PN
Physiology News

Issue 99 / Summer 2015

Invertebrate physiology

The Physiological Society

Physiology News

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Contents

Welcome to the Summer 2015 edition of *Physiology News*

Introduction

News in brief

News in depth

Meetings & events

Features

Membership

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Invertebrates as experimental animals

Roger Thomas

Editor, *Physiology News*

This issue features articles on invertebrate physiology. I was myself introduced to the snail brain for my PhD studies at Southampton University, where my supervisor Gerald Kerkut was inspired by a 1960 paper (in French) by Tauc and Gerschenfeld concerning cholinergic transmission in the CNS of 'l'Escargot', which we took to be either *Helix aspersa* or *pomatia*. (The two species are shown in the picture adjacent taken by me in Paris in the year of student unrest, 1968). The zero cost, and minimal upkeep needs, of land snails were also a factor. Since snails are so easy to maintain the Head of Department gave me the additional duty (times were hard and the department had few staff) of looking after the department's crabs, collected weekly from the local power station intake. To enable me to be away for the weekend, I had to double their sea-water filter capacity. Otherwise they overflowed. It taught me useful plumbing skills I suppose.

Neurophysiological research on molluscs was greatly stimulated by J.Z. Young's in the 1930s. He realised that what had been thought to be a blood vessels in the squid mantle were in fact giant axons. Hodgkin and Huxley exploited this preparation brilliantly, and many others since have focussed on it. Other molluscs too have a long history as experimental animals. The sea-slug *Aplysia*, like snails, has very large and colourful nerve cells, and these were widely used 50-30 years ago for a variety of basic studies. The introduction of the Aplysia nervous system to study the cellular and molecular basis of organized neuronal interactions has been described as one of Tauc's essential contributions to neuroscience. Specimens from California were (of course) much larger than European specimens. There was a time when labs in Paris imported Aplysia from California by air, and took advantage of the separation between ganglia to make several preparations from one animal.

In those early days crustacean muscles and nerves were also popular. Indeed the first discovery that GABA and Glutamate were synaptic transmitters of inhibition and excitation were made using crustacean preparations, notably the crustacean stretch receptor. Alas I have been unable to include in this issue anything about recent crustacean research, but readers keen to look into this should read Harold Atwood's chapter in Volume 3 of The Natural History of the Crustacea, edited by Derby and Thiel. OUP, published in 2014. The articles I have included cover nematodes, leeches, snails, flies, spiders and tunicates. Many other classes of invertebrate have been investigated by physiologists, of course, but we have too little space to do them justice.

Mammalian preparations are much more widely used now. The invention of the whole-cell patch clamp method in the early 1980s allowed single-cell recording from much smaller cells than before. Over the same period the development of methods of cutting and nurturing viable slices of parts of mammalian brains and culturing isolated neurones led to many neurophysiologists working on mammals instead. Grants are easier to obtain, since relevance to human physiology is more convincing. Previously, sharp microelectrodes had to be pushed through the cell membrane to record membrane potentials, inevitably creating an often-fatal leak. As pioneered by Eccles in the 1950s large cells such as mammal motoneurones could tolerate this method, but smaller cells could not. The whole-cell patch clamp involves a smooth-tipped electrode being gently sucked onto the clean cell membrane to make a seal, then the membrane under the electrode tip being broken by a pressure pulse to gain access to the cell interior. The cost of mammals, however, in bureaucracy and animal house fees is considerable. Land snails cost nothing, and other invertebrates are relatively cheap. And there are advantages to the relative simplicity and short generation time of many invertebrate systems, as the various contributors explain. This applies particularly to *Drosophila*, whose genetics have been investigated for over a century. Whereas the move in the 1990s was from invertebrate to mouse, the high cost of the latter is driving a move back to flies. Since research grants are likely to be harder to obtain in the UK in future, one might hope that money is saved by experimenting on flies. No licence is required, either.

I write this after the opinion pollsters have been discredited by the UK election result. This new Conservative government may lead to Scotland leaving the UK, but following the precedent of Irish independence in 1922, The Physiological Society will not need to change. It is not as if its official name includes any geographical element. The objects of The Society do include the term 'at home and abroad', but as far as I can see membership is not restricted at all. It is open to 'those persons who are interested in Physiology in accordance with requirements determined by Council and shall be eligible…' Standing order B1(d) sets out the criteria, which do not include any nationality or residential restrictions. On the other hand I suspect Charity Commission and/or Companies House rules require at least the Annual General Meeting to be held in the British Isles.

I end by repeating the plea made frequently by my predecessors, to urge you the reader to offer material for publication. Letters to the editor, articles featuring a topical problem, comments, reports etc. Suggestions, books to review, jokes, cartoons, even ideas for cartoons will be welcomed. I am even prepared to publish negative comments if phrased tactfully. Letters and emails not for publication will also be read, cautiously.

A man is as young as his endothelium

Pasquale Pagliaro
Department of Clinical and Biological Sciences, University of Torino, Italy

I have read the interesting Features Article by Raya All-Maskari & Yasmin entitled 'A man is as old as his arteries: a scientific journey of ageing and aortic function', published in issue 98 of *Physiology News*. The article is enjoyable and easy to read. However, it is necessary to point out that the effects **on coronary flow** of arterial stiffening described are true only in chronic unhealthy conditions, when pulse pressure increases for some non-physiological condition, such as marked atherosclerosis, for example. A casual reader may take the misconception that increased pulse pressure (increased systolic and reduced diastolic pressure) necessarily implies a reduction of coronary blood flow. Unfortunately this false idea is sometimes reported in the textbooks of physiology: when the arterial diastolic pressure decreases myocardium is less perfused, because it is mainly perfused in diastole. This is not correct. In a healthy individual an acute increase in pulse pressure (increased systolic and reduced diastolic pressure at the same mean pressure) may induce an increase in coronary flow in the left coronary artery (Pagliaro *et al.* 1999; Paolocci *et al.* 2001). Actually, during intense dynamic exercise, in a healthy subject pulse pressure may double and mean pressure does not change appreciably (Lind & McNicol, 1967). In these conditions coronary flow may increase greatly.

No one doubts that exercise is a primary stimulus for the increased demand of myocardial oxygen, which may increase by about 5 times during intense effort. This increased oxygen demand is met mainly by increasing coronary blood flow (approximately 5 times). In fact, the extraction of oxygen (which is already ~ 70% at rest) can increase only slightly. As a result, in an healthy young subject the coronary blood flow increases **because of vasodilatation** and is closely coupled to the myocardial oxygen consumption. This tight coupling has been proposed to depend on tissue oxygen tension, signals released from cardiomyocytes, neuro-humoral influences, and the endothelial factors. Although the interactions of these regulatory factors to determine exercise-induced coronary hyperaemia is not completely understood, there are plethora of articles pointing out the importance of endothelium in coronary regulation during acute exercise and in training (Westerhof *et al.* 2006, Duncker & Bache, 2008).

When pulsatility increases in unhealthy individuals for a long time the raised systolic pressure and the reduced diastolic pressure may compromise coronary perfusion, because of an unmatched increase in cardiac oxygen demand, especially in the presence of cardiac hypertrophy. In these conditions it is likely that the increase in pulsatility is a cause and/or an effect of endothelial dysfunction. However, in a young individual with a healthy endothelium, when there is an acute increase in pulsatility, it is likely that coronary flow increases because the vasodilator endothelial factors concur to determine coronary flow. In fact, during exercise the pulsatility may increase considerably and in these conditions the increased oxygen demand is matched by increased coronary flow with the fundamental help of endothelial factors.

Duncker DJ, Bache RJ (2008). Regulation of coronary blood flow during exercise. *Physiol Rev* **88**, 1009-1086.

Lind AR, McNicol GW (1967). Muscular factors which determine the cardiovascular responses to sustained and rhythmic exercise. *Can Med Assoc J* **96**, 706-715.

Pagliaro P, Senzaki H, Paolocci N, Isoda T, Sunagawa G, Recchia FA, Kass DA (1999). Specificity of synergistic coronary flow enhancement by adenosine and pulsatile perfusion in the dog. *J Physiol* **520**, 271-280.

Paolocci N, Pagliaro P, Isoda T, Saavedra FW, Kass DA (2001). Role of calcium-sensitive K(+) channels and nitric oxide in *in vivo* coronary vasodilation from enhanced perfusion pulsatility. *Circulation* **103**, 119-124.

Westerhof N, Boer C, Lamberts RR, Sipkema P (2006). Cross-talk between cardiac muscle and coronary vasculature. *Physiol Rev* **86**, 1263-1308.

In response to Pasquale Pagliaro's letter 'A man is as young as his endothelium'

Raya Al-Maskari & Yasmin
University of Cambridge, UK

We are delighted that our Feature Article has sparked interest and prompted some constructive feedback from Prof. Pagliaro. Given the complex nature of age associated stiffening of the arteries we aimed to highlight its pathological consequences with particular emphasis on the remodelling of the extracellular matrix and not the distending pressure or vascular smooth muscle tone. As such, we maintain that Prof. Pagliaro's argument complements the discussion in our article. Pulse pressure (PP) has long been recognized as a surrogate marker for central artery stiffness as Bramwell and Hill noted '...other things being equal, [pulse pressure] will vary directly as the rigidity of the arterial walls' (Bramwell and Hill, 1922). In support of Prof. Pagliaro's views on exercise and endothelium, we showed that PP increases with habitual exercise in younger (<30 years) but not in older (>50 years) adults compared with their age-matched sedentary counterparts. The decline in endothelial function with aging also associates with increased stiffness in healthy people and in isolated systolic hypertensives. Enhanced endothelial function with regular exercise underlies this improved vascular profile in habitually active individuals (McDonnell *et al.* 2013, McEniery *et al.* 2006, Wallace *et al.* 2007).

As a corrigendum to our article in *PN*98, Figure numbers 4 and 5 should be reversed.

Bramwell JC & Hill AV (1922). Velocity of transmission of the pulse-wave: and elasticity of arteries. *The Lancet* **199**, 891-892.

McDonnell, BJ, Maki-Petaja KM, Munnery M, Yasmin, Wilkinson IB, Cockcroft JR & McEniery CM (2013). Habitual exercise and blood pressure: age dependency and underlying mechanisms. *Am J Hypertens* **26**, 334-41.

McEniery CM, Wallace S, Mackenzie IS, McDonnell B, Yasmin, Newby DE, Cockcroft JR & Wilkinson IB (2006). Endothelial function is associated with pulse pressure, pulse wave velocity, and augmentation index in healthy humans. *Hypertension* **48**, 602-8.

Wallace SM, Yasmin, McEniery CM, Mäki-Petäjä KM, Booth AD, Cockcroft JR & Wilkinson IB (2007). Isolated systolic hypertension is characterized by increased aortic stiffness and endothelial dysfunction. *Hypertension* **50**, 228-33.

'Zero Gravity' – a weighty problem?

David Miller
Hon Res Fellow, University of Glasgow and History & Archives, Physiological Society

The fascinating article by Harridge *et al.* 'Space Flight and ageing' in *PN*98 (pp26-29) deployed the subtitle (or standfirst) 'Zero gravity mimics some of the effects of ageing', but in the text itself referred to microgravity: these terms refer (in subtly different ways) to the status of those a few hundred km 'up' in orbit around our planet. But we struggle to find a term other than 'free fall' that covers the physics without risking a serious muddle of comprehension for the unwary: every utterance of 'zero gravity' will have Newton and Einstein spinning in their graves.

OK, so we all know that gravity is everywhere; that its magnitude falls with the inverse square of the distance between massive objects, etc. So how come that, a mere stone's throw above the Earth in

space-travel terms, we can talk, even loosely, of 'zero gravity'? Well, you might argue, everyone understands 'zero-gravity' as shorthand for free-fall and thus the *appearance* of weightlessness generated in sustained orbit … right? Wrong! In a MCQ-style poll of my 400-strong physiology Year 2 class ten years ago, nearly half believed that humans would be weightless on the Moon, never mind in orbit. Should we blame the press, or the schools? (See e.g. Gürel & Acer, 2003 for a full discussion of the educational issues). In my classes, only a minority chose the correct answer for Moon gravity (i.e. one eighth that on Earth … and NO, that's not 'eight times less' … don't get me started).

Recognising that gravity very much acts on people in Earth orbit was dinted into me by my late colleague in Glasgow, Tris Roberts (see e.g. Roberts, 1983). He sought to understand the special case for proprioceptive and central sensory physiology in which 'free-fall' astronauts are *actually* operating. How do we know what way is down? Could astronauts perhaps perceive that gravity (…the acceleration due to…) is still there, together with a constant velocity orthogonally to it? Is it principally the 'contact force' that we perceive through our feet (when standing) plus the relevant proprioception (within postural muscles opposing gravity's pull) that delivers the relevant sensation?

Finally, the authors of the *PN*98 article were following space science convention in referring to the 'microgravity (μG) environment' within their text. (Confusingly for those used to microbalances, microgravity is more routinely symbolised as 'μg'). Strictly, μg or μG refer to the truly micro-gravitational forces generated when, for example, astronauts move around relative to the centre of gravity of an orbiting vehicle. In free-fall, these tiny forces become of potential physiological significance. But even in orbit, 'Big-G' is still there: we really do need new words.

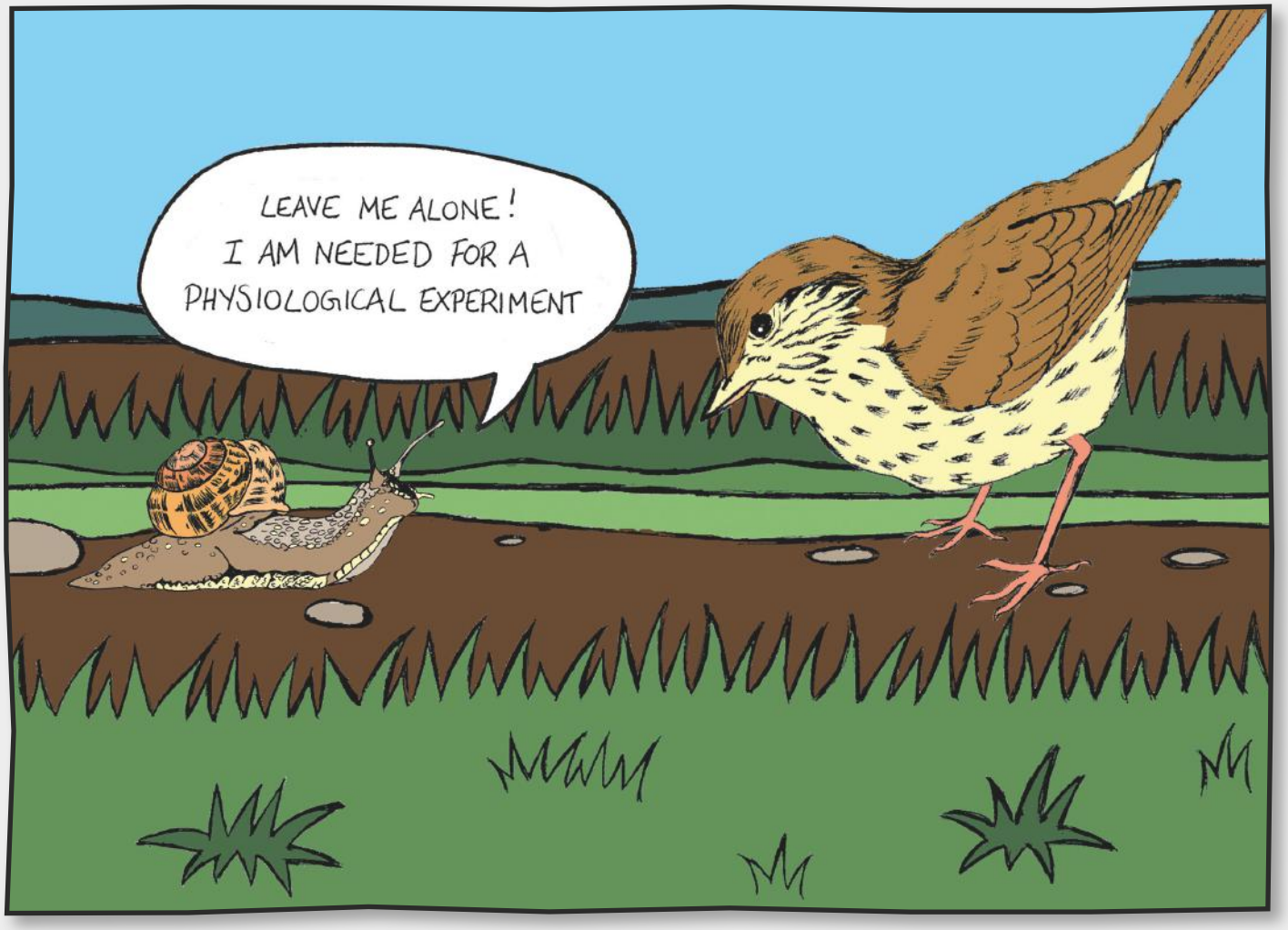
Gürel, Z & Acer, H (2003). *Astronomical Education Review* **2** (3) 2003

Roberts, TDM (1983). *Nature* **306**, 742-742

Editor's note: The zero was inserted by the Editor, not by the authors.

Question forthe Editor or comment on a recent *PN* article?

Please send your correspondence to **magazine@physoc.org**



Drawn by Elizabeth Querstret after a discussion with RCT. For more examples of her work see **http://querstret.co.uk/**

Vote now: 2015 Council elections

The Society has three vacancies on the Council of Trustees from July 2015. Trustees are legally responsible for the overall governance, management and policy of The Society, ensuring that the charitable objects for which it has been set up are met. The Trustees are also the Directors of The Society. Following a call for nominations earlier in the year, eight members are standing for election:

- Damian Bailey, University of South Wales
- Guy Smith Bewick, University of Aberdeen
- Jane Cleal, University of Southampton
- Raheela Khan, University of Nottingham
- Anthony Lewis, University of Portsmouth
- Frank Sengpiel, Cardiff University
- Holly Shiels, University of Manchester
- Francis Stephens, University of Nottingham

All candidates have been proposed by five Members or Honorary Members. The candidates' proposers, supporting statements and instructions to vote can be found at **http://bit.ly/councilelec15**

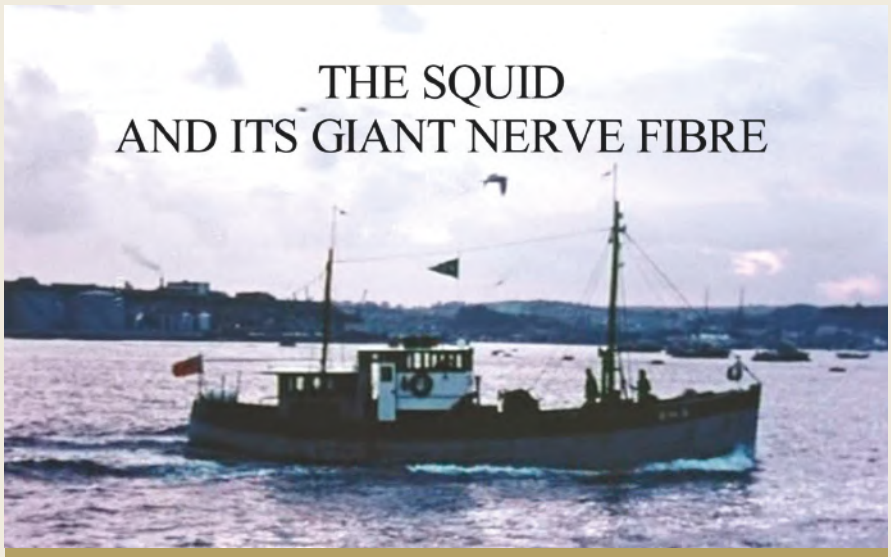
All Members and Honorary Members are encouraged to vote for the individuals they wish to fill these vacant roles. You may vote for up to three candidates. Voting closes at noon on Thursday 02 July. Please note Affiliates, Associates and Undergraduates are not eligible to vote.

The Affiliate Representative 2015 elections are also open until Thursday 02 July and all Affiliates are encouraged to vote for the individuals they wish to fill these vacant roles at **http://bit.ly/affiliateelec15**

The squid and its giant nerve fibre

The Society now owns several copies of a DVD of *The squid and its giant nerve fibre*, a film made in the 1970s at the Marine Biological Association in Plymouth. It shows how recordings of the action potential and currents were done by Hodgkin, Huxley and colleagues, and other classic experiments on the giant axon in Plymouth over 50 years ago. These DVDs were made recently by the MBA and Wellcome Trust from the one good copy of the original 16mm film found at the British Film Institute. Members interested in borrowing this DVD for teaching should contact The Society at **news@physoc.org**

Members might also be interested in the book of the Plymouth course Microelectrode Techniques for Cell Physiology. This book is now a downloadable pdf at **http://www.mba.ac.uk/microelectrode-techniques-for-cell-physiology/**



The classic Plymouth Film about research on the Squid Giant Axon

Report from the History & Archives Committee (HAC)

HAC is developing new projects aimed to increase the profile of physiology and physiologists. We are planning two seminar-style meetings to be held at Hodgkin Huxley House. One of the outcomes of the First World War was a development in physiological knowledge triggered by the circumstances of that conflict and the weaponry deployed. We are considering themes such as poison gases, fluid replacement after trauma and related areas. Another project seeks to enhance the representation of physiologists on Wikipedia. We aim to host a combined training and writing session with support and advice from those with Wikipedia editing skills and experience. Our routine work continues to add to the growing collection of Oral History interviews. Recent interviewees include Ron Whittam, Lynn Bindman, Andrew Packard, Charles Michel, Tony Angel and Linda Rimmer. An innovation this year was to make pdf versions of selected interviews available on the website (physoc.org/society-history under 'Other Resources'). The current list of seventeen will continue to grow. Updates about these initiatives will appear on the Society's website. Members with interests in these specific topics, or others within our remit, are warmly encouraged to email us at **history@physoc.org**

At a dinner on 2 March, Prof Max Headley was presented by The Society's President and CEO with a framed certificate recognising his inclusion in the list of 100 Leading Practising Scientists 2014, named by the Science Council.

News from the Publications Committee

Roger Thomas

Editor, Physiology News

PubComm, as it is known, met in April 2015 in Hodgkin Huxley House, chaired by Prem Kumar. After the usual approval of minutes etc, we carefully went through the 'Action Points' of the last meeting 6 months ago. Terms of reference were amended to address the change of *PN* editor and it was noted that more significant changes in terms of reference and voting rights had to be approved by Council. We then spent most of the time discussing The Society's two main publications, *The Journal of Physiology* and *Experimental Physiology*. Key Performance Indicators, referred to as KPI, were reported as steady in most areas. This led to a discussion about the effectiveness of 'web crawler' blocking by the data collectors. As I understood it downloads by computer programs (crawlers) seeking to corrupt advertiser feedback may also corrupt our journal data. The Editors considered how best to ensure that the Journals were well positioned to accommodate the anticipated increased submissions that would arise from increased activity in and quality of Chinese physiological research. Greater engagement with China through, for example, webinars was discussed.

After a brief discussion of *Physiology News* under my editorship, there was a very interesting presentation by Paul McLoughlin on future policy for reporting study design and statistical analysis. Apparently funding agencies and pharmaceutical companies are becoming worried about the lack of reproducibility of too much basic research. There was a major risk to credibility. To help address this authors needed to make clear whether the work they were describing was exploratory, needing little or no statistics, or hypothesis testing, needing large numbers. The Committee discussed a scheme introduced by Nature (see the 17 April issue) that imposes a new MS submission form requiring authors to answer specific questions about design and statistics by reference to text from their MS. Such an approach would minimise the burden for editorial staff and reviewers. PubComm generally supported this approach, suitably modified for physiological journals and Editorial Boards would consider how to approach the need for more rigour. We then discussed the need and purpose for Society monographs before deciding not to reintroduce them. The Committee also reported on the closure of development of an e-learning resource and noted that such developments were most likely better placed within the remit of the Education and Outreach Committee. The meeting lasted 3 and a half hours.

Both The Society's and the Wellcome Library's own collections of PS monographs are both far from complete. Members might like to consider lightening their shelves if they can offer certain numbers.)

Physiology Feed

Bringing you snippets of the latest intriguing research

Communication link in brain areas implicated in schizophrenia

An inhibitory connection between two brain areas has been discovered in mice that can control the timing of information flow into the prefrontal cortex, which may help explain what goes wrong in schizophrenia and indicate a path to new treatments.

DOI: 10.1523/JNEUROSCI.4565-14.2015

Telomeres and cancer mortality: The long and the short of it

Long telomeres may represent a survival advantage for cancer cells, allowing multiple cell divisions leading to high cancer mortality.

DOI: 10.1093/jnci/djv074

Sea slug brains

Researchers introduce new methods for pulling apart neural circuits to expose their inner building blocks to study brain function.

DOI: 10.1016/j.neuron.2015.03.005

Synaesthesia: Why some people hear colour, taste sounds

Researchers have shed new light on synaesthesia -- the effect of hearing colours, seeing sounds and other cross-sensory phenomena.

DOI: 10.1016/j.concog.2015.02.019

Blue blood on ice

An Antarctic octopus uses modulated blood oxygen transport to facilitate cold compensation and eurythermy. The study suggests that the octopus' specialised blood pigments could help to make it more resilient to climate change than Antarctic fish and other species of octopus.

DOI: 10.1186/s12983-015-0097-x

Oxytocin enables maternal behaviour

Oxytocin enables pup retrieval behaviour in female mice by enhancing the salience of acoustic social stimuli. Neural responses to pup calls were lateralised, with co-tuned and temporally precise excitatory and inhibitory responses in the left cortex of maternal but not pup-naive adults.

DOI: 10.1038/nature14402

continues overleaf

Physiology Feed

Bringing you snippets of the latest intriguing research

Insightful Locust brains

Researchers are studying locust olfaction to use the results to determine if the brain processes signals similarly for other senses.

DOI: 10.1038/ncomms7953

World's first genetic modification of human embryos

Chinese scientists have genetically modified human embryos for the very first time to modify the gene responsible for β-thalassaemia, a potentially fatal blood disorder, using a gene-editing technique known as CRISPR/Cas9.

DOI: 10.1007/s13238-015-0153-5

Scientists watch living taste cells in action

Scientists have captured live images of the process of taste sensation on the tongue for the first time. Molecular kinetic analysis suggested that intravascular taste sensation takes place at the microvilli on the apical side of taste cells after diffusion of the molecules through the pericellular capillaries and tight junctions in the taste bud.

DOI: 10.1038/srep08661

Dogs sniffed prostate cancer with more than 90% accuracy

Trained German Shepherd (Alsatian) dogs were able to sniff out the chemicals linked to prostate cancer from urine samples with remarkable accuracy.

DOI: 10.1016/j.juro.2014.09.099

How a bacterial cell recognizes its own DNA

CRISPR adaptation biases explain preference for acquisition of foreign DNA.

DOI: 10.1038/nature14302

Fruit fly eyes shed light on adaptability of nerve cells

Neurons in the eye change on the molecular level when they are exposed to prolonged light, new research demonstrates, which may be a tool to protect neurons from degeneration or cell death in the future.

DOI: 10.1016/j.neuron.2015.03.046

Spotted some interesting research? Send it to us at **magazine@physoc.org**

Policy Focus

General Election 2015

The Election results confounded everyone with a Conservative Party majority albeit a small one. At the time of going to print we know that Jo Johnson has been appointed Minister for Universities and Science. The Society is in the midst of writing to Mr Johnson welcoming him into his new role and we hope to know the formation of relevant committees in due course, i.e Science & Technology.

The Government is likely to conduct a spending review over the summer, and this will determine departmental budgets and how much money the government will invest in science and engineering.

The Conservative manifesto commits them to balancing the overall budget by 2018, and not just everyday spending, giving them less room to borrow for investment . This enables them to finish their 'long-term economic plan' to eliminate the deficit and reduce national debt. Regarding Science and Innovation the Prime Minister and Chancellor have repeatedly asserted they are at the heart of their plan (sparing them from heavy cuts in the last term), however the 2015 manifesto contains no new money for science or commitment to continue the Science Budget ring-fence.

For investment in science and engineering, the manifesto commitments are:

- Continue with the measures in the Science and Innovation Strategy, including investing £1.1 billion in science capital each year, rising with inflation up to 2020/21
- Direct further resources towards the Eight Great Technologies – among them robotics and nanotechnology
- Seek to ensure that the UK continues to support world-leading science, and invests public money in the best possible way through the Nurse Review of the Research Councils
- Put the 'NHS at the frontier of science' and prioritise funding for dementia research in the NHS

European Citizens' Initiative – Stop Vivisection

The last few months have been dominated by a new effort to ban animal research in Europe.

The Stop Vivisection European Citizens' Initiative (ECI) called for 'the European Commission to abrogate directive 2010/63/EU on the protection of animals used for scientific purposes and to present a new proposal that does away with animal experimentation'.

Under the Citizens' Initiative programme, EU citizens can propose changes in legislation to the European Commission. To warrant formal attention from the Commission, the ECI must gain one million signatures across from at least seven member states.

Stop Vivisection collected 1,173,130 signatures across 26 of the EU's 28 member states. The majority are from Italy, where anti-animal research sentiment is high. It is the third citizens' initiative to receive a public hearing at the European Parliament, and took place on the 11th May 2015.

Speakers at the hearing included initiative organisers Gianni Tamino and Claude Reiss, as well as Dr Ray Greek of Americans for Medical Advancement (AFMA), Emily McIvor of Humane Society International and Françoise Barré-Sinoussi, winner of the 2008 Nobel Prize for physiology or medicine.

Ahead of the hearing and in response to the initiative, over 120 organisations—including The Physiological Society, other learned societies, patient groups and leading universities signed a joint statement supporting European Directive 2010/63/EU. The statement calls on the European Parliament to oppose the 'Stop Vivisection' initiative, explaining that *repealing the Directive would represent a major step backwards both for animal welfare in the EU and for Europe's leading role in advancing human and animal health.*

Furthermore the Society prepared a template letter for members to help them contact their MEPs, articulating the necessary use animals in their research and why, urging their MEPs to oppose the Stop Vivisection Initiative. While we do not have exact figures, we can confidently say a significant number of members were mobilised to take action, and many also received encouraging responses from their MEPs.

At the time of going to print, we are cautiously awaiting a formal response from the European Commission on 3 June 2015.

Interested in these or any other policy related issues? Please contact us via **policy@physoc.org**

Science Slam Leeds 2015

Participants of the first Science Slam in Leeds, 18 March 2015

Charlotte Haigh
University of Leeds, UK

The University of Leeds held an inaugural Science Slam as part of the Leeds Festival of Science 2015, culminating in a competitive show at The Carriageworks Theatre in Leeds on Wednesday 18 March 2015. Science undergraduates teamed up with performing arts school students to be trained by science communication experts. They developed short pieces about the human body, which they then performed to an 80-strong public crowd. The project was funded by the Wellcome Trust ISSF fund and an Outreach grant from The Physiological Society. It received overwhelmingly positive feedback by our audience, who were all interested in attending a similar event in the future.

Members of the co-ordinating team had been involved with traditional slams in the past. A science slam is a method of science communication where researchers can present short talks on their work in an engaging and out-of-the-box way. These are normally done through the medium of spoken word poetry, where researchers/performers present their own work on a given topic. Speakers aren't allowed to use PowerPoint presentations in order to avoid lecture style shows and a time limit is usually given. They showcase their pieces, perform them on stage and an audience votes on a winner. The STEM team at the University of Leeds worked together with Charlotte Haigh, an academic in the Faculty of Biological Sciences, to co-ordinate the project and recruit science undergraduates. Selected students from Cathedral Academy Performing Arts (CAPA), Wakefield, were selected to form teams with these undergraduates. Students at CAPA have extensive experience of working with older students and take part in many theatrical productions whilst following their enriched performance curriculum at the school. The team also worked with Helen Bamber and Sarah Farrar, staff from CAPA who supported the students. Helen provides drama provision at the school and was on hand at rehearsals and training sessions to ensure all the performances were of a very high standard.

Teams also received professional training from science communication and performance experts as most of the undergraduates had no previous experience of working on theatrical productions.

Sam Illingworth, a lecturer on Science Communication from Manchester Metropolitan University, ran the first two facilitation sessions. In these sessions, the teams learnt about the three most important aspects of communication and performance: the narrative, the audience and the self.

Lewis Hou delivered the next training session and encouraged the teams to derive three main learning points from their performances. The teams picked the most important take-home messages they wanted to convey to the audience and developed these to be more prominent in their pieces.

The final training session was delivered by Victoria Pritchard, a professional actress, communication trainer and voice coach at production company Screenhouse, who recorded the whole show, which can be found on the 'STEM at Leeds' YouTube channel. All the trainers were thoroughly impressed with the growth and development each student made on their performance skills throughout the project and the students hugely benefited from the cross-pollination of mixing the sciences and arts in their teams.

Due to its success, the science slam will be run again in next year's Leeds Festival of Science working with the CAPA students but perhaps involving other local schools in a head to head competition!

Outreach grants

If you'd like to run your own Science Slam or have another idea for engaging schools and the public with physiology, you can apply for an Outreach grant of up to £1,000 to support your activity. For more details, please visit: **www.physoc.org/grants**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2015 *Forthcoming events*

26–29 Aug

Joint Meeting of the Federation of European Physiological Societies (FEPS) and the Baltic Physiological Societies
Lithuanian University of Health Sciences, Lithuania

www.feps2015.org

3 Sept

Translational Electrophysiology in Neuroscience
Hodgkin Huxley House (H³), London, UK

13 Nov

Life Scientists' Symposium - Modelling approaches in molecular signalling
Hodgkin Huxley House (H³), London, UK

www.yls2015.org.uk

7 Dec

H³ Symposium - Physiology, pathophysiology and future treatment options for diabetic complications
Hodgkin Huxley House (H³), London, UK

Meeting Notes

The 23rd Northern Cardiovascular Research Group meeting

21 April 2015,
The Copthorne Hotel,
Newcastle upon Tyne, UK

Iffath Ghouri & Simon Bamforth

Newcastle University, UK

The 23rd Northern Cardiovascular Research Group (NCRG) meeting was held on a beautiful sunny day at the Copthorne Hotel on Newcastle's iconic Quayside, the first time that the meeting had been held in the city. The NCRG meeting showcases the high quality cardiovascular research that takes place in the north of the UK, and around 120 delegates attended from England, Scotland and Northern Ireland to participate in this one-day gathering. The Physiological Society's Cardiac & Respiratory Physiology and Vascular & Smooth Muscle Physiology Themes were well-represented at the meeting.

Traditionally, the relatively small size of the NCRG meeting has provided a great opportunity for students and early career researchers to present their work in a supportive environment. This year was no different, with the majority of talks and posters being presented by PhD students and post-docs. However, unlike previous meetings where the focus has been mainly on excitation-contraction coupling aspects of cardiovascular research, the scope of this year's meeting was broadened to also encompass genetic and developmental aspects of cardiac and vascular tissue. The result was an eclectic mix of topics up for discussion, including cardiovascular adaptation to exercise, angiogenesis, in silico modelling, cardiac proteomics and ischaemic-reperfusion injury. Indeed, the important role of physiology as an integrative science was evident throughout the day. Although the topics were varied, many people commented that it was great to be exposed to research just outside their field, without it being pitched at a level beyond their understanding.

In keeping with the northern geography of the meeting, The Cairn Research Keynote Lecture was delivered by Dr Morten Høydal from the Norwegian University of Science and Technology, Trondheim. His talk, entitled 'Can exercise training teach us how to treat cardiac disease? From patients to cells and back', discussed his group's experimental approach of using animal models to understand the underlying molecular and cellular mechanisms of cardiac disease, and the improvements that can be made with exercise training. Knowledge from this is being developed to improve the quality of life of cardiac patients, and this was a great example of how physiology research can lead on to clinical therapeutic strategy.

Prizes were on offer for the best oral presentation and best poster. Michael Boylan from the University of Manchester won the prize for best talk for his eloquent description of how a point mutation in the Prpf8 gene leads to abnormal heart formation and defective left-right axis establishment. The prize for best poster – a pair of binoculars kindly donated by Zeiss – was won by Charlotte Smith, also from the University of Manchester. Her poster described postnatal T-Tubule development in ovine cardiac muscle. Congratulations to both!

The day would not have been as successful without the participation of all the delegates, who made the effort to attend from the Universities of Dundee, Glasgow, Manchester, Salford, Leeds, Liverpool, Hull, Queens University Belfast (and of course, Newcastle). The meeting provided an excellent forum for discussion, which was carried on afterwards at the conference meal in Sachins Punjabi Restaurant. We would like to extend our appreciation and thanks to our supporters and sponsors – The British Heart Foundation, Newcastle University, Badrilla, Cairn Research, Clyde Biosciences, IonOptix, Life Technologies, Olympus, Radnoti and World Precision Instruments. Photos of the event can be found at our Twitter page **@NCRG2015**.

Meeting Notes

Ageing and Degeneration: a Physiological Perspective

10–11 April 2015,
Royal College of Physicians,
Edinburgh, UK

Roger Thomas

Editor, *Physiology News*

This, the second of the Society's Topic Meetings, dominated by a symposium, took place in the opulent premises of the Royal College of Physicians of Edinburgh, who were obviously extremely prosperous in the 19th Century. More recently they had built a splendid 300-seat lecture theatre, with spacious seats and excellent audio-visual system. The symposium organisers were Marc Poulin (Calgary), Simon Gandevia (Sydney), Ylva Hellsten (Copenhagen), Paul Greenhaff (Nottingham) Brian Head (San Diego), Mandy Jackson (Edinburgh) and Ian Forsythe (Leicester).

The first session was introduced by the meetings secretary, Ken O'Halloran. It began with the plenary lecture: 45 minutes on Healthy Cognitive Aging, given by Ian Deary of the University of Edinburgh. He compared the results of cognitive tests of virtually all 11-year olds born in 1921 and 1936 in Scotland with tests of some of the same individuals as adults many decades later. Of course almost all properties tested declined with age, but it was remarkable how high scores at age 11 often corresponded with good results in 70-90 year olds.

After coffee in the impressive Great Hall, where some 40 posters were displayed, there were talks covering aspects of ageing, exercise and brain health. Kirk Erickson from Pittsburgh reported that brain scans showed that in the elderly moderate exercise, but not simple stretching, increased the size of the hippocampus and frontal cortex. He concluded that physical activity is beneficial for learning, memory and brain health for people of all ages. The audience clapped, but otherwise hardly moved a muscle till lunchtime.

Lunch was served in the space below and behind the lecture theatre, while coffee or tea was available in the Great Hall, with the posters. Two of these had my initials as an unexplained acronym. Enquiries of other viewers led me to understand that RCT means Randomly Controlled Trials, or something similar. I quote the first part of the first sentence of the abstract of poster 21, from Oxford, 'Evidence from experimental RCTs of regular exercise programs in previously sedentary adults….'. The main conclusion seemed to be that the relationship between aerobic fitness and cognitive performance remains equivocal.

The first session on Friday afternoon was about falling in the aged, with two talks from Australia, two from the USA, and one from the Netherlands. The main take-home message again was that all exercise is good, while falling is bad. I did learn a new word from the last talk before tea: 'assortativity'. According to Wikipedia it means a preference for a network's nodes to attach to others that are similar in some way. Sadly I am no wiser, but the talk by Arthur Kramer of Illinois in which it appeared was about cross-sectional and intervention studies of fitness differences and fitness training.

After tea the talks covered skeletal muscle oxygen supply in ageing. Apparently with age red blood cells are less able to release ATP, and the arteriolar response to acetylcholine is reduced. Briefly, everything deteriorates with age, but training regimes can offset some responses. After these five talks and an attempt to take a group photograph on the stairs up to the Great Hall, drinks were served in the magnificent library.

On Saturday the first session covered ageing, physical activity and neurodegeneration. Mitochondrial DNA deletions were implicated in neuromuscular degeneration, but the aging brain is still surprisingly adaptable, as shown during recovery after strokes. After coffee there were five talks featuring membrane biology and function, with membrane lipid rafts playing a key role. Such rafts in nerve cell membranes establish cell polarity by clustering pro-growth receptors and tethering cytoskeletal machinery. They require a scaffold protein called caveolin. Remarkably, Brian Head of San Diego showed that overexpression of caveolin had multiple effects on mice dendritic arborization and even prolonged the lifespan of the nematode C. elegans. The last session of the symposium covered cerebellar ataxia and neurodegeneration. This was followed by a final poster session. The Society Dinner was held in the Scotsman Hotel, with drinks for an hour, before dinner was tardily served at 20.15 in a low-ceilinged basement room. The meetings secretary made a brief speech thanking all concerned, and guests departed into a howling cold wind. Total attendance at the meeting was about 150 people, with 60 attending the dinner.

I left determined to take more exercise while I still remember how good it is for almost all physiological properties.

Drinks in the Library with Danielle Thomas & Anneka Antoniak

Poster session in the Great Hall

View from the front of the lecture theatre

Physiology 2015: welcome to Cardiff / *croeso i Caerdydd!*

6–8 July 2015, Motorpoint Arena, Cardiff, UK

Sarah Hall, Paul Kemp & Frank Sengpiel

School of Biosciences, Cardiff University, UK

Cardiff University's Main College Building

We were surprised, and then delighted, to realise that the main meeting of the Society is to take place here in Cardiff this July. The last time the Society met in Cardiff was in 1999, when Vincenzo Crunelli was head of the Physiology Unit. Back then, the meeting was a very hands-on affair, with the Cardiff physiologists acting as hosts and organisers. Local members were involved in all aspects of the meeting, including arranging venues for talks and dinners, inviting speakers, chairing sessions and even negotiating terms with the B&Bs around the city. This time, the Society staff in Hodgkin Huxley House has managed every aspect so smoothly that all we Cardiff physiologists are required to do is to turn up with everyone else!

Cardiff claims to have more green space per person than any other UK city, more hours of sunlight than Milan and more castles than any other city in the world (that's four, for the trivia aficionados). It's not surprising, then, that *National Geographic* magazine recently selected Cardiff as one of the 10 best places to visit in the world! Although you don't need to speak Welsh to get around in Cardiff, you might be intrigued to learn that the Welsh word for '*physiology*' is '*ffisioleg*'. Visiting physiologists may also find it useful to know that the local beer is called Brains (you could meet a man with several Brains!) and that the type of seaweed used to make the local delicacy laverbread is unusual because its fronds are only one cell thick.

Cardiff's roll call of distinguished physiologists includes John Berry Haycroft (Professor of Physiology from 1893-1920), whose obituary in Nature in 1923 stated that he had 'devoted his life to physiology', and Vernon Pickles (Head of Department from 1966-1981), who made significant discoveries relating to the role of prostaglandins in uterine function. There are currently around 30 members of the Society scattered across Cardiff University, predominantly in the Schools of Biosciences, Medicine and Pharmacy. The School of Biosciences is housed in the Sir Martin Evans Building, named after the first Head of School, who won the Nobel Prize in Physiology or Medicine in 2007 (together with Mario Capecchi and Oliver Smithies) for 'discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells'. The theme of stem cells in Cardiff has been expanded over the last decade, to include significant strengths in pluripotent stem cell-based modelling and potential treatment of neurodegenerative diseases, particularly Huntington's and Parkinson's Diseases, as part of an integrative approach across the School and the Neuroscience & Mental Health Research Institute. MRC Prof Ole Petersen, who has recently stepped down as Director of the School, pioneered studies of calcium signalling in epithelial cells and is a former President of The Physiological Society. His team investigates physiological and pathophysiological signalling mechanisms in the pancreas. Daniela Riccardi, Paul Kemp and their group have been in the news recently, having identified a potential role for the calcium-sensing receptor (CaSR) in asthma. Their work demonstrates the effectiveness of calcilytic drugs in manipulating CaSR to reverse all symptoms associated with the condition. The calcium focus extends to the Institute of Molecular and Experimental Medicine, where physiology-related research encompasses molecular investigation of the structure and function of the ryanodine receptor, and events during fertilisation and early embryo development. The prestigious ARUK Biomechanics and Bioengineering Centre is leading interdisciplinary studies of joint biomechanics and disease, and Cardiff University scientists have also recently developed a novel anti-cancer stem cell agent capable of targeting aggressive tumour-forming cells common to breast, pancreas, colon and prostate cancers. Researchers in Cardiff are employing an array of molecular, electrophysiological, immunohistochemical and imaging techniques to understand physiological and pathophysiological processes.

Cardiff University has also had a strong presence in cellular and integrative neuroscience for many years. Graham Brown, who made significant contributions to

School of Biosciences Sir Martin Evans Building

understanding the neural control of locomotion, was head of the Institute of Physiology in Cardiff from 1920 to 1947. Under Brown's chairmanship, The Society met for the first time in Wales and, afterwards, the Great Western Railway Company provided a special coach to take the delegates back to London. Delegates in 2015 should be warned not to expect such treatment! Current neuroscience research foci in the School of Biosciences include developmental plasticity of the visual and somatosensory systems, and the thalamocortical circuitry underlying sleep-wake cycles and epilepsy. The methods employed range from classical slice recordings and *in vivo* extracellular recordings to map receptive field properties to recordings with implanted tetrodes in conscious, behaving animals, and from optical imaging of intrinsic signals of cortical areas to two-photon calcium imaging of the activity of individual neurons. Cardiff hosted a themed meeting on sensory systems in 2009 and Frank Sengpiel was the Neuroscience theme lead from 2010 to 2014. Since 2010, the Neuroscience & Mental Health Research Institute has provided a new focus on neuropsychiatric, neurodevelopmental and neurodegenerative conditions; here new discoveries of genetic causes of mental illness are translated, in animal models, into greater understanding and diagnosis of these conditions. This work also encompasses a wide range of cellular and integrative neuroscience approaches.

In addition to its research activities, the School of Biosciences is home to over 1,300 undergraduate students. We offer a range of degrees across the spectrum of biosciences, from molecular biology to ecology; although only some of these students are enrolled on standard Physiology or Neuroscience degree programmes, physiology is a core component of the first year of all our degrees. In addition, we contribute to teaching medical and dental students, as well as medical engineers. Post-graduate provision is an important aspect of the School's purpose. There are currently around 150 PhD students, and another 15 MRes students, enrolled for post-graduate degrees. A small team of PhD students and post-doctoral researchers have worked together to produce a programme for the Early Career Physiologists symposium in July which builds on Cardiff's research profile to explore the diverse ways cellular signalling is involved in health and disease.

The School of Biosciences has made a significant commitment to the development of innovation and engagement activities to enhance the traditional academic pursuits of learning, teaching and basic research. Annual events for schools include the 'Learn About Life' days for local primary school pupils, the 'Wales Brain Bee' for secondary school pupils and sixth-form workshops aimed at widening access to health-related sciences. Undergraduate students are encouraged to participate in such outreach sessions and can even undertake final year research projects based on the design and development of engagement activities or tools.

Since the Cardiff meeting has a packed programme, not to mention the other competing tourist attractions, you may not find time to venture all the way across town from the Motorpoint Arena to the University, but we can provide a map and directions if you want to visit. It is only a 15-20 minute walk, and the postcode is CF10 3AX. In any event, we look forward to welcoming you to the Welsh capital in July - *Croeso ffisiolegwyr!*

For more information please visit
www.physiology2015.org

The nematode 'worm' >> fast forward for physiology

Forward genetics in *Caenorhabditis elegans* has provided new insight into a broad range of physiological processes spanning synaptic physiology, to behavioural plasticity and ageing

Lindy Holden-Dye

Fernando Calahorro, Anna Crisford, James Dillon, Lindy Holden-Dye, Vincent O'Connor & Robert J Walker

University of Southampton, UK

Forward genetics, an approach which maps and characterises genetic mutations that confer specific phenotypes, is one of the most powerful approaches in biology providing new, often unexpected, insights into physiological processes. It has been deployed to great effect in the nematode worm *Caenorhabditis elegans*. Here we introduce the 'worm' to the uninitiated and, focussing on forward genetics, describe a few of our favourite studies that have supported advances in physiology.

The 'worm' is a nematode

For those working with *C. elegans* it is often referred to endearingly as the 'worm'. This can cause some confusion as to many people 'worm' means an earthworm – as exemplified by our marketing department which supplied a photo of an earthworm dangling from a fisherman's hook to illustrate our research in *Caenorhabditis elegans*! To be clear, *C. elegans* is a nematode; it is much simpler than an earthworm: An earthworm has thousands of cells and ~10, 000 neurones but *C. elegans* (adult hermaphrodite) has precisely 959 somatic cells of which 302 are neurones. The connectivity between neuronal cells was beautifully described and mapped by John White and colleagues in their paper with the running title 'Mind of a Worm'. This was the first ever, and still is, the only complete connectome for any animal.

C. elegans belongs to the phylum Nematoda- all of the organisms within this phylum are round worms like *C. elegans* and have the same overall body plan but they have a variety of lifestyles, some are free-living bacteria-eating worms like *C. elegans*, others are parasitic and infect humans, livestock, pets and even crops. Their sizes span an amazing range; *C. elegans* is just 0.08 mm wide and 1 mm long but the largest known nematode, a parasite of the sperm whale, *Placentonema gigantissima*, is the width of a garden hose and the length of a bus. The dimensions of the latter raise an interesting physiological dilemma in terms of long-distance neural signalling given that nematodes are not known to be able to generate fast Na^+-dependent action potentials!

A 1960s debut for the worm

C. elegans was the inspired model organism of choice of Sydney Brenner in the 1960s. He pioneered its use as a genetic model for biology and his early emphasis on developmental and neurobiological processes quickly drew others into the fold. Over the next couple of decades *C. elegans* established itself as an exceptionally powerful animal in which to address fundamental questions in biology. A world-wide community of *C. elegans* researchers has continued to grow over the years, characterised by a willingness to share ideas and resources exemplified by the informal publication *The Worm Breeder's Gazette* and co-ordinated cataloguing of a wealth of resources available for the investigation of this animal (Box 1). The worm has had an added significance by inspiring

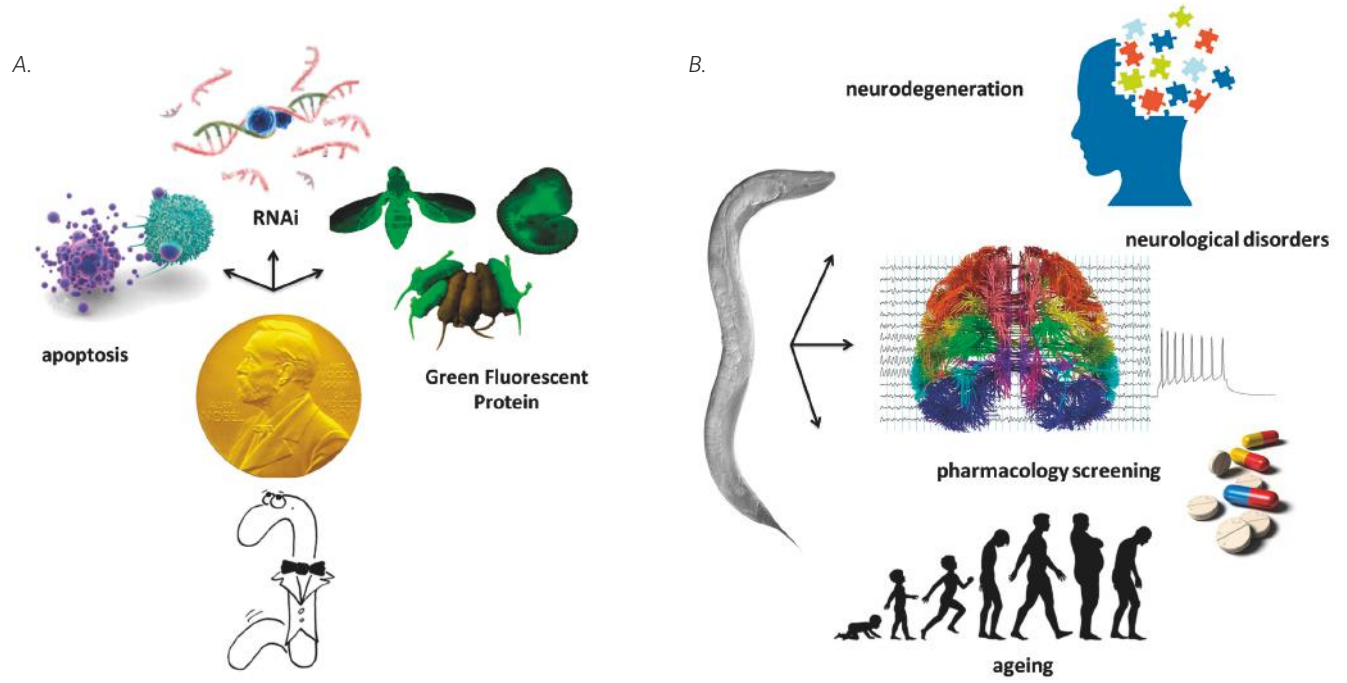

Figure 1. **Prize winning nematode worm as a model for disease and drug discovery**
A. The worm has 'won' the Nobel prize in Physiology or Medicine three times. In 2002 it was awarded to H Robert Horvitz, Sydney Brenner and John Sulston for 'discoveries concerning genetic regulation of organ development and programmed cell death''. The citation rather coyly mentions the generic term 'organ', rather than specifying the organ in question, the worm's vulva. In 2006 it went to Andrew Z. Fire and Craig C. Mello 'for their discovery of RNA interference - gene silencing by double-stranded RNA' and in 2008 it was won by Osamu Shimomura, Martin Chalfie, and Roger Y. Tsien 'for the discovery and development of the green fluorescent protein, GFP'. Will it win again? And what for?
B. C. elegans provides a genetically tractable platform for the investigation of a wide range of physiological processes, disease and neurological disorders including neurodegeneration, addiction and autism spectrum disorders. It is also increasingly being deployed in drug screens and as a quick route to mode of action that addresses the 3R's principles (reduce, refine, replace) of vertebrate animal use.

ground-breaking technical and conceptual advances most notably the discovery of RNAi and the development of green fluorescent protein as experimental tools (Figure 1*A*).

Worm watching catches on

Watching worms down a binocular microscope might not seem like a very sophisticated scientific pursuit but this has been at the forefront of some major discoveries in biology. Worms develop and behave in a very stereotyped manner which means that a skilled worm watcher can readily detect aberrance. Not only that, for an animal with just 302 neurones their behaviour might surprise some in terms of its refinement. In addition to basic behaviours, eating, moving and mating they can make simple associations between environmental cues and show classic conditioning and aversive learning. For example, they will migrate on a thermal gradient to a specific temperature they have learnt to associate with food. They can also learn to avoid eating bacteria that made them feel 'sick'. Recent papers have even investigated 'decision-making' in *C. elegans*. All these endeavours were set in train by Sydney Brenner who first demonstrated in 1974 that exposure of worms to a chemical mutagen enables one to generate and propagate multiple behavioural mutants within the space of just a few weeks. These mutants were named through a three letter code according to the aberrant phenotype, hence '*unc*' for uncoordinated, '*egl*' for egg-laying, *eat* for feeding etc. Combining this with techniques for genetic mapping, transgenesis which permits cell specific expression of genes of interest and importantly the provision of the complete genome sequence in 1998 (Box 1), the first for any animal, ultimately led the way to decoding the function of specific genes with key biological roles. This is what is meant by forward genetics; it has proven to be an exceptionally important and unbiased approach to liberate meaningful information from the genome. There have been many successful applications of this for physiology (Figure 1*B*). Below are a few examples of work using the worms where this has been used to best effect.

What makes a worm wriggle?

The physiological depiction of vesicle-mediated neurotransmitter release onto cognate postsynaptic receptors made Sir Bernard Katz a Nobel 'vordenker' (1970) and established a field that focussed on a molecular explanation of synaptic transmission. The studies that allowed identification of synaptic proteins were largely biochemical in nature. Forward genetics in *C. elegans* supplemented this revolution in 'molecular physiology' by providing a comprehensive supply of individual genes including many *uncs* that were suspected of an essential role in neurotransmission because the mutant worms had an abnormal wriggle. An important part of the *C. elegans* efforts was the identification of molecular components defining the transmitter release site or active zone. Insight into this process was provided by the *C. elegans* mutant *unc-13* which was first characterised to encode a novel class of phorbol ester receptor by Ahmed *et al.* in 1992. This was picked up by Nils Brose and colleagues who recognised that *unc-13*'s domain structure, harbouring Ca^{2+} -sensing and phorbol ester binding that predicted lipid-dependent membrane association, was highly suggestive of a role in regulated neurotransmitter release. They cloned and functionally characterised the corresponding mammalian gene family, for which the term *munc-13* was coined, and showed it has a fundamental role in vesicle priming and defining the substructure of the active zone (Brose *et al.* 1995); it was among the first of the truly active zone proteins for the mammalian nervous system . Thus, an

‘Forward genetics has proven to be an exceptionally important and unbiased approach to liberate meaningful information from the genome’

awkwardly moving worm prompted mammalian studies that led to the identification of an evolutionary conserved gene family pivotal in synaptic physiology.

Social worms

Mario de Bono noted that different isolates of *C. elegans* would either feed in clumps of worms together, so-called ‘social’ feeding whilst other isolates, including the N2 strain used as the wild-type by most *C. elegans* research groups, is a ‘solitary’ feeder. (Some comment has been passed on the fact that one of the social strains derives from Australia, compared to the less social N2 which hails from a Bristol compost heap.) This natural variation in behaviour is due to a single residue difference in a neuropeptide-Y like receptor, *npr-1* (de Bono *et al.* 1998). How does this determine whether a worm is social or solitary? Chasing this question is providing a systems level interpretation of how the worm’s nervous system can integrate multiple environmental cues to bring about flexible behaviour. Important here has been an understanding of the worm’s natural environment, rotting fruit, and the cues it has evolved to respond to especially the relative concentrations of O_2 and CO_2. The excitement is that these sensors which detect molecular gases are embedded in a simple nervous system. The behavioural consequence of this sensing can be quantified in the whole worm by tracking changes in locomotion and scoring aggregation. Dovetailed with cell specific rescue approaches, this is mapping out the individual cells and microcircuits that underpin the behavioural responses to these environmentally salient gaseous cues (Busch *et al.* 2012). The ability to visualize the activity of these networks using genetically encoded Ca2+ sensors in the intact behaving worm opens a window to the dynamics of these networks. In addition to defining novel sensors of key physiological cues these paradigms, which began with the identification of *npr-1* through a forward genetic approach, provide a whole organism view of how a changing environment determines behaviour. It is driving our conceptual understanding regarding the true nature of physiological control as the interface between molecules and behaviour.

Lurching worms

Villu Maricq’s group were led into previously uncharted territory by a specific application of forward genetics, suppressor screens. When a worm is on a bacterial lawn it exhibits a behaviour, called dwelling, in which it frequently changes between backwards and forwards movement so that it stays in pretty much the same location on the food patch to eat. This behaviour is regulated by a *C. elegans* glutamate receptor, GLR-1 which is an orthologue of mammalian AMPA/Kainate receptors. In a clever piece of model hopping, Maricq’s lab introduced a gain-of-function mutation into *C. elegans glr-1* that was known to confer constitutive channel activity in the mouse receptor, originally identified in a mouse locomotor mutant called ‘lurcher’. The worms expressing the gain-of-function channel showed an extremely high frequency of reversals. By subjecting the ‘lurcher’ worms to chemical mutagenesis it was possible to identify mutations in other genes that suppressed this behaviour, so-called suppressor-of-lurcher, or *sol* genes. *sol-1* and *sol-2* were found to encode auxiliary AMPA receptor subunits and are important in regulating receptor mediated currents. These studies are helping to elucidate the constituents and dynamics of evolutionary conserved multi-molecular complexes that co-assemble with AMPA receptors to regulate their function. The beauty of this approach is that it has allowed identification of proteins in the GLR-1 signal transduction cascade with no *a priori* assumption about their identity (Wang *et al.* 2012).

Ageing worms

C. elegans lives for just two to three weeks so finding mutants that can live longer and healthier can be achieved in a much shorter period of time than for other genetic animal models of ageing. As it grows older the worm becomes progressively slower and more ‘wrinkly’. Intriguingly, worms that are about to die show a wave of intense blue autofluorescence propagating from the anterior to the posterior end of the gut. This is a biomarker of calcium-dependent necrosis and is a precise predictor of their forthcoming death (Coburn *et al.* 2013). The first investigations of the genetics of *C. elegans* ageing, by Klass and Johnson in the late 1980s, were apparently greeted with some surprise when it was shown that it is possible to extend lifespan through mutation in a single gene, *age-1*. It was counterintuitive to the view of the day that such a complex phenomenon could be manipulated in this way. The gene *age-1* was subsequently shown to lie in a signalling pathway involving the insulin-like growth factor receptor *daf-2*. Mutations of genes in this pathway have shown that it is possible to extend *C. elegans* life span more than five-fold. Using epistasis analysis the worm has also provided insight into the organisation of the signalling cascade. Importantly, mechanisms for life-span extension are conserved in mouse and human with key roles for insulin-like growth factor signalling, dietary restriction and even exercise (yes, you can make a worm do exercise!). Not only is the lifespan increased but many of these long-lived worms often appear to remain healthy for longer. Nonetheless, the longest-lived worm at Southampton which notched up 107 days under the careful husbandry of Neil Hopper (Hopper, 2006), equivalent to 400 human

years, earned the name 'Steptoe' as in latter-life it was rather decrepit.

The attraction of the worm is that it provides the chance to answer core questions in physiology even when the related biology is complex and poorly understood. The world-wide community of worm researchers, first instigated by Sydney Brenner, have a wealth of resources at hand which are carefully catalogued and readily available (you can order your mutant of interest and receive it in the post for $7!). Clever use of forward genetics and model hopping between the worm and mammalian systems has greatly facilitated answering important fundamental physiological questions. We have reported on but a few of the >25,000 papers and the brilliant minds that have been hooked by *C. elegans*. The quality and resonance of the overall outputs indicates fishing in biology with these worms is actually very smart - the Mad Men in our advertising department were clearly onto something after all!

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Worm facts & resources

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'An awkwardly moving worm prompted mammalian studies that led to the identification of an evolutionary conserved gene family pivotal in synaptic physiology'

Drosophila – a model for all reasons

This tiny fly is too useful to be the preserve of developmental biology

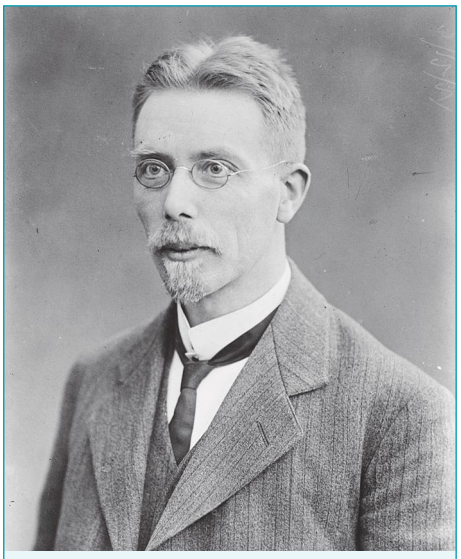

Figure 1. August Krogh

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For many years, physiology and genetics were on orthogonal trajectories. However, combining the two approaches can provide valuable general insights. Nowhere is this better illustrated than in the fruit fly.

The Krogh principle and comparative physiology

August Krogh (Nobel prize in Medicine, 1920, photo Fig 1) is widely considered to be the founding father of modern comparative physiology, and his eloquent exposition of the comparative approach '*for such a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied*', is now known as the Krogh principle. In the current era, where biomedical relevance is key, it is easy to forget that physiological advances have come from anything other than mouse; however, Krogh's principle of choosing the best species for the question being studied shines through some of our biggest advances – the squid giant axon, the electric ray electric organ, frog skin and snail neurones have all advanced our understanding of the human condition more than we might think. Indeed, this principle of using a simple model for a general insight goes further back in history; the great natural philosophers of the enlightenment would draw, not just on different species, but different sciences, as they saw fit.

Drosophila as a 'Krogh' organism

On the surface, it might seem that *Drosophila* has very little to commend itself as an 'ideal' physiological subject. It is small –tiny, in fact as shown in Fig 2 – and an utterly harmless and undistinguished member of one of the largest Orders of insects. The rapid generation time (1 week) and fecundity of this little fly (one mating can produce several hundred offspring) means that genetics is really powerful, and their small size and the low cost of rearing them means that a modest fly group can keep hundreds of mutant lines – something that is not so easy with transgenic mice! So *Drosophila* is a genetic model par excellence. As it develops from fertilized egg to larva in just 22 hours, it has become the darling of developmental biologists (and indeed, underpinned a Nobel prize). Although this explains the continued fixation of most of the *Drosophila* community with development, the organism is really far more versatile! Indeed, the fruit fly is already embedded in our physiological lexicon. The reason is that, with genetic power comes the ability to see mutations, or to perform screens, that provide overwhelming insights with great speed and agility, compared with our bigger models. Below, I give a few examples.

White

Back in 1910, Thomas Hunt Morgan, working in the new field of Genetics at Columbia, spotted the first *Drosophila* mutant, *white*. Instead of the rather lovely red eyes characteristic of the species, these eyes were brilliant white. The mutation was a classical Mendelian recessive; and so the floodgates opened on *Drosophila* genetics. But what does white actually encode? It's a broad-spectrum organic solute transporter of the ABC family, instantly recognisable today as an orthologue of our own ABC transporters. The

Figure 2. Drosophila is small, easy and cheap to rear. In fact, you can raise them on pennies.

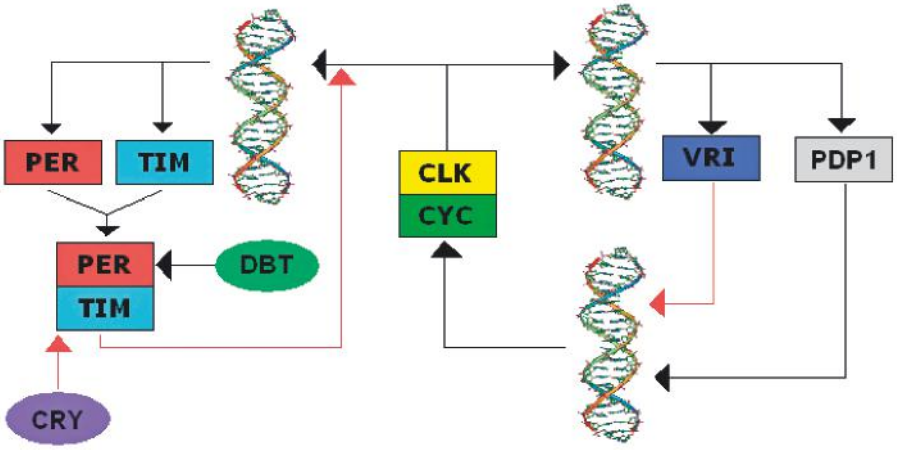

Figure 3. Genetic timing network in Drosophila.

precursors of the bright red eye pigments are stored and metabolized in the kidney tubules of the rapidly growing larva, then released into the pupal blood space to be taken up by the maturing compound eye. White plays a role in these transport processes; in mutants, the kidney tubules fail to accumulate pigment precursors (and so are white instead of yellow) – and in turn, the eye never gets any pigment to absorb, and so also looks white. So, long after Morgan's discovery, we can trace out a physiological odyssey of transporters, tissues and metabolites. Better yet, although there are now hundreds of alleles of white, the direct descendants of the very first fly that Morgan spotted are still available from *Drosophila* stock centres by post. For $25 – just ask for *white*[1]. Talk about walking in the footsteps of giants...

Shaker and voltage-gated ion channels

Another spontaneous *Drosophila* mutant transformed our understanding of neurobiology (Bellen HJ, Tong C, & Tsuda H, 2010). In 1944, Catsch described a line of flies bred from a single fly observed to shake its legs under ether anaesthesia (before boring old carbon dioxide anaesthesia, *Drosophila* labs were quite fragrant –and dangerous – places). The mutant was named *Shaker*, and subsequent decades of neurophysiological research showed that it was defective in rapidly inactivating (A type) potassium conductance – hence the shaking. One of the great early triumphs of molecular biology had been the sequencing of the first sodium channel, with its highly suggestive structure of four repeats of 6 transmembrane domains. The internal similarity of these four repeats suggested that the gene had evolved by two successive tandem gene duplications of an ancestral channel of just 6 transmembrane domains. So, instead of making a 24-helix tetramer of 4 separate 6-helix proteins, we had evolved a single gene encoding all 24. When *Shaker* was sequenced, this turned out to be the 'living fossil' ancestral condition – it encoded 6 helices that operated as a subunit of a homotetramer. However, it would be quite incorrect to think of *Shaker* as the primitive condition – flies are no less 'evolved' than us! We diverged from flies 400 M years ago, and we are both quite successfully alive today. And in due course, a six transmembrane domain *Shaker* orthologue was identified in humans. Today, of course, we know of the Sh, Shal, Shab and Shaw families of 6-transmembrane potassium channels, common to flies and humans.

Dunce, cabbage, rutabaga – the molecular basis of learning and memory

These first two stories were about chance discoveries of spontaneous mutants. However, it is relatively easy to mutate thousands of flies with X-rays or chemical mutagens, to screen them for individual mutants with a desired phenotype, then to painstakingly identify the mutated gene – this is known as classical, or forward, genetics. Such screens paved the way for our understanding of learning and memory. *Drosophila* are not as stupid as one might think, and can be trained to associate particular odours or tastes with a mild electric shock, and this was the basis for a screen that identified the first learning and memory mutants in any species. Flies learn the odour shock pairing, in a T-maze, in relatively few trials, then forget relatively slowly; it is thus possible to screen either for flies that are defective in learning separate from those that learn normally, but forget quickly. Such screens identified dunce, the archetypal memory mutant. *Dunce* encodes a cyclic AMP phosphodiesterase, firmly pointing the finger at the cAMP signalling pathway as key in associative learning. Other learning mutants – wittily named after vegetables – support this theme: *rutabaga* encodes an adenylate cyclase, for example. Critically, these findings resonated with those of Nobel laureate Kandel on the simple sea slug *Aplysia*, confirming a broad phylogenetic base for cAMP in learning and memory.

Circadian rhythms and clock genes

Now that the principle of identifying genes based on an informative screen is established, we can have more fun. *Drosophila* adults tend to emerge from their pupae at a particular time of day, and adults show clear diurnal locomotor patterns. These behaviours persist in constant darkness. So screening mutants for locomotor behaviour that fails to show circadian rhythmicity in darkness could identify genes that are important in intrinsic clocks. That describes nicely the discovery of the archetypal clock gene, *period*, and its partners in crime, such as *clock* and *timeless*, see Fig 3. Such genes have been found from plants to flies to humans, so the principle established in a simple model is of general application.

Reverse genetics and the *Drosophila* 'toolbox' for integrative physiology

The forward genetics method is powerful, but slow; until the advent of next-generation sequencing, it could take a decade to identify the DNA change associated with a mutation. Reverse genetics is the opposite; a gene is intentionally mutated in the hope that the resulting phenotype will tell us what the gene does. This is vital for modern biology, because surprisingly, we have no idea of the function of about a fifth of our 20 000 or so genes. Now, creating mutations in humans is not a good path to tread; so reverse genetics of novel gene orthologues in genetic model organisms is a hugely important process. Indeed, this is precisely why mouse is now such an important model.

'*Drosophila* is not important just because we can model human function or disease'

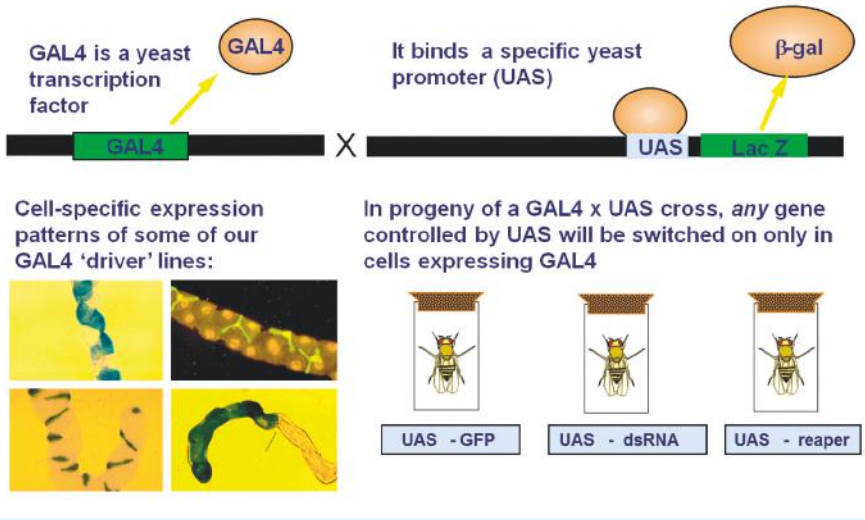

Figure 4. Introduction to the GAL4/UAS system of Drosophila.

In *Drosophila*, much use has been made of transposons, autonomous bits of DNA that insert in our genomes randomly. Transposons encode transposase, the enzyme that catalyses their excision and reinsertion with relatively high efficiency. *Drosophila* research makes heavy use of one such transposon, the P-element. The transposase can be replaced with a genetic payload of choice; and when co-injected into fly embryos with a separate source of transposase, the P-element can insert into the genome with high efficiency. As well as a simple method for high-efficiency transgenesis, P-element insertions near to a gene of interest can be identified by PCR, providing a rapid means to mutate a specific gene.

One day in the early 1990s, my late colleague Kim Kaiser explained to me a method that allowed expression of any transgene in any tissue or cell population of the experimenter's choice, and so convinced me to jump ship to *Drosophila*. As a comparative physiologist, I had wanted to tinker with genes in just the way that we can pharmacologically intervene with some signalling pathways – but without the clumsiness associated with simply mutating the whole gene. Classical mutations can have multiple effects on multiple tissues (this is called pleiotropy); but the GAL4/UAS binary system allows beautifully precise interventions to be made (Duffy JB , 2002). Essentially, the yeast transcription factor GAL4 is put under control of a gene with a tissue or cell-specific expression pattern, and so is expressed in the same pattern of cells; such GAL4 lines are typically identified as part of a large-scale P-element screen. GAL4 has no major effects in the fly genome; but when such GAL4 'driver' lines are crossed to flies transgenic for P-elements containing a genetic payload downstream of UAS, the sequence bound by GAL4, something magical happens. In the progeny of the cross, the genetic payload is expressed in only those cells in which GAL4 is being expressed. So all *Drosophila* labs keep a panel of GAL4 driver fly lines that can direct expression of transgenes of choice, simply by performing a cross, see Fig 4.

Better yet, generating such UAS lines is not a long job; there are off-the-shelf vectors for most purposes, and injection services that will produce transgenic flies from your plasmid in about 3 months, for less than $500. So (for example) a graduate student in my lab could fuse their gene of interest to a GFP gene in a UAS vector and send off the construct to be injected, in about a week, and three months later could be studying the subcellular localization of their protein in specific cells of an otherwise normal organism. By contrast with mouse transgenics, I would not necessarily need to approve such a modest expense! This freedom to perform cell-specific interventions in an otherwise normal organism is what I call the 'toolbox' of integrative physiology. Of course, the GAL4/UAS system is only one simple example of what can be achieved, and the book is being rewritten all the time; many *Drosophila* labs, for example, are currently excited about genome editing with CRISPR/Cas9. No matter the detailed technology, the physiological uses are limited only by one's imagination.

Harnessing the toolbox for real physiology

My lab, together with that of Shireen Davies, focusses on integrative physiology and functional genomics of osmoregulation and renal function. Scaling arguments suggest that terrestrial insects live their lives in continual danger of drying up, and so osmoregulation is critical to their success. Paradoxically, then, insect renal tubule cells can secrete their own volume of primary urine every 6 seconds, the fastest rate known for any epithelium (Beyenbach, Skaer, & Dow 2010). So both the transport mechanisms

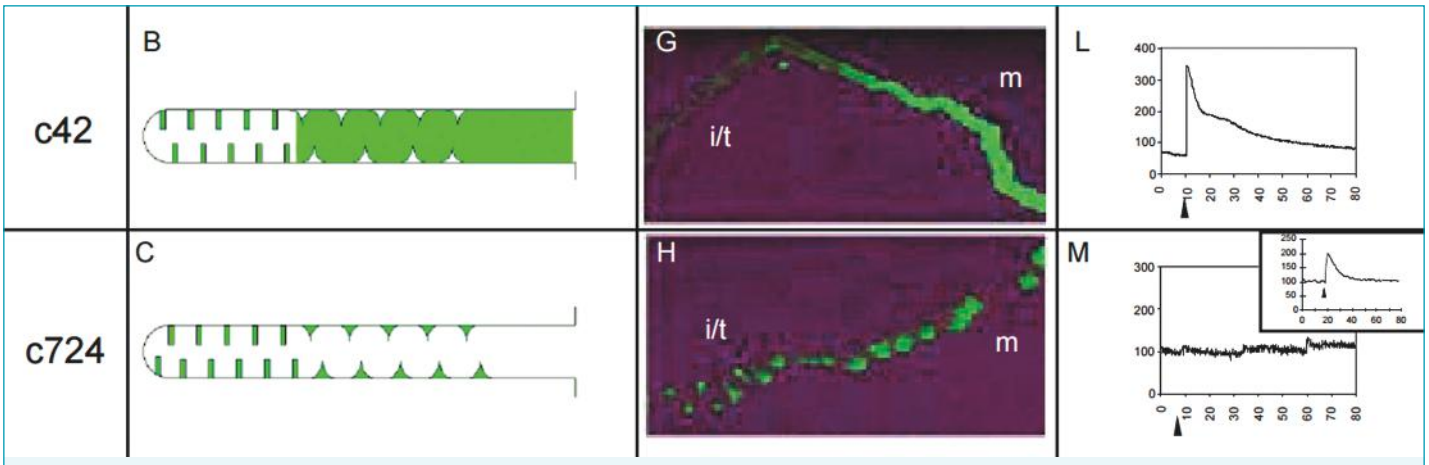

Figure 5. By driving a transgene for the calcium reporter aequorin with GAL4 drivers specific for principal cells (c42) or stellate cells (c724), the neuropeptide CAP2b can be seen to signal through intracellular calcium in only the former cell type.
(From Rosay P, et al. 1997).

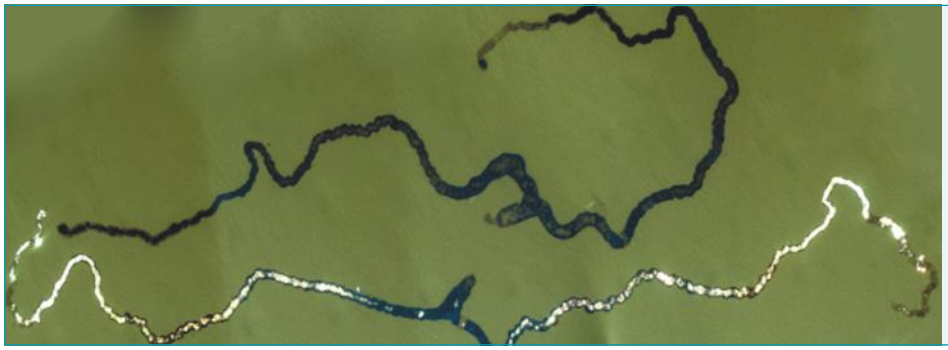

Figure 6. Polarizing microscopy of Drosophila renal tubules, acutely dissected from flies fed normal (top) or high oxalate (bottom) diets for just 1 day. Oxalate crystals are visibly birefringent. Pablo Cabrero, pers. Comm.

and their tight neuroendocrine control are of great interest, both from the basic science viewpoint, and because disruption of these control mechanisms could provide us with valuable new insecticides. Our GAL4 driver lines demarcate the major cell types and regions in this tiny tissue of 150 cells, and we have used it, for example to make the first animals transgenic for a calcium reporter, allowing us to show that a particular neuropeptide signals through calcium in only one cell type in the tissue, see Fig 5.

Biomedicine

Across 400 My of divergent evolution, it might seem that there would be little in common between *Drosophila* and human renal function. Indeed, you won't find renin or angiotensin in flies. However, the two tissues perform analogous tasks, with orthologous genes, and so there can be some surprises. For example, simply by feeding flies oxalate, we have been able to model in flies the most common form of kidney stones, with the advantage that they are visible *in situ*, as shown in Fig 6, and not hidden in an opaque kidney capsule (Dow & Romero MF, 2010). This unique advantage opens the door to a screen for agents that block stone formation.

However, it is important to appreciate that *Drosophila* is not important just because we can model human function or disease; *Drosophila* is a highly successful organism, and understanding how it works is important in the bigger scheme of things. Although biomedical imperatives have distorted the picture, human physiology is but a special case of physiology. Great minds have appreciated this: indeed, the famous codebreaker Alan Turing studied pattern formation in *Drosophila*, and proposed in 1952 that diffusible morphogens provided a self-organising system. And our system, the Malpighian tubule, is named after Marcello Malpighi, in the 17th century the physician to the pope and the father of microscopic morphology. He studied insect and human kidneys with equal enthusiasm; and several structures, discovered by him, bear his name today.

Acknowledgements

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Are medicinal leeches still a useful model for studying neurophysiology?

From medical tool to model organism

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A question I am frequently asked, as an investigator who works on leeches, is 'can further studies of these simpler animals generate sufficiently interesting information to justify their continued study?' The answer is 'yes', studying the CNS of leeches can still make a useful and relevant contribution to modern neuroscience research.

The medicinal leech has several advantages for studying many general principles of nervous system function. These include the organization of neural networks that generate behaviors and the mechanisms of decision-making used to select appropriate behaviors. Historically, the European medicinal leech (which I will refer to as the leech in this article, for the sake of brevity; species: *Hirudo medicinalis* and *Hirudo verbena*, see Fig 1) has been studied to understand better the properties of mechanosensory neurons, motoneurons, synaptic communication as well as the organization of neural circuits capable of generating behaviors (Reviewed by: Kristan *et al.* 2005). More recently, some researchers have given less attention to model systems like the medicinal leech in favor of more complex and human-like animal models such as rodents and zebrafish. I will explain the continued value of the leech by briefly describing some of the features of its central nervous system (CNS) that make this animal well-suited for studies of the neural mechanisms of behavior. I will also give some examples of recent work done to understand better the generation and control of behavior at the levels of neural networks and individual neurons.

The CNS and behavioral repertoire of leeches have features that facilitate addressing many important questions in neuroscience. The ventral nerve cord (i.e., the CNS) of the leech contains approximately 10,000 neurons divided between a compound cephalic ganglion, 21 segmental ganglia and a compound tail ganglion (Fig. 2). Each segmental ganglion contains approximately 350-400 neurons or about 200 bi-lateral pairs. Compared with the CNS of insects and vertebrates, which have millions or billions of neurons within their CNS, leeches are a potentially more tractable system. This is especially true when the research goals are to understand the contributions of individual neurons in generating a behavior, which is often among the goals of studies involving leeches. One of the principle features making these types of studies possible is that single neurons in the CNS are uniquely identifiable between individuals based on the size and location of the neuron's soma, its electrophysiological signature, neurotransmitter phenotype, connectivity with other neurons and its morphology (Fig. 3). Furthermore, the CNS, which resides inside of the animal's circulatory system, lacks a formidable blood-brain barrier. This fact makes delivery into the CNS of many neuroactive substances, *in vitro* and *in vivo*, all but trivial in most cases. Lastly, although the CNS of these animals contains far fewer neurons compared to insects and vertebrates, leeches exhibit an impressive array of dynamic and adaptable behaviors common to nearly all animals. These behaviors include (but are not

Figure 1. Photo of a leech *Hirudo verbena*. Image credit: Cynthia M. Harley

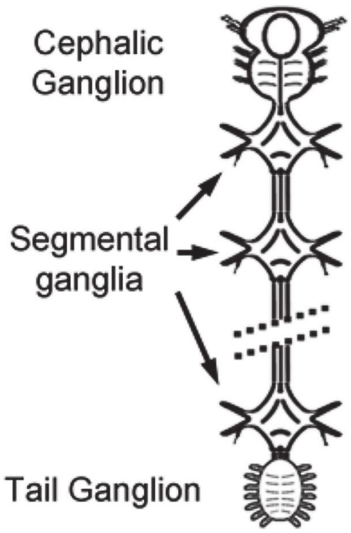

Figure 2. A condensed schematic diagram of the CNS of the medicinal leech showing the cephalic ganglion, the first, second and twenty-first segmental ganglia and the tail ganglion. The repeated interposed segmental ganglia are not shown for the sake of compactness.

limited to) hunting and searching, feeding, mating as well as two primary forms of locomotion, crawling and swimming.

These features, along with over 75 years of research, have established a solid base from which to ask questions relevant to contemporary thinking on the neural mechanisms of behaviors. For example, the locomotor behaviors have been well studied at the level of identified neurons and the role of many such neurons has been established in the generation of these behaviors. These studies include cells located within the segmental ganglia that generate timing and motor commands, as well as descending cephalic neurons which act in a command or executive capacity to control the segmental neural networks. Work done on swimming and crawling has established that the neural circuits within the segmental ganglia underlying these behaviors, called central pattern generators, have overlapping and multifunctional elements. Many of the identified descending cephalic neurons can control and modulate both behaviors. Recent work has established that the decision to swim or crawl is influenced by a number of factors. Briggman *et al.* (2005) showed that during the decision-making period just prior to activation of locomotion the activity level of a single identified neuron (named cell 208; shaded black in Fig. 3) biased the CNS to produce one form of locomotion over the other. Biogenic amine modulators like dopamine and serotonin, which act at both the segmental and cephalic levels, predictably influenced the type of locomotion expressed in isolated CNS preparations. Finally, signaling from a multifunctional identified descending cephalic neuron onto cells housed in the segmental ganglia (named R3b-1) is both necessary and sufficient for crawling but only activates and modulates swimming (it is not necessary for swimming; Puhl *et al.* 2012). These results and others like them have inspired new projects where investigators are probing how higher-order, decision-making cephalic neurons interact with segmental locomotor circuits as well as how neuromodulators influence their interactions. These new projects aim to elucidate how higher-order 'command' systems control multifunctional central pattern generators, at a level of detail focused upon individual neurons. This would be a difficult or impossible task in nearly all currently studied organisms, but is likely to be a tractable one using leeches.

In addition to locomotion, recent studies of leeches have contributed to other areas of active investigation in neurophysiology. Engineers have enlisted the help of those who study medicinal leeches to assist in the development of new technologies for physiology research. For example, leech CNS preparations were employed to test and validate voltage-sensitive dye imaging (Miller *et al.* 2012) and nanowire intracellular electrode fabrication methods (Ferguson *et al.* 2012). These technologies are being adapted for use in other animal models.

Leeches also possess cells with interesting electrophysiological properties worthy of study in the context of the neural mechanisms of behavior. One such neuron is the segmental non-spiking (NS) neuron which does not generate normal sodium-dependent action potentials, but does communicate with many other cells within a segmental ganglion (mostly motoneurons). Researchers at the University of Buenos Aires recently found that these NS neurons modulate the activity of motoneurons during locomotion and may help to regulate the electrical properties of these cells during ongoing behavioral expression and switching between behaviors. Their current work is probing these very ideas.

A team at Emory University is looking at the variability in the strength of synapses among identified neurons and how this variability affects the function of neural circuits such as the well-characterized leech heart central pattern generator. They found that, between individual leeches, there is a great deal of variability in the synaptic coupling between the identified neurons of the heart central pattern generator. In the face of this variability this neural circuit is still able to generate predictable and stereotypical outputs. Using a combination of 'wet' biological and computational modeling techniques these researchers and their collaborators are beginning to describe, in detail, the mechanisms which underlie these observations. Along this same line of inquiry, another recent report from a lab at the University of Minnesota, Twin Cities established that acute removal of descending cephalic signaling, via transection of the ventral nerve cord just below the cephalic ganglion, led to the degradation of crawling in intact leeches. Curiously, without restoration of the damaged neural fibers, the disrupted crawling behavior spontaneously

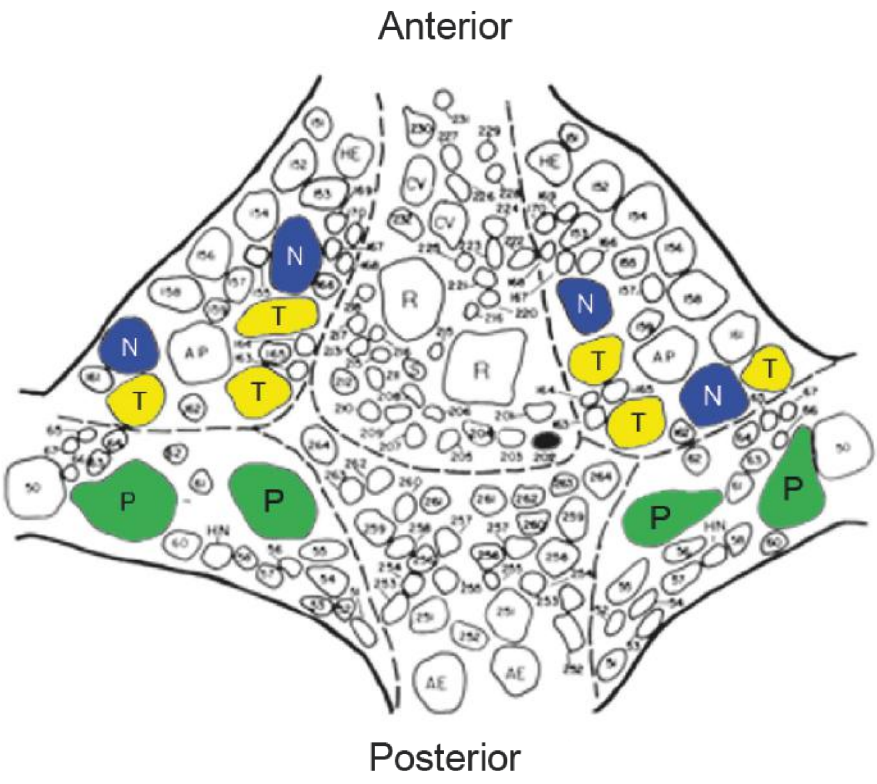

Figure 3. A classic drawing depicting the ventral aspect of a typical segmental ganglion. The somata of the touch (T, yellow), pressure (P, green) and nociceptive (N, blue) mechanosensory neurons are color-filled. The small soma of cell 208 is filled in black. Adapted from Muller KJ (1981), *Neurobiology of the leech*. Cold Spring Harbor Laboratory.

returned days to weeks after chronic removal of these descending signals. These results indicate that the crawl-generating neural networks were able to adapt to restore function without the cephalic inputs normally required for crawling. Presumably, this adaptation arises through changes in the cell-to-cell signaling or in the electrical properties of crawl-generating neurons. Follow-up work may lead to new insights into mechanisms of how neural circuits adapt to restore behavioral function after catastrophic injury.

The last example I will share is work done by a group at the University of California at San Diego who studied competitive behavioral selection of the leech feeding behavior. Their paper proposed a mechanism for the preferential selection of feeding over other behaviors via modulation of the synaptic strengths from sensory cells onto premotor neurons. They determined that this modulation was mediated by serotonin.

On top of their many contributions to scientific research, leeches are often used to help train new scientists; a task that should never be taken lightly. Because of their accessibility features these animals are well suited for use in behavior and basic neurophysiology teaching labs. Often, with some determination and a little luck advanced undergraduate and graduate students can perform their first independent recordings of identified neurons in just a day or two. Within a relatively short time period, these students can design and perform sophisticated experiments that confront ideas in neurophysiology and the neural bases of behavior (with a bit of guidance from their mentors, of course). A well-established example which supports this assertion comes from the Neural Systems and Behavior course offered each summer at the Marine Biological Laboratories in Woods Hole, MA, USA (http://www.mbl.edu/education/summer-courses/neural-systems-behavior/). Leeches can be even more useful when engaging inexperienced undergraduate and high school students. Using hands-on demonstrations and lab exercises, they can see concepts they learned in lectures and classroom discussions come alive right in front of them. In many cases, these students can participate in novel neuroscience research during laboratory exercises without the months of training required to do so in more complex models.

In this short article I have described some of the features of medicinal leeches that make these simpler animals useful for addressing important questions in neurophysiology along with providing some examples of current research which exploit these features to elucidate the mechanisms underlying dynamic and adaptive behaviors common to nearly all animals. Although I was not able to mention every significant study using these animals, my hope is that I have inspired some readers to do some follow-up reading. I have provided some interesting examples that establish that the CNS of these animals is a relevant and useful model for studying the neural bases of behaviors and behavioral selection in the context of modern neurophysiology teaching and research.

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Why do physiologists work on snails? A personal perspective

A mighty brain in a simple organism

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By the early 1970s it became clear that snails are excellent subjects for physiologists interested in relating neuronal electrical properties to behaviour. Individual large neurons (up to 150µm in diameter) were identified in the central ganglia of the snail brain (Fig. 1) as parts of defined behavioural circuits and their synaptic connections determined by paired intracellular recordings. Since then physiologists working on snails have made significant progress in solving a wide range of fundamental physiological problems from membrane biophysics and peptidergic signalling, through motor pattern generation to learning and memory. I have chosen examples that illustrate some of the advances that have been achieved by exploiting snails.

Not such a simple system

In my choice of the pond snail, *Lymnaea*, I was strongly influenced by a paper published in Science by Dennis Willows in 1967 demonstrating that stimulation of single giant neurons in sea slugs reliably elicited sequences of muscular movements underlying rhythmic swimming. It made me believe that if I could elucidate the neural circuits ('wiring' diagram) underlying examples of these stereotyped behaviours then I could understand how the brain works. This assumption turned out to be extremely naïf and I spent the next thirty years trying to understand how the 'simple' brain of *Lymnaea* is organized (Benjamin, 2008).

Multi-channel model of the nerve cell membrane

Early work on snails focussed on the biophysics of snail giant neurons whose large size facilitates multi-electrode impalement. Two novel types of potassium current, distinct from the classical Hodgkin-Huxley delayed rectifier, were discovered that were of general significance.

One type is the voltage-sensitive potassium A current, sometimes called the transient potassium current. This current is activated at the sub-threshold region of the membrane potential and helps to control the frequency of repetitively firing neurons. Although it is largely inactivated near the resting potential and completely inactivated during the action potential, the inactivation is removed by the after-hyperpolarization that normally follows an action potential. The A current is active for a short period after each action potential and delays the return of the membrane potential to spike threshold. This determines the duration of the inter-spike interval and thus the frequency of firing.

The other is the calcium-dependent potassium current. This type of ion current is also involved in controlling the pattern of firing of snail neurons. Some neurons that fire

‘How neurons communicate with each other is still a major question in physiology’

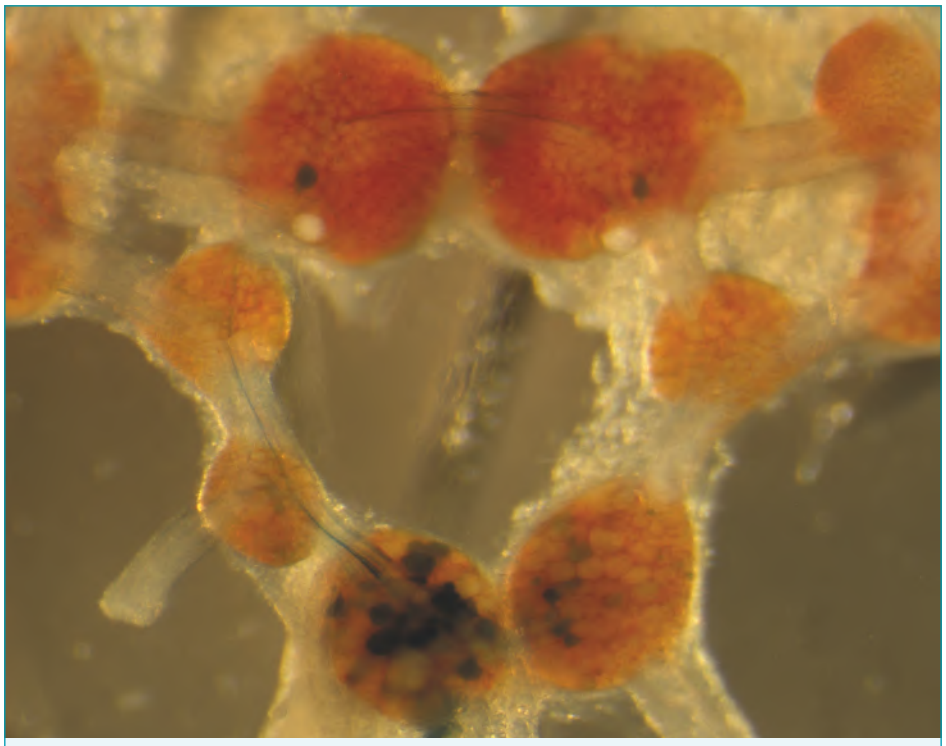

Figure 1. Neurons in the brain of the snail *Lymnaea*. There are about 20,000 neurons in the ring of nine ganglia that form the central nervous system and many can be identified on the basis of their cell body size, colour and location on the surface of the individual ganglia. In this preparation the axonal projections of individual neurons were determined by back-filling a nerve with nickel chloride and the blue stain precipitated with rubeanic acid. Neurons that project along this nerve are widely distributed in different ganglia. Image produced by Zsolt Pirger.

spontaneously do not fire at regular intervals but instead generate regular bursts of action potentials, separated by hyperpolarizations of the membrane potential. During the spiking phase of bursting neurons there is an influx of calcium into the neurons that progressively increases the intracellular calcium concentration. This activates the calcium-sensitive potassium current resulting in a delayed hyperpolarization of the membrane potential and a consequent cessation of firing. The calcium-dependent current thus contributes to the ‘silent’ phase of bursting neurons.

The electrogenic sodium pump in neurons was first investigated in the 1960s by Roger Thomas in the snail *Helix aspersa*. An active ion transporter, the sodium-potassium ATPase or sodium-potassium pump, mediates the pumping of sodium ions out of and potassium ions into the neuron to maintain the ion concentration gradients across the cell membrane. The stoichiometry of the ATPase, that determines the transport ratio for sodium and potassium, is 3:2 and so three sodium ions are transported out of the cell for every two potassium ions that are transported inwards. As a result the pump produces a net outward current. This kind of pump is said to be electrogenic because its activity causes the neuron to hyperpolarize and thus contributes to the setting of the resting potential.

Why so many neurotransmitters?

How neurons communicate which other is still a major question in physiology. It has been made more complex by the realisation that large numbers of neuropeptides modulate the basic neuron to neuron communication systems that use ‘fast’ transmitters such as acetylcholine and L-glutamate. It has been suggested that neurons are bathed by a ‘chemical soup’ of multiple peptide transmitters that indirectly modulate the more direct local inhibitory and excitatory synaptic pathways between neurons. In the snail, neuropeptides are distributed throughout the central nervous system, and although there are about 100 different types, they can be located in identified neurons and their functions investigated by a combination of molecular and electrophysiological techniques. A major advance in understanding the complexity of peptide signalling, was the use of mass spectrometry to identify multiple neuropeptides in a single neuron; for example in neurons expressing the FMRFamide gene a total of 13 different FMRFamide-related peptides were identified. A pair of identified cardio-excitatory motoneurons were found to co-release five different peptides, each with a distinct function in heartbeat control. This work revealed the sophistication of peptidergic signalling in the nervous system controlled by a single gene. The

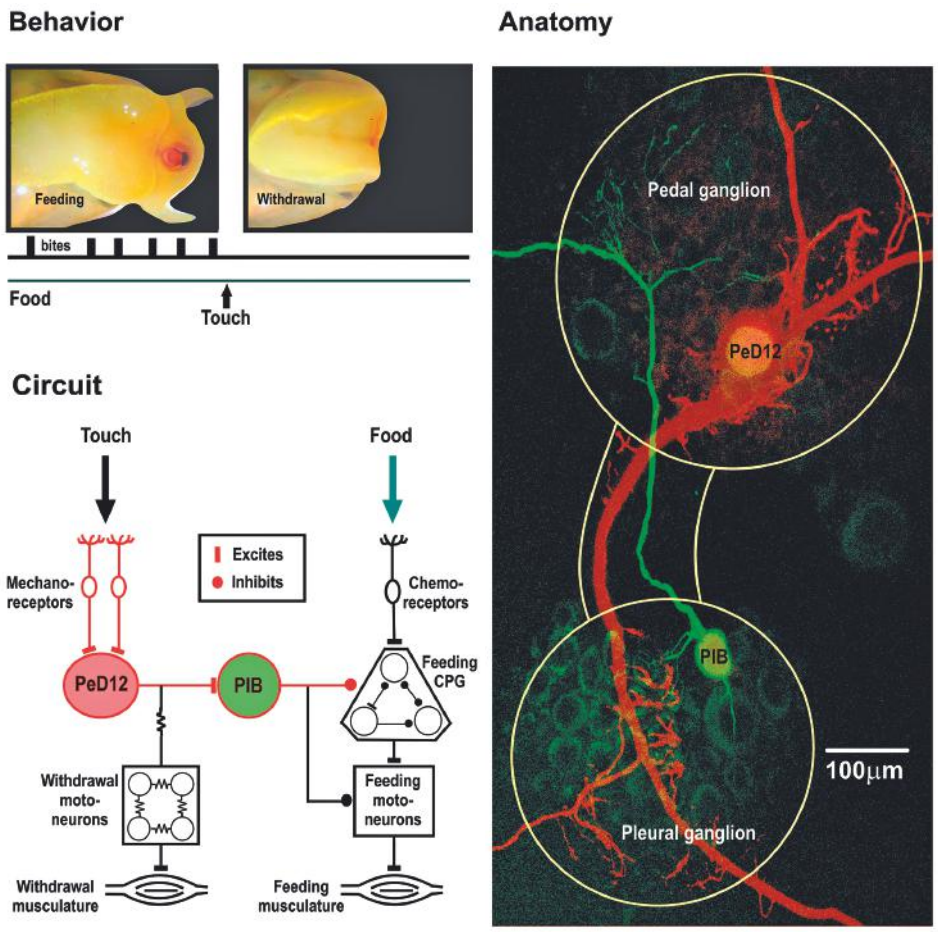

Figure 2. Interneuron mechanism for Tinbergen's model of behavioural choice. This model depends on hierarchically-based competition between autonomous networks. Whole body withdrawal (escape) dominates feeding in this example from *Lymnaea*.

Behavior Strong touch to the skin activates withdrawal and at the same time inhibits food-induced rhythmic feeding.

Circuit Strong touch to the skin induces a burst of firing in PeD12 (pedal dorsal 12) interneuron which in turn excites the PlB (pleural-buccal) interneuron. PlB inhibits the feeding network at multiple and stops on-going feeding. However, PlB does not play a role in triggering withdrawal in response to touch. Thus there is a clear asymmetry in the function of the two cells, with PeD12 co-ordinating activation of withdrawal with inhibition of feeding. PlB is only involved in the inhibition of feeding.

Anatomy The close apposition of the fine neuritic processes of PeD12 and PlB suggest that they are monosynaptically connected.

Reproduced from Pirger *et al.* (2014) *Current Biology* **24**, 2018-2012.

neuropeptides involved in heart-beat control target G protein coupled receptors (GPCRs) and use different second messenger pathways. For instance, FMRF/FLRFamide increase the rate and amplitude of heart beats by mobilising the inositol phosphate pathway whereas the co-released EFLRamide peptides are mediated by a cyclic AMP-mediate pathway and these produce more prolonged excitatory effects on heartbeat compared with FMRFamide. FMRFamide, however, can elicit excitatory effects on snail neurons without activating G-proteins. FMRFamide application directly gated sodium channels with pharmacological properties similar to that of mammalian epithelial sodium channels (ENaCs): they were highly selective for sodium, blocked by amiloride and were insensitive to blockers of other sodium ion channel types. This was the first type of peptide-gated ion channel to be described in any system (Cottrell, 1997)!

How are neural networks organized?

Classifying neurons into types eg motoneurons, interneurons and sensory neurons is a classic way of trying to understand the organization of neural circuits. We investigated whether this method of classification is still useful in a snail feeding circuit where about 100 neurons control the rhythmic feeding movements that the snail uses to ingest food. This analysis of function is possible because we can identify all the neurons and their synaptic connections in *Lymnaea* (Benjamin, 2012). Rhythmic motor behaviour in feeding and other behaviours like respiration are generated by central pattern generators (CPGs) and the emphasis has been on identifying the basic rhythm generating machinery, identifying the interneurons that form the CPG networks and describing their firing patterns and synaptic connectivity. A first was the isolation and growing of the three neurons of the respiratory CPG in culture to form a functional reconnected network to recapitulate the rhythmic firing pattern seen in the intact ganglia. Subsequently it was realized that the feeding CPG was controlled by several types of modulatory interneuron, which form a part of a highly interconnected network with properties that underlie flexible responses to internal and external stimuli.

Recent work indicates that many of the feeding neurons are multifunctional and that properties traditionally attributed to one class of neurons are distributed across several neuronal types. For instance, feeding motoneurons play a role in rhythm generation via their coupling to CPG interneurons. Many different neurons are involved in the initiation of feeding due to the wide neuronal distribution of chemosensory inputs that drive feeding. The epitome of multi-functionality is a single interneuron that as well as being part of the rhythm-generating CPG circuit is also involved in switching of behaviour from quiescence to feeding. It mediates the effects of hunger and satiety as well. It fires continuously in a single spiking pattern to inhibit feeding when the animal is satiated but fires in bursts to be part of the CPG pattern-generating mechanism when the snail is hungry.

This 'distributed' organization of network function has been successfully modelled in a recent computational study. This use of neurons for more than function may be a type of 'economy' measure in the snail networks with a small numbers of neurons available but on the other hand it is also observed in other animals including vertebrates where much larger numbers of neurons are involved.

Snails are quite brainy: how do they store memories and why do they have memory lapses like us?

Snails have been used extensively for studying the neural mechanisms underlying a type of associative learning (reward classical conditioning) first described by Pavlov in dogs. Reward classical conditioning of feeding is performed in *Lymnaea* by pairing amyl acetate (the CS, conditioned stimulus) with sucrose (the US, or unconditioned stimulus).

'Snails have been used extensively for studying the neural mechanisms underlying a type of associative learning (reward classical conditioning) first described by Pavlov in dogs'

Amyl acetate is not a feeding stimulus in naïve snails, unlike sucrose, but after CS+US training the snail perceives it as food. Remarkably, a single training trial leads to a long-term memory that persists for up to 3 weeks. As well as these simple forms of associative learning, snails are capable of other forms of learning whose features are similar to those found in vertebrates. For instance stimulus generalization (responding to related stimuli), goal tracking (moving towards the reinforcing food stimulus) and context dependency (increased learning in a novel environment) have been demonstrated in *Lymnaea*.

By taking advantage of the ability to record from identified neurons in the snail, it is possible to record electrical correlates of memory formation in single neurons of the feeding network following single-trial reward chemical conditioning. Mechanistic studies were carried out on single neurons and their synaptic connections. Changes at multiple synaptic sites within the feeding network were found, yielding a surprisingly complex picture of the mechanisms involved in learning and memory. Increases in strength of excitatory synapses that facilitate interneuron and motoneuron responses to the CS have been found. Conditioning also results in a reduction in inhibitory synaptic inputs to the feeding CPG, also directly contributing to the conditioned response. The previously favoured model for explaining associate conditioning in molluscs involved restriction of changes to a single site. Pioneering work on snails has shown that non-synaptic neuronal changes such as increases in excitability also play an important role in learning. These result from a learning-induced change in intrinsic ion currents. An example of this comes from our own work using one-trial chemical conditioning of feeding. The modulatory interneurons, the Cerebral Giant Cells (CGCs) are persistently depolarized by about 10 mV between 16-24 hours after conditioning. This increases the strength of post-synaptic responses to CGC stimulation by a process that involves an increase in the intracellular calcium concentration in the proximal dendritic processes of the CGCs. The conditioning–induced depolarization is due to an increase in the amplitude of a cyclic AMP-sensitive persistent sodium current.

An intriguing recent aspect of our work on learning and memory in snails has revealed specific time points after training when temporary lapses in memory expression occur (Marra *et al.* 2013). These coincide with transitions between different molecularly-defined phases of memory. Reports of memory lapses during memory consolidation are widespread. They have been observed in many types of organism including human subjects raising general questions about function. By application of novel sensory stimulation during the lapses (but not at other time points), we found that memory consolidation becomes vulnerable to these 'disturbing' stimuli and leads to the blocking of the subsequent progress of consolidation. We have speculated that lapses represent choice points that allow the memory trace to be expressed adaptively according to the variety of novel external stimuli that the animal is exposed to in the environment. More recently we have shown that the initial memory trace can be replaced by another memory trace if another type of training is carried out at the lapse point, suggesting that the consolidation of memory is an extremely adaptive process.

How does a snail decide what to do next?

We have to make decisions about what to do next on a moment by moment basis. Often these decisions are between incompatible behaviours. Early in the field of neurophysiology and behaviour, the Nobel Prize-winning ethologist, Niko Tinbergen (1951), suggested that choices between incompatible behaviours involve hierarchically-based inhibitory interactions between autonomous neural circuits providing priority to actions more important for survival. Although this model is still very influential there is little supporting evidence for it in either vertebrates or invertebrates. In our *Lymnaea* snail system, we were are able to provide direct electrophysiological evidence for this model taking advantage of the ability to identify neurons responsible for behavioural choice (Pirger *et al.* 2014). As predicted by the Tinbergen model, defensive escape behaviour (withdrawal into the shell) takes precedence over feeding. By recording neurons from the feeding and escape networks, we found no direct synaptic connections between the interneuronal and motoneuronal elements that generate the two behaviours. Instead, we discovered a novel interneuronal pathway that when activated by an aversive stimulus (a strong poke), triggers escape but at the same time inhibits feeding. This asymmetrical inhibitory interneuronal pathway allows one behaviour to dominate the other (Fig. 2). This is the first cellular level analysis of the original Tinbergen's hypothesis proving the existence of a hierarchical decision-making process at the level of a simple circuit.

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Tunicates: not just little squirts?

These marine invertebrates have some properties of vertebrates, and many interesting physiological aspects related to their tadpole-like larval stage and sessile adulthood

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Over the last 20 years whole-animal physiology has focused on an increasingly limited number of vertebrate models. However pressure to apply the 3R principles, a wish to uncover universal mechanisms, and a curiosity to understand where we come from (evolution), should mean that an occasional glance over the shoulder to what is available as alternatives is justified. Where better to look then than in the marine environment?

As animal life evolved first in the sea, it still contains a far greater diversity of key phyla than the land, and many of these are exclusively marine. Furthermore, the recent 'genome revolution' means that there are many more experimental resources available for what until recently, had been considered phylogenetic oddities. The good news then is that the occasional trip to the seaside to explore the use of alternatives is not an unreasonable activity for the serious physiologist! It is also worth pointing out that access to European Marine Laboratories for short visits to test out ideas has been greatly facilitated by the appearance of Research Infrastructure Initiatives funded by the European Union framework programmes. In the case of marine models, EMBRC (**http://www.embrc.eu/**) funds short term, timely projects. As support is available on site there is no need to become an expert on a particular organism to access it. Here, to encourage this process, I discuss some of the recent advances and opportunities in tunicates.

Tunicate relations

Tunicates occupy a key phylogenetic position, they are invertebrates, but unlike other invertebrates they belong to the phylum Chordata (Figure 1). Despite some fundamental differences, tunicates share several important common features with vertebrates; such as a basic chordate *bauplan*, including a dorsal nervous system with neural canal, notochord, hypophysis-pituitary complex, pineal eye and simple single chambered heart. The privileged phylogenetic position of ascidians is supported by most modern molecular phylogenies, establishing them as the sister group of vertebrates (implying that we shared a common ancestor around 500-600 million years ago). This makes tunicates particularly interesting for the study of the basic mechanisms that underpin the physiology of both groups.

What are tunicates?

Tunicates are a group of exclusively marine animals that include over 2,000 species divided into Ascidiacea (Aplousobranchia, Phlebobranchia, and Stolidobranchia) Thaliacea (Pyrosomida, Doliolida, and Salpida) and Appendicularia (Larvacea). Probably the most popular for experimental studies is the ascidian *Ciona intestinalis*, commonly known as the 'sea squirt'. It is a solitary sessile tunicate that can be found growing in great clumps under piers around the world (Figure 2a). It can be collected with ease and maintained in simple seawater aquaria. *Ciona*

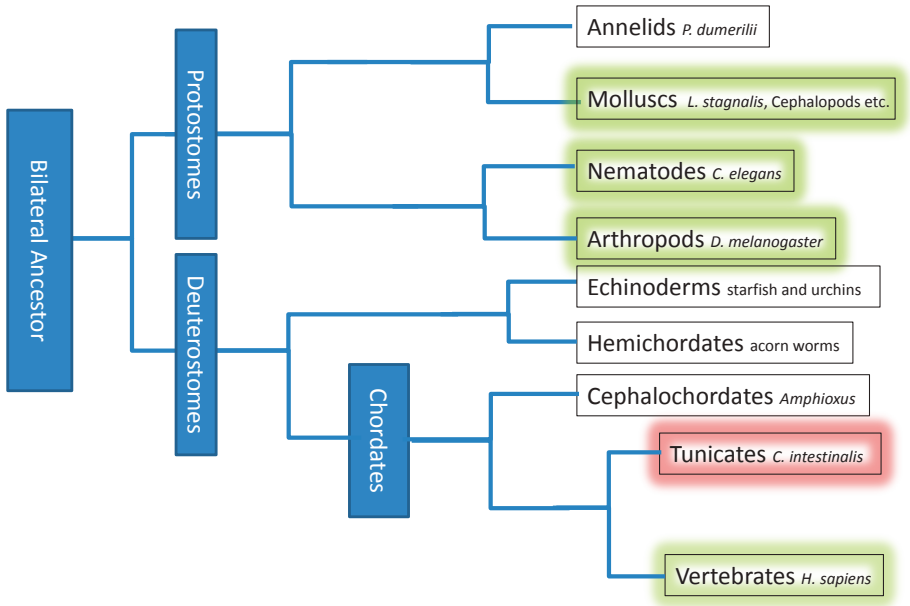

Figure 1. Tree of the relationships between some commonly used model invertebrates and vertebrates. Note the close kinship between vertebrate and invertebrate chordates.

is hermaphrodite (each individual contains eggs and sperm) and has a two stage life cycle. 18 hrs of development produce a free swimming vertebrate-like tadpole (Figure 2b) that transforms through metamorphosis within 6-12 hours after hatching into a miniature version of the tube-like adult (Figure 2c). Since the genome was sequenced in 2002, *Ciona* has become increasingly popular for evo/devo research. The genome is very compact (180 Mbp/haploid size), with around half of the number of genes estimated for vertebrates (~15,000). While vertebrate genomes have undergone at least three complete rounds of duplication (resulting in multiple copies of genes), invertebrate chordate genomes did not, and as a result are useful in single gene knock -down studies using morpholino, and siRNA techniques.

The larval nervous system

The nervous system, of ascidians and vertebrates seem to have followed two separate evolutionary trajectories. While the vertebrate CNS is has many cells and is anatomically complex, the CNS of the ascidian tadpole shows extreme parsimony, consisting of fewer than 100 identified neurones and no glia, perhaps holding the current 'model animal record' for fewest neurons in a CNS (Imai & Meinertzhagen 2007). Despite this, the larval nervous system conforms to the basic chordate CNS arrangement. A 'forebrain' or sensory vesicle integrates sensory information and contains a rudimentary 'pineal-eye' (~ 16 hyperpolarizing vertebrate-like photoreceptors and a tripartite lens) and a single otolith or gravity sensing organ. These organs can be clearly seen in the transparent larva as two black spots as they are heavily pigmented (Figure 2 b). The sensory vesicle is connected via a 'midbrain' neck region to a motor ganglion of ~10 cholinergic motoneurones that innervate the muscle of the tail. Rhythmic alternating activation of the tail drives a limited number of high frequency (30-40 Hz) swimming 'gaits'. A number of neurotransmitter systems have been identified in the CNS (Figure 3). Hyperpolarising photoreceptors interact with dopaminergic interneurons that may prefigure retinal ganglion cells. Glutamatergic drive by interneurons impinges on cholinergic motoneurons in the motor ganglion which in turn activates muscle fibres by cholinergic synaptic transmission. GABAergic interneurons modulate the system at different levels. Probable glycinergic interneurons in the tail interact to control left-right alternation of swimming by acting on glycine receptors (Nishino *et al.* 2010). While the swimming output is experimentally tractable, unfortunately the cells of the nervous system have not proved to be amenable to microelectrode or patch clamp recording techniques (although cells can be dissociated and successfully cultured and patch clamped). A likely way forward for network analysis is the use of fluoro-genetic encodable voltage sensors targeted to specific neuronal classes.

Adult nervous system

It was thought until recently that the adult 'brain' ganglion was a primitive cell cluster unrelated to the larval chordate body plan, however recent work has established that it partly arises from stem cells that reside in neck region of the larva. Interestingly, these cells have some similarities to vertebrate cranial motoneurons as they express Ci-Phox2 and Ci-Tbx20, whose vertebrate orthologues define vertebrate cranial motoneurons (Dufour *et al.* 2006). Thus, the adult nervous system which has long been known to possess remarkable powers of regeneration also makes an attractive model for physiological studies.

Muscular system

The larval tail and adult body muscles are controlled by cholinergic synaptic transmission as are most chordates including vertebrates. In this respect they differ from the majority of invertebrate neuromuscular junctions which utilise glutamate as the excitatory transmitter. The adult muscles are involved in a strictly limited series of coordinated actions relating to filter-feeding and expulsion of eggs and sperm via the siphons. The larval neuromuscular system is organised into two 'myotomes' consisting of three rows of muscle on each side of the midline. Alternate activation of these muscle fibre groups drives rapid alternating swimming (Nishino *et al.* 2010).

Cardio–vascular system

The adult ascidian contains a vasculature and a simple single chambered heart that, curiously, pumps haemolymph in one direction then reverses periodically. It is much simpler than the single atrium and ventricle of fish heart and the four chambered mammalian system. It is absent in the tadpole larvae as there is no circulation. However in larval development, two cells that give rise to the heart can be clearly identified and followed during metamorphosis and into adult development (Davidson *et al.* 2006).

Because of rapid development, and destruction and remodelling of the neuromuscular system during and after metamorphosis and the rapid development

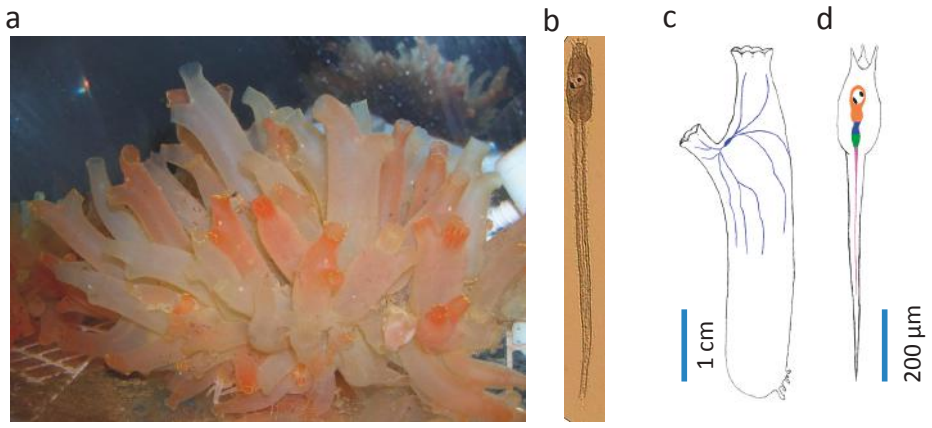

Figure 2. The tunicate *Ciona intestinalis*. *A*, cluster of adults (Photograph courtesy of Dr Stefania Piscopo). *B*, larval form (approximately 750 µm in length). *C*, diagram of the adult body plan showing the nervous system (blue). *D*, diagram of the larva showing the nervous system consisting of sensory vesicle (brown), neck (blue) and motor ganglion (green).

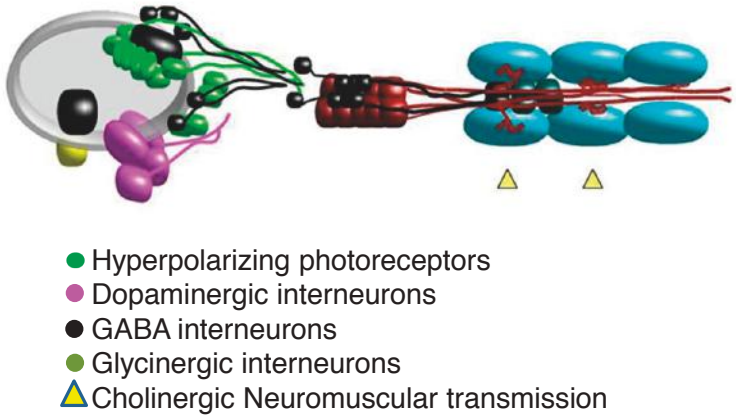

Figure 3. Diagram of the main neurotransmitter systems in the larval nervous system (real cell numbers). (Diagram modified after Razy-Krajka *et al.* 2012).

of the single chambered heart, the muscular system is a potential model to study the physiology of heart and NMJ development.

Biophysics/biotechnology

The study of ion channels in ascidians has resulted in the recent striking discovery of two entirely new membrane protein classes –the voltage-sensing phosphatases (termed Ci-VSP) and the voltage sensitive proton channels (Ci-VSOP *Ciona* voltage sensing only protein) (Okamura 2007). These proteins were detected *in silico* in the *Ciona* genome sequence and subsequently found in mammals and most other organisms. In recent work, the voltage-sensing domain of Ci-VSP has been fused to a pair of fluorescent reporter proteins to generate genetically encodable florescent voltage reporting proteins.

In a recent extensive survey of the genome for evidence of theoretical proteins, it was found that the genome of *Ciona* contains genes coding for a minimal 'chordate set 'of ion channels and receptors. There was some evidence for gene loss and the appearance in tunicates of potentially chordate specific genes. There has been also apparent expansion of certain receptor families (e.g. GABA and ACh receptors).The main inhibitory neurotransmitter systems in vertebrates are GABAergic and Glycinergic. In invertebrates a more diverse system also includes inhibitory glutamate receptors which are so far unknown in chordates. All other receptors form part of the cys –loop superfamily class of ion channels which have been shown to have an ancient pre-bilateran origin. GABA and GABA receptors and their transporters have been identified in *Ciona* and the expression pattern of the GABA transporters indicates a presence in the motor ganglion.

Conclusions

Tunicates provide a simple chordate system with limited cell numbers and a single copy genome. This makes them ideal for the investigation of single gene issues. Their rapid development and small size render them ideal for imaging and for some whole animal techniques. Their close phylogenetic relationship to vertebrates makes them particularly useful in understanding the first steps in vertebrate evolution. Finally they have provided some biotechnologically useful proteins for cell physiology.

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Some recent advances in spider sensory physiology

Silk spinner, sharp-sensed predator, ecological police… – an arthropod with superpowers

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The world of the typical spider is dominated by mechanical events such as vibrations generated by prey, predators or courting partners. Spiders also sense their own body position and movements. Consequently, they possess an array of sophisticated mechanosensory organs.

Spiders are arachnids, arthropod animals that lack wings or antennae, but have eight legs plus a body divided into two distinct regions. Like other arthropods (insects, crustaceans, millipedes and centipedes) arachnids have segmented bodies with jointed limbs. Their hard exoskeletons require frequent and major reconstruction as the animal grows and develops, they have open circulatory systems, and lack the oxygen-carrying hemoglobins or myoglobins of mammals, instead using copper-based hemocyanins. Considering all these similarities among the arthropods, what functional differences would give particular interest to spider physiology? Some are obvious, such as their ability to secrete extremely tough silks and lethal venoms, essential for the ecologically important function of spiders; capturing insects. It is less obvious that spiders' lives depend on detection of mechanical signals; some generated by the insect prey and others by their partners during often elaborate courtship behavior (Foelix 2011). Because of this, spiders have highly developed mechanosensory systems. While spiders use other senses, including chemical senses and vision, particularly important for the impressive visual prey detection of jumping spiders, the archetypal spider image is the patient predator waiting for mechanical vibrations in the web that signal approaching prey.

Not all spiders build webs, some dwell in the ground or on leaves and subdue passing prey, but they all use mechanical senses for detecting prey and predators, as well as for proprioception and communication with potential mates (Foelix 2011). Detection of touch, stress and vibration are performed by an array of specialized mechanosensory organs (Barth 2002), and the unique and advantageous structures of some of these organs have made them important physiological models of mechanical sensation with widespread application to other animals, including humans (French & Torkkeli, 2012). One limitation to research in spider physiology has been a lack of the molecular and genetic information that is available for more common model species such as mouse and *Drosophila*. This situation is changing rapidly with sequenced genomes now available for several arachnids, including the medically and agriculturally important ticks and mites, and the first complete spider genomes (Sanggaard *et al.* 2014). Transcriptome sequencing has also been applied to spiders, including the Central American wandering spider, *Cupiennius salei* (French *et al.* 2014). This large species (leg span 10–20 cm, typical adult weight 5 g) has been used as a model of spider sensory physiology, behavior and development since the 1960s (Barth 2002), with breeding colonies in Europe and North America. For more information see http://asf-pht.medicine.dal.ca/Pictures/Cupiennius_2003/Cupiennius.html

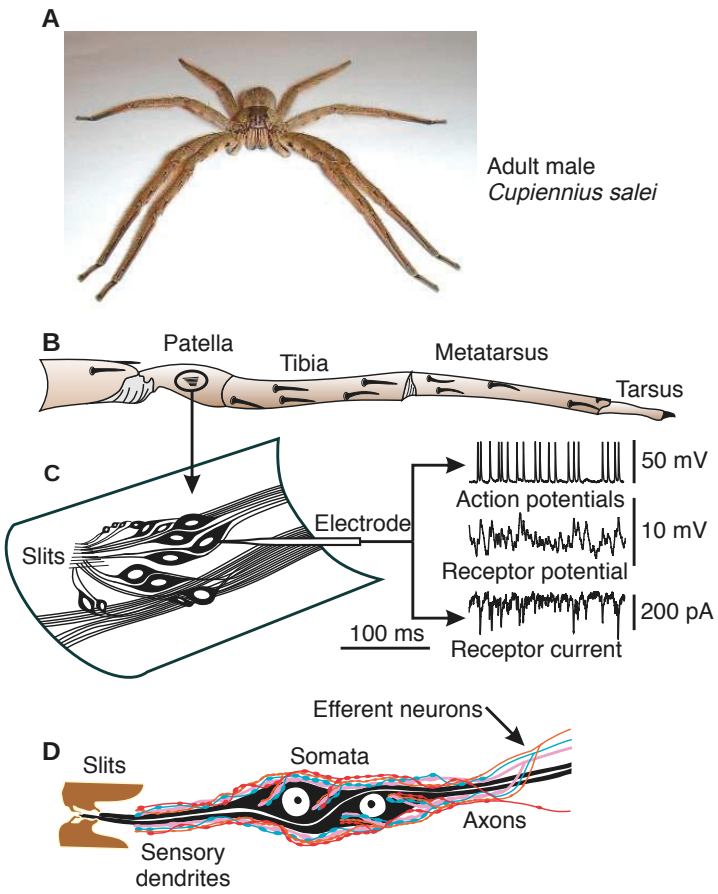

Figure 1. VS-3 slit-sense organ of the tropical wandering spider, *Cupiennius salei*. Adult spiders (**A**) have a leg span of 15–20 cm. Slit sensilla occur in all body regions. The VS-3 organ in each leg patella (**B**) consists of 7–8 cuticular slits, each innervated by two mechanosensory neurons (**C**). The neuron cell bodies are ~100 µm by diameter. Intracellular electrical recording, including single-electrode voltage clamp, is possible during mechanical stimulation of the slits. The traces show receptor current, receptor potential and action potentials during random mechanical stimulation. The axons, cell bodies and dendrites of the sensory neurons are surrounded by numerous efferent neurons (**D**) that originate in the CNS and release transmitters, including GABA, glutamate and octopamine to modulate the dynamic sensitivity of the neurons. Spider photograph by courtesy of Dr. Ulli Höger.

Mechanoreceptors and sensory transduction

Mechanotransduction is a process where a sensory neuron detects a mechanical stimulus, such as touch or vibration, and converts it to electrical signals that can propagate along the nerve toward the central nervous system. This process is thought to involve mechanosensitive ion channels in the sensory dendrites. Additional protein molecules probably tether these channels to extracellular matrix and to the cytoskeleton. This arrangement makes it difficult to isolate and investigate mechanotransduction channel proteins, and although several have been identified as good candidates, no complete mechanoreceptor structures have been identified in any animal model. This is partially due to the difficulty of performing physiological experiments on vertebrate mechanoreceptors because their sensory endings are small, often in difficult locations, and are at long distances from their cell bodies in the dorsal roots, cranial, or autonomic ganglia.

Spider mechanoreceptors

Thousands of touch detecting hairs cover the spider exoskeleton, and their legs have extremely flexible hair sensilla (trichobothria) that detect air movements. Research into both types of sensory organs in *Cupiennius* have provided a wealth of information about how sensory stimuli are detected and transformed into neural signals (Barth 2002). Spiders also have thousands of slit sensilla that detect cuticular strains and vibrations. Slit sensilla are unique to arachnids, with some functional similarities to the strain detecting campaniform sensilla of insects. They occur singly or in groups and are located in all body regions, but particularly near joints in the legs (Barth 2002). A compound lyriform slit sensillum VS-3 (Fig. 1) on the anterior side of spider leg patella provides a particularly useful mechanoreceptor model for research. The mechanosensory neurons are large enough to be seen during electrophysiological and optical experiments, and are accessible to pharmacological manipulations. Each VS-3 organ contains 7–8 slits, with a pair of large sensory neurons innervating each slit, allowing intracellular recording and voltage clamp during mechanical stimulation of the slits, so that receptor current, receptor potential and action potentials can all be observed (French & Torkkeli 2012).

The mechanotransduction channels in VS-3 neurons are selective to Na^+, impermeable to Ca^{2+} and open more easily at acidic pH. The receptor current through these channels is inhibited by the epithelial Na^+ channel blocker amiloride. Most of the receptor current decays rapidly, with a time constant of ~6 ms, but there is also a smaller, slower component with a time constant of 100 ms. The single channel conductance estimated by steady noise analysis was ~7.5 pS, and there are only ~500 mechanotransduction channels per cell. Open probability of the mechanotransduction channels changed from near zero at rest to near unity just after a step stimulus. Most of these properties are quite different to the channels responsible for mechanotransduction in vertebrate auditory hair cells, but similar to channels found in some mammalian and nematode touch sensitive neurons. The spider mechanotransduction channels are particularly similar to the roundworm (*Chaeorhabditis elegans*) MEC channels that are members of the epithelial sodium channel/acid sensitive ion channel family (ENaC/ASiC) (French & Torkkeli 2012). Current research aims to identify and locate the *Cupiennius* mechanotransduction channel molecules and to understand their functional properties.

Modulation of spider mechanoreceptor neurons

Animals are bombarded by vast amounts of sensory information from their external and internal environments but only relevant information is delivered to the brain and used to modify behavior. Sensory organs detect stimuli in their own modality, but transmission of that information can be intensified, attenuated, or even completely blocked along the sensory pathways. Mechanosensory afferent nerve terminals entering the central nervous systems of all animals receive presynaptic, largely inhibitory, modulation but

‘One of the most fascinating properties of spider peripheral mechanoreceptors is the abundant and complex efferent innervation that reaches even the most peripherally located parts of the sensory neurons’

mechanoreceptors and pain receptors can also be modulated in the periphery, either by direct efferent innervation or by circulating neurochemicals. Well-known specialized examples are the fusimotor control of muscle spindles and the efferent innervation of hair cells in the inner ear.

One of the most fascinating properties of spider peripheral mechanoreceptors is the abundant and complex efferent innervation that reaches even the most peripherally located parts of the sensory neurons. Efferent neurons form synapses with the axons, cell bodies and dendrites of the mechanosensory neurons (Fig. 1; French & Torkkeli 2012). There are also synapses between neighboring efferent neurons and between efferent neurons and glial cells. These presynaptic sites have multiple types of synaptic vesicles and the efferent fibers are immunoreactive to antibodies against γ-aminobutyric acid (GABA), glutamate, acetylcholine and octopamine. There is also evidence that more than one neurotransmitter can be released from a single efferent nerve. Each of the above mentioned transmitters modulates the sensitivity of VS-3 neurons by acting on a variety of ionotropic and metabotropic receptors, providing fine control of mechanosensation by tuning their sensitivity to different, behaviorally relevant vibration frequencies. VS-3 neurons provide an exceptional model of mechanoreceptor modulation because pharmacological agents can be applied during intracellular recordings and mechanical stimulation to investigate which agonists bind to these receptors, how this binding affects signal propagation within the cell, what types of signaling pathways are involved and how they can be inhibited.

The discovery of genes for several transmitter proteins in the *C. salei* transcriptome (French *et al.* 2014) is advancing research into sensory neuromodulation. The transcriptome contains genes for numerous adrenergic G-protein coupled receptors as well as ligand gated cys-loop receptor subunits, homologous to human and insect counterparts (French *et al.* 2014). Some of these receptors are already known to have a multifaceted role in modulating spider mechanoreception. Cys-loop receptors are targets of psychoactive drugs such as benzodiazepines and general anesthetics in humans as well as insecticides and antiparasitic agents, such as avermectins and neonicotenoids in invertebrates, with importance for human health and environment. Electrophysiological work has revealed differences in the ways that various transmitter receptor agonists modulate the sensitivity of VS-3 neurons. Even the same agonist can produce different effects in different neurons or with different stimulation. Future work will try to discover which receptors are located in the various spider mechanosensory neurons and how they modulate their functions.

Calcium-based sensory feedback in VS-3 neurons

When VS-3 neurons are mechanically stimulated their membrane potential depolarizes and they fire action potentials. Depolarization leads to opening of voltage activated Ca^{2+} channels that are widely distributed through all regions of the neurons. Ca^{2+}-sensitive dyes have been used to investigate changes in intracellular Ca^{2+} concentration during mechanical stimulation, action potential firing and when neurotransmitters are applied. The effects of increased Ca^{2+} concentration were explored using controlled release of ‘caged’ Ca^{2+} molecules by flash photolysis, and were found to almost completely eliminate the mechanically-activated receptor current (Höger *et al.* 2010). The dose response relationship for receptor current reduction versus Ca^{2+} concentration indicated that two Ca^{2+} are needed to inhibit each mechanotransduction channel. This mechanism presumably provides a negative feedback control that limits the depolarization produced by mechanical stimulation. GABA application also caused a large increase in intracellular Ca^{2+} concentration, suggesting that this transmitter mediates its effect at least partially via Ca^{2+} and the feedback control mechanism.

Dynamically varying biomaterial properties – the metatarsal vibration receptor

Arthropod cuticles are variable and complex materials. The basic building block is chitin, a polymer of N-acetylglucosamine, but the hardness, elasticity and other physical properties are varied by the addition of proteins and other molecules in a process called sclerotisation to provide the range from soft, flexible joint membranes to the hard materials of fangs and carapaces. Spider cuticle typically consists of many layers of chitin, whose organization and thicknesses also affect the biomechanical properties. Recent work on the spider metatarsal vibration detector provides a striking illustration of dynamically changing material properties contributing to physiology.

The metatarsal vibration detector is another compound lyriform slit-sense organ, located on the tarsal-metatarsal joint and called HS-10 (Fig. 2). Substrate vibration is directly transmitted to the tarsus, as the most distal leg segment, and then used to compress the 21 slits via a cuticular cap structure at the end of the metatarsus. The organ is extremely sensitive to vibration; movements in the nanometer range at frequencies up to 1000 Hz elicit action potentials in the sensory neurons. However, behavioral and physiological measurements show that the HS-10 organ also responds to lower frequencies ranging from 0.1 - 40 Hz if the

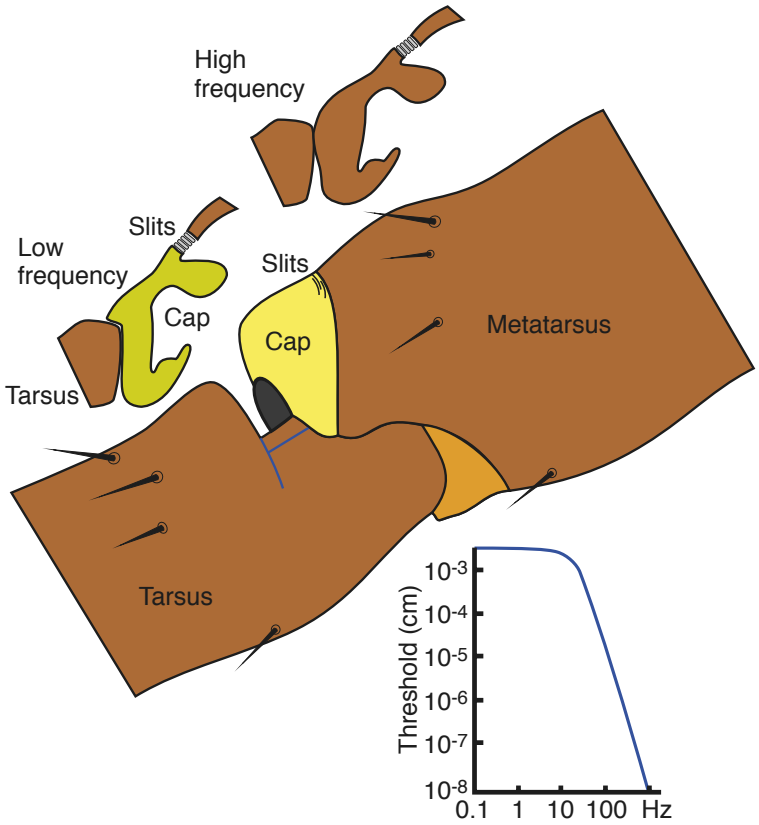

Figure 2. Metatarsal vibration detector of *Cupiennius salei*. Vibrations are transmitted from the tarsus resting on the substrate through a specialized cuticular cap to compress the HS-10 slit-sense organ at the distal end of the metatarsus. The organ is exquisitely sensitive to high frequency vibrations but also responds to lower frequency proprioceptive stresses (Threshold vs frequency plot is shown lower). Upper two cartoons show the changing properties of the cap from rubbery and compressible at low frequency that attenuates large movements, to hard and glassy at high frequency that conducts small vibrations. Redrawn from Barth (2002) and Erko *et al.* (2015).

movements are significantly larger, in the range of 10 - 100 µm. This transition from high to low frequency sensitivity occurs at about 40 Hz, and probably allows the organ to be used for proprioceptive feedback of rotation in the tarsal-metatarsal joint (Barth 2002).

How can a delicate sensory structure cope with such a large range of displacements without damage? Experiments using a wide range of biomechanical measurement techniques, including atomic force microscopy, X-ray microtomography and scanning acoustic microscopy, have demonstrated that the structure of the cuticular metatarsal cap is crucial (Fig. 2). The material of the cap, which transmits tarsal movements to the closely adjacent slits, transitions between rubbery and glassy states depending on a range of factors that include temperature, vibration frequency and hydration state (Erko *et al.* 2015). Large, low frequency movements find the cap in a rubbery, compressive state that strongly attenuates pressure from the tarsus, while higher frequency movements cause transition of the cap to a more rigid, glassy state that can transmit them without significant attenuation (Fig. 2). These mechanical properties of the cap depend critically on the chemical composition and layered structure of the cuticle to create the correct level of hydration in the normal temperature range that the animal experiences.

Conclusions

This essay has only covered a few of the recent findings in the sensory physiology of spiders, and much clearly remains to be learned. The emphasis on mechanical processes reflects the particular lifestyle of these animals, with its reliance on mechanical signaling for major behavioral modes, but the information being gathered promises to be important for understanding the physiology of other species, including humans. Spider mechanoreceptors have also provided inspiration for technical advances, such as creation of an artificial vibration sensor based on slit sensilla with ultrahigh sensitivity that can detect physiological signals (Kang *et al.* 2014), so contributions to prosthetics and robotics may also arise in the future.

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Careers beyond academia: consulting in the pharmaceutical industry

Our colleagues agree – university students do not know our jobs exist

(L-R) Dr Katja Stettin and Dr Patricia Keegan

Patricia Keegan & Katja Stettin

Covance Market Access, London, UK

Patricia Keegan, Senior Consultant (PhD in Physiology, Cambridge 2009), writes:

When I was a student, I had no idea that there was a big group of specialist consultancy companies servicing the pharmaceutical and medical device manufacturer markets.

Originally, I started working for a healthcare ThinkTank, believing this was the only place I could use my science background and apply my skills to topics that really mattered to me: shaping the healthcare industry and finding affordable ways to bring innovation to the NHS. Gradually I became aware of a large number of consultancy companies specialising in exactly that. I was offered a job at the level of Senior Analyst (one above entry level due to having some prior work experience) and I now work as a Senior Consultant in a room full of people with biology-based PhDs, including a few people who were on the Downing Site with me. We all survived the days and nights looking down a microscope in the dark room and now make use of the skills we learnt during our PhDs to further the development of novel products for the healthcare industry.

We are market access consultants, medical research writers, "value communication" experts and health economic modellers. Our clients come to us with questions like:

- *We are developing a product and have so far achieved these phase II clinical trial results. Please can you let us know if we should keep moving forwards with the development of this product, or if you think it will never be able to compete with other products currently on the market?*
- *We are thinking of buying one of these three biotechnology companies. Please can you evaluate their product portfolios and let us know which one to buy?*
- *We have been manufacturing this product for years. It is off patent and much cheaper than its competitor. Why are all the clinicians in France still prescribing the more expensive competitor?*
- *We have evidence showing that our product has significantly fewer side effects than the current product prescribed on the NHS. How can we communicate the value of our product to clinicians?*
- *We are putting together a launch strategy for our new product in Europe. Please can you help us decide in which country we should launch first?*
- *We are developing our clinical trial program. Which competitor do you think we should use as a comparator?*

In order to answer these questions, we carry out literature searches in MEDLINE and Embase databases through the Ovid interface as well as PubMed, research clinicaltrials.gov, review data from our clients, interview experts in the field, attend conferences and NICE appraisals. We also keep up to date with changes in the healthcare field by attending lectures – colleagues, who are alumni from Imperial, LSE, UCL and KCL, let us know when interesting speakers are lecturing and now and again we will all head out to one of the universities after work to attend a lecture together.

The best thing about our jobs is that we make use of all the skills and scientific background knowledge we learnt at university. I find myself reading through papers to understand the mechanism of action of a new cancer therapy, or reading through some early trial data and checking out the p-values to decide if one therapy has a significant advantage over another. I use my maths background when asked to help build a cost-effectiveness model to demonstrate that the use of a new drug will save the NHS millions through reduced hospital readmissions and I certainly make use of my thesis-writing skills. If you have kept up your language skills while at university, this will help when you find yourself on the phone with a world expert in France or Germany and want to discuss their views on how effective a new drug is likely to be compared with the drugs already in use today.

Katja Stettin, Analyst (PhD in Zoology, Cambridge 2014), recently completed her PhD at Cambridge, gives her thoughts on moving on from academia and her tips for current students:

When I was approaching the end of my PhD – and therefore the end of my funding – I started to apply for all kinds of different jobs. I panicked a little when I received the invitation to interview for my current position because I only had a really vague idea what the job required. This motivated me to contact Patricia via GradLink (the service offered by the Cambridge Careers Service) and she gave me a great introduction to what the job would entail – and is now my team leader. Still, moving from academic research to this new world of pharmaceutical consultancy was a scary prospect. But I have not regretted it once. From day one, I have been involved in interesting projects that have broadened my knowledge and skills immensely. Since starting about a year ago, I have:

- *Built a user-friendly Excel model to demonstrate how new drugs will affect payers' budgets*
- *Developed content for an eLearning tool to be used by a big pharmaceutical company to train new employees*
- *Produced documents that demonstrate the value of new and existing products*
- *Interviewed stakeholders over the telephone in multiple countries to understand their view on new products and where these products might fit into the current treatment landscape*
- *Researched the healthcare environment of different countries*
- *Carried out all kinds of work related to project management*
- *This is only a selection of the things that I have worked on.*

All the time I am surrounded by intelligent and open-minded people who like me, have a passion for science. The best thing is that I use my scientific background – a good understanding of science is surely a prerequisite for this job. I think this job is a good option for everyone who feels that academia is not their future but still does not want to cut all ties with science. Also, due to the growing pressure on healthcare budgets, not only in the UK but all over Europe and the US, the healthcare industry increasingly relies on experts like us to help them understand the challenges of the current environment and better communicate the value of their products. Healthcare Pharmaceutical consulting and value communication are surely industries with a promising future, something one might want to consider when choosing a new job path.

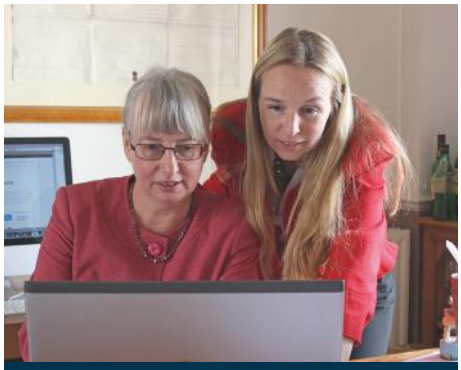
My day at the office usually starts with me checking my emails to see if I have received anything important which requires my immediate attention. I then start working on one of my projects, which might involve drafting content for the project lead to review, implementing feedback received from a client or a colleague, or researching clinical papers or other kind of information. Usually, I am assigned multiple projects at the same time, which means that there is a lot of variety in what I do throughout the day. I also have regular meetings with clients, where we discuss project progress or present deliverables. In between, I discuss aspects of the projects with colleagues; depending on the project this might also involve our in-house programming and design team. Another aspect I really like about this job is that everything we do is a team effort. The responsibility for the project is shared and even in a junior position like mine your input is highly valued.

I am hopeful that this article will help more students become aware that the industry I am working in exists and that this might be an ideal option for someone with extensive scientific training, who is keen to enter a world founded on science that is different to academia.

'When I was a student, I had no idea that there was a big group of specialist consultancy companies servicing the pharmaceutical and medical device manufacturer markets'

Careers beyond academia: from biochemistry to bioengineering

One woman's career journey in science and society



Liz Bell with one of her EdAd spin out company colleagues

Liz Bell

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Partner and Director, EdAd Ltdl
l.bell@edadsolutions.com

I've been fascinated about science and its applications since I was a teenager who was captivated by an ex-industrial chemist teacher at my secondary school nearly blowing up our lab during one of our practical sessions. The arts and humanities just couldn't compete with explosions, so I packed my bags in 1980 to go to Lancaster University to study biological sciences, which then migrated to my leaving with a BSc in biochemistry instead. Friends in Physoc have told me over the years that this meant that I have always really been a physiologist but didn't know it!

I'd realised during my first degree studies that my fascination with science had metamorphosed into a passion for scientific research, its applications and its importance for society. I was very fortunate to get a rare research council funded place on an MSc course at Aston University in 1984, which was one of the first ever courses on science and society. As a science graduate this led to me getting an MSc and PhD in this area from the Aston Business School (the PhD thesis focusing on the use of co-citation analysis to map scientific research fields from citation databases). Just as well that I had that management grounding, as after getting my PhD in 1989 I found myself embarking on a career managing and making policy about research, its supporting education systems, and its applications.

This area, where scientific research meets its applications, has become a great source of employment for people interested in the "Bigger Picture", and I would really encourage younger readers to look at career opportunities here if you are planning to leave research after getting your doctorate. My career has involved working for most types of organisations supporting the research and education ecosystem, including a funder research council, a science based charity, the diplomatic service, a learned society (yes, I mean our beloved Physoc), four universities and two shared service delivery companies for education and research. I hope that by giving you below a flavour of some of the jobs I've had you might be inspired to join our community.

Science still needs you after you have left the lab, it couldn't survive without its managers and policy advocates.

I did a couple of short research contracts at Aston and Humberside University in Hull in parallel with, and just after my PhD studies, respectively looking at the role of biotechnology in the production of food flavours and colours and New Chemical Entity innovation in the pharmaceutical industry. Then my first "proper" job (as my family saw it) was commissioning and managing innovation research programmes and centres in the Industry, Economy and Environment Research Development Group at the Economic and Social Research Council (ESRC) in 1990. Then, presumably because I might have learnt a couple of things about innovation issues from working with those leading social science academics, I was then seconded to The Oxford Trust in 1991 to lead a two year research project working with the universities and research labs in Oxfordshire to make their expertise and technologies accessible to local high tech companies. The Oxford Trust was, and is, a charity set up by one of the first really successful university spin out entrepreneurs in the UK, Sir Martin Wood (of Oxford University and Oxford Instruments). His work as an engineer is of great interest to physiological research as his spin out company made magnets that revolutionised medical imaging. I was lucky to work very closely with Sir Martin as the Chair of my Steering Group, learning loads from him about the effective commercialisation of research, and producing reports and papers on the development of innovation systems. These were lapped up by the then Department of Trade and Industry in formulating some of their initial forays into initiatives supporting innovation.

ESRC then claimed me back as Deputy Director of Postgraduate Training in 1993, so I was thoroughly immersed in all things to do with the support of Masters and PhD programmes in the social sciences (including developing new national accreditation and studentship allocation systems), but still managed to play in the field of innovation by

setting up their first CASE studentship scheme, and representing the Research Council for four years on a Whitehall committee dealing with innovation and technology transfer (the TCS Sponsors Forum). I'm afraid that brought me to the attention of the diplomatic service, which needed a scientist to spend five years in Moscow from 1997 leading the British Council Science Team that was busy building links between the UK and Russian research communities following the end of the Cold War. We also helped the Russians to take their first steps in reformulating their science policies to build a national innovation system, the lessons learned from our pilot projects in venture capital, intellectual property legislation and management, innovation training for research institutes, support for high tech companies etc being absorbed into President Putin's science and innovation plan for Russia.

At the end of my fixed term diplomatic contract, I, like a lot of expats, had to quickly search for a job to re-establish myself in the UK. I arrived at Brunel University in 2002, where I worked for two years as their Technology Commercialisation Manager (learning skills such as intellectual property contract management and the dark arts of briefing patent attorneys!), followed by six years at the Physiological Society as Head of Policy and External Affairs (doing such things as lobbying in support of animal research, debating with parliamentary contacts about biomedical issues, and setting up our links with organisations such as Sense About Science).

Like many mums I then said goodbye to the commuting lifestyle and took a career break for a year during 2011, spending a lot of time with my daughter and working closely with her primary school as a governor (I'm still Vice Chair of the Governing Body for my sins). I then looked around for a more local job and found a fixed term contract in 2012 as Head of Policy and Public Affairs at Janet UK, the UK's research and education ICT network based at Harwell, where I worked with the Chief Technology Officer to build the business case for the next iteration of the network, Janet 6, and secure a very large amount of money from the Cabinet Office and Treasury to build it. This work was also vital to our physiological community as research is increasingly reliant on the crunching of big data, and universities need a very high tech network to support it.

This led to my being headhunted for another famous shared service delivery company, UCAS in Cheltenham (so back to commuting again, hey ho!). I was Head of Policy leading the team that horizon scanned for policy developments affecting UCAS' products and services in education admissions and which engaged with Government stakeholders throughout the devolved UK countries. Highlights included being a member of the Welsh Government's Steering Group that designed the new Welsh Baccalaureate, and a chance to really understand the policy issues affecting the talent pipeline for research. Sadly major cuts bit, and I had to lead my team through a painful business review and redundancy process. This is a reality that you may well face in your own careers in our economically uncertain world. But I would say that new opportunities will be there if you look for them, and I left UCAS with 3 senior colleagues, to set up our own education admissions advisory company (EdAd) in 2014. This is in the throes of its first contract with a Welsh University. It is a judgement on me. I've spend so many years working with, and thinking about spin out entrepreneurs, that it was perhaps inevitable that I would become one. Sir Martin would laugh!

And where does bioengineering come into this? Well, not quite sure how it happened, but as well as being a Partner and Director of EdAd, I'm now also working with Queen Mary University of London to develop their new Institute of Bioengineering from their science and engineering departments, medical and dental schools, and associated hospital (Barts). The main focus of my work there is getting them organised (cat herding skills useful) and building up their research and industrial partnerships to conduct medically guided advanced bioengineering research focused on developing medical solutions. I have made a very personal commitment to bioengineering research, having had my hip replaced in January as part of a clinical trial with their rivals at Oxford, and donating my old hip to the Oxford BioBank!

I think I probably just can't help myself when it comes to the biomedical sciences and innovation!

For more information please visit:

https://www.linkedin.com/profile/view?id=61648992&trk=nav_responsive_tab_profile

http://www.edadsolutions.com/

Heroic experiments in man by Abraham Guz: breathing and not breathing

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Abraham Guz (1929–2014) was a respiratory physician who frequently observed the great distress in patients with dyspnoea – breathlessness – gasping for breath. He wanted to understand the mechanisms which mediated this very unpleasant sensation.

'I want to deflate lungs as well!'

Prof Abraham Guz's early work on lung reflexes was performed in London between 1964 and 1974. His death a year ago was followed by a memorial meeting on 5 November at Hodgkin Huxley House, under the kind auspices of the Physiological Society. Very little was said at the memorial meeting about these early studies, in which some heroic experiments on human volunteers were described in the days before ethical committees. This work surely deserves to be better known.

Lung reflexes: How to get at the vagus nerves in humans

As the lungs are innervated by the vagus nerves, i.e., the Xth cranial nerve, it was natural to be interested in what afferent fibres in these nerves enter the brain stem and in the central connections giving rise to reflex effects and cerebral perceptions. By the 1960s, it had already been shown that inflation of the lungs in anaesthetised humans elicited a much weaker inhibition of breathing compared with animals, in whom the participation of the afferent vagus was proved. In 1963, Guz, with his colleagues Diana Trenchard and Mark Noble first tried to establish whether the brief stop in breathing in humans, induced by lung inflation was indeed mediated by the vagus nerves (the Hering Breuer inflation reflex). For this he needed a surgeon willing to allow him to put local anaesthetic on the nerves with the patients' consent.

Transporting a laboratory to another hospital's operating theatre

The willing surgeon worked at Charing Cross Hospital in the Strand, so the experiments involved much practical and logistic difficulty. Guz had his laboratory in the grounds of the Fulham Hospital near Hammersmith Broadway, but the patients undergoing thyroidectomy had their operations performed at Charing Cross Hospital in the Strand. Therefore his team had to transport all their recording equipment and Douglas bags (for the pressurised inflating air), etc., to the Strand in central London. There they met the theatre sister who looked askance at the cleanliness of this stuff and started damp-dusting it. For some of it, this was not a source of anxiety, but the recording equipment consisted of large cabinets filled with thermionic valves and cabinets containing moving coil galvanometers and photographic recording paper. Guz was worried about water getting into this electronic equipment and also about jolting of the delicate parts, particularly the thermionic valves and moving coil galvanometers.

The weak Hering-Breuer lung inflation reflex in man

The inflation reflex was indeed abolished by local anaesthesia on the vagi. The experiment was repeated using intra-bronchial intubation with a Carlens tube. That established that there was no cross over of signal from one lung through the contralateral vagus nerve.

Then Guz asked, 'How can I study the individual afferent fibres?'

Abe's collaborator John Widdicombe could do that in animals by shredding the nerve and recording directly from single fibres in the cut peripheral end. Even Guz would not have considered doing this in humans! He required a recording method that did not damage the nerve in any way; the antidromic method could fulfill this requirement, but could only explore afferent activity in large myelinated fibres that signal lung volume. It could not be

used in thyroidectomy patients as a length of nerve was required, but was possible in radical operations to remove cancerous tissue. The results were very simlar to those obtained in dogs and cats, suggesting that there is a central nervous loss of the lung inflation reflex sensitivity in humans compared with animals.

Then Guz said, 'I want to deflate lungs as well'

In humans study of the deflation reflex, which accelerates breathing, was beset by methodological difficulties until it was attempted by Guz by allowing a lung to collapse in patients being treated for pneumothorax. That treatment in 1971 was by inserting a tube into the air filled pleural space and putting the other end of the tube under water in a bedside bottle (sometimes negative pressure was applied to the bottle). When this tube was opened, air went back into the pleural space and the lung collapsed. This was accompanied by an immediate increase in breathing rate and electrical activity recorded in inspiratory muscles. This confirmed that humans did have a deflation reflex, but uncertainty persists in the absence of a repeat of the experiment with vagal block, and it is difficult to be sure how the strength of the reflex in humans compares with other species. In the 1960s and seventies, the Guz group also carried out a series of animal experiments to probe these and other lung reflexes.

Perception of events in the lungs and breathing: breath-holding

Paradoxically, Guz's first investigation of perception of breathing was to experiment with breath holding! He persuaded his colleagues Mark Noble (MN) and John Widdicombe (JW) to undergo vagal and glossophayngeal block with local anaesthetic injected at the base of the skull (having persuaded an anaesthetist to put needles into the right place!). The pattern and sensation of normal breathing was unchanged in both subjects. The first subject (MN) held his breath after breathing air. Breath holding time at total lung capacity was prolonged from 100 seconds control to 230 seconds during block; so long indeed that he became cyanosed. Therefore, in the second subject (JW), the experiment was performed with him breathing 100% oxygen. His breath holding time at total lung capacity increased from 110 seconds to 215 seconds. Thus, there was a clear diminution in the unpleasant sensation that increases during breath holding until one is obliged to take a breath. But was this due to block of the vagus or glossopharyngeal nerves (containing afferent pathways from chemoreceptors)? We plumped for attribution to the vagus nerves because the first breath after breatholding gives relief even if the inspired air is a mixture of nitrogen and carbon dioxide.

Not breathing due to paralysis

An alternative approach was then taken by Campbell, who paralysed himself with curare and found that he could stand lack of inflation from a ventilator for much longer than he could hold his breath under normal conditions. His hypothesis was that respiratory sensations arose from the muscles that produce breathing. This idea arose from the results of Agostoni, who recorded increasing frequency and intensity of activity in the diaphragm, (recorded by electromyogram) during breath holding. Campbell then persuaded Mark Noble to be his second subject because he had also been a subject for the vagal block experiments of Guz. The result was the same in MN as it was in Campbell. The idea that diaphragmatic contractions were involved in breath holding sensation seemed to be confirmed by the results of phrenic nerve block by the Guz group, and by the absence of the sensation in a patient with diaphragm paralysed by poliomyelitis. There was then an attempt to find if muscles other than the diaphragm were a source for this sensation. The intercostal muscles seemed not to be involved because the sensation was still present during block of these muscles. This was compatible with studies in patients with mid or low cervical spinal cord transection, who had normal breath holding sensation. However, a patient with a C3 transection maintained his breathing with his sternomastoid muscles only; this patient had no breath holding sensation. One needs an intact vagus nerve and a contracting diaphragm to ellicit this sensation.

Not a travelling laboratory full of metal barrels please!

Perhaps this concentration of studies on breath holding is not relevant to that which is sensed during breathing and breathlessness. Other sensations that were intensively studied during these years were the detection of added elastic loads and the detection of added resistive loads to breathing. These probably share the same mechanism as both cause sub-atmospheric airway pressure during inspiration. Elastic loads consist of metal barrels which are switched into the patients airway with a Douglas tap. The wide range of elastic loads of varying magnitude and thus different sized barrels required Guz to fill the operating theatre at Fulham with all these barrels, when he was doing vagal plus pharyngeal nerve block at the base of the skull (more extensive damp dusting required). The ability to detect added elastic loads was not impaired by these blocks. Guz then had the good idea of doing this test on patients with cervical cord transections, but this necessitated travelling all the way to Stoke Mandeville Hospital in the Chilterns. Even he could not contemplate transporting a lorry load of metal barrels all that way, so he switched to resistive loads, which are a set of tubes of varying linear resistance. The ability to detect these added resistive loads was not affected by cervical transection at C3 level, thus excluding the possibility that the sensation was mediated by chest wall receptors. In patients with tracheostomies, resistive loads could be detected, but became more sensitive if the tracheostomy cuff was deflated and the ability detect added resistive load is impaired by local anaesthesia of the mouth. The ability to detect added loads in subjects with vagus nerve blockade could therefore have been mediated by receptors in the upper airways, such as afferents running in the laryngeal nerve.

‘The type of heroic experiments on man became very difficult or impossible with the advent of ethical committees’

Guz, ‘I’ll make my friend John Widdicombe breathless with asphyxia’

Guz was primarily interested in breathlessness, but the studies described above contribute little to the understanding of this sensation. The loads used in the foregoing paragraph are very low and not unpleasant. Breathing through much higher resistances is required to cause unpleasant breathing. Breathing CO_2 causes hyperpnoea that becomes unpleasant at high minute ventilation. This was studied in the period of interest in the subject JW, using 7% CO_2 93% O_2 rebreathing, with blocked vagus and glossopharyngeal nerves at the base of the skull. Peripheral chemoreceptor block was confirmed by the abolition of the ventilatory response to hypoxia, and hyperoxia inhibits the peripheral chemoreceptor response in any case. So it was the central drive from hypercapnia that was recorded. The lower ventilatory response to CO_2 after block, compared with control, was due to the loss of the peripheral chemoreceptor response to CO_2. The subject continued to breathe the hypercapnic inspired gas to a much higher end-tidal PCO_2, because the distressful sensation was absent. Guz stopped the experiment at a PCO_2 of 70mmHg for safety reasons. The authors concluded that the results were compatible with the hypothesis that the loss of unpleasantness was related to loss of lung afferent activity in the vagus nerves. However, it has to be said that the actual peak minute ventilation was lower during block than during control, so that it is possible that the lower level of unpleasant respiratory sensation was related to the lower level of hyperneoa.

Partial paralysis

Another type of experimental respiratory distress was expressed by MN after the curare experiment. Prior to complete muscular paralysis, as the curare concentration was gradually increased, there was a phase of partial curarisation when the vital capacity became near to and below tidal volume. This caused considerable distress that was relieved by switching on intermittent positive pressure ventilation. It appears that respiratory distress induced in normal humans could be mediated by lung afferent or chest wall afferent neural activity until someone does the partial curarisation experiment during vagal block!

Ethical experiments with no ethical committees

The type of heroic experiments on man described above became very difficult or impossible with the advent of ethical committees. I consider that these experiments were ethical in that the subjects consented to them and came by no harm from them. I prefer to trust the scientist rather than a committee. The later work of Guz and his expanding team diversified into many aspects of pulmonary pathophysiology, but he did show that lung inflation is sensed in the cerebrum. However, without vagal, phrenic and chest wall blocks, it is not possible to be sure of the afferent pathway, although Guz postulated that it resided in lung receptors and vagal afferent fibres. Of the many studies by Guz after 1974, which are outside the scope of this article, the enthusiasm to understand respiratory sensation was his continued main concern leading to identification of the area of the cerebral cortex associated with inspiration and other breathing sensations.

Further reading

Widdicombe JG (2006). Reflexes from the lungs and airways: historical perspective. *J Appl Physiol* **101**, 628-634

Parkes MJ (2005). Breath-holding and its breakpoint. Exp Physiol **91**, 1-15

Obituary:

Carole Mavis Hackney 1955 – 2015

Carole was born in Preston, Lancashire and went to the University of Manchester, gaining a B.Sc with 1st class Honours in Genetics and Cell Biology, and then a PhD in Cell Biology before becoming a New Blood lecturer at Keele University in 1983, appointed to the Department of Communication and Neuroscience by the then head, Professor E.F. (Ted) Evans. This department, founded by Donald Mackay, was known for its excellent research into hearing, speech and vision

At Keele, Carole worked on cochlear hair cells. Whilst more anatomist than physiologist, she became a member of the Physiological Society and with me as her first post-doc , provided novel data on the recently discovered tip link, a tiny mechanical gating structure that underlies hair cell transduction. Carole built up an electron microscope unit and became head of department, then school, when the department merged with Biological Sciences to form the School of Life Sciences, after which she was given a Personal Chair. Carole was noted for her collaboration with Professor Kirsten Osen in the University of Oslo. They provided the first detailed anatomical map of the guinea-pig cochlear nucleus, still used by auditory physiologists today. She made beautiful camera lucida drawings of cells in the nuclei, following on in the tradition of classical neuroanatomists like Lorente de No.

Carole won several awards, among them the Thomas Simm Littler Prize from the British Society of Audiology, and the BAAS Charles Darwin Award Lecture (1996). Her research excellence is evidenced by the 89 research articles and numerous book chapters on hearing, including a revision of Gray's Anatomy Inner Ear chapter. Carole acted as occasional spokesperson and advisor to various deafness charities in the 1990s, and was on the Wellcome Trust Neurosciences Panel and International Committee for the ARO. She was certainly well known and well thought of in the auditory field.

Carole was a keen wildlife enthusiast and photographer, rehabilitating injured birds of prey such as barn owls, for release to the wild. After divorcing her first husband, Carole moved to the University of Wisconsin-Madison to focus on research with her long-term collaborator Professor Robert Fettiplace. Family concerns made her return to the UK where she held a post at Keele and then a lectureship at Cambridge University. However, she developed a desire to return to the Midlands, coming back to South Cheshire when we got married.

Carole's career then took a completely different turn. Although retaining her research links, she started a company called Advanced Imaging and Microscopy, setting up two electron microscopes of her own in the garage to provide consultancy and research support. Carole died in a tragic accident on the 17 February, an untimely passing, leaving me and her two sons from her first marriage. All who knew her were touched by her determination and supportive, caring attitude. The help she gave to many people in her career will never be forgotten.

David N Furness

Husband and colleague

*PN*99 crossword answers

Neurophysiology

1. Homeostasis 2. IPSP 3. Saltatory 4. Diastole 5. Radioactive 6. Zymogen 7. PMCA 8. Integratory 9. Acidosis 10. Motoneurone 11. Mylean 12. Passive 13. Calcium 14. Adrenaline 15. Cytoplasm 16. Crayfish 17. Strychnine 18. Atpsynthase 19. Vagus 20. Pressure 21. Myosin 22. Systole 23. Caffeine 24. Nernst 25. Synapse 26. Vesicle 27. Atpase 28. Flux 29. Cyanide 30. ATP 31. Aorta 32. Eccles 33. Henrydale

Expert Cardiac

1. P wave 2. White 3. Cusps 4. SCN 5. EAD 6. Aorta 7. Sinus 8. Vena 9. Acidosis 10. RYR 11. Beta 12. ACE 13. Purkinje 14. Nav 15. Alkaloid 16. Axis 17(a). RAP 17(d). Rheumatic 18. Pleura 19. LVAD 20. Taurine 21. Cord 22. Actin 23. Stress 24. J point 25. APD 26. Treppe 27. PV 28. LQT 29. BPM 30. Dub 31. Dog 32(a). ACH 32(d). ANP 33. Hz 34. Septum 35. No 36. Gap

Experimental Physiology

New Editors

Greti Aguilera received a MD degree and postdoctoral training in clinical endocrinology and experimental endocrinology at the University of Chile. After a Fogarty International fellowship, she became visiting scientist and then tenured investigator at the National Institute of Child Health and Human Development, NIH, USA. Research interests and contributions include physiological, cellular and molecular regulation of adrenocortical mineralocorticoid and glucocorticoid secretion; neuroendocrine mechanisms of adaptation to stress, with focus on the regulation of the hypothalamic pituitary adrenal axis, the mechanisms of regulation of the neuropeptides, corticotropin releasing hormone, vasopressin and angiotensin II, and their receptors

Dawn A. Lowe is an Associate Professor in the Department of Physical Medicine & Rehabilitation, Medical School, at the University of Minnesota. She earned her PhD at the University of Georgia in Exercise Science and completed post-doctoral studies muscle biology and then muscle biophysics. Broadly defined, her research interests include muscle physiology, aging, muscular dystrophy, and exercise science. The focus of this research is cellular and molecular mechanisms underlying skeletal muscle deterioration that occur with age, injury, and disease. Current studies are also aimed at preventing or reversing this muscle deterioration through exercise, pharmacological interventions.

Virtual issues

- *Experimental Biology Meeting - Editor's Choice* for the *Experimental Biology* Meeting
- *Ageing and Degeneration* for The Society Topic Meeting in April
- *Atrial Fibrillation* for the Heart Rhythm society meeting in May

Video Slideshows

Watch introductions to Symposium issues on:

- *The role of renal nerves in cardiovascular and renal function in normal and pathophysiological states* by Clive May
- *The heart is lost without the brain- The autonomic perspective* by Mark Chapleau

Can you reproduce your data? Physiology 2015, 6 July 2015

There is concern about the inability to reproduce results in many research papers published in pharmacological and biomedical journals. Indeed, some recent publications in prominent journals have provided evidence that only a minority of published results can be reproduced and have attributed these problems to widespread poor experimental and reporting practices.

This problem has caused such concern that the National Institutes of Health in the USA and the Nature Publishing Group have initiated new submission requirements to improve the quality of information published on study design and analysis in research articles. A group of pharmacology journals are proposing a similar strategy aimed at enhancing reproducibility.

Experimental Physiology is hosting this session at Physiology 2015 to highlight the key problems and involve the community of physiological researchers in a discussion of how to address these issues in physiology research. We hope you will get involved.

Organised by **Paul McLoughlin**, University College Dublin, Ireland, and Editor-in-Chief, *Experimental Physiology*. With speakers **Gordon Drummond** (University of Edinburgh, UK, and Emeritus Statistical Advisor to The Physiological Society journals), **Martin Michel** (Boehringer Ingelheim, Germany, and Managing Editor, Naunyn-Schmiedeberg's Archives of Pharmacology).

Principles and standards for reporting animal experiments in *The Journal of Physiology* and *Experimental Physiology*

The Journal of Physiology and *Experimental Physiology* have always used UK legislation as the basis of their policy on ethical standards in experiments on non-human animals. However, for international journals with authors, editors and referees from outside the UK the policy can lack transparency and is sometimes cumbersome, requiring the intervention of a Senior Ethics Reviewer or advice from external experts familiar with UK legislation. The journals have therefore decided to set out detailed guidelines for how authors should report experimental procedures that involve animals.

As well as helping authors, this new clarity will facilitate the review process and decision making where there are questions regarding animal ethics.

The new guidelines, which include a checklist for authors, are set out in an Editorial written by Senior Ethics Editor David Grundy, that outlines the rationale for this change and provide authors with detailed information on how to conform to this new policy. We hope that authors of both journals, and the wider community, will adopt this rigorous way of conducting and reporting on their animal experiments.

http://onlinelibrary.wiley.com/doi/10.1113/JP270818/full

The Journal of
Physiology

Physiology meets evolution – A conversation with Denis Noble and Michael Joyner

Following on from the special issue published in *The Journal of Physiology* last June (volume 593, issue 11) entitled 'The integration of evolutionary biology with physiological science', we were pleased to host a session, moderated by Editor-in-Chief David J. Paterson, at Experimental Biology on 30 March 2015 that addressed many of the key points raised in the issue.

Denis Noble FRS (Emeritus Professor of Cardiovascular Physiology, University of Oxford, UK) and Michael J. Joyner (Professor of Anesthesiology, Mayo Clinic, USA) addressed their views on this important topic in a casual, engaging and accessible manner. As this was an unusual and interesting event, we filmed the session so we would be able to disseminate it to the wider community. The video is free for all to view on The Society's YouTube channel so we encourage you to watch and share. The session was attended by over 250 people and we are hoping many more will take on board the salient points by watching this video.

Feedback is also welcome! jphysiol@physoc.org

Symposium: Cutting-edge approaches towards the functioning mechanisms of membrane proteins

Following on from the highly successful 45th joint symposium of The National Institute for Physiological Sciences (NIPS) and *The Journal of Physiology*, 25–28 November 2014, Okazaki, Japan, we are pleased to publish an Editorial and six reviews related to the presentations in the 15 June issue of *The Journal* (volume 593, issue 12).

Organised by Deputy Editor-in-Chief Yoshihiro Kubo, the symposium addressed four key points on this topic:

1. Dynamic imaging of functioning membrane proteins.
2. Novel regulatory mechanisms involving the local environment.
3. Understanding structure and function of membrane protein complexes.
4. Aspects of systems physiology, pathophysiology and translational or clinical research.

The reviews help to highlight recent developments in current knowledge and indicate future directions for this rapidly-moving research field. http://onlinelibrary.wiley.com/doi/10.1113/jphysiol.2015.593.issue-12/issuetoc#group2

The Journal of Physiology at Heart Rhythm, 13–16 May 2015

In continuing our efforts to identify a suitable international cardiovascular conference to complement our existing schedule, *The Journal of Physiology* had at stand at the Heart Rhythm Society's 36th Annual Scientific Sessions meeting in Boston, MA. We were glad that the sun was shining and the excessive snow fall from early 2015 had finally melted.

Previous meetings that we have attended focusing on this important area of research for *The Journal* (the AHA Scientific Sessions in 2013 and the ESC Congress in 2014), have proved to be too clinical for us, so we were hoping that the Heart Rhythm meeting would offer us greater access to basic science researchers.

From day one, we could tell that this meeting had received significant financial support from major device and pharmaceutical companies hoping to target clinicians. Although this meant that they laid on lots of free drinks and snacks in the exhibition hall to drive delegate flow to the stands, we suspected early on that this would mean the vast majority of delegates would be clinicians, and not the basic scientists we were after. Indeed, this was the case, and although *The Journal* content was of interest to many delegates who stopped by, their main focus was on reading our content, rather than submitting research. As one of our main aims at conferences is to attract new, high-calibre, international authors, it was disappointing that there were very few active researchers wanting to talk to us about submitting.

Our Reviewing and Senior Editors that attended the meeting reported that the basic science sections of the programme were of a very high standard, but were poorly attended compared to the clinically-focused sessions. This was a shame as some of the research being presented was of world-class quality by some leading scientists. However, when basic science was combined with more clinically-relevant research, the sessions were well attended and well-received, perhaps because they could more readily see how important understanding the underlying physiology is to their clinical practice.

We are glad that we went to the meeting, but it has shown us that attending a large international cardiovascular meeting is not the ideal strategy for attracting new authors in this area. Instead, we will focus our efforts on attending or sponsoring some smaller, more focused meetings where we can be sure that the delegates are familiar with our content and will be keen to submit their best research to us.

Although unsuccessful in our mission to secure more papers, we were very successful (and lucky) in spotting over a dozen humpback whales off the Boston coast. Perhaps we would have had more success by targeting marine biologists rather than cardiovascular researchers!

Sally Howells, Managing Editor, and David J. Paterson, Editor-in-Chief of The Journal of Physiology at the Heart Rhythm Society annual meeting in Boston, MA, May 2015

Physiology 2016

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