinant in the aetiology of hypertension.

Autonomic balance is integral for cardiac health

The autonomic nervous system (ANS) plays a fundamental role in the regulation of cardiac parameters, including heart rate and myocardial contractility. The ANS comprises the sympathetic and parasympathetic nervous systems; two opposing branches that act to increase cardiac excitability or decrease cardiac work, respectively. The highly regulated integration of these two lines of communication is crucial for homeostatic responses to physiological, emotional and environmental stimuli, such as exercise, excitement, stress and sleep. As such, a disrupted balance of sympathetic and parasympathetic activity (dysautonomia) can have devastating effects on human health (Figure 1).

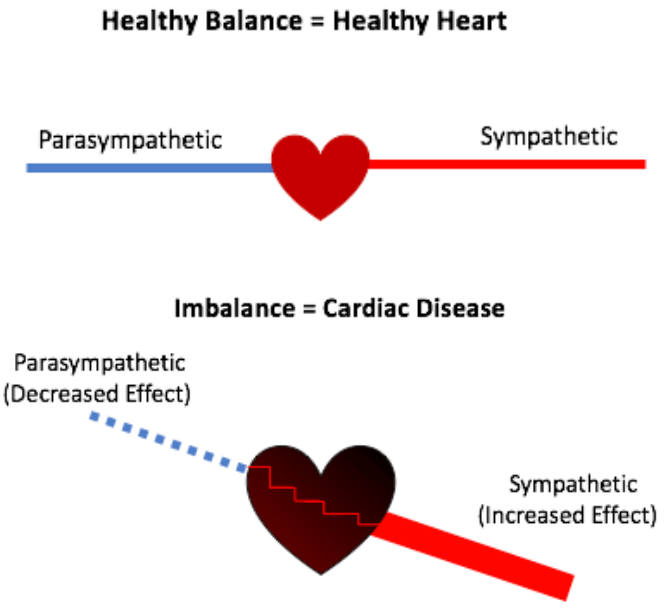

Figure 1. The effects of a disrupted balance in the autonomous nervous system on human health.

Neuronal pathology in heart disease

In the Paterson group, we are interested in the development and progression of sympathetic neuronal pathology in relation to hypertension and cardiovascular diseases. We predominantly use neurons cultured from the spontaneously hypertensive rat (SHR), a multifactorial model of heart disease (Figure 2). Importantly, neuronal pathology manifests prior to the onset of hypertension. Moreover, renal, vascular, and cardiac complications

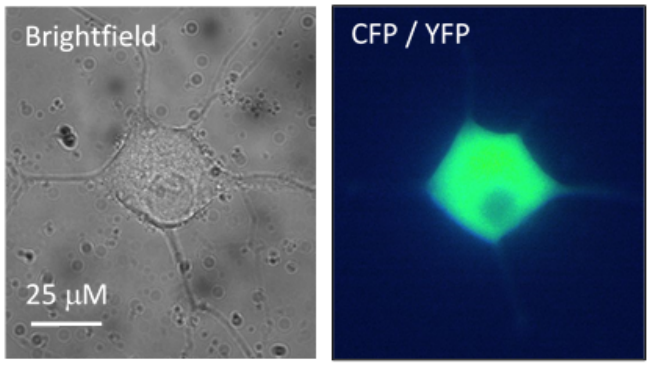

Figure 2. Sympathetic stellate neuron in culture. This neuron is expressing a fluorescent marker and was imaged using visible (left panel) and fluorescent light (right panel).

develop progressively over time, akin to pathogenesis in humans.

In the last decade, the Paterson group has been at the forefront of ANS research in cardiac disease. Early work from the group demonstrated that impaired neuronal nitric oxide synthase (nNOS) activity and downstream reductions in nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) signalling results in enhanced intracellular calcium ($[Ca^{2+}]_i$) transients and end-organ neurotransmission (1). We have also shown that cyclic adenosine monophosphate (cAMP) signalling is compromised in these models in response to particular pharmacological stimuli, suggesting that there are differences in the transcription and activity of certain phosphodiesterase (PDE) isoforms involved in cyclic nucleotide breakdown (Figure 3) (2).

Recently, the importance of sympathetic cyclic nucleotide dysfunction became apparent. We demonstrated that sympathetic hyperactivity in the stellate neuron may occur as a result of increased N-type Ca^{2+} channel activity ($Ca_v2.2$), which is regulated by cyclic nucleotides (Figure 3). Moreover, the larger Ca^{2+} currents measured in SHR neurons could be abolished by pharmacologically increasing cGMP signalling, supporting a significant physiological role for the N-type Ca^{2+} channel in neural modulation associated with cardiovascular disease (3).

What next?

Pharmacological, surgical and genetic interventions aimed at rectifying dysautonomia have been successful in improving and/or reversing the development

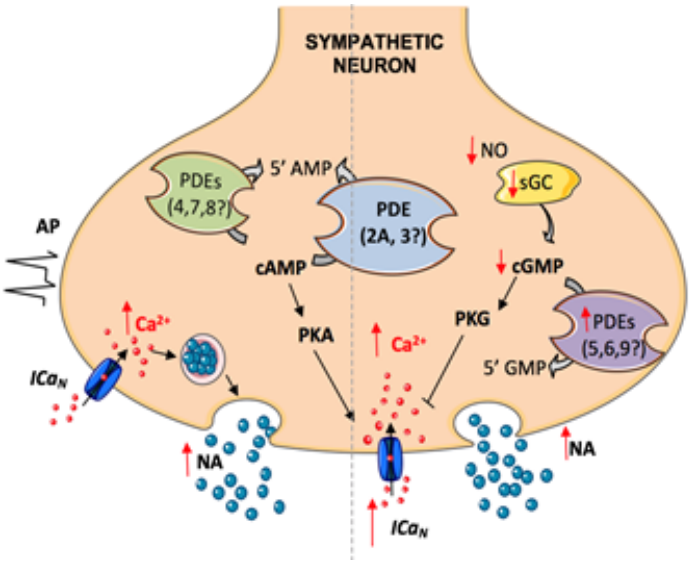

Figure 3. Representation of the axon terminal of a sympathetic neuron. Red arrows indicate increases and decreases in signalling molecules.

of cardiovascular disease in animal models. Stellate sympathectomy (removal of cardiac sympathetic nerves) and vagal nerve stimulation have been tested in a wide range of clinical paradigms with varying levels of success, highlighting the importance of translational research for the development of effective clinical strategies. This year, we have fostered a close collaboration with researchers at UCLA and are now receiving sympathetic ganglia from control donors and patients with cardiovascular disease, with the aim to sequence the transcriptome of human postganglionic sympathetic stellate neurons. Targeting specific aberrant signalling pathways that contribute to the development and progression of hypertension is a safer and more precise approach, and we hope that the identification of disease hallmarks will provide the opportunity for effective prophylactic treatments in the not-too-distant future.

References

1. Li D & Paterson DJ (2016) Cyclic nucleotide regulation of cardiac sympatho-vagal responsiveness. *J Physiol* 594(14):3993–4008.

2. Bardsley EN, *et al.* (2016) Impaired cAMP-cGMP cross-talk during cardiac sympathetic dysautonomia. *Channels* 11(3):178–180.

3. Larsen HE, *et al.* (2016) Dysregulation of neuronal Ca2+ channel linked to heightened sympathetic phenotype in prohypertensive states. *J Neuroscience* 36(33):8562–8573.

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Crush the resistance: new strategies to save antibiotics

by Henry Stennett

In his 1945 Nobel lecture, Alexander Fleming recounted the discovery of penicillin, the antibiotic that would revolutionise medicine and save countless lives. His speech ended with a stark note of warning: 'It is not difficult to make microbes resistant to penicillin.' Antibiotics are our best weapons against bacteria, stopping their growth and reproduction, or killing them outright. Despite Fleming's caution, antibiotic resistance - the ability of bacteria to overcome these drugs - has become a global problem of growing concern (1).

WHO has detailed how "this serious threat is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country". By 2050, antibiotic resistance could cause more deaths than cancer and cost the world $100 trillion. Multidrug resistant bacteria currently cause half of the deaths due to hospital-acquired infections in Europe, and bacteria resistant to all approved antibiotics have already claimed lives.

Dollars and cents

Although better-regulated use of antibiotics, improved sanitation, and alternative treatments are important, development of novel antibiotics is essential (2). From 1940-60, twenty antibiotic classes were established, but since then only two new classes have reached the clinic. The main reasons for this alarming decline are economic.

Less than five percent of the $38 billion spent on pharmaceutical research and development between 2003 and 2013 went towards antibiotics discovery. Major pharmaceutical companies discontinued antibiotic research because the market for these drugs is uniquely unappealing. Companies want their new products to capture a large market share and sell at high volume while still on patent. However, public health authorities limit the use of new antibiotics to slow the emergence of resistance. Additionally, developing new antibiotics is technically challenging, and even when a promising lead is found, the high rate of failure at each stage of clinical trials makes investment high risk. Although small biotech companies and academia are more financially limited, they have identified some innovative solutions.

"With a sustained effort, perhaps we can outsmart our oldest foe once again."

Exploring new worlds

Historically, antibiotics have been discovered using Selman Waksman's screening platform: soil bacteria are cultured with test species, and agar plates examined for inhibition zones where secreted antibiotics stopped the growth of test bacteria (3). As pathogens became resistant to early antibiotics, chemists created derivatives with restored efficacy. Unfortunately, soil bacteria produce a finite number of antibiotics, and a limited set of chemical modifications can be made to these, so discovery eventually shuddered to a halt.

"Nature is the best chemist, but we have only seen a fraction of its potential: there is ample exploring to be done!"

One solution is to exploit untapped sources of natural products. Soil is just one of our planet's many varied environments, any of which could harbour new antibiotic-producing species. Less than five percent of the ocean has been explored; the inhabitants of this mysterious environment and their biosynthetic potential are almost entirely unknown. Marine bacteria have already yielded novel antibiotics. Sporolides A and B are 'chemically unprecedented' molecules with elaborate multiple ring structures and heavily oxidised carbons, while abyssomicin C is synthesised by the only known enzyme capable of performing a Diels-Alder reaction, "the Mona Lisa of organic chemistry".

Less than one percent of soil bacteria can be grown in the lab - far from being exhausted, the vast majority of this resource remains unexplored. Devices like the iChip allow characterisation of 'unculturable' bacteria. Environmental samples are diluted to isolate single cells in each of the device's channels, which are covered by membranes to trap bacteria but allow diffusion of nutrients and growth factors. The iChip is then placed back into the natural environment where conditions are optimal for bacterial growth (4). This technology allows about half of the bacteria in soil to be grown and screened, and recently resulted in the discovery of teixobactin, an antibiotic that kills many clinically relevant drug-resistant bacteria by an entirely new mechanism.

Some researchers are taking unorthodox approaches to sampling: *Swab and Send* is a citizen-science project that encourages members of the public to swab environments around them - toilet seats, shoes, pets - and send in

samples. The bacteria captured are grown in the lab and tested for activity - so far the project has identified eighteen species that kill multidrug resistant *Escherichia coli*. Nature is the best chemist, but we have only seen a fraction of its potential: there is ample exploring to be done!

One-two punch

Bacteria have so far developed resistance to all of our treatments - but is it possible to design "evolution-proof" antibiotics? If an antibiotic targets a single protein, resistance quickly develops through mutations to the coding gene. However, if several targets are inhibited by multiple antibiotics, the pathogen would need to accumulate multiple mutations simultaneously, which is far less likely. Synergy between antibiotics might also enhance their activity, allowing for lower doses and shorter courses of treatment, and further reducing the rate of resistance. Combination therapy is already in use for diseases like malaria, HIV, and cancer to slow the development of resistance (5).

Some groups have already identified powerful cooperative effects between antibacterial drugs. The outer membrane of Gram-negative bacteria is highly impermeable, and acts as a barrier to many antibiotics that are effective against Gram-positives, which lack this structure. A search for compounds that disrupt the outer membrane identified pentamidine, an antiparasitic drug, as an agent that grants certain antibiotics the ability to kill Gram-negatives. The drug unexpectedly binds lipopolysaccharide, a key component of the outer membrane, and disrupts electrostatic interactions between neighbouring molecules, allowing antibiotics free passage. In mice infected with multidrug resistant Gram-negative pathogens, the combination of pentamidine and a Gram-positive-specific antibiotic saved the animals and cleared infection, even at doses much lower than those normally given during therapy (6). Since pentamidine is already an approved drug, clinical trials of antibiotic combinations may be easier to complete.

Another avenue of research is the design of single antibiotic molecules with multiple mechanisms of action. Resistance to vancomycin, a glycopeptide antibiotic, has emerged remarkably slowly, after sixty years of clinical use. Glycopeptides bind dipeptide groups in cell wall precursors to inhibit cell wall maturation - because these targets are metabolites, resistance cannot occur due to simple genetic mutations. Pathogens have not evolved vancomycin resistance themselves, but acquired the necessary genes from glycopeptide-producing organisms. Vancomycin resistance seems intrinsically difficult to evolve, involving coordinated antibiotic sensing, intracellular signalling, and remodelling of precursor dipeptides. To overcome this mechanism and make antibiotics more durable against future resistance, a vancomycin analogue has been designed with three different modes of action (7). Functional groups were added that:

1. Bind both the normal and modified dipeptide to inhibit cell wall maturation,
2. Inhibit enzymes involved in cell wall biosynthesis, and
3. Induce cell membrane permeability.

Intriguingly, these mechanisms synergise, so that the analogue is ten thousand times more potent than vancomycin, and resistance develops so slowly that it was not observed during the study.

Cause for optimism?

Antibiotic resistance is a sobering problem, and there is a desperate need for novel drugs to fight bacterial infections. Pioneering research is tackling this problem in inventive ways: past strategies are inspiring exploration of new natural product sources, existing drugs are being trialled in powerful combinations, and antibiotics are being designed to prevent future resistance. With a sustained effort, perhaps we can outsmart our oldest foe once again.

References

1. Venter H (2017) Essays In Biochemistry. *Essays Biochem* 61(1):1–10.
2. Coates A (2011) Novel classes of antibiotics or more of the same? *Brit J Pharmacol* 163(1):184–194.
3. Lewis K (2013) Platforms for antibiotic discovery. *Nat Rev Drug Discov* 12(5):371–387.
4. Ling L, *et al.* (2015) A new antibiotic kills pathogens without detectable resistance. *Nature* 517(7535):455–459.
5. Silver L (2007) Multi-targeting by monotherapeutic antibacterials. *Nat Rev Drug Discov* 6(1):41.
6. Stokes JM, *et al.* (2017) Pentamidine sensitizes Gram-negative pathogens to antibiotics and overcomes acquired colistin resistance. *Nat Microbiol* 2:17028.
7. Okano A, *et al.* (2017) Peripheral modifications of [Ψ [CH2NH] Tpg4] vancomycin with added synergistic mechanisms of action provide durable and potent antibiotics. *P Natl A Sci* 201704125.

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Patch-clamp electrophysiology

by Matthew Cooper

The development of patch-clamp techniques since the 1970s has revolutionised neuroscience by enabling researchers to record from individual ion channels. Use of these techniques has improved our understanding of the contribution of different ion channels to fundamental neuronal cell processes, and as a result, its developers Erwin Neher and Bert Sakmann received the Nobel Prize in Physiology or Medicine in 1991. However, its development would not have been possible without centuries of scientific discovery and technological advancement.

Some of the first research into electrical currents in animal tissue were performed in the 1700s by Luigi Galvani, who found that electrical excitation of a nerve stimulated muscle contractions in a frog neuromuscular junction preparation (1). The first recordings of electrical activity within tissue were performed by Leopoldo Nobili in 1825, using a device called a galvanometer to record contraction-associated currents. Subsequent research throughout the 19th century detected what we now know as action potentials, signal propagation along neurons, and the resting membrane potential.

These findings fuelled the emergence of a theory, developed across several decades, that currents in nerve cells are carried by charged ions moving across a lipid membrane. Following the discovery of the giant squid axon (a very large and accessible neuronal process), mini-electrodes that could record from inside the axon, directly detecting resting and action potentials, were produced. Furthermore, Kenneth Cole found it was possible to experimentally control the cellular membrane potential using two electrodes and a feedback circuit, resulting in the first known use of a voltage clamp (2).

Subsequent work by Alan Hodgkin and Andrew Huxley, utilising this technique, demonstrated the passive flow of ions down electrochemical gradients across membranes, with the specific permeability properties of such membranes determining the excitation profile of a given cell, laying the foundations for electrophysiology. Ultimately, their work culminated in the Hodgkin-Huxley model, a mathematical description of the action potential that won them the 1963 Nobel Prize in Physiology or Medicine (3).

The electrodes used by the likes of Cole were large and crude, and as such were limited to use in large cells such as the giant squid axon. The ability to record from smaller neurons required finer equipment; ultimately, the creation of microelectrodes with sub-micrometre tips signalled the beginning of single cell, and eventually single channel electrophysiology. Using such fine-tipped electrodes to isolate a tiny 'patch' of membrane and eliminate background noise, Neher and Sakmann were able to record single channel ion currents for the first time in 1976 (Figure 1) (4).

With the ensuing development of the gigaohm high-resistance seal, formed with a combination of super-smooth microelectrode pipette tips and negative suction, patch-clamp electrophysiology was born. The capacity to record from single channels, as well as from individual cells both internally and externally in both *in vivo* and *in vitro* settings has revolutionised modern neuroscience.

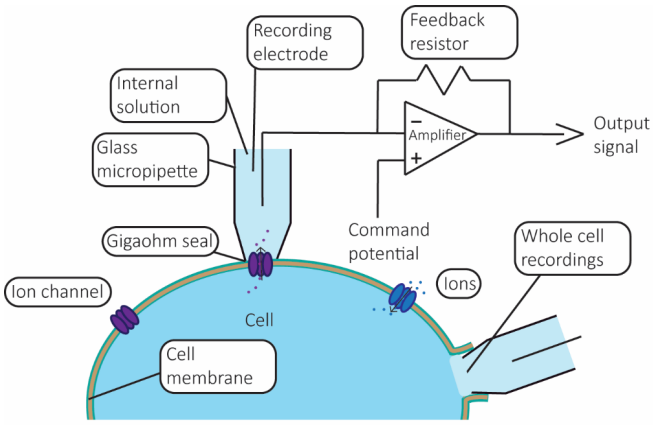

Figure 1. Diagram of the set-up for patch-clamp electrophysiology. A microelectrode pipette isolates a patch of cell membrane by forming a gigaohm seal, and either records from single channels (top), or 'breaks through' the membrane to record from the whole cell (right). A command potential is set, and current is injected to maintain this potential at the recording electrode relative to the ground electrode. When ion channels open, charged ions flow through them. Resultant changes in membrane potential are detected by the amplifier, which generates an error signal between the command potential and actual cell voltage, and compensatory current is injected. The output signal from this circuit can be used to determine ionic currents, as the compensatory current will be equal in amplitude.

References

1. Galvani L (1791) De viribus electricitatis in motu musculari commentarius. *Bon Sci Art Inst Acad Comm* 7:363–418.
2. Cole KS (1949) Dynamic electrical characteristics of the squid axon membrane. *Arch Sci Physiol* 3(2):253–258.
3. Hodgkin AL, Huxley AF (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. *J Physiol* 117(4):500–544.
4. Neher E, Sakmann B (1976) Single-channel currents recorded from membrane of denervated frog muscle fibres. *Nature* 260(5554):799–802.

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Animal research: good or evil?

by Mariangela Panniello

Would you be willing to be the first living creature on Earth to try out a new medical compound, not knowing the risks its interaction with our complex bodies might bring? This is a question that animal research advocates may ask to convince their audience of how important it is to use animal models in biomedical research. However, while it is easy to understand that it is not ethically acceptable to use humans for certain experiments, researchers in the medical sciences hold the responsibility to carefully explain to members of the public why it is important to overcome ethical issues for experiments involving animal models.

Animal research is challenging, imperfect and often frustrating for researchers. However, there is an overwhelming consensus in the scientific community that it is still essential for medical progress.

Animal experiments were necessary to develop effective vaccines and treatments against diseases that represented a death sentence just a few decades ago, from polio to tuberculosis, smallpox and meningitis. Penicillin was discovered by Alexander Fleming on bacterial cultures, however its efficacy as an antibacterial agent in the body was proven only after Howard Florey and Ernst Chain tested it in mice, here in Oxford in the early 1940s. These may seem like tales from a forgotten past, when the molecular basis of life was not yet known and scientists didn't have computers to complement their work at the lab-bench.Indeed, the number of animals used in laboratories has fallen since then, and nearly halved in the last 30 years. Whenever possible, scientists are replacing animal tests with other techniques, such as cell cultures, large-scale statistical analyses and computer simulations. However, we simply cannot yet use a machine to simulate how the full human body will react to chemotherapy, or explore the interactions between the nearly 100 billion nervous cells in our brains by looking at the networks created by a few hundred cells growing on a dish. The best way we have to understand our bodies is to use 'models' of them: bodies of other animals sharing molecular, physiological or behavioural features with us.

Despite its essential role in medical progress, animal research does not come without restrictions. All animal procedures carried out in Europe are strictly regulated by the Directive 2010/63/EU. According to this directive, animal research projects are scrutinised by an ethical commission and allowed only when a convincing scientific justification is present, when the potential benefits outweigh the risks for the animals involved, and when no alternative methods exist. This set of laws is based on the '3Rs' principle of replacement, reduction and refinement, and its final aim is to replace all animal research with non-animal methods. Before this becomes possible, the directive ensures that every experiment is conducted in the species with the lowest complexity necessary for that specific study and by using as few animals as possible.

Many complex diseases must be investigated at a range of different levels, from their genetic basis to their consequences for the whole organism. Take Alzheimer's disease: despite the much lower complexity of the fruit

fly brain compared to the brains of mammals, it contains tau, a key protein involved in the pathogenesis of the neurodegenerative disorder in humans. This similarity has allowed researchers to use this small insect to study the molecular mechanisms at the origin of Alzheimer's disease. On the other hand, insights into its cognitive consequences come mainly from non-human primates, whose brains are wired to function very similarly to ours.

Animal tests not only involve the models, but the researchers too. Each scientist working with non-human species undergoes mandatory training, and is constantly supported by teams of veterinarians and animal technicians working within the same institution. They perform their personal 'race for life', spending endless lab hours making sure experimental animals are healthy and treated in the fairest way possible, in order to guarantee reliable and unbiased scientific results. They also get their own 'ice-bucket challenge' every time their experiments do not work and need to be designed again. After all, animal researchers, patients, and animal rights activists share a similar passion: that for life.

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Children's mental health: my internship at POST

by Jacqueline Gill

The latest national statistics for children's mental health in the UK from 2004 are disappointing. They showed that 1 in 10 children and young people aged between 5 and 16 suffers from a diagnosable mental health condition (1). Since then, evidence suggests that demand for children and young people's mental health services has been increasing.

Although we will have to wait until 2018 for the next national prevalence survey on children's mental health, the government has recognised that significant changes to children and young people's mental health services are required, through increased funding and changes in policy (2).

During my internship at the Parliamentary Office of Science and Technology (POST), I produced a parliamentary briefing document, called a 'POSTnote', about new models of mental health services for children and young people. This will be published at the beginning of the next Parliamentary Session, in September 2017, to be used by parliamentarians (MPs and peers) to inform their policy decisions surrounding this topic.

POST is Parliament's "in-house source of independent, balanced and accessible analysis of public policy issues related to science and technology" (3). It aims to improve parliamentarians' understanding of key science and technology policy issues by providing peer-reviewed, evidence-based information, enabling them to make informed decisions supported by accurate information.

I interviewed many experts in children's mental health services to inform my POSTnote. These included academics, members of public health bodies, medical professionals, and a variety of mental health charities. Many of them had developed their own initiatives to improve children's mental health services, using evidence from their own experience in the field. They were able to explain to me many of the reasons behind the current problems in children's mental health services, and the evidence to support the changes that are currently being made.

Children and young people's mental health services in the UK have been undergoing significant changes since the announcement of an investment of £1.25 billion over five years by the UK government in 2015 (4). This has resulted in many new 'models' of children's mental health services, which are currently being developed across the country. As most mental health services are managed locally in the UK, each region can focus on the most relevant improvements for its own mental health services. For example, central Manchester faces different problems in its mental health services than those faced by rural Devon. It was really interesting to find out how the different areas of the UK are responding to the varied mental health services challenges, how committed they are in creating evidence-based change, and how they plan to use the funding to improve their services.

During my internship, I was also able to attend parliamentary sessions on mental health, as this is currently an important topic for politicians and for the general public. Many sessions were streamed live on ParliamentTV. It was eye-opening to see how the scientific research done by many of these experts was presented to parliamentarians, to inform real-world policy decisions.

I had a fantastic time during my internship at POST, learning about how science is used to inform policy, and experiencing the parliamentary process. My internship was provided through the Research Council Policy Internship Scheme, and POST has a variety of other fellowship schemes available for PhD and early career researchers (5). All published POSTnotes can be found on the POST website (6).

References

1. Office for National Statistics (2005) Mental health of children and young people in Great Britain 2004. Available at http://content.digital.nhs.uk/catalogue/PUB06116 [Accessed 31st July 2017]
2. Department of Health and NHS England (2015) Future in mind. Available at https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/414024/Childrens_Mental_Health.pdf [Accessed 31st July 2017]
3. Parliament.uk (2017) POST. Available at www.parliament.uk/post [Accessed 31st July 2017]
4. Gov.uk (2015) Press release: Deputy PM announces billion pound boost over 5 years for children's mental health services. Available at https://www.gov.uk/government/news/deputy-pm-announces-billion-pound-boost-over-5-years-for-childrens-mental-health-services [Accessed 31st July 2017]
5. Parliament.uk (2017) POST fellowships. Available at https://www.parliament.uk/mps-lords-and-offices/offices/bicameral/post/fellowships/ [Accessed 31st July 2017]
6. Parliament.uk (2017) POSTnotes. Available at http://www.parliament.uk/mps-lords-and-offices/offices/bicameral/post/publications/postnotes/ [Accessed 31st July 2017]

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Science activism

by Phoebe Oldach

The past year has been rife with political turbulence, from the Brexit referendum to general elections across the globe, opening possibilities for dramatic perturbations to the world order. In step with these elections has come a burgeoning activism. One of the most visible manifestations of 2017's activism was the proliferation of marches. On 21 January – the day after the inauguration of Donald Trump as US president – Washington DC was inundated with more than half a million people in support of the Women's March on Washington. While this march has been described as the largest protest in US history, participation was not limited to the states; the three million people marching across the USA were joined by an estimated 300,000 marchers in cities across the world (1).

A litany of reasons, from disgust at Trump's character and disappointment over the failure to elect America's first female president to apprehension of Republican stances on social issues including reproductive rights and equality, motivated people to join the Women's March. While civil rights have long been catalysts for activism in American history, this year prompted the unprecedented galvanisation of scientists to collective action.

This was perhaps surprising as many scientists shy away from politicisation of research. This may be motivated by individual reputation, as the research of activist scientists could be perceived as being biased by strong political views. Others have argued that individuals staying apolitical is important to the reputation of science as a whole. Coastal geologist Robert Young stated that activism by scientists works to "reinforce the narrative from skeptical conservatives that scientists are an interest group [that politicises] their data, research and findings for their own ends" (2). For others, the hesitation to become an activist may arise from fears over losing funding. Environmental Science and Technology editor David Sedlak cautioned that by becoming "allies of a particular cause, no matter how just, we jeopardize the social contract that underpins the tradition of financial support for basic research" (3).

However, the growing animosity towards science seen broadly throughout the campaigns and elections of the previous year was enough to outweigh concerns about activism for many scientists. This animosity, expressed in discussions of radical funding cuts, dubious cabinet picks, and striking outright statements from political leaders – Brexiteers claiming suspicion of experts, and the prospective US president repeatedly expressing disregard for the Environmental Protection Agency and even calling climate change a hoax – left some scientists wondering whether their research was in danger. Even beyond explicit dismissal of the scientific method and research findings, populist movements across the globe posed the threat of increased insulation stunting progress.

In the face of the backlash against science, a remarkable resistance was born. More than one million people in over 600 cities around the world marked Earth Day with the first ever March for Science. In addition, there have been mass movements in letter writing and petition signing. Frustrated by the apparent deaf ear of the government to petitions and marches, some academics and researchers have been inspired to become directly involved by committing to run for office. Many in the US are finding support from 314 Action, a newly-founded non-profit focused on supporting the election of STEM individuals to public office.

It remains to be seen whether this wave of increased political activism by scientists will be maintained into the next election season, and whether the misgivings of the activism sceptics are in fact justified. But while the March for Science had the explicitly political goals of sending a message to governments about the importance of considering scientific findings in policymaking and reiterating the importance of investing in society by funding research, the march organisers equally stressed public goals of improving science outreach, advocating science education, and fostering a diverse scientific community. Regardless of the political climate or an individual's stance on activism, these goals will remain important to a thriving scientific society.

Q&A: The Oxford Climate Society

To learn more about how some of the Oxford population responded to last year's political climate, we got in touch with the 2016-17 and 2017-18 presidents of the Oxford Climate Society, Rupert Stuart-Smith and Alice Boyd. Comprised of students, professionals, and members of the general public, the Oxford Climate Society is a university-based organisation focused on offering the education, connections, and inspiration critical for developing informed climate leaders, thinkers, and activists.

What would you pinpoint as some of the main changes or challenges of the year?

The election of Donald Trump as President and the resulting uncertainty for international cooperation in tackling climate change was a considerable challenge. Although Trump's regressive rhetoric on energy and climate issues has threatened progress on mitigation of climate change and its impacts on a global scale, it has also led to a productive discussion on the role of science activism, which has been increasingly debated at our events. As a society, we have been considering how best to give ourselves a unique and successful campaigning

edge, and our new outreach team has been developed to achieve this goal.

Did you see a change in membership numbers or focus in this year relative to others?

We found that the creation of our own programme of lectures meant that the number of attendees grew dramatically. This was a result of both securing a range of well-known speakers and of more widespread marketing. The number of people following our social media accounts and our weekly mail-out also grew. However, the society should look towards reaching beyond those who attend the talks, to engage a wider audience in the future.

What actions did you organise or take?

Our reaction to developments in international climate diplomacy have been twofold: academic discussion and debate, and public protest and awareness-raising. Oxford Climate Society's (OCS) key action was organising 'Bridges not Walls, Oxford' on the day of President Trump's inauguration in January, which brought together ten of Oxford's student groups campaigning on social and environmental issues. Attracting hundreds of students and Oxford locals to Radcliffe Square, we opposed Trump's attacks on marginalised groups, the natural world on which we rely, and science itself. Shortly before this event, we held a panel event with scientific and political experts, anticipating Trump's climate agenda. This was our most popular event ever, clearly demonstrating the concern of our members for the implications of his Presidency. Following the announcement of Trump's decision to begin the process of withdrawing the US from the Paris Agreement, we organised another panel event in June.

What sort of actions do you prioritise as a society?

The society has previously focused on academic lectures and events as well as attending climate marches, but we now see public outreach as one of our biggest opportunities to make a difference. We recently recruited an outreach team to produce engaging videos and blogs on contemporary climate issues. Our new team will also advocate on behalf of Oxford's students for strong climate policies, including through national consultation responses and investigative reporting. We remain committed to educating future climate leaders through a high quality lecture series. This is complemented by the opportunity for Oxford students to become involved in advocacy on tackling climate change by investigating political issues, reaching out to politicians and engaging peers in tackling climate change.

Do you think that students and academics have a special obligation, or a special role to play in activism?

Students and academics have the opportunity to dedicate themselves to learning and generating knowledge and it should be their duty to ensure that this knowledge is employed for the benefit of society. Climate change threatens the wellbeing of everyone on Earth and it should be the responsibility of the scientific community to ensure that government policy is in line with the best scientific evidence. Students also have the opportunity to use their knowledge to advocate for progressive climate policies, and as the generation that will feel the greatest impacts of climate change, we have a vital role to play in defending our own futures.

References

1. Broomfield M (2017) Women's March against Donald Trump is the largest day of protests in US history, say political scientists. *Independent*. Available at http://www.independent.co.uk/news/world/americas/womens-march-anti-donald-trump-womens-rights-largest-protest-demonstration-us-history-political-a7541081.html [Accessed 1st September 2017].
2. Young R (2017) A Scientists' March on Washington is a bad idea. *New York Times*. Available at https://www.nytimes.com/2017/01/31/opinion/a-scientists-march-on-washington-is-a-bad-idea.html [Accessed 1st September 2017].
3. Sedlak D (2016) Crossing the Imaginary Line. *Environ Sci Technol* 50(18):9803–9804.

Phoebe Oldach is a DPhil student in Conrad Nieduszynski's research group in the Dunn School of Pathology.

5' WITH...

Professor Victoria Bajo Lorenzana

by
Stefania Monterisi

Victoria Bajo Lorenzana is Associate Professor of Neuroscience at the University of Oxford. She completed an MD/PhD at the University of Salamanca and came to Oxford in 2000 as postdoctoral fellow. In 2009 she was awarded University Research Lecturer status and is Lecturer at Balliol College. She is based in the Department of Physiology, Anatomy and Genetics, where she investigates sensory perception, with the goal of identifying and manipulating the neural circuits involved in auditory plasticity related to hearing loss and tinnitus.

When did you first decide that you wanted to be a scientist?

My first thought was to be a physician, and this was the reason why I graduated with a bachelor degree in Medicine and Surgery. During my medical degree I realised that knowledge of human physiology and of the pathophysiology of many diseases was not as complete as I expected. This lack of knowledge, alongside my own curiosity, led me to become a scientist instead.

How did you decide you wanted to work on auditory neuroscience?

In my second year of medicine, when I was studying neurophysiology, I read about the action potential and neuronal activity in the brain and knew that this would be the field I wanted to stay in. It is magical to listen to neurons fire and to know that this is the language that commands your actions and thoughts. Regarding auditory neuroscience, it combined my two loves very well: music and the brain. If I were not a scientist, I would love to be a singer!

What is the best advice you received in order to pursue your career?

It was from Jeffrey A. Winer, Professor of Molecular and Cell Biology at the University of California, Berkeley, simple and wise: "Always be yourself and do things in the way that you would do. Every person has a particular way to resolve a problem, the only way that will work for them. This variety makes science thrive."

The biggest challenge in your career?

The big challenge in academia is to be able to move to the next level owing to its pyramidal structure. The higher you go up the pyramid, the fewer people you have. So obviously, it is very competitive. I think that this competitiveness is often measured in the wrong way, just by papers or citations, whereas other parameters should be taken into account in career progression. At a personal level, it was challenging to reconcile the academic career with family life. It is extremely difficult for a woman because, if you want to have a family, the peak of your career is at the moment you are deciding to have children. Having raised two, it has been difficult not just during maternity leave but also afterwards: with schools, education, etc. You have to find a balance in the time you spend at work and the time you spend with them.

Is there any moment in your career that you recall as memorable?

My contribution on how the descending pathways mediate learning-induced auditory plasticity is one of the highlights of my career. For example, we demonstrated that removing a specific circuit, which sends information from the cortex back to the midbrain, impairs animals' ability to localize sounds when the values of the cues normally used have changed.

Is there any big discovery you think we will see in 20 years' time in neuroscience?

In my field, I hope to see a treatment for tinnitus, including my scientific contributions toward its cure. We will be able to identify the changes in brain activity leading to the phantom perception of sound, and the hub centres in the brain where changes occur.

In your opinion, what makes a good scientist?

The combination of intellectual curiosity and resilience. This moves us to ask questions and find answers. Hard work, good mentoring and a little bit of luck also helps. To love what you do is essential.

The Brain Atlas: A Visual Guide to the Human Central Nervous System, 4th Edition

Thomas A. Woolsey, Joseph Hanaway, Mokhtar H. Gado
ISBN: 978-1-118-43877-0 Wiley-Blackwell (2017)
272 pages: Paperback, £45.00 / eBook, £40.99
Reviewed by Jack Cooper

Santiago Ramon y Cajal, viewed by many as the father of neuroscience, once said "the brain is a world consisting of a number of unexplored continents and great stretches of unknown territory". Though much is still unknown in neuroscience, it is safe to say that those continents have been better mapped since Cajal's times. There is certainly no textbook where the brain is more beautifully presented than in '*The Brain Atlas: A Visual Guide to the Human Central Nervous System, 4th Edition*'.

The book's content is organised into five sections: Background Information, The Brain and its Blood Vessels, Brain Slices, Histological Sections, and Pathways. With over 350 high quality images and diagrams, complemented by a good level of descriptive detail, this textbook provides a resource that is not only useful to medical professionals and students, but accessible to interested members of the public.

It is worth bearing in mind that *The Brain Atlas* serves as a reference for physical structures. Its aim is to explain the anatomical basis for neurological diseases, not to further the understanding of systems. The full-colour images cover all relevant modes of display, from MRI and MRA scans to histological sections, in all three planes. Indeed, the design and layout of these images are the main strength of the book. Subtle colour choices, clockwise labelling of features and the use of overlays all combine to give the reader images that are easy on the eye and easy to interpret. Navigation between the five sections is aided by colour coding and extensive cross-referencing.

The main improvement of the 4th edition on older ones is the addition of the *Wiley Digital Companion: Powered by VitalSource*, an interactive digital version of the book. Its features include show/hide for self-testing, hyperlinks within the text for fast navigation, colour coding and highlighting, and even the ability to copy and paste any of the images for professional use. The electronic resource is compatible with desktops, tablets, and smartphones; this is the one companion you won't be able to do without.

Delusions of Gender

Cordelia Fine
ISBN 978-0-393-34024-2 W.W. Norton & Company (2011)
368 pages: Paperback, $17.95
Reviewed by Ellen Pasternack

That women's brains are different from men's is often treated as common knowledge. Confidently proclaimed by respected academics, the tabloid media, an avalanche of self-help books and 'that guy' in every psychology seminar, one could easily be forgiven for assuming that this is established scientific fact. Separate but equal: men have evolved to be better at maths, creating great art and thinking rationally, whilst women excel in other areas, like helping people and tidying up. One can't argue with science, and that's what the science says.

In this razor-sharp critique, Cordelia Fine examines the science behind neurological and psychological sex differences, and offers an alternative view. She argues that much of the research used to back up these claims is poorly conducted, often self-contradictory and wildly over-interpreted. For instance, a 2001 study by Connellan *et al.* (1) is regularly cited as conclusive evidence that baby girls have an innate preference for social interaction, whilst boys prefer mechanical objects. Both sexes looked for the same length of time at Connellan's face, but male babies spent on average about six seconds longer looking at a mobile: a difference found across a few dozen individuals in a single hospital, and not repeated elsewhere. The 'staring baby' protocol is subjective and open to interpretation at the best of times (2), but this study is particularly full of grave methodological flaws. For instance, Connellan was not blind to the infants' sex whilst carrying out the experiment. What Fine terms 'neurosexism' – sexist stereotypes couched in the scientific language *du jour* – has a history as long as science itself. Victorians were certain that the smaller size of their brains meant that women could never be men's intellectual equals, despite the absence – glaringly obvious once pointed out – of any correlation between absolute brain size and intelligence. Since then, researchers have investigated and cast aside the size of the upper portion of the spinal cord, the ratio of skull length to width, and the verticality of the face as candidates for the seat of women's inferiority. In recent years, foetal testosterone exposure and the size of the corpus callosum have been seized upon as explanations. Fine explains the many difficulties inherent in drawing any conclusions about how the mind works from physiological factors, and advocates a healthy dose of scepticism with the warning that "wrapping a tape measure around the head was [once] considered modern and sophisticated, and it's important not to fall into the same old traps."

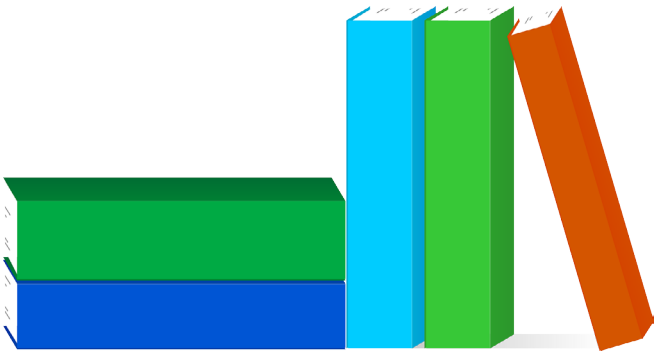
In the final chapters of the book, Fine presents persuasive evidence that gendered stereotypes are far more pervasive and insidious than most of us appreciate, taking hold before a baby is even born. She suggests these stereotypes should be considered much more carefully as an explanation for gendered differences in behaviour, rather than leaping to the conclusion that any behavioural differences observed must be innate.

This book is an important and witty cautionary tale about the consequences of over-interpreting bad science. Everyone should read it.

1. Connellan J, *et al.* (2001) Sex differences in human neonatal social perception. *Infant Behav Dev* 23(1):113–118.
2. Prinz JJ (2014) *Beyond human nature: How culture and experience shape the human mind.* WW Norton & Company.

Neuroprotective Natural Products: Clinical Aspects and Mode of Action

Edited by Goutam Brahmachari
ISBN: 978-3-527-34186-3 Wiley-Blackwell (2017)
376 pages: Hardcover, £120.00 / eBook, £108.99
Reviewed by Alice Lightowlers



Pharmacological medicines often begin life in the natural world. Two familiar examples that have passed the rigour of scientific testing are aspirin from willow trees and artemisinin from wormwood plants. There are many more such molecules with medicinal potential whose efficacies are still being evaluated.

Neurological diseases are of particular interest to those studying natural medicinal products, as many natural molecules are neuroactive. The nervous system and the diseases that affect it are among the most complex and least understood areas of medicine. However, investigating compounds that affect the nervous system may help to elucidate key biochemical pathways involved in its normal function and pathophysiology.

Research in this field is active, with over 1000 articles published on the neuroprotective effects of cannabinoids in 2017 alone. It is, therefore, incredibly useful to have a thorough and up-to-date review of bio-active products with neuroprotective potential. '*Neuroprotective Natural Products*', edited by Dr Goutam Brahmachari, is a good example of one such review.

The book is divided into 13 sections, each authored by a specialist in that area. The first few chapters comprise a clear overview of the general modes of action of neuroprotective products. Later chapters go on to outline the specific modes of action of key natural products on individual diseases. Chapters read like individual reviews and are diverse in the depth of their coverage: from the more general chapter describing the effect of anti-oxidants on neurological disorders, to the specific chapter on the effects of Ayurvedic preparations on Parkinson's disease. Perhaps because each chapter has a different main author, the book sometimes has a lack of cohesion, but is nevertheless very informative on each individual subject.

The book is well-written, with clear language that makes it accessible to both neurological and non-neurological biologists. However, it would benefit from additional figures and diagrams to illustrate some of the more complicated concepts. While '*Neuroprotective Natural Products*' is not for the casual reader, I would recommend it to those with some prior knowledge of neuroscience who are looking for a current analysis of potential natural treatments for neurological diseases.

Neural Dynamics of Neurological Disease

Christopher Shaw
ISBN: 978-1-118-63457-8 Wiley-Blackwell (2017)
408 pages: Hardcover, £104 / eBook, £93.99
Reviewed by Jenna Hebert

In our brains, billions of neurons are wired together, each programmed to perform a specific job. Genes, proteins, and ions work together seamlessly in a complex but fragile system.

When a computer crashes, the cause of the breakdown is not always clear. Similarly, when a component of the brain fails, as in neurological disease, we often only see the superficial symptoms – memory loss, tremors, and/or cognitive deficiencies. By that point it's likely too late; neurons do not regenerate. Brain damage due to a "cascading failure", which causes the entire system to fall apart, is permanent.

This is the theme of Christopher Shaw's book *Neural Dynamics of Neurological Disease*. Shaw takes a sharply critical look at neurological disease research. The aetiology of many neurodegenerative diseases is unknown because, according to Shaw, the approach to research has been narrow-minded. Studies have mostly focused on identifying causal gene variants, but few have been identified. Researchers then design animal models carrying these genetic mutations to test potential therapeutics, but Shaw argues that most of these models are poor representations of the actual diseases, partly due to the fact they assume an unrealistic "one-hit" model of disease progression, where a single mutation is responsible for the cascading failure.

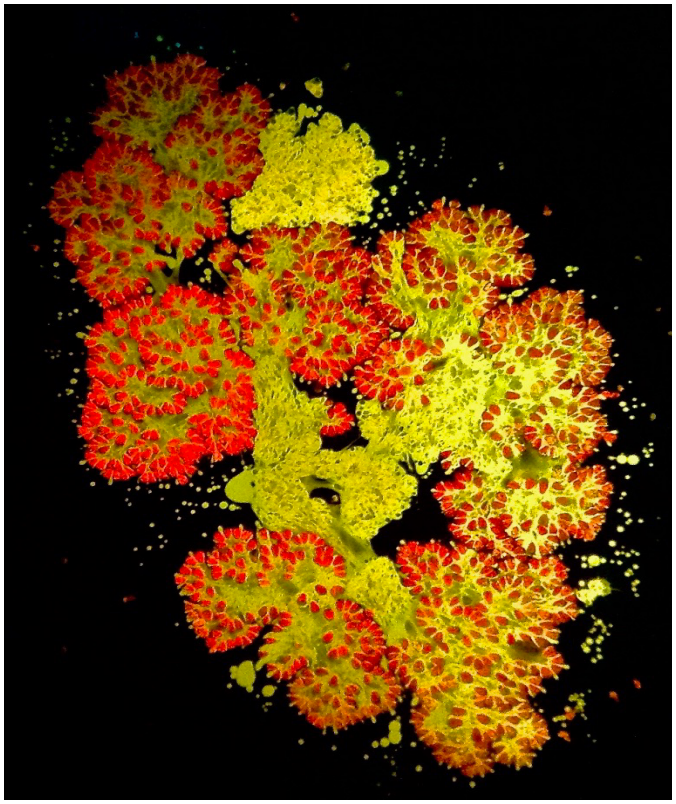
Shaw's critique of neurodegenerative disease research at times feels harsh, but it is hard to find fault with his opinions. Research is like a freight train moving ahead at full speed; when scientists have based their entire careers on a particular animal model or drug, the momentum is hard to stop.

Shaw suggests that researchers open their minds to the contribution of environmental toxins and gene-environment interactions. He also believes scientists need to look for therapeutics that stop disease progression much earlier in the process, when the patient is still asymptomatic. Shaw's book leaves the reader with mixed emotions: optimism, because there appears to be a path to success, but also unease, because this path requires a major overhaul of the research framework.

SNAPSHOT
Research Image Competition

This issue's winner is...

Sahutchai Inwongwan,
Department of Plant Sciences



City lights

The canary golden luminescence from a Nile red-stained colony of *Botryococcus braunii*, a lipid-rich freshwater green alga, illustrates the accumulation of lipid inside the algal cells, while the red light of the teardrop-shaped cells comes from the autofluorescence of its natural chlorophyl. The gold and the red conjure up the image of a finely-crafted, ruby-ornamented golden tiara. These golden lipid drops have long been the focus of bioenergy research and may become as valuable as real gold in the not too distant future.

Sahutchai has been working on phycological research for more than six years, with a background in molecular genetics and microbiology. His passion for algal research gradually formed during his academic journey involving many research topics, including algal biosynthesis and cultivation, bio-energy, genetic modification, algae-bacteria microbiome and metabolic network reconstruction. Alongside his research, he always seeks for beauty in his experiments as a hobby.

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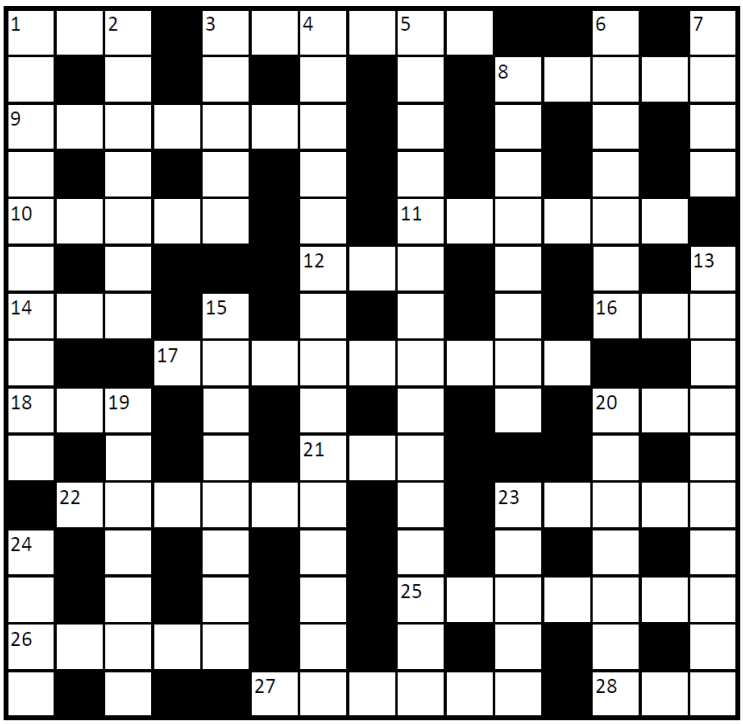
PHENOTYPE crossword

Fish challenges you to this latest cryptic crossword! Can you crack it? Answers to last issue's crossword are given at the bottom of the page. Enter this term's competition by sending your answers to stefania.kapsetaki@new.ox.ac.uk. Entries received before the 17th November 2017 will be entered into a prize draw to win one of the books reviewed in this issue.

The winner of the crossword competition will receive their choice of one of the books reviewed in this issue, kindly provided by WILEY-BLACKWELL



SPECIAL INSTRUCTIONS: All the DOWN clues conclude with the same word; while this word is included in the constructions of the clues, it is not entered into the grid. For example, if the answer was "USE YOUR BRAIN", with "BRAIN" being the common word, only "USE YOUR" would be entered in the grid.

Answers to the crossword from Issue 27, Trinity 2017:

Across: **8.**Mouthpiece **9.**Rash **10.**Unnerved **11.**Hamlet **14.**Oink **15.**Out on a limb **18,28.**Lymph node **19.**Too **20.**Orfeo **21.**Bone marrow **22.**Acid **24.**Louise **26.**Cytokine **28.***See 18.* **29.**Intestinal

Down: **1.**Immunoglobulins **2.**Burning man **3.**Churn **4.**Wire cutters **5,27.** Act one **6.***See 23.* **7.**White blood cells **12.**Amazon **13.**Homopolymer **16.**Infections **17.**Thymus **23,6.**Booty call **25.**Undo **27.***See 5.*

ACROSS

1,14. Hat doffed at finish (3,3)

3. A way that beam can be diverted from its correct path (6)

8. Even Schliemann is tidy! (5)

9. Setting agent made from a **6** flower (7)

10. Desire for more good grass (5)

11. Raising a single expression of disgust is sufficient (6)

12. Talk about sack (3)

14. See **1**

16. One with pain avoiding hospital (3)

17. It's OK to put lettuce filling in pasta (9)

18. Decline Elizabeth Barret Browning's initial offering (3)

20. Hit a party (3)

21. Spanish river flowing through half of Spanish neighbourhood (3)

22. Halo turns up in Catalan or Occitan iconography (6)

23. Elderly relative, in short, is endlessly eating cereal (5)

25. Oblige head writer to drink so she spills the beans (5,2)

26. Creepers grow in six new directions (5)

27. What Oxford student wants for describing alphabetical order? (1,5)

28. Elderly relative, in short, is, at heart, foolish (3)

DOWN

1. Decide differently, amending chosen form (6,4,4)

2. Calm while discussing id or ego, perhaps?

3,7. I'm a damn'd loony! Confused, with lots to think about (1,4,2,2,4)

4. Conservatives plan in soft-hearted Michigan and North Dakota to encourage "correct thinking" (3,5,5,2,4)

5. Half-remembered where your cerebellum is? (2,3,4,2,4,4)

6,7. Ray Charles' nostalgic state? (7,2,3,4)

7. See **3,6** and **8**

8,7. James Taylor's nostalgic state? (8,2,3,4)

13. Look back over article about lack of exercise; take care to be open to new interpretations (4,2,4,4)

15. Know what one is thinking: unending new, randomised trials! (4,4,4)

19. Agree to disagree: mob need info (2,2,3,4)

20. Is aware of remembering Yogi and Baloo, perhaps? (5,2,4)

23. Genius' crush eats meat accidentally (5,4)

24. After I've entered H&M, I nod emptily to the collective intelligence (4,4)