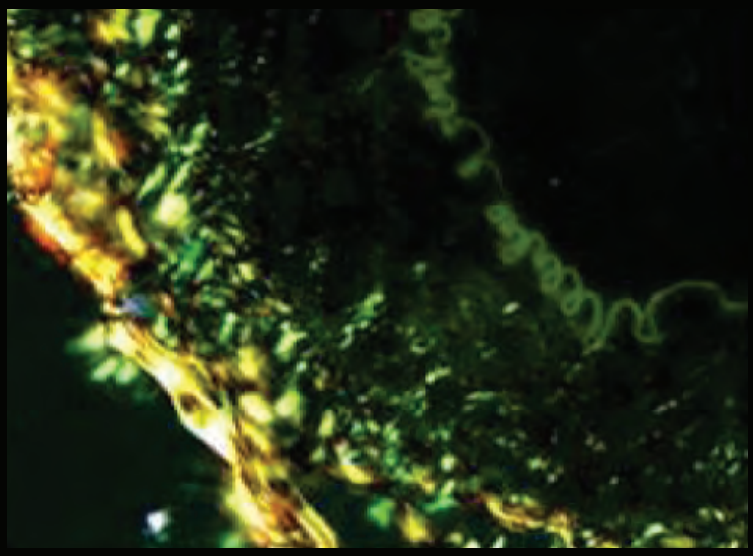
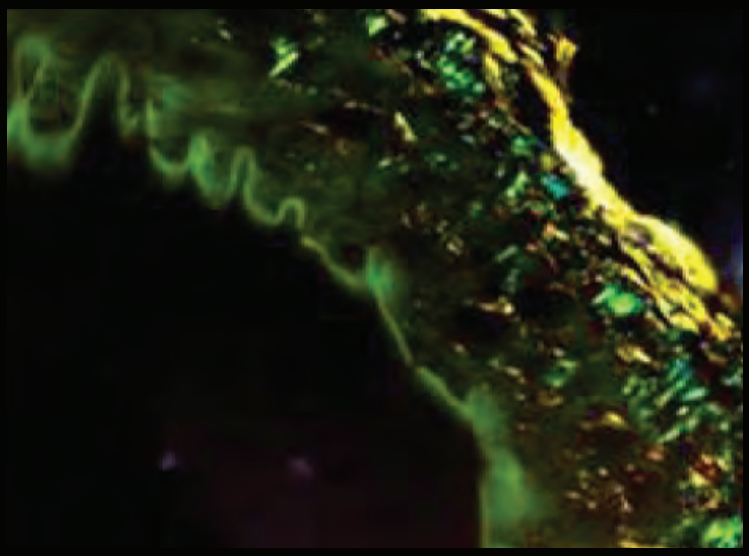
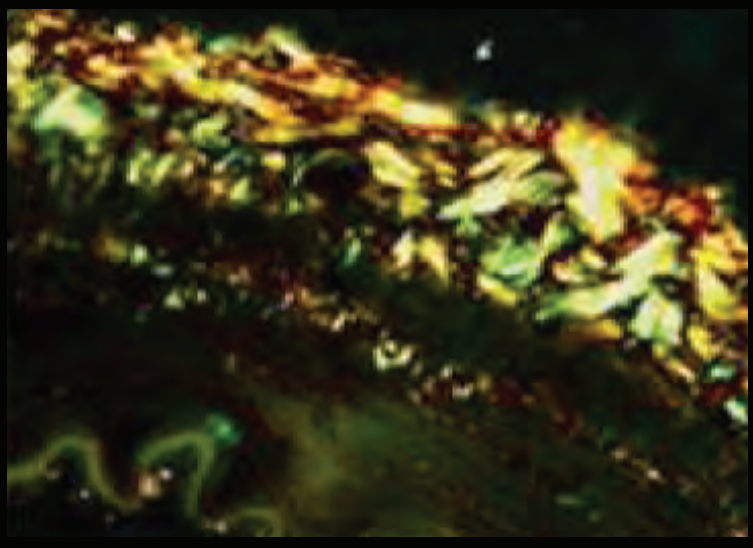
PHYSIOLOGY NEWS

summer 2011 | number 83







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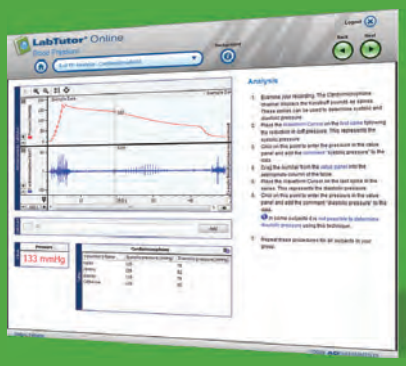

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The Society's dog. 'Rudolf Magnus gave me to Charles Sherrington, who gave me to Henry Dale, who gave me to The Physiological Society in October 1942'

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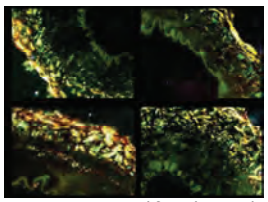
The Physiological Society

Advancing the science of life



Cover image: Surviving life in the womb and the implications for vascular health in adulthood, from p. 29.

PHYSIOLOGY NEWS

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Action points

Grants
The Society offers funding through the following grant schemes: Travel Grants, Non-Society Symposia Grants, Outreach Grants, International Teaching and Research Grants and the Vacation Studentship and Departmental Seminar Schemes. For full information, please visit: www.physoc.org/grants

Membership applications
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Physiology News

Deadlines
Letters and articles and all other contributions for inclusion in the Autumn 2011 issue, No. 84, should reach the Publications Office (magazine@physoc.org) by **7 July 2011**. Short news items and letters are encouraged, and can usually be included as late copy if space permits.

Suggestions for articles
Suggestions for future articles are welcome. Please contact either the Editorial Administrator or a member of the Editorial Board of *Physiology News* (see contents page for details).

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Physiology News online: www.physoc.org

Guidelines for contributors

These guidelines are intended to assist authors in writing their contributions and to reduce the subsequent editing process. The Editorial Board of *Physiology News* tries to ensure that all articles are written in a journalistic style so that they will have an immediate interest value for a wide readership and will be readable and comprehensible to non-experts. Scientific articles should give a good overview of a field rather than focus entirely on the authors' own research.

Format of articles
The main message or question posed should be introduced in the first paragraph. The background for the topic should then be established, leading up to the final conclusion.

Length of articles
This will be determined by the subject matter and agreed with the Senior Production Editor.

Submission of articles
Authors should submit articles as a Word document attached to an email. Illustrations should be sent as separate attachments (see below) and not embedded in the text.

Illustrations and authors' photographs
Authors are encouraged to submit diagrams, drawings, photographs or other artwork with their articles and a photograph of the author(s) should accompany submissions. Illustrations and photographs may be colour or black and white, and preferably TIFF, JPEG, PNG, PDF or AI files with a **minimum resolution of 300 dpi**.

References
Authors are requested to keep the number of references to a minimum – preferably no more than two or three. Please cite all references in the style of *The Journal of Physiology* (see *Information and Guidance for Authors* at http://jp.physoc.org).

In this issue

Welcome to the Summer 2011 *Physiology News*.

This issue has, completely serendipitously, somewhat of a historical flavour. William Van der Kloot tells us about the WW1-era activities of famous physiological brothers-in-law Bayliss and Starling on p. 36, J Prakasa Rao gives us a snippet of diuretic history on p. 38, and even my editorial has had a history outbreak. Meanwhile, the winning entrants for the Paton Prize Photographic competition (p. 42) show that in history, as in other things, a picture can be worth an awful lot of words.

One of the things *Physiology News* seeks to do is air views – and not just views from the magazine editorial staff, or from The Society's Council or officers, but from you – The Society's Members. This issue we have two Members expressing strong opinions – Roger Thomas is on his soapbox (should I say 'again'?) on p. 12, while Richard Naftalin explains how and why he thinks physiology teaching has been in retreat on p. 8. And another thing magazines try to do is encourage debate, so please do write in and tell us if you agree – or disagree – with either of our soapbox-ers.

Moving to our regular features, this issue has a wide-ranging selection of Science News and Views that takes us from the womb to adulthood (p. 29), appropriately taking in learning to walk (p. 20), 'fight or flight' (p. 15) and endurance (p. 18). We can all agree that modern scientists need plenty of the last quality, and probably the one before that too.

Finally, we say farewell to, and salute the life and work of, four stalwarts of UK physiology in our obituaries.

Austin Elliott
Editor

Then and now

Pondering a suitable topic for this Editorial, I used the internet to ask some of my colleagues and friends what might make a good subject. A range of ideas were offered, but there was a common theme. 'Not more gloom'. 'People', another suggested 'need cheering up'. Among the positive suggestions for how to be more upbeat, the one that caught my eye was 'There must be an anniversary of some important discovery – talk about that'.

They had a point, I conceded, about the gloom. When one editorializes about the issues facing scientists in 2011, it can be hard not to sound gloomy. The grim economic climate, grant success rates at historic lows, rocketing university tuition fees, rising student expectation, governments that don't listen to scientists – I could go on. But there is a danger that, in talking about these topics, one ends up repeating oneself. Gloomily.

So I have decided to take my friends' suggestions, and tell you about an anniversary. Though if I find contemporary messages there too, you will have to forgive me. Old habits.

Among the many changes in science in the modern era, 'one-person discoveries' have become increasingly rare. This year marks a significant anniversary for a famous past one, however; it is 50 years since the 1961 Nobel Prize for Physiology or Medicine was awarded to Professor Georg von Békésy (1899–1972) 'for his discoveries of the physical mechanism of stimulation within the cochlea'. For those unfamiliar with von Békésy and his work, a quote from the Nobel presentation speech by Professor Bernhard of the Karolinska Institute gives a good summary [1]:

"Von Békésy's distinction is to have recorded events in a fragile biological miniature system. Authorities in this field evaluate the elaborate technique which he developed as being worthy of a genius. By microdissection, he reaches anatomical structures difficult of access, uses advanced tele-technique for stimulation and recording, and employs high magnification stroboscopic microscopy for making apparent complex membrane movements, the amplitudes of which are measured in thousandths of a millimeter..."

Von Békésy's work thus had many of the typical hallmarks of the scientific process: identifying a problem to address; working out a strategy to attack it; devising and refining the methods to do it; making the most accurate observations possible; and finding the appropriate theoretical framework to interpret them. Von Békésy's background in physics was also key, and his being able to obtain tissue from obliging pathologists useful, so perhaps we could also add 'interdisciplinarity'. A final and perhaps less obvious ingredient, to which I will return, was also present: the necessary time to work all this out.

Von Békésy's life took a decidedly 'non-standard' route to scientific greatness. He must surely be the only Nobel Laureate in physiology to have done his research whilst working (for over two decades) for the national telephone company. Having completed a physics PhD in Hungary in the 1920s he initially struggled to find a scientific job.

"After a certain time I decided to look around systematically and find out which was the best equipped laboratory in Budapest. I found that it was the laboratory of the government controlling the research in long distance telephones, telegraphy, and radio stations. Hungary was in the middle of Europe and therefore communication was a very important feature. The government was forced by peace treaties to spend a certain amount of money to keep the transmission lines in good shape. To do so, they constructed a laboratory and gave the laboratory a certain amount of money to buy the necessary equipment. It was this financial support which started my research. There was a fixed income to the laboratory with no questions asked as to how it was spent. I still think that this is the basis of every big discovery." [1]

So to the list of things that underpinned von Békésy's discoveries we could add good research facilities; a secure job; and minimal interference or need to justify his research. And thus, in turn, time to try things – and fail – on the way to success.

Apart from the central place of the scientific method, what other 'contemporary resonances' can we find in von Békésy's story? One is the key role of physical sciences, and numeracy, as a prerequisite for much physiological and biophysical investigation. It is a regular complaint of today's university scientists that numeracy among students is ever-declining – though this has been a regular complaint for at least the last half-century.

Another point that comes across strongly is the necessity for time, and a free hand, and a supportive environment – even if mainly supportive by lack of interference – for scientific discovery. Though there is far more science, and many more scientists, now than in von Békésy's day, many of them can be heard saying they are 'too busy chasing funding', 'too busy doing administration' or just 'too busy to think'. This situation, more and more evident in the last few years, has been masked by the funding that allows scientists to employ more junior scientists (or 'scientists in training') to do the hands-on work. The danger now is that, as funding becomes ever scarcer, there will be fewer of these pairs of hands to do science, but still just as much other stuff to distract scientists' attention. If we are to see more von Békésys, I would suggest, we need to strive much harder to keep scientists doing science – not filling in forms.

Austin Elliott

1. Von Békésy G (1974). Some biophysical experiments from fifty years ago. *Annu Rev Physiol* **36**, 1–16.

Epithelia & Membrane Transport Themed Meeting

Royal Free Campus, University College London, UK

1–3 September 2011

September's Themed Meeting is an event for all those working in Epithelia & Membrane Transport. It is hosted by Anselm Zdebik, Robert Unwin and Ted Debnam (University College London) at the Royal Free Campus, University College London. Themed Meetings bring together basic scientists and clinicians working in the Theme to share the latest ideas and cutting-edge knowledge. Submit your research, from any topic within the Theme, when abstract submission opens on 23 June as there are many opportunities to present your work.

The focused symposium '*Ion channels: Insights from disease and animal models*' will explore recent new developments in transport physiology that have surfaced with the recent analysis of newly identified genetic diseases and animal models. Insights arising from this approach integrate into a wider perspective in many newly developing fields. These include TRP channels and epithelial polarity, but also their role in calcium and magnesium handling by epithelia. Advances in the understanding of genetic diseases has also changed traditional views on potassium transport, sodium transport and has identified diseases where these two processes show considerable cross-talk.

By tying in speakers from related, but less traditional fields, such as ion transport regulation, inner ear transport and even developmental biology, this opens the epithelial field to other physiologists with an interest in ion transport. The understanding of genetic disease and their analysis using animal models will be of a broad interest, not only to physiologists in the epithelial field, but also to others with an interest in translational research. This approach is emphasised by combining presentations from basic scientists and clinicians for every one of the main topics covered.

http://www.physoc.org/em2011

Topics covered

- Calcium and magnesium transport and paracellular permeability
- Luminal sensing in the kidney and gastrointestinal tract
- Novel aspects of phosphate homeostasis
- Potassium transport, sodium transport and cross-talk – from disease to structure/function studies
- Ion transport protein trafficking and modulation

Young Physiologists' Symposium 2011

Royal Veterinary College London, UK

31 August 2011

Epithelial physiology across species: can other species inform your epithelial research?

A one-day Young Physiologists' Symposium (YPS) is designed specifically for early career researchers in the field of epithelial physiology to meet and exchange ideas. Explore the differences and similarities in epithelial physiology across species, with specific focus on epithelial transport, sensory epithelium, naturally occurring animal models to facilitate epithelial research, and epithelium in health and disease.

Early career researchers can present the same abstract at the YPS and the Themed Meeting.

http://www.rvc.ac.uk/Meetings/YPS/Index.cfm

Invited speakers

Frances Ashcroft
University of Oxford, UK

Jonathan Ashmore
University College London, UK

Detlef Bockenhauer
Great Ormond Street Hospital, London, UK

Ted Debnam
University College London, UK

Annette Dolphin
University College London, UK

Dominique Eladari
Centre de Recherche des Cordeliers de Jussieu, France

Ian Forster
University of Zurich, Switzerland

Pascal Houillier
National Institutes of Health, USA

Christian Huebner
Medizinisch Theoretische Institute Universitätsklinikum Jena, Germany

Edith Hummler
University of Lausanne, Switzerland

Martin Konrad
University of Münster, Germany

Michael Pusch
National Research Council, Italy

Bernard Rossier
University of Lausanne, Switzerland

Blanche Schwappach
University of Manchester, UK

Victor Sorribas
University of Zaragoza, Spain

Robert Unwin
University College London, UK

Oliver Wrong
University College London, UK

Anselm Zdebik
University College London, UK

Networking at Meetings may help your career

My Physiological Society journey began in my hometown of Dublin at Physiology 2009, just before starting my final year of my joint BSc (Hons) Physiology & Pharmacology degree at University College Dublin (UCD). A last minute decision, some classmates and I decided to attend the Meeting in support of our lecturers who were giving talks at the various symposia. It was here our interest was sparked, and not too long after we had all signed up as Undergraduate Associate Members of The Society.

The following year, with the financial support of bursaries from the Young Physiologists' Bursary Scheme (YPBS), I encouraged even more of our class to attend Physiology 2010 in Manchester. This time round, my classmate, Naadiya Carrim, and I had been accepted to present our research at the Young Physiologists' Symposium YPS), much to our surprise. So you can only imagine my utter shock when I discovered I had been placed second in the oral communications section despite being the only undergraduate presenting a talk. With a great start to the Meeting and new YPS friends, we launched into the week's many talks, presentations and socials.

On the night of the Society Dinner at Manchester United, our former lecturer Stuart Bund advised us to take advantage of this opportunity and network, not only with our peers but other senior scientists and perhaps meet a potential supervisor. So with this in mind on the last day of the Meeting, I chose to attend a symposium on my favourite subject and passion, renal physiology. It was here I met Kevin O'Shaughnessy, University of Cambridge, and his talk on renal salt transport and blood pressure really caught my imagination and I decided to hang about after the session to ask him a few questions and get some career advice for this area of research.

I figured it was now or never and decided to chance my arm, dropping into conversation my earlier success at the YPS and my recently obtained first class honours UCD degree. As luck would have it Dr O'Shaughnessy was in search of a new PhD student to recruit to his lab. Fast forward several emails, phone conversations and trips to Cambridge and I've since been awarded funding by the British Heart Foundation for a three year PhD studentship at the University of Cambridge, starting this October. If there is one piece of advice I can give to those of you attending this year's Meeting in Oxford, it is to never be afraid to ask a question and always be prepared for when opportunity comes a-knocking.

UCD Physiology Class of 2010 from left to right: Louise Cully, Naadiya Carrim, Keith Siew, Philip Lewis and Naoise O'Ciardha.

Keith Siew
University College Dublin, Republic of Ireland

Annual Public Lecture of The Physiological Society

Light, Clocks and Sleep

Russell Foster (University of Oxford, UK)

18.00 Tuesday 12 July

The Sheldonian Theatre, Oxford, UK

An internal 24 hour biological clock (circadian clock) controls, modulates and fine-tunes our sleep patterns, alertness, mood, physical strength, blood pressure, and every other aspect of our physiology and behaviour. Russell's lecture will look at how circadian rhythms are generated, regulated by light and why we can't ignore our internal time in both medical treatments and in the way we organise our 24/7 society.

If you wish to participate and are NOT attending Physiology 2011 in Oxford, please email Sarah Bundock at The Society (sbundock@physoc.org)

Physiology 2011 – Early Career Social

Monday 11 July from 19.30

A chance for all Early Career scientists to make new friends before Physiology 2011 kicks off. It is free to sign up, so please email Sarah Bundock (sbundock@physoc.org)

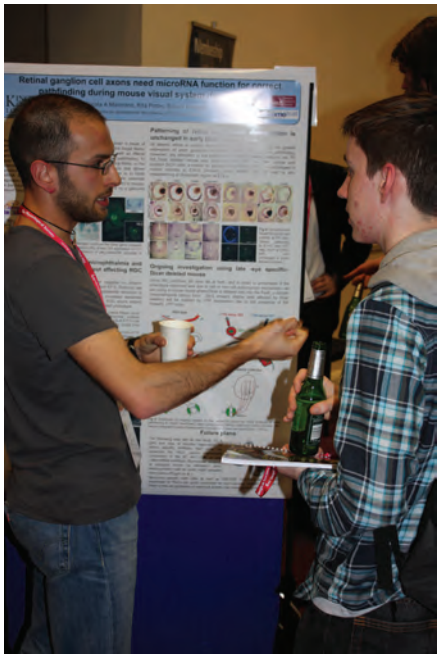
Cellular & Integrative Neuroscience Themed Meeting

6–8 April 2011 King's College London, UK

As this was my first Cellular & Integrative Neuroscience Themed Meeting, I wasn't entirely sure of what to expect. In addition, I had never visited the Guy's Campus of King's College London and I was left extremely impressed by its facilities, as the room layouts, of both the lecture theatre and the poster presentation room, worked incredibly well. The organisers of the Meeting, Jon Robbins, Pat Doherty and Jeremy Ward (King's College London), had clearly researched everything thoroughly and the result was a meeting that ran smoothly from start to finish. To be honest, having read through the programme a few times, I wasn't sure how relevant some of the various speakers would be to me, as there was a wide breadth of subjects covered, and none of the symposia were on chronic pain, which is my specific research interest. However, my fears were unfounded because, as the Meeting progressed, the quality of the invited speakers and selected oral communication presentations shone through. One speaker that I was particularly impressed with was Chris Anacker, a PhD student from King's College London. He presented a poster and, at extremely short notice, stepped in for his ill supervisor, Carmine Pariante (King's College London), to give a 30 minute extended presentation. The presentation, on exploring the role of the gluco-corticoid receptor in depression and hippocampal neurogenesis, was given so well that I never would have guessed he filled in to give the presentation at the last minute. I also particularly enjoyed James Fawcett's (University of Cambridge, UK) thought-provoking presentation on how to increase the regenerative ability of axons by providing the right micro-environment for the axons.

Left to right: Jeremy Ward, Roger Nicoll and David Wyllie. Roger delivered the Hodgkin–Huxley–Katz Prize Lecture at the Meeting.

All the talks were of a very high standard and were very well attended, which was extremely impressive given that the weather could not have been any nicer, as there was glorious sunshine for three days straight. This provided thoroughly enjoyable breaks sitting in the grassy quad outside New Hunt's House and quite a lot of gratitude from the various speakers for everyone who attended their talk, rather than sitting in the sun.



Participants discussing a poster.

After the talks on the first and second days, there were two poster sessions. These were incredibly useful, as they were held in an informal setting and everyone was able to walk around and engage in light conversation over research they were interested in. All of the posters were of a high quality and one that particularly stood out to me was Jean-Pierre Lin's (King's College London), as he had brought his computer along and had some videos to supplement his poster on how potent a treatment of deep brain stimulation (DBS) can be to treat dystonia. Walking around the posters, it was quite amazing to see how far some of the research groups had travelled from. Just to name a few of the locations: Porto, Portugal; Brescia, Italy; Cairo, Egypt and Dunedin, New Zealand. I think a lot of those names also appear on my holiday wish-list, so maybe there will be some new post-doc positions in a year's time.

On Thursday evening, The Society Dinner was held at the Little Ship Club and although I wasn't able to attend myself, I heard great things the next day. There were fabulous views over the River Thames, as the sun set after belting out heat all day long. In addition, the food and opportunity for networking were both top notch.

Overall, the Meeting did exactly what every scientific meeting should do, as novel results were presented, friendships were formed, and ideas were discussed. For budding and experienced neuroscientists, the three-day meeting was beneficial to all who attended. Let's hope in the future, the next Cellular & Integrative Neuroscience Themed Meeting can bring the same high calibre of scientific research and maybe, if we're lucky, some sunny weather.

Trevor Smith

University of Liverpool, UK

Biochemical Society Conferences

13–15 July 2011
Robinson College, Cambridge, UK

DEADLINES:
Abstract submission 11 MAY 2011
Earlybird registration 13 JUNE 2011

A Biochemical Society Focused Meeting

Nuclear envelope disease and chromatin organization

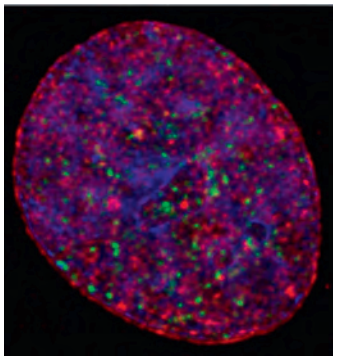


Image kindly supplied by Sue Shackleton (University of Leicester, UK)

Organizers:
Juliet Ellis
Sue Shackleton

Overview:
Laminopathies are a diverse group of diseases associated with nuclear envelope proteins, ranging from muscle wasting to premature aging disorders. This meeting brings together researchers and clinicians working on diverse aspects of nuclear structure and associated diseases, to review recent progress and define strategies for future research.

Topics:
* Nuclear envelope proteins
* Disease models and mechanisms
* Nuclear envelope protein-dependent stem cell aging
* Chromatin interactions
* Nucleo-cytoskeletal connections and mechanics

For a full programme please visit
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The decline of physiology teaching in British universities

Physiology teaching in most British medical schools has decreased over the last quarter century. Precise figures on the amount of physiology now taught are hard to obtain as it is no longer as clearly demarcated as it once was. However, first year physiology teaching for medical students at King's College London has been cut by more than fifty per cent, as Table 1 below shows. During the same period medical student numbers have doubled. Currently eight thousand students annually enrol in UK medical schools. King's College London is the largest medical school in Britain, but is fairly representative of other UK city universities e.g. those in the rest of London, Glasgow and Newcastle. This decline in the amount of physiology teaching along with the bloating of student numbers reflects a general trend in the UK.

Where medical teaching practice leads, biology teaching in general soon follows. The reduction in medical physiology practical teaching is symptomatic of a decline in practical teaching extending to other disciplines, like pharmacology, biochemistry and microbiology, where practical teaching once had a preponderant role.

So what? All academic subjects wax and wane according to contemporary needs and vagaries. Why bewail physiology's relative decline any more than that of *Ozymandias*?

What is so important about physiology that it needs to occupy the ascendant position in the time and thoughts of our ostensibly hardworking British medical students?

History of academic physiology

The foundations of the physical–chemical approach to physiology were laid in Germany and France in the early nineteenth century and it developed in parallel with physical and organic chemistry. Jan Purkinje (1787–1869) held the first

Richard Naftalin

chair in Physiology at Breslau (now Wroclaw) from 1823 to 1850 and there opened the first independent Physiology Department in 1839 and was the first to introduce a course of experimental physiology. His teaching was much influenced by the ideas of the Swiss educationalist and social reformer, Johann Pestalozzi (1746–1827), which were based on the concept of *'Anschauung'* – direct concrete observation from experiment – *'A man learns by action ... have done with mere words'*. Pestalozzi's main concerns were to maintain an equilibrium between head, hands and heart, and with what was 'right' as opposed to 'correct'. Purkinje is remembered today by physiologists mainly because of his innovative use of the microtome in preparation of histological specimens which enabled him to discover the large nerve cells in the cerebellum and the conducting fibres within the heart, both of which are now named as Purkinje cells.

In Britain the first chairs in Physiology were founded in 1836 at both University College London and King's College London, then in Cambridge and Oxford in the 1870s. The Physiological Society was founded in 1876. One of its *raison d'êtres* then, as now, was as a lobby against the Anti-Vivisectionist movement, which was as vociferous, although rather less menacing then (Sharpey-Schafer, 1927).

Experimental physiology in Britain was initially practised by a very small number, whose financial support was sourced mostly from their income from their own clinical work and occasional anatomy and histology teaching. Most British physiologists gained their research training from visits to the established laboratories of eminent German physiologist–physicians or physicists; for example, Carl Ludwig (1816–1895), discoverer of glomerular filtration; his student Otto Frank (1865–1944), who worked on the mechanics of muscle; Rudolf Virchow (1821–1902), the populariser of the cell theory – *'every cell originates from another'* – and 'father' of modern pathology; and Hermann von Helmholtz (1821–1894), a physician and scientist, who made seminally important contributions to both thermodynamics and optics, and invented the ophthalmoscope. No doubt Helmholtz would take a very jaundiced view of the current vogue for 'Translational Medicine'. He wrote, *"Whoever in the pursuit of science, seeks after immediate practical utility may rest assured that he seeks in vain"*.

The life, scientific works and philosophy of the French physician and physiologist, Claude Bernard

Table 1

Year	Med School	Physiology lecture hours (numbers attending)	Tutorial hours (group size)	Practical hours (group size)	Total hours
1990	KCL	72 (120)	20 (6–8)	36 (2–4)	128
1992	Glasgow	62		34	
2011	KCL	46 (450)	8 (8–10)	6 (6–8)	62
2011	Glasgow	15 (PBL plenary lectures on physiological topics)	—	9	

PBL, Problem-Based Learning.

(1813–1878) were one of the other important formative influences on the ethos of twentieth century physiology research and teaching. Bernard insisted that all science must be based on 'facts' established by rigorously controlled experiments. 'Active' experimental physiology was differentiated from 'passive' observation of unvarying phenomena, e.g. histological appearances. '*An 'experimenter' observes the changes in phenomena, whilst applying a controlled variable, thereby uncovering an otherwise hidden process'*. He conceded, nevertheless, that one could take advantage of opportunities presented in medical practice, as in the case of Alexis St Martin, who as a result of a shooting accident had a gastric fistula. Fortuitously, he became the personal servant to Dr William Beaumont (1785–1853), a US army surgeon. Beaumont put St Martin's gastric fistula to good use.

Claude Bernard asserted that whilst Beaumont's observation and description of the condition of St Martin's gastric mucosa were merely acts of passive inspection, examining of the process of digestion by insertion and removal of digestible materials via the fistula was the act of an experimentalist – an altogether nobler endeavour.

The accident that befell St Martin and his subsequent fortuitous employment by Beaumont is an exceedingly rare coincidence as '*it would be immoral to experiment on humans*', and since physiological science must be based on experiments on living animals, Bernard reasoned that animal vivisection was a necessity for both advancement of physiological knowledge and teaching (Bernard, 1865). Mme Claude Bernard, amongst others, vociferously disagreed.

Bernard (Claude) considered that experimental hypothesis must always be based on prior observation – '*Theories of how systems function are only hypotheses, verified by more or less numerous facts. Those verified by the most facts are the best, but even then they are never final, never to be absolutely believed'*. So we see that Bernard's scientific philosophy was far ahead of his time and closely aligned with the views of Karl Popper (1902–1994).

Bernard was sceptical about the Darwinian theory of evolution, viewing it as speculation unsupported by proper experimental evidence. He was, as was Darwin, unaware of Gregor Mendel's (1822–1884) work, which remained unrecognised until its rediscovery and translation into English (Bateson, 1902).

A modern criticism of Bernard's views and by extension to all contemporary physiology, is that he ignored species differences when applying results from animal models to human clinical conditions. The main differences between the physiologies of mice and men were assumed to be quantitative rather than qualitative. We know now that genetic variations in protein structure can alter enzyme specificities and activities, so both qualitative and quantitative intra- and interspecies differences exist (LaFollette & Shanks, 1994). These differences must make us cautious about extrapolation from animal to human models of physiological and pathological processes. However, these real differences do not invalidate, as anti-vivisectionist propaganda would like us to believe, the tried and tested corpus of results based on animal experimentation.

Because Purkinje's and Bernard's views were strongly influential on medical and biological thought throughout the twentieth century, physiology teaching became the core topic from which medical and biological sciences stemmed.

What is physiology?

Physiology is the study of functional relationships of living tissues with each other, using the methods and precepts of physics and chemistry. It attempts to understand the integration between living states at all levels – molecular, subcellular organelles, cells, tissues and body organ systems. From its beginnings experimental physiologists have tried to eliminate as many extraneous factors as possible from the processes under investigation and so improve the precision of their conclusions. This reductionist trend has meant, wherever possible, that physiological processes are examined in isolation – after removal of the living tissues from the animal. In most cases this requires acquisition of skills and development of expensive and sensitive instrumentation. This atomization of physiological function has led to growth of sub-specialities and to a growing divide between the immediate research interests of physiologists and the essential topics required for the training of future clinicians and most biologists.

Clinicians work mainly at the level of interrelations between the various body systems and their disorders, e.g. the cardiovascular, respiratory, gastrointestinal, genito-urinary, reproductive and nervous systems, rather than in the cellular and subcellular domains, where reductionist studies have led.

It is claimed that physiology *"lost its position as the exclusive paradigm of biology ... when behavioural biology, ecology, population biology, and other branches of modern biology developed, it became even more evident how unsuitable mechanics was as the paradigm of biological science... In contrast, clinical medicine – based on observation and comparison (as opposed to the determinists' controlled experiments) – is emerging as a genuine science, and epidemiological studies, while irredeemably statistical in nature, have provided much valuable information about human pathology"* (LaFollette & Shanks, 1994).

This argument is analogous to the statement that the classical mechanical paradigm has been overturned by the discoveries of quantum mechanics. Although quantum physics overrules Newtonian physics in the particle

physics domain, this is both untrue and misleading when applied in macroscopic terrestrial domains, where Newtonian mechanics still rules okay.

Distrust of statistics and of 'empirical' clinical studies were ingrained in Bernard's philosophy that dominated early twentieth century physiological thinking. These aversions stemmed from Bernard's view that trustworthy facts can only be legitimately derived from experimental data. In fairness, modern statistical methods were not fully developed until the 1920s (Fisher, 1925), about forty years after Bernard's death. However, the canard that contemporary physiology rejects the statistical approach to biology is completely false and misleading. For example, the 'modern' theory of chemical transmission of signals between nerves or nerve and muscle is based on a statistical theory of neurotransmitter release (Fatt & Katz, 1952).

Molecular biology, including specifically molecular genetics and genetic modification, has made an immense impact into understanding of physiological control mechanisms of growth and development at the cellular and subcellular levels. These studies have brought huge dividends to the understanding of many genetic diseases and physiological processes. Physiology has always been totally promiscuous in adopting any and every method that is appropriate towards elucidation of living processes – it is not a scientific discipline relying on a specific set of methodologies. So these discoveries are not seen as a threat, but as an important adjunct to physiology (Noble, 2011).

The threats to physiology from changes in the medical curriculum

As a consequence of the disproportionate increase in student numbers relative to the available teaching laboratory accommodation and staff, the shape and design of the medical curriculum has undergone a fairly radical change. These changes might have occurred in any case, as the rise in student numbers coincided with adoption in the late 1990s of a new Problem-Based Learning (PBL) teaching model imported from North America. It was evangelised in the UK, at first in Manchester, with the avowed aim of encouraging the students towards 'self-directed learning', but had the added advantage that it was seen as a means of re-distributing the increasing teaching load to 'specialist teaching' staff and relieved the increased burden on research staff. PBL introduced students to an integrated or 'holistic' approach to medicine from the outset. As another of the aims of PBL is to break down the 'artificial barriers between the -ologies', widespread adoption of PBL has had a major impact on the academic disciplines that have been co-opted into this venture.

Encroachment of PBL into the early medical teaching curriculum has meant that clinicians are now integrated within these early years and have a major say in early training. However, only a few scientists are expected to take part in the converse, that is of clinical teaching. Consequently, any perceived need to make good losses from natural attrition in pre-clinical staff numbers has been overridden by the higher priority that the Medical School Administration gives to the 'health' needs of patients – and for clinical duties.

PBL is more appropriately suited to medical teaching in North America, where every medical student already has a degree in biological sciences before entering medical school. British students mainly do not have equivalent experience in physiology, cell biology, biochemistry or pharmacology before coming to medical school. Realistically UK students can only be expected to integrate knowledge and solve problems once they have acquired a firm basis in medical sciences. This requires that physiology should be taught and examined prior to the students applying this knowledge to problem solution.

Threats to physiology from loss of academic departmental structures

Heads of university Physiology departments, as with other scientific disciplines, were traditionally the regulators of new appointments, promotions, allocators of financial resource for shared equipment and teaching and the ones answerable to higher university authorities. Peer pressure from national and international academic organizations, such as The Physiological Society and International Union of Physiologists generally ensured a progressive drift in research and teaching.

The years of faltering economic growth in the UK (1975–90 and 2008–2015) with reduction in financial support from central government for academic research and consequent 'greying of academia' together have eroded the traditional pyramidal structure of academic departmental authority. General adoption of information technology throughout universities in the 1990s meant that it became feasible for the central administrations to take closer charge of departmental finances and of student progression. Resource allocation within the pre-clinical schools, formerly controlled at the pre-clinical faculty level, was transferred to central committees composed of administrators and senior clinicians, whose perspectives were based on the global needs of teaching and research in the whole medical school and university rather than the local needs of pre-clinical science departments. Naturally in a hospital environment in which most medical schools are based, these perceived needs militate against science in general and physiology in particular. Physiology was seen as being old-fashioned, based on outdated methods – antagonistic to clinical science and engaged in expensive and controversial vivisection, which required an expensive protective umbrella of security precautions. PBL was viewed as a cheaper, cleaner and more contemporary option and had the

added advantage that it could be controlled by clinicians.

Perhaps the most important consequence of physiology's loss of autonomy has been the virtual abolition of practical physiology teaching (see Table 1). Whilst physiology and indeed clinical medicine can be illustrated and illuminated by using well-chosen clinical problems, the premature use of the PBL approach leads to exclusion of important aspects of physiology and loss of the kind of understanding for which, as Pestalozzi's teaching demonstrated, no substitute exists.

The main goal of practical physiology classes – giving the student direct personal experience of how deductive reasoning is based on experimental observation – has been abandoned. Practical demonstrations on key methods in clinical physiology, such as blood pressure and electrocardiograph and lung function, and widespread adoption of computer-aided learning (CAL) programs used as surrogates for animal experimentation, although better than nothing, cannot replace experience gained from real experiments on living tissue.

The lessons that Bernard and Purkinje taught so successfully have been abandoned and forgotten. Practical classes are now viewed as an optional and expensive 'extra' for medical and basic medical science courses. Human or animal experimentation, like nothing else, imprints the need for precision in preparation, accurate observation, recording, collation, analysis and eventually reporting the precious data. Inadequacy of any of these essential elements will lead to wasted time, money and in some cases life. This is exactly the kind of lesson which is needed by all intending clinicians and animal researchers.

Although this 'old fashioned approach' of incorporating extensive laboratory courses in physiology into medical and medical science courses is both a time-consuming and labour-intensive process – its omission may prove to be even more costly. British science graduates will become less competitive and attractive to employers than more thoroughly trained European competitors, all of whom still take laboratory teaching seriously.

It is not simply because of elitism or conservatism that Oxford and Cambridge have not yet completely complied with the trend towards integration of departmental structures and teaching. For example, in Oxford medical students in their first two years undertake both human and animal experimental work, carried out in relatively smaller groups (e.g. of 48 students working in 12 separate groups with four staff members present); and in their third years, all work in a laboratory (usually joining an experimental group taking part in 'cutting edge' experimental work). As a result these students enter their clinical training with at least some of the mental concept of the scientific method and of data at the centre of the intellectual challenge. Preservation of pre-clinical science departmental autonomy appears necessary for maintaining their presence within the curriculum and in conserving the rational approach to medicine which the GMC prescriptive document '*Tomorrow's Doctors*' are required to follow.

When, as now, medical education and National Health are the playthings of politicians, it is particularly important that clinicians should be well trained in rational thinking and know what 'evidence' actually means. They should be trained to examine with suspicion all claims from authority, including those that suggest that particular measures will 'save money' in the long term by offering short-term financial 'inducements' to some.

But fashions also recur, *Ozymandias* (*aka* Rameses II) arguably has more enduring posthumous celebrity than anyone. Certainly his works will remain Egypt's biggest attraction for awhile longer; maybe in a future age Jan Purkinje and Claude Bernard's precepts will be rediscovered.

Richard Naftalin

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The Physiological Society Oxford Debate – is physiology relevant?

If you enjoyed this article, there's more to come – Richard Naftalin will be arguing in The Society debate at the upcoming Oxford meeting in July (Monday 11th at 16.30), where he and Giovanni Mann (KCL) will be opposing the motion:

'Physiology as a separate discipline is no longer relevant to modern science.'

The proposers of the motion will be Maynard Case (Manchester) and Ian McGrath (Glasgow), and the debate will be chaired by David Eisner (Manchester) and Godfrey Smith (Glasgow). Come along to hear the views, to give your own and to ask questions – or just to see the fur fly.

Abstracts of Communications: people were faster than computers

It is almost 35 years since the Centenary Meeting of The Society. This leads me to compare the way printed abstracts were dealt with then and now. Forty-odd years ago abstracts could be submitted up to 7–9 weeks before a meeting; nowadays the window closes 14 weeks before. The Centenary Meeting in 1976 was the largest ordinary meeting of The Society to date. It was held in Cambridge with much celebration, and five or six parallel sessions. It ran from the 1st to the 3 July, and the window for submitting abstracts was, I recall, 3–14 May. I remember the first of these dates because I managed to do an important experiment just in the nick of time.

In those days there were no posters. If the number of submissions exceeded the time available the Meetings Secretary operated a first-come first-accepted policy. As a result, one aimed to post the abstract to arrive on the day acceptance opened. I managed a complete experiment late on Thursday 29 April, showing that intracellular pH regulation by a snail neurone was inhibited by the removal of Na^+ but not K^+. On the Friday I cut up my pen recording, assembled the figure on a sheet of card and labelled it by hand as shown here, in part, on the left of Fig 1. I wrote the abstract text in long hand, had it typed up by a departmental secretary, and sent it off with the original figure by post to the Meetings Secretary (D. Noble). My submission was accepted and sent with the rest of the abstracts to Cambridge University Press for figure labelling and printing. The finished book of abstracts and the programme were posted to all Members a week or so before the meeting. I reproduce part of the figure as printed.

After 35 years of amazing developments in computers and the internet, we now have to upload abstracts for the Main Meeting (in Oxford) 14–18 weeks in advance, with the text and any figures ready for printing as submitted. (The actual submission process often requires, in my experience, much wrestling with the ScholarOne computer somewhere in the USA.)

It used to be that one attended a Meeting to learn of the latest advances. Now most of the programme is full of symposia covering work often done some years ago, and even the voluntarily submitted material is staler than it used to be because of the long submission time and paucity of Meetings. The authors have no chance to correct minor errors, which used to happen after the abstract was accepted by the Meeting for publication.

The Oxford Meeting of 2011 is bigger than the Centenary Meeting, but much of the programme was fixed many months ago. The space for free communications may be somewhat larger than in 1976, and there will be many posters, but there must be other factors dictating the long submission time. Ethics scrutiny and checking by Special Interest Group convenors only takes a week, and the submitted material simply needs sorting into suitable sessions. What takes so long? The Society has a much larger administration than 35 years ago, and many powerful computers, but perhaps too few people are involved with the programme preparation. Is it simply that people can do things much more efficiently if computers are not involved?

Roger Thomas

I thank Ann Silver for comments and corrections.

Thomas RC (1976). Comparison of the Na^+ and H^+ pumps in a snail neurone. *J Physiol* **263**, 212*P*–213*P*.

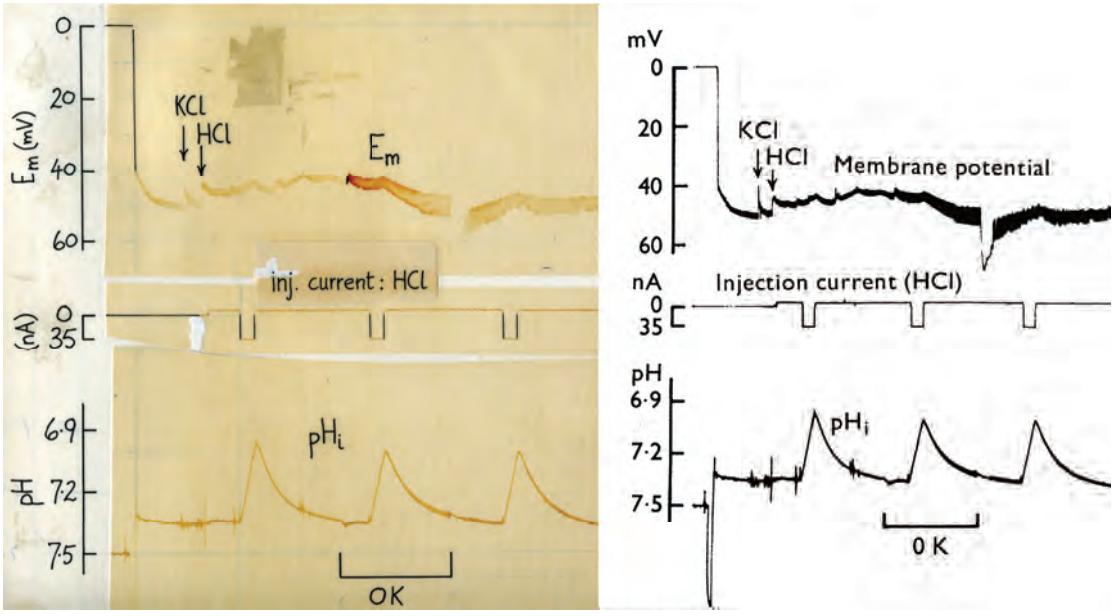

Figure 1. Left: part of my figure as submitted, though now rather faded. Right: the same part of the published version re-labelled by the printers (Thomas, 1976).

Do peripheral chemoreceptors in the carotid body serve as sites of glucose sensing?

The peripheral chemoreceptors in the carotid body are well known for their role in oxygen sensing and control of respiration. Recently, cellular evidence has shown that these cells are also responsive to glucose. Our recent work is supportive of carotid body involvement in glucose sensing in humans.

The brain lives on oxygen, adequate blood pressure and glucose. If any one of these three essential elements for brain function falls below a critical level, unconsciousness and even death can occur. The carotid arteries are located bilaterally in the neck and deliver oxygenated blood and glucose to the brain. They contain strategically located sensory organs that monitor the blood oxygen level (carotid chemoreceptors) before it enters the brain and also blood pressure (carotid baroreceptors). If the carotid chemoreceptors sense a drop in oxygen level, ventilation is stimulated. If a fall in blood pressure is detected by the baroreceptors, there are adjustments in the heart and blood vessels to raise it. The teleological argument is that the carotid chemo- and baroreceptors serve to ensure that blood is delivered to the brain at an appropriate pressure with enough oxygen for neuronal survival. Do the chemoreceptors in the carotid bodies also function to ensure an adequate supply of glucose to the brain?

The carotid body structure is conserved among vertebrates and has also been studied extensively for its role in oxygen sensing. In fish, there are 'glomus-like' cells in the first gill arch that serve as an oxygen sensor between the 'inhaled' water and blood flow. However, in the case of fish these cells sense the external environment and are unlikely to respond to hypoxaemia and serve a homeostatic mechanism similar to that described above. In diving birds, there is evidence that the carotid bodies and their sensing of oxygen is related to dive duration (Milsom & Burleson, 2007). Furthermore, in diving ducks the carotid bodies mediate at least part of the dive


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reflex so that oxygen consumption is temporarily slowed and the brain and heart are protected from hypoxic damage. In humans, the carotid bodies play an important role in oxygen sensing as evidenced by the loss of hypoxic ventilatory response in carotid body-denervated patients (Timmers *et al.* 2003*a*,*b*). The latter two are great examples of the role the carotid bodies serve in an integrative physiological response of the neural, respiratory and vascular systems, and underpin the notion that the carotid bodies are important homeostatic control points for key physiological parameters.

Recently cellular evidence has emerged that Type I glomus cells in the carotid bodies are in fact sensors for glucose in addition to their roles in sensing oxygen and carbon dioxide. There is also animal work in support of this notion. In dogs, carotid body resection drastically impairs the animal's ability to sense and respond to hypoglycaemia (Koyama *et al.* 2000). During a hypoglycaemic clamp the glucose infusion rate was higher in denervated dogs, consistent with the idea that endogenous glucose production was blunted. There were also blunted glucagon and cortisol responses to hypoglycaemia (Koyama *et al.* 2000), suggesting that the carotid bodies are integral to the neuro-hormonal response to hypoglycaemia. In a study in exercising dogs, intact carotid bodies were also required for an appropriate neuroendocrine response to exercise and carotid body-resected dogs did not show the normal exercise-induced increase in glucagon, noradrenaline (norepinephrine) or cortisol (Koyama *et al.* 2001). Given these compelling findings and the strategic location of the carotid bodies, we sought to address the role of the carotid bodies in glucose sensing in conscious humans.

Our recent work in *The Journal of Physiology* entitled 'Hyperoxia blunts counterregulation during hypoglycaemia in humans: possible role for the carotid bodies?' focused on the interactions of oxygen and glucose sensing in the carotid bodies (Wehrwein *et al.* 2010). Hyperoxia was used to 'desensitize' the carotid bodies since exposure to 100% oxygen results in a depression of minute ventilation (Downes & Lambertsen, 1966) and immediately drops chemoreceptor activity in the carotid bodies to zero and initiates a brief apnoea in cats (Lahiri & DeLaney, 1975). When we inactivated the carotid body chemoreceptors, neuro-hormonal counterregulation to hypoglycaemia was blunted (Fig. 1) suggesting: (1) that the carotid bodies play a role in glucose sensing and regulation in humans, and (2) there is an interaction between glucose and oxygen in the carotid bodies. These observations are also consistent with studies from isolated carotid glomus cells showing an additive effect of oxygen and glucose signalling such that the glomus cells respond to hypoglycaemia and that this response can be enhanced with concurrent exposure to hypoxia (Pardal & Lopez-Barneo, 2002).

Before our study there was evidence in healthy humans and in several patient groups for a

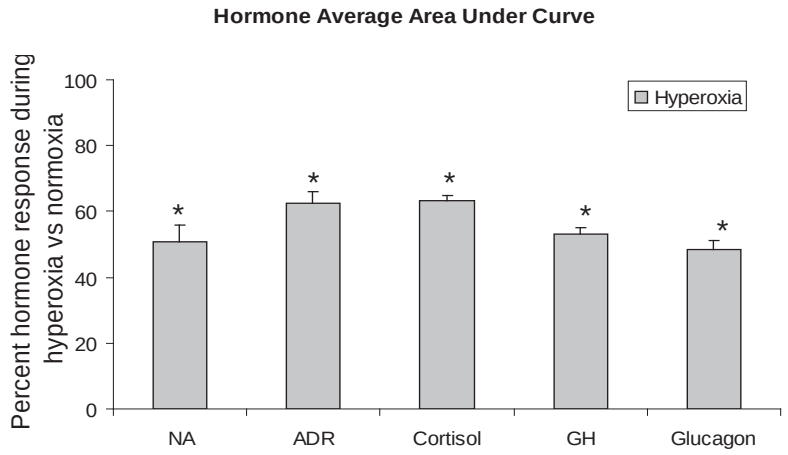

Figure 1. Counterregulatory hormone response to hypoglycaemia was blunted during carotid body deactivation under hyperoxic conditions. Area under the curve for counterregulatory hormones was calculated during the entire 3 h hypoglycaemic clamp: noradrenaline (NA), adrenaline (ADR), cortisol, growth hormone (GH) and glucagon. Area under the curve during normoxia (carotid body active; bars not shown) was normalized to 100% and hyperoxia (carotid body deactivated; grey bars) is shown as a per cent of normoxia. Significance denoted as $^{*}P < 0.05$ for differences between normoxia and hyperoxia. All hormone profiles are significantly reduced under hyperoxic conditions (carotid body desensitized). $N = 7$.

fundamental link between oxygen and glucose sensing. Although these examples have not been linked to the carotid bodies, it is tempting to speculate that multi-modal sensing in the carotid bodies may be involved. Some examples include: (1) Acute reductions in oxygen saturation in healthy subjects causes glucose intolerance (Oltmanns *et al.* 2004); (2) In chronic obstructive pulmonary disease (COPD) patients, chronic hypoxia is associated with impaired glucose tolerance while COPD patients with more normoxic blood gases have normal response to oral glucose tolerance testing (Hjalmarsen *et al.* 1996; Jakobsson & Jorfeldt, 2006); (3) Hypoxic COPD patients acutely placed on supplemental oxygen have an immediate improvement in glucose tolerance and insulin sensitivity during euglycaemic glucose clamps (Jakobsson & Jorfeldt, 2006); (4) Two days of treatment with continuous positive airway pressure improves glucose tolerance and insulin resistance in metabolic syndrome patients with sleep apnoea (Czupryniak *et al.* 2005; Dorkova *et al.* 2008); and (5) Diabetic patients receiving insulin and exposed to hyperbaric oxygen for treatment of diabetic ulcers frequently experience unexpected reductions in blood glucose and/or hypoglycaemia during treatment (Al-Waili *et al.* 2006). Taken together these observations are consistent with the concept that oxygen and glucose homeostasis are linked, and our recent publication supports the notion that one site of interaction is the carotid bodies. These observations along with our findings also have implications that extend into a variety of diseases in which there is an apparent interaction of oxygen and glucose homeostasis.

In summary, our findings support the cellular, animal and anecdotal clinical evidence that the cells of carotid bodies function as glucose sensors. We have shown an interaction between oxygen and glucose sensing in the carotid bodies with systemic implications. The anatomical location of the carotid bodies makes them ideal for the strategic control of what the brain needs to function in humans and other vertebrates.

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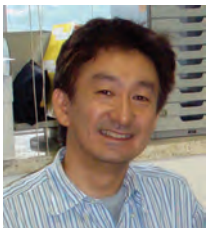
A key role of orexin (hypocretin) neurons in the fight-or-flight response

Stress increases cardiac function, ventilation and body temperature. These changes increase metabolic rate, oxygen supply and conduction velocity of nerve impulses and prepare the body for a fight-or-flight response. A part of the hypothalamus, called the 'defense area', has long been known to play a key role but the precise mechanisms are largely unknown. Our recent research suggests that orexin (hypocretin) neurons act as a master switch of the fight-or-flight response.

Research on neural mechanisms of state-dependent adjustments of central autonomic regulation has been sparse, despite the importance of this event from the perspective of quality of life. In addition to calm and resting states, our daily life involves many perturbations that induce active conditions such as locomotion, eating and communication. During such active periods, cardiovascular, respiratory and body temperature regulation needs to be adjusted according to the situational demands, which differ from those during resting states, by modulating or resetting homeostatic points (Kumada *et al.* 1990). One of the neural substrates regulating such adjustment appears to be located in the hypothalamus because stimulation of the so-called 'defense area' in the dorsal hypothalamus elicits behavioural 'rage' along with the specific autonomic responses; this was termed the 'defense response' (Hess, 1954).

Several neurotransmitters have been proposed to be involved in the modulation of the efferent pathways of defense responses against stressors (for review see Kuwaki & Zhang, 2010). For example, the activation of serotonin (5HT)-1A receptors in the medullary raphe reduces cardiovascular changes, and the inhibition of 5HT-3 receptors in the nucleus tractus solitarii prevents the baroreflex bradycardia inhibition during the defense response. Microinjections of adenosine into the rostral ventrolateral medulla augment the increase in blood pressure induced by electrical stimulation of the hypothalamic defense area. The pros and cons of glutamate participation in the cardiovascular component of the defense response have been a topic of debate. However, there is no



Tomoyuki Kuwaki

report on the molecular basis of the defense response underlying the multi-faceted nature of simultaneous and coordinated changes in the cardiovascular, respiratory, sensory, thermal and behavioural parameters. Localization of orexin-containing cell bodies in the perifornical area (PFA) and dorsomedial hypothalamus (DMH), which overlap the 'defense area', prompted us to investigate the possible role of orexin in the defense response against stressors.

Orexins (orexin-A and orexin-B), also known as hypocretins (hypocretin 1 and hypocretin 2, respectively), are recently discovered hypothalamic neuropeptides (de Lecea *et al.* 1998; Sakurai *et al.* 1998). Although orexins were first described as hypothalamic neuropeptides

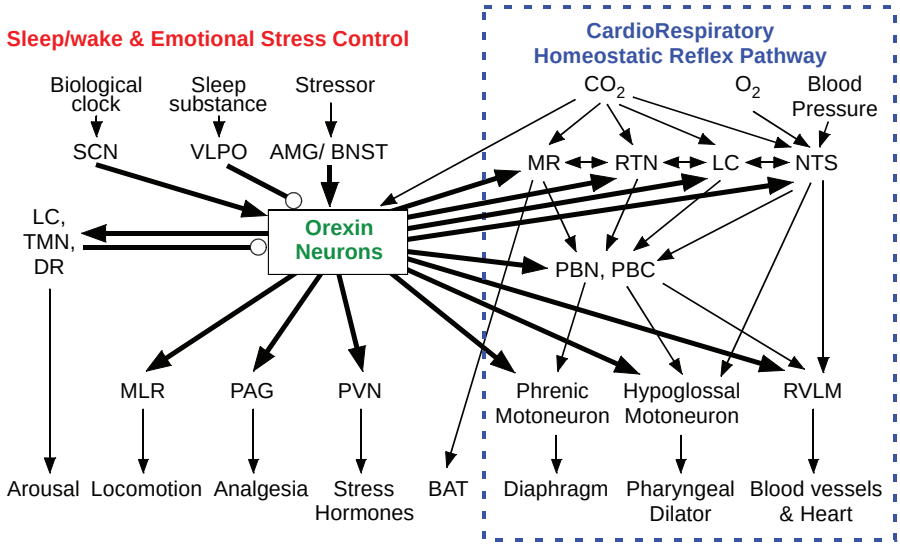


Figure 1. Pivotal role of orexin neurons in linking the state-dependent behavioural regulation system and cardiorespiratory homeostatic reflex pathways. Among known connections from/to orexin neurons in the hypothalamus, selected brain nuclei that are relevant to our study are indicated by thick lines. Many nuclei located at both input (MR, RTN, LC, NTS) and output (cardiorespiratory motor neurons) interfaces in the homeostatic cardiorespiratory reflex pathway receive projections from the orexin neurons (right half). Simultaneously, orexinergic connections are engaged in sleep–wake regulation and emotional stress-induced behavioural changes (left half). Thus, orexin can modulate cardiorespiratory homeostasis in a state-dependent and feedforward manner. Arrows indicate a probable excitatory connection, and circles indicate an inhibitory connection. Connections shown with thin lines are either direct or indirect. Abbreviations: AMG, amygdala; BAT, brown adipose tissue; BNST, bed nucleus of the stria terminalis; DR, dorsal raphe; LC, locus coeruleus; MLR, medullary locomotor region; MR, medullary raphe; NTS, nucleus tractus solitarii; PAG, periaqueductal grey; PBC, pre-Bötzinger complex; PBN, parabrachial nucleus; PVN, paraventricular nucleus; RTN, retrotrapezoid nucleus; RVLM, rostral ventrolateral medulla where sympathetic cardiovascular premotor neurons are located; SCN, suprachiasmatic nucleus; TMN, tuberomammillary nucleus; VLPO, ventrolateral preoptic nucleus. From Kuwaki & Zhang (2010).

that influenced appetite and consciousness, it was later found that orexins also modulate the reward process, the pain process, and autonomic regulation of the cardiovascular, respiratory and neuroendocrine systems (Kuwaki & Zhang, 2010). Orexin-containing cell bodies are restricted to the

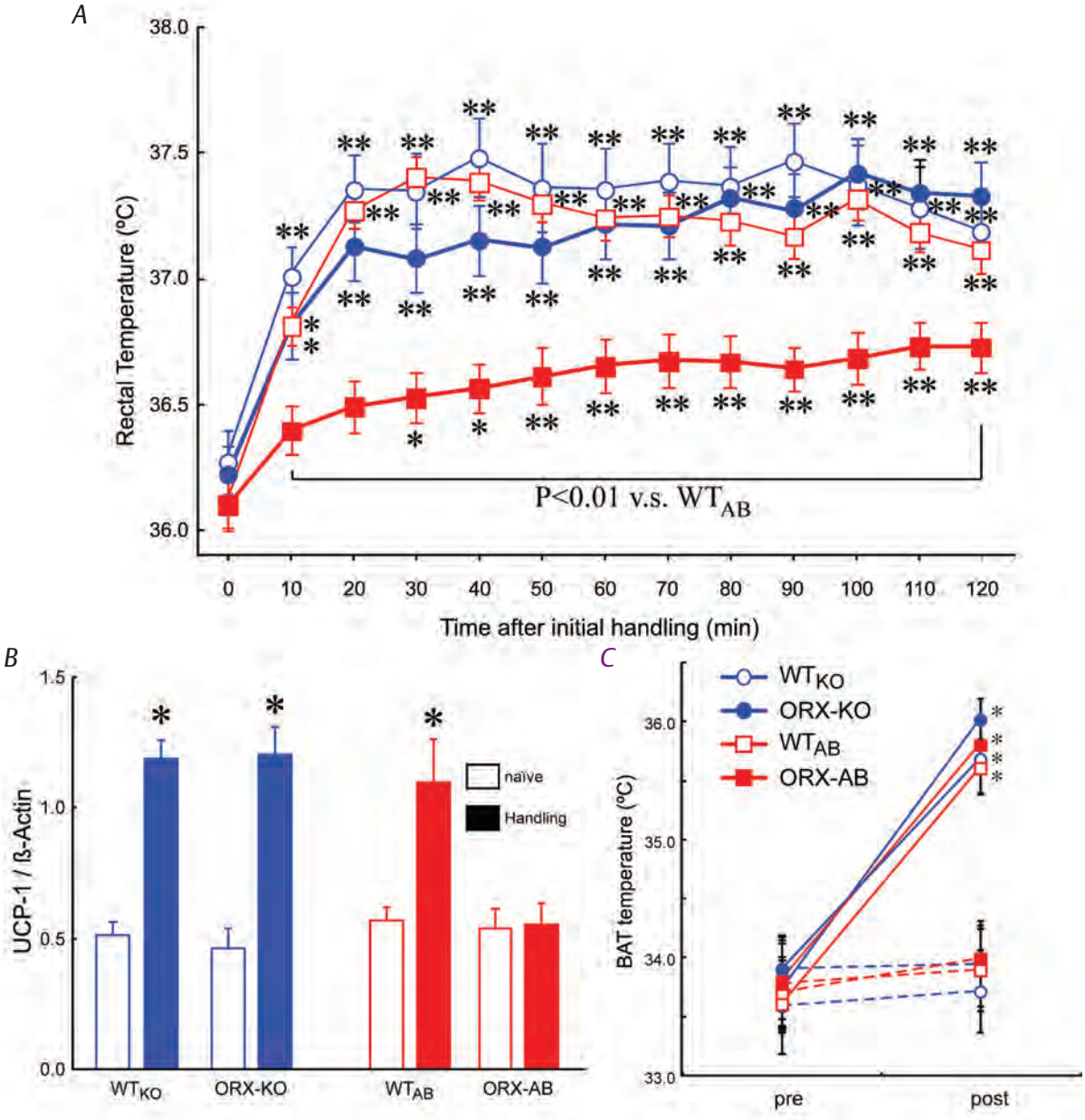


Figure 2. *A*, effect of repeated handling stress on rectal temperature of mice of 4 genotypes. The temperature measurement (insertion of thermistor probe into the animal's rectum) itself was used as a stressor and repeatedly applied at 10 min intervals for 2 h. Data are presented as mean ± SEM of orexin knockout mice (ORX-KO, n = 37), orexin neuron-ablated mice (ORX-AB, n = 32), and their corresponding wild-type littermates (WT_{KO}, n = 22 and WT_{AB}, n = 21). *B*, expression of uncoupling protein (UCP)-1 in the brown adipose tissue (BAT) of stressed or naïve mice. BAT was dissected from stressed mice (4 rectal temperature measurements at 10 min intervals) and from naïve (unstressed) mice of the same genotypes. The total RNA extracted from the BAT and cDNA was reverse transcribed. UCP-1 mRNA was determined by quantitative real-time PCR in triplicate and normalized with β-actin mRNA. Data are presented as means ± SEM of 7–9 animals. Note that handling stress increased the expression of UCP-1 in wild-type and ORX-KO mice but not in ORX-AB mice. *C*, BAT function test. Change of BAT temperature in response to a β3-agonist, CL316243, was examined in chloralose- (75 mg kg^{-1}, i.p.) and urethane- (750 mg kg^{-1}, i.p.) anesthetized mice. The animals received intraperitoneal injection of saline (dashed lines) or CL316243 (1 mg kg^{-1}, continuous lines). Data are presented as means ± SEM of the peak values during the observation period of 120 min (n = 5 for each group). These results indicate that blunted stress-induced hyperthermia in ORX-AB was caused by the loss of orexin neurons and abnormal BAT regulation and not due to an abnormality of BAT *per se*. From Zhang *et al.* (2010). $^{*}P < 0.05$, $^{**}P < 0.01$.

lateral hypothalamic area (LHA), PFA and DMH. On the other hand, orexin-containing nerve terminals and receptors are widely distributed in the hypothalamus, thalamus, cerebral cortex, circumventricular organs, brainstem, cerebellum and spinal cord, suggesting that the orexin neurons have widespread connections with other regions in the brain. Specifically, the following cardiorespiratory-related areas receive orexinergic innervation: nucleus tractus solitarii; pre-Bötzinger complex; periaqueductal grey; rostral ventrolateral medulla; intermediolateral cell column of the spinal cord; and the retrotrapezoid, hypoglossal, medullary raphe, parabrachial/Kölliker–Fuse and phrenic nuclei (Fig. 1). Moreover, orexin neurons receive inputs from the regulatory sites of the circadian clock, sleep–awake cycle, and emotional stress, such as the ventrolateral preoptic area, locus coeruleus, dorsal raphe, amygdala, bed nucleus of stria terminalis (BNST), and suprachiasmatic and tuberomammillary nuclei.

We have previously shown that orexin plays an important role in cardiorespiratory excitation during stress in orexin knockout mice (ORX-KO) and orexin neuron-ablated mice (ORX-AB) (Kuwaki & Zhang, 2010). The results of the study are summarized as follows. (1) In response to stress, both ORX-KO and ORX-AB exhibited an attenuated defense response, including increase in respiration and blood pressure and stress-induced analgesia. (2) Stimulation to the amygdala or BNST, both of which are implicated in the stress-induced autonomic responses, induced long-lasting cardiorespiratory excitation in wild-type mice but not in ORX-AB. On the basis of these results, we proposed that orexin serves as a central node between the state-dependent behavioural regulation system and the cardiorespiratory homeostatic reflex pathways (Fig. 1).

In line with the above-mentioned hypothesis, we thought that stress-induced hyperthermia would also be influenced by orexin. On the contrary, we found that ORX-AB but not ORX-KO mice had blunted stress-induced hyperthermia (Zhang *et al.* 2010) (Fig. 2*A*). The brown adipose tissue, which is a major thermogenic organ in rodents, did not respond to handling stress (Fig. 2*B*), although it did respond to a direct pharmacological stimulation (Fig. 2*C*). These abnormalities in ORX-AB were not observed in ORX-KO, in which orexin peptide is deficient but the neurons are preserved. Therefore, the integrity (orexin and other co-existing neurotransmitter/modulators) of the orexin neurons is indispensable for the complete expression of multiple facets of the fight-or-flight response (Fig. 3). Although the concept of co-transmitters in one neuron is well known and the related anatomical evidence has been frequently reported, the synergic/comprehensive role of the co-transmitters has been scarcely reported. From this point of view, our report is, I believe, an exceptional one.

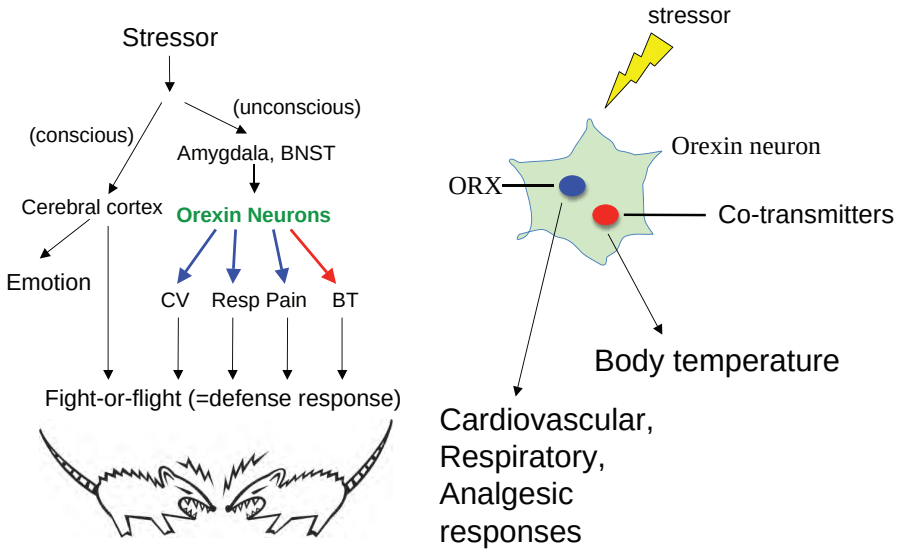


Figure 3. Hypothetical diagram showing the key role of orexin neurons in the fight-or-flight response. When animals confront a stressor, orexin neurons mediate cardiovascular (CV), respiratory (Resp) and analgesic (Pain) responses by using orexin as a transmitter. At the same time, orexin neurons regulate body temperature (BT) by using co-transmitters other than orexin. These responses are collectively called the 'defense response' that prepares the body for fight-or-flight behaviour.

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Endurance training–overtraining: a fine-tuned balance that depends on Ca^{2+}

The razor-thin margin between beneficial effects of endurance training and the deleterious effects of overtraining is a central dilemma in endurance sports. We show here that modest changes in muscle cell Ca^{2+} homeostasis can cause both these effects.

Endurance training is of fundamental importance in all types of endurance sport activities. Moreover, endurance exercise is probably the best way to counteract the large and rapidly growing health problem referred to as the metabolic syndrome: the combination of central obesity, increased blood lipids and glucose, and increased blood pressure, which often develop into type 2 diabetes and cardiovascular disease. It is then natural that there are an increasing number of studies into mechanisms by which endurance exercise mediates its beneficial effects.

From left: Joseph Bruton, Håkan Westerblad and Niklas Ivarsson

Endurance exercise results in numerous adaptations in skeletal muscle, including increased aerobic capacity and improved metabolism with increases in insulin sensitivity and fat oxidation. Studies addressing mechanisms underlying these beneficial adaptations have to a large extent been focused on cellular signalling events, such as activation of kinase cascades and transcription factors. Relatively less attention has been paid to primary effectors, i.e. factors that are directly affected by endurance exercise and subsequently activate signalling pathways and transcription factors. One tentative primary effector is increased free Ca^{2+} concentration in the cytosol ($[Ca^{2+}]_i$), because $[Ca^{2+}]_i$ must increase to drive contractions during exercise, and *in vitro* experiments have shown that increased $[Ca^{2+}]_i$ can increase mitochondrial biogenesis in adult skeletal muscle fibres (Wright *et al.* 2007).

Recent studies in our laboratory were performed on cold-acclimated mice. Serendipitously, we observed adaptations in their muscles resembling both the beneficial effects observed with endurance training and the impaired muscle function which can be the result of excessive training, i.e. overtraining (Aydin *et al.* 2008; Bruton *et al.* 2010). Both adaptations could be explained by changes in the cellular Ca^{2+} handling due to modification of the sarcoplasmic reticulum (SR) Ca^{2+} release channel complex, i.e. the ryanodine receptor 1 (RyR1)

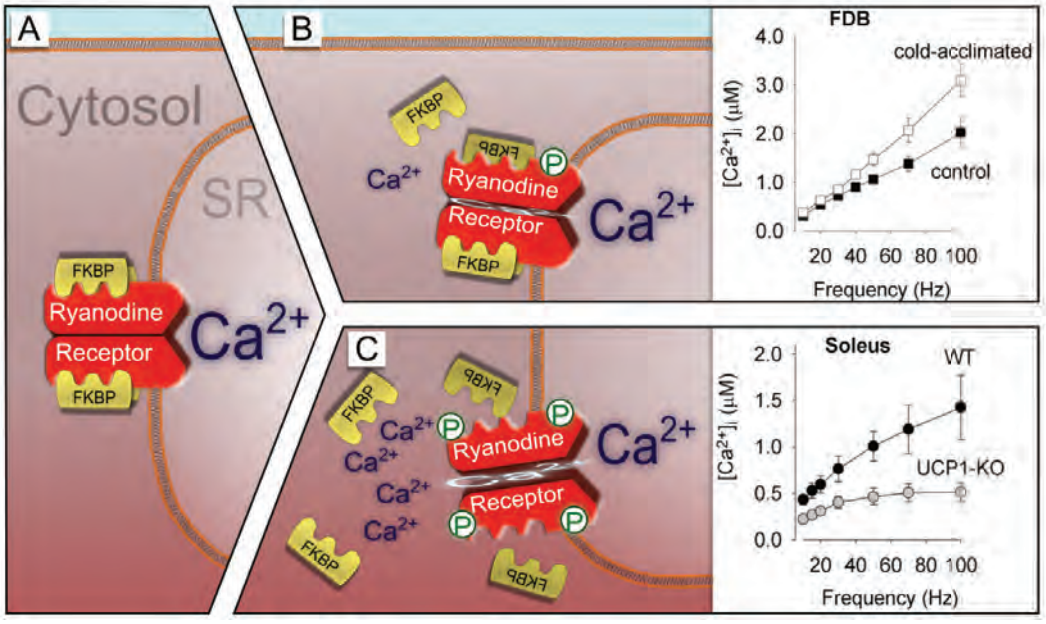

Figure 1. *A*, the ryanodine receptor (RyR1) complex and one of its associated proteins, FKBP12, can be modified by various stressors. For instance, RyR1 can be phosphorylated (P) and this, possibly together with other modifications, can lead to release of FKBP12. *B*, minor changes of this sort are accompanied by increased $[Ca^{2+}]_i$ during tetanic stimulation in flexor digitorum brevis (FDB) fibres of cold-acclimated mice (mean ± SEM, n = 10–11). *C*, conversely, major changes in the RyR1 protein complex are accompanied by decreased tetanic $[Ca^{2+}]_i$ in shivering soleus fibres of cold-acclimated UCP1-KO mice (n = 3–5); these mice cannot generate heat in their brown adipose tissue because they lack uncoupling protein-1 and therefore they are constantly shivering in the cold. *B* and *C* adapted from Bruton *et al.* (2010) and Aydin *et al.* (2008), respectively.

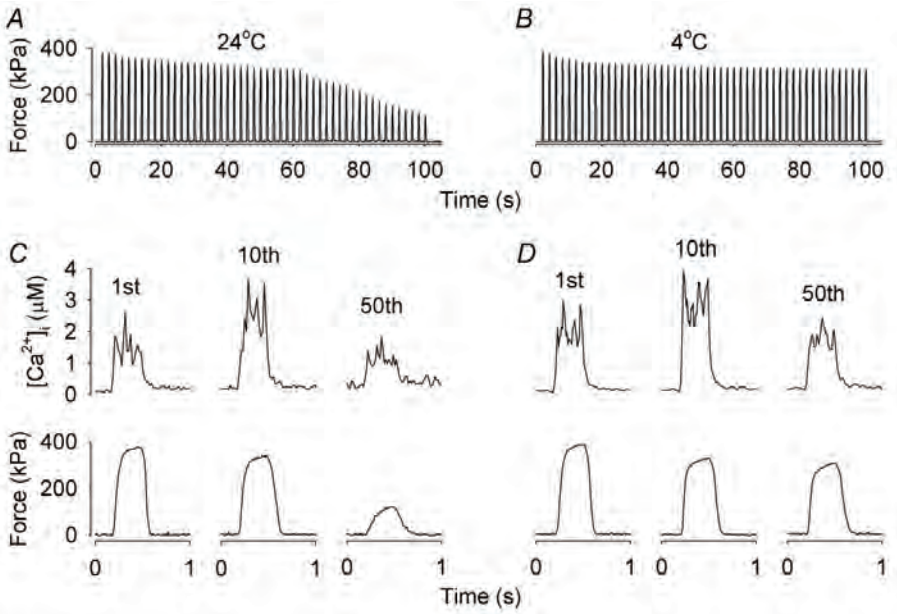

Figure 2. The slightly increased SR Ca^{2+} leak in FDB fibres of cold-acclimated mice was accompanied by increased fatigue resistance. Continuous force records from fatigue induced by repeated tetanic contractions of a fibre from a room-temperature (*A*) and a cold-acclimated (*B*) mouse, respectively. *C* and *D* show $[Ca^{2+}]_i$ (upper) and force (lower) from the first, tenth and last (50th) contractions of the fatigue runs. From Bruton *et al.* (2010).

protein complex (Fig. 1). The RyR1 protein complex can be modified by a wide range of stressors (Bellinger *et al.* 2008*a*), including endurance exercise and exposure to a cold environment. A critical component of the RyR1 modification is the release of the 12 kDa FK506 binding protein (FKBP12, also called calstabin 1), which results in a destabilized RyR1 channel complex and increased SR Ca^{2+} leak.

Toe muscles (flexor digitorum brevis, FDB) do not participate in the shivering response induced by exposure to a cold environment. FDB fibres of cold-acclimated mice show minor changes in the RyR1 protein complex with some increase in the RyR1 phosphorylation and depletion of FKBP12. These changes were found to be associated with increased SR Ca^{2+} leak and increased $[Ca^{2+}]_i$ both at rest and during tetanic contractions. An increased mitochondrial biogenesis has previously been shown when caffeine was used to increase $[Ca^{2+}]_i$ in muscle fibres *in vitro* (Wright *et al.* 2007). Accordingly, FDB muscles of cold-acclimated mice displayed increases in: (i) the protein expression of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α, a key regulator of mitochondrial biogenesis); (ii) the mRNA expression of the mitochondrial transcription factor A (Tfam, required for transcription and replication of the mitochondrial genome); (iii) the cellular mitochondrial content; (iv) the capacity for fatty acid oxidation. Furthermore, FDB fibres of cold-acclimated mice were more fatigue resistant and maintained much higher forces during fatigue induced by repeated tetanic stimulation than control fibres of mice kept at room temperature (Fig. 2). Interestingly, there was no change in fibre type (i.e. myosin heavy chain isoform) composition between FDB fibres from cold-acclimated and room-temperature mice. This is consistent with results from endurance training studies where major increases in endurance are accompanied by little or no fibre type changes, especially not between slow-twitch type I and fast-twitch type II fibres. To sum up, FDB fibres of cold-acclimated mice show increased $[Ca^{2+}]_i$ and adaptations similar to those observed with endurance training. This occurs despite them performing no obvious endurance exercise and hence the results indicate a key causative role of increased $[Ca^{2+}]_i$.

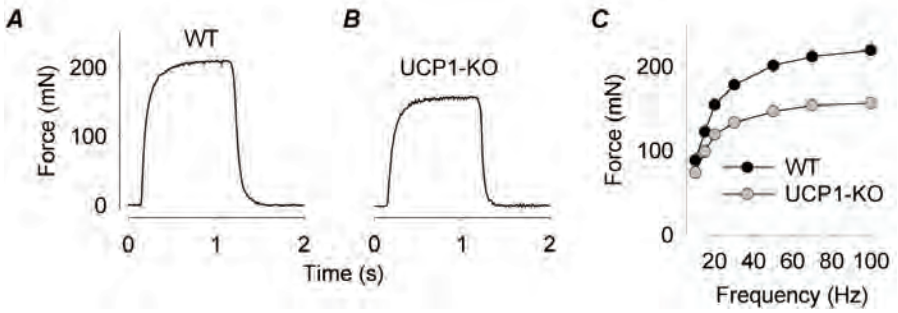

Figure 3. The marked modifications of the RyR1 channel complex in soleus muscles of cold-acclimated UCP1-KO mice were accompanied by decreased force production. Original force records from tetanic contractions (100 Hz, 1 s duration) of soleus muscles from a cold-acclimated wildtype (*A*) and a UCP1-KO (*B*) mouse, respectively. *C*, the force–frequency relationship in soleus muscles of cold-acclimated mice (mean ± SEM, n = 6–7). Adapted from Aydin *et al.* (2008).

Genetically engineered mice lacking uncoupling protein-1 (UCP1-KO) cannot generate heat by increasing the metabolism in brown adipose tissue. When kept in the cold, UCP1-KO mice shiver to maintain their body temperature. Soleus muscles, which participate in the shivering response, of cold-acclimated UCP1-KO mice show major changes in the RyR1 protein complex, with hyperphosphorylation and a major FKBP12 depletion (Aydin *et al.* 2008). These changes were associated with decreased $[Ca^{2+}]_i$ during contractions (see Fig. 1*C*) and hence also lower forces (Fig. 3). Thus, the combination of shivering and the general stress induced by exposure to a cold environment resulted in severe changes in the RyR1 protein complex and impaired contractile function. This resembles the contractile dysfunction observed with overtraining, which is a frequent problem for the elite in

many endurance-type sports. This notion is supported by the fact that similar changes in the RyR1 protein complex accompanied by contractile dysfunction have been observed after a period of intense exercise (Bellinger *et al.* 2008*b*). Furthermore, similar changes may also occur in patients with disorders afflicting muscle function where muscles have to be used closer to their maximal capacity even in everyday activities.

Another interesting aspect related to exercise-induced modifications of the RyR1 channel complex and increased SR Ca^{2+} leak is that this would lead to accelerated energy-dependent Ca^{2+} reuptake into the SR and hence increased heat production. Thus, this is likely to contribute to the increase in basal metabolism and the sensation of being hot that remain for prolonged periods after many types of endurance exercise.

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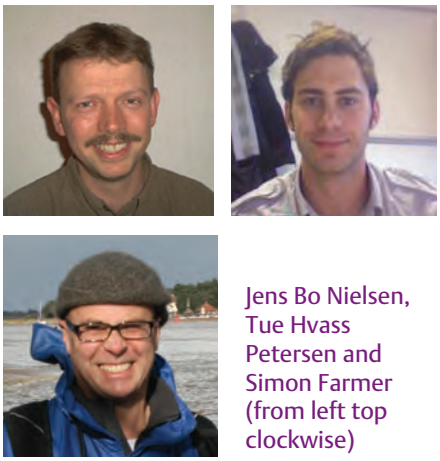
The way we learn to walk

Learning to walk is a milestone in children's motor skill development. Newly published research suggests that emergence of oscillatory drive in the corticospinal pathway plays an important role in developing and shaping this motor skill.

The world of walking

Parents regard the first few steps taken by their child as a huge milestone in their child's motor skill development. Learning to walk independently usually occurs at around 1 year of age with some degree of variance. However, children can be taught to walk at a slightly earlier age by means of motor practice. One of the most striking examples comes from an anthropological study by Super (1976). He studied children from a particular tribe in Kenya and found that they learned to walk slightly earlier than American children and this was likely to be due to the fact that these Kenyan mothers spent a remarkable amount of time teaching their children how to walk by means of jumping-like movements and supported walking practice. Furthermore, he reported that these children seemed to spend less of their waking time lying down and hence learned to crawl later than their American counterparts (this runs contrary to the notion of a child needing to learn to crawl before it learns to walk). The final motor outcome of teaching children to walk independently a few weeks earlier than normal is unclear. However, understanding the maturation and plasticity processes of the underlying neural networks responsible for generating the characteristic pattern of gait will prove helpful in developing rehabilitation strategies for adults and children learning to walk following damage of the central nervous system.



Jens Bo Nielsen, Tue Hvass Petersen and Simon Farmer (from left top clockwise)

Infant stepping – the first few steps

If you happen to have an infant in your near vicinity while reading this, now would be the right time to lift him or her and perform one of the regular exercises performed by African mothers – simply place the legs on a surface and perhaps jump the infant gently up and down. You would be likely to experience the legs performing stepping-like movements. These movements occur if the infant is younger than 2–3 month of age, for reasons we will touch upon later. Scientists have wondered which part of the nervous system generates these movements since cerebral projections and hence the brain's control of the leg muscles has not fully developed at this stage.

Work done by Hans Forssberg (1985) showed that when very young infants (0–18 months of age) perform stepping-like movements, they place the foot on its forepart straight under the body. This is unlike adult human walking which is characterised by the foot being placed in front of the body with a clear initial heel strike. In adults, the soleus and gastrocnemius muscles act on the ankle joint in the later parts of the stance phase to propel the body forward and the tibialis anterior (TA) muscle is responsible for lifting the forefoot during the swing phase of gait to prevent it from striking the ground. However, when Forssberg investigated the individual muscle activation patterns of the infants he found that the

electromyographic (EMG) activity displayed less modulation than in adults through the gait cycle and that the different muscles were often activated in parallel rather than in a sequence. He argued that these premature stepping movements are generated by pattern generators located in the spinal cord. Such pattern generators can produce alternating muscle activity in flexor and extensor muscles in the same leg and even coordinated activity between flexor and extensor muscles of both legs. In other human studies, indirect evidence of spinal pattern generators is provided by studies such as that of Yang *et al*. (2004). In this experiment infants stepped on a treadmill during which reciprocal organisation of muscle activity between the two legs was demonstrated and this activation pattern was modulated by the speed of the treadmill, suggesting the presence of a spinal central pattern generator that is modulated by proprioceptive input. Whilst in babies there are important biomechanical constraints on the actual output of spinal pattern generators during reflex stepping, it is acknowledged that there exists significant spinal organisation for walking which is increasingly modulated by cortical circuitry as the child grows and develops. The process of this 'corticalization' of lower limb control and the role of the *pattern* of descending neural drive is only just beginning to be understood.

Maturation of gait – the role of motor network oscillations

Although children are able to walk independently after the first few years of life, the adult pattern of

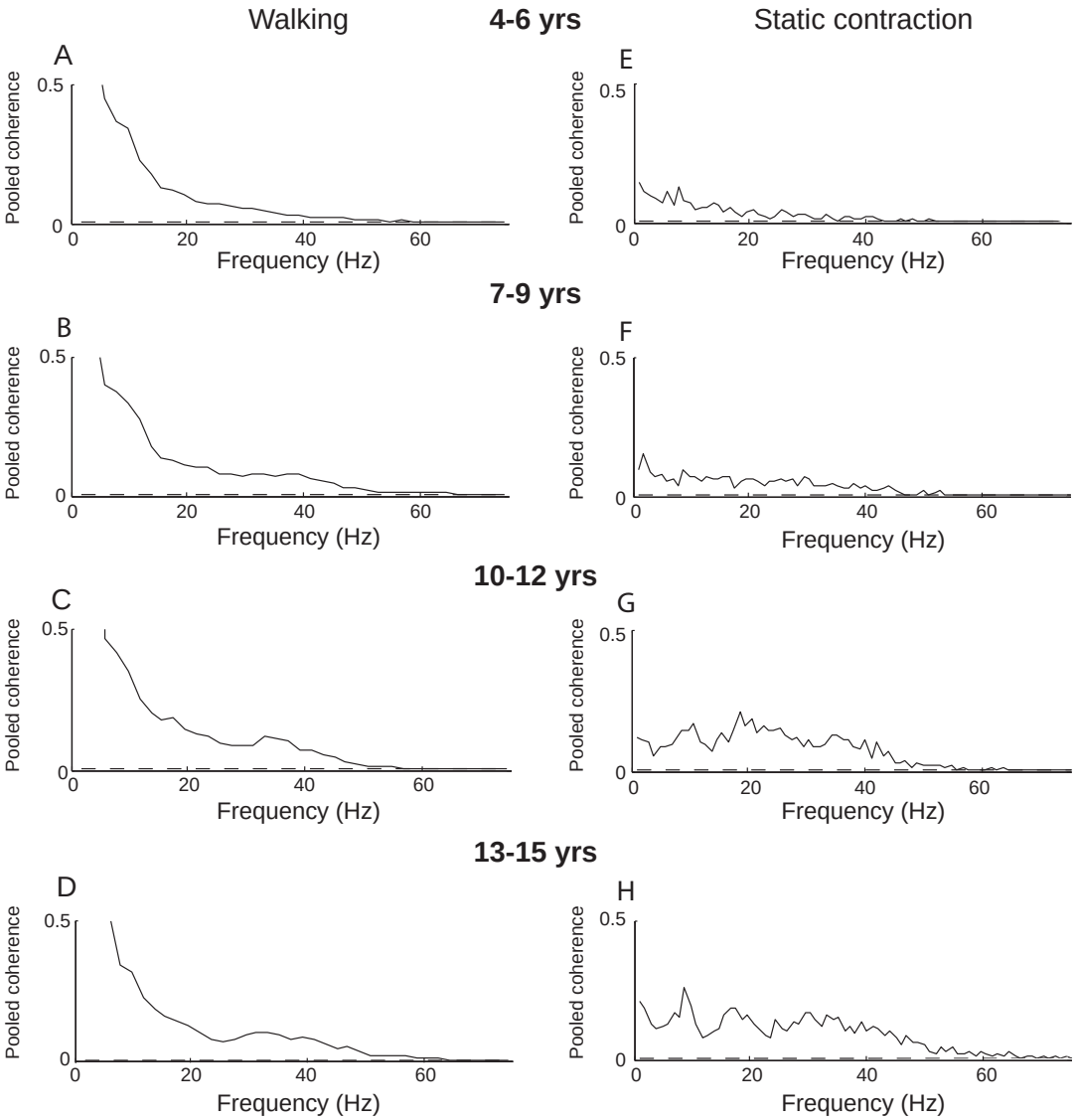


Figure 1. Pooled coherence for the 4 groups of children during walking and static contraction. *A–D*, pooled TA–TA EMG coherence at frequencies from 1 to 75 Hz for the 4–6 years age group (*A*, *n* = 11), 7–9 years age group (*B*, *n* = 11), 10–12 years age group (*C*, *n* = 10) and 13–15 years age group (*D*, *n* = 10) during walking. *E–H*, pooled TA–TA EMG coherence at frequencies from 1 to 75 Hz for the 4–6 years age group (*E*, *n* = 9), 7–9 years age group (*F*, *n* = 7), 10–12 years age group (*G*, *n* = 8) and 13–15 years age group (*H*, *n* = 9) during static contraction. The dashed horizontal lines denoted the upper 95% confidence levels.

gait may not be fully established until 12 to 15 years of age. The likely reason for this is that different neural pathways mature at different times during development. One important part of this is the development of the corticospinal pathway. White matter density changes in this tract continue until the age of 17 and the neurophysiological properties follow the same trajectory of development i.e. there is maturation of lower extremity muscle response to transcranial magnetic brain stimulation (TMS) (Muller *et al.* 1991; Eluvathingal *et al.* 2007). The most striking evidence of the importance of the corticospinal tract is the fact that patients with damage to the corticospinal tract either within the brain or the spinal cord display varying degrees of gait impairment depending on the extent and location of the lesion. The severity of gait impairment seen in spinal cord damage in adults suggests that once connections from brain to the spinal cord have developed the functional relevance of a central pattern generator is reduced because the system now relies on complex interactions between cortical and spinal activity.

The TA muscle motoneurones receive prominent drive from the corticospinal pathway and damage to this system, for example, in stroke, is clinically evident in the upper motoneurone 'dropped foot syndrome' where the forefoot is lifted poorly during the swing phase. Activity in TA and the role of corticospinal drive is easy to study both through examination of the TMS evoked response during static contraction and walking and through study of the common drive to TA motoneurones. Individual motoneurones fire at frequencies from 8 to 13 Hz; however, their activity is commonly modulated by the cortical oscillations at a frequency of around 20 Hz (beta frequencies). When looking at signals from individual pools of motoneurones it is possible to

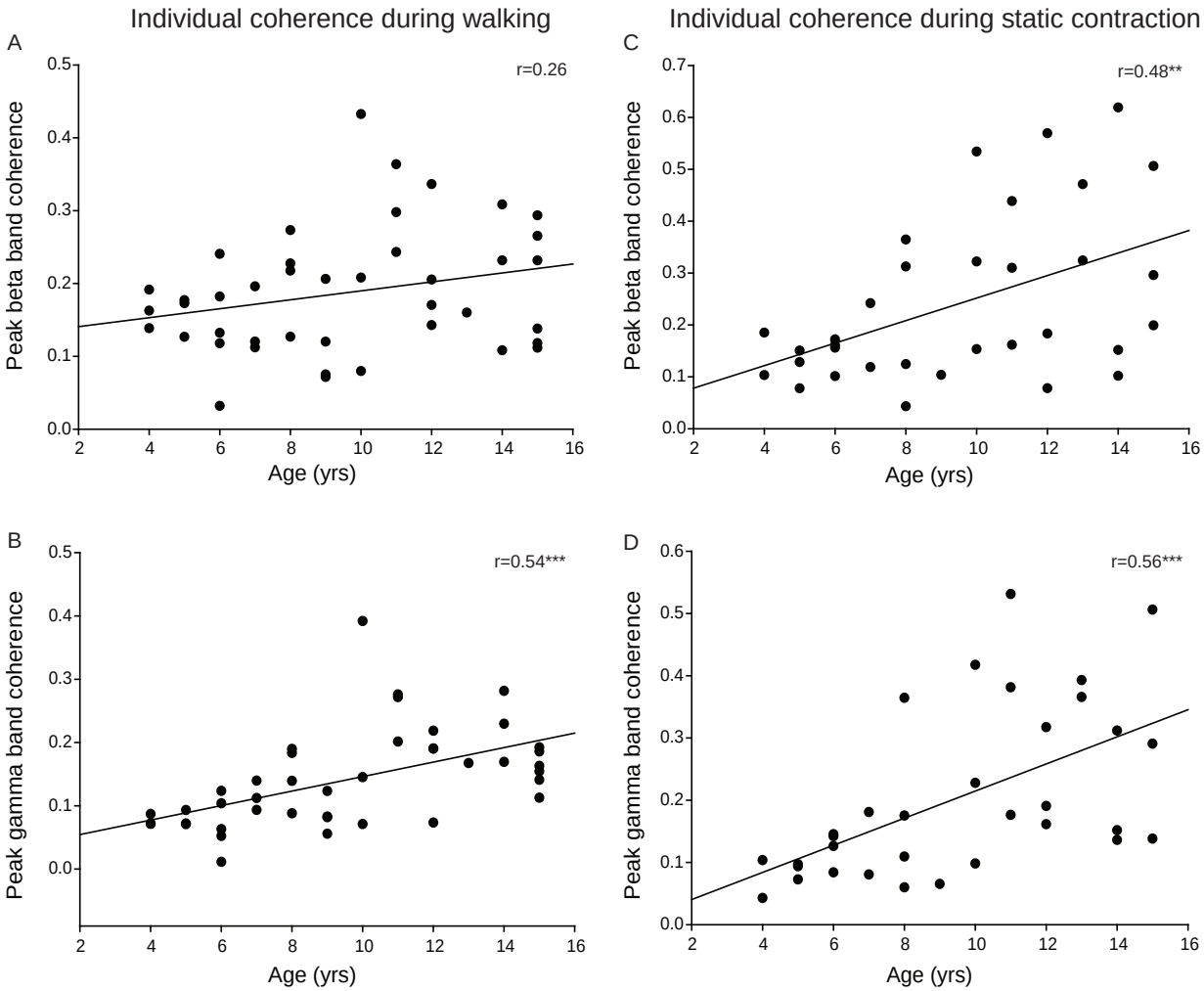


Figure 2. Individual peak coherence estimates plotted against age. Individual subjects' peak beta and gamma band coherence in the walking experiment (*A* and *B*, respectively) and the static contraction experiment (*C* and *D*, respectively) plotted against their age. Statistical analysis showed a significant correlation between age and beta band coherence estimates for the static contraction experiment ($r = 0.48$, $P < 0.01$) but not for the walking experiment ($r = 0.26$, $P = 0.097$). Statistical analysis showed a significant correlation between age and peak gamma band coherence estimates in both the walking experiment ($r = 0.54$, $P < 0.001$) and in the static contraction experiment ($r = 0.56$, $P < 0.001$).

calculate how the signals are correlated in the frequency domain. Coherence analysis provides an estimate of the magnitude of common input to the different pools of motoneurones and during static muscle contraction coherence is present in the ~20 Hz beta band. Beta rhythms are coherent between the EEG/MEG and the EMG, and damage to the corticospinal tract (see, for example, Barthelemy *et al.* 2010) reduces or abolishes the ~20 Hz coherence between pairs of motoneurones and between the EMGs of synergistic muscles indicating that beta band coherence within and between the EMGs of muscles reflects the oscillatory modulation of corticospinal drive to spinal motoneurones

We have recently asked whether the 'corticalization' of muscle control during development can be reflected as an increase in the strength of the ~20 Hz common drive to motoneurones. A developmental increase was recently demonstrated for EMG–EMG and EEG–EMG coherence in upper limb muscles (Farmer *et al.* 2007; James *et al.* 2008). In a new set of experiments (Petersen *et al.* 2010), we investigated the development of oscillations in the corticospinal pathway during human treadmill walking in forty-two children of different ages. Firstly we showed an age-dependent increase in the ~20 Hz oscillatory input to different pools of TA motoneurones during steady muscle contraction (a result analogous to that described for the upper limb) (see Fig. 1*E–H*). More importantly we found strong age effects for activation of TA muscle during treadmill walking. These effects were detected for oscillatory drive at higher frequencies – the 35-40 Hz gamma band which in a number of studies has been associated with important motor cortex network functions and which can also modulate motoneurone firing. In contrast to static muscle contraction in which beta band coherence dominates, during walking the gamma band coherence dominates (Fig. 1*A–D*). The gamma band coherence increase was most obvious when comparing the youngest group of children (aged 4–6) with children aged between 7 and 15 years. Interestingly, individual values of peak coherence in the beta and gamma bands display a linear correlation with age (see Fig. 2). In addition we found a relationship between the strength of gamma band coherence and a measure of the efficacy of TA activation during walking, suggesting that oscillations in this frequency range are helpful in stabilizing the ankle during the swing phase of gait. This behavioural measure matures with increasing age. We argued that this age-related increase in oscillations is related to functional maturation of the corticospinal network involved in controlling the muscles during gait. The functional role of development of beta and gamma oscillations still requires investigation, and direct recordings of EEG–EMG coherence during the development of gait remain to be performed.

Gamma band coherent oscillations are important in processes requiring attention and prediction. Beta band coherence oscillations are important for maintenance of a motor state. The findings that coherent oscillatory drive to TA muscle in beta and gamma ranges changes significantly across childhood leads us to speculate that these frequencies play a crucial role in motor control. Their emergence correlates with the onset of the adult pattern of walking in which the interaction between spinal and cortical circuits becomes more fixed. Seeing how this oscillatory drive changes more precisely at the crucial transition points of motor development and their relation to learning and plasticity will be an interesting topic for further research.

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CO_2 emissions of locomotion: innovative automobiles do better than humans

Four male friends plan to support their favourite soccer team at the stadium located at the opposite side of the town. Would they emit more CO_2 to reach their destination by jogging or by boarding a hybrid car and driving there? Despite the hybrid character of both human and innovative automobile locomotion, modern vehicle technology allows transportation of four passengers (in town) with lower CO_2 emissions than if they were running.

Hybrid locomotion in biology and in mechanical engineering

Hybrid vehicles use different energy forms to move around. Petrol-based cars accelerate mainly by converting fuel combustion into mechanical work. During the inevitable braking frequently occurring in town, though, most of the car kinetic speed is not 'traditionally' dissipated as heat, but it enters the battery after being converted by an electric motor. That motor is successively used to convert electrical energy back into motion thus, human locomotion and hybrid car technology have something in common.

During walking at constant average speed, humans (and other bipeds/quadrupeds) behave like inverted pendula. The inherent constraint with the ground, causing inevitable raising–lowering and acceleration–deceleration of the body centre of mass within every stride, makes the pendulum an effective strategy to continuously exchange (part of the) body potential energy with kinetic energy. This maintains almost 60% of mechanical energy inside the body, and leaves the remaining 40% to be supplied by the propelling muscles (Cavagna *et al*. 1963).

Also, the walking cost of transport shows low sensitivity to greater inter-strides speed changes. The mechanics of more ample, multi-stride accelerative–decelerative oscillations can be seen as the cumulative, separate sums of

Alberto Minetti and Gaspare Pavei

single-step kinetic energy increases and decreases, respectively (Fig. 1*A*), which can occur at no extra cost with respect to the same 'constant' speed, equal-strides walking (Minetti *et al*. 2001). This seems to happen for controlled oscillating cycles of 6 seconds, up to ±40% sinusoidal change of walking speed.

Human running (and legged terrestrial animals trotting/galloping) uses another strategy to keep CO_2 emission low. During the first half of the contact phase, part of the inevitable mechanical potential and kinetic energy decrease is temporarily stored into elastic structures, tendons, which release it to power the next take-off, again reducing the work muscles have to provide (Fig. 1*B*).

Thus, both walking and running dynamics incorporate a sort of regenerative braking, the same strategy of the most modern fuel-saving cars.

Further analogies include the efficiency of muscles (Woledge *et al*. 1985) and internal combustion engines (Vogel, 1998), about 25–28%, which makes the production of heat and the related water vapour (the most relevant determinant of the greenhouse effect) similar in the two 'motors'. Tendons and electric motors, namely the hybrid parts in the two 'vehicles', also share similar efficiencies (95 and 88%, respectively).

From the metabolic cost of transport to CO_2 emission

The carbon dioxide emission of a 'standard' male runner can be

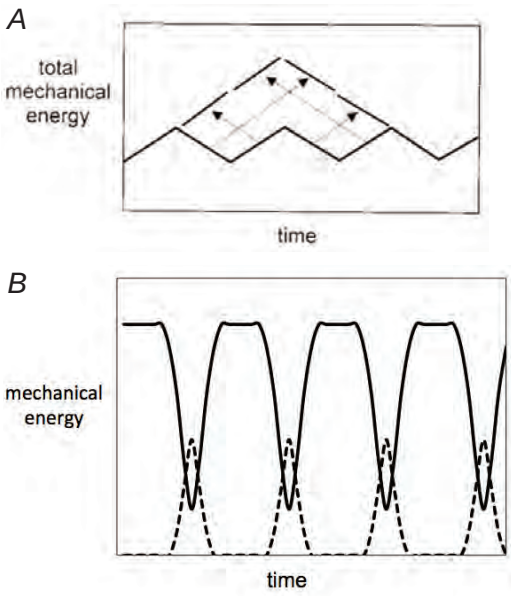

Figure 1. *A*, a single walking stride is here represented as a raise followed by a decrease of the total mechanical energy of the body centre of mass. In a series of consecutive 'constant' speed strides the total energy changes are mechanically (and metabolically) equivalent to longer accelerative/decelerative sequences, as illustrated by adding all the positive and the negative changes together. *B*, in running, part of the mechanical energy of the body centre of mass (on the ordinate) during landing is stored, (almost) free of charge, into the elastic proteins of tendons (dashed curve), for it to be released as kinetic + potential energy before taking off. This corresponds to a sort of regenerative braking of human locomotion, very similarly to modern hybrid cars.

calculated by knowing his metabolic cost of transport. Experimental measures (di Prampero, 1986) and theoretical models (Minetti, 2004) analysing world records in the distance range of 1 km to the marathon (42.2 km), usually achieved by athletes of about 65 kg mass, lead to a value of about 270 J per metre travelled, independent of the running speed. This has been inferred (Minetti, 2004) by assuming a maximum aerobic metabolic power of 1.9 kW and realistic values of anaerobic energy stores (21 kJ). When we correct the cost of running according to a mass of 75 kg, with a respiratory quotient of 0.9 (corresponding to 80% of the maximum metabolic power sustainable for 2–3 hours) and, consequently, a unit conversion of 20.6 J = 1 ml O_2, the production of carbon dioxide is calculated as 13.7 ml CO_2 m^{-1}. By considering that 1 mol CO_2 (= 44 g) occupies 24,056 ml at standard barometric pressure and at an ambient temperature of 20ºC, the emission of a 'standard' male runner is 25.1 g CO_2 km^{-1}. In order to compare human to car emission we need to consider four runners, a total of 100 g CO_2 km^{-1} (a reference value we named ECO_2R).

CO_2 emission per unit distance in fuel-saving automobiles

The most fuel-efficient car available at present produces about 87 g CO_2 km^{-1}, but the reported value refers: (1) to a 'combined cycle', which is less expensive than the 'urban cycle', and (2) to transporting a load of 180 kg, corresponding to 2.4 passengers. When those data are corrected according to 'urban' cycle and 4 passengers, only hybrid car emission is lower than ECO_2R (i.e. the 4 runners, see Fig. 2). Their superior economy with respect to competitors (e.g. diesel vehicles) is based on the very low correction factor for the 'urban' cycle, since the regenerative brakes and the 'Stop&Go' expedient make hybrid car economy less sensitive to the frequent speed changes occurring in town. Today there are about a dozen models on the market and for the most fuel saving of these, the emission is about 95 g CO_2 km^{-1}.

Humans *vs.* automobiles

It seems that we have hit a wall: four passengers in a hybrid car produce less CO_2 per unit distance travelled in town than if they all were running. A similar rationale brought David Miller (2008) in this magazine to conclude that running was still marginally less polluting than driving (he used the value of 150 g CO_2 km^{-1} for cars moving at 100 km h^{-1} on a motorway). It seems a 'physiological' coincidence that one of the EU's goals in terms of containment of pollution is to have automobiles producing just below ECO_2R (95 g CO_2 km^{-1}) by the year 2020.

Two other interesting limits could be approached in the future, namely four walkers and four cyclists, who are expected to aerobically produce 50 g CO_2 km^{-1} (ECO_2W, at 5.5 km h^{-1}) and 25 g CO_2 km^{-1} (ECO_2B, at 30 km h^{-1} on race bikes), respectively (Fig. 2). It is likely that at least ECO_2W is within reach by considering that, at the Shell Eco-Marathon 2010 (www.shell.com/home/content/ecomarathon/europe/), an UrbanConcept petrol-driven car designed at the Lycee Louis Delage, Cognac, France, reached a distance of 303 km with 1 litre of fuel (31 g CO_2 km^{-1} when calculated for 4 one-passenger vehicles). In the same event the Polyjoule team from Polytech Nantes University achieved an economy record of 4,896 km travelled with (the equivalent energy from their hydrogen fuel cell) 1 litre of gasoline, corresponding to about 2.8 g CO_2 km^{-1} (calculated for 4 one-passenger vehicles).

Despite all the strategies mentioned to contain the metabolic cost of human locomotion, wheels are wheels are wheels. Our mode of being in contact with the ground forces continuous raise–descent, acceleration–deceleration sequences of the body centre of mass, inherently requiring a significant amount of work, and thus quite a high metabolic energy. However, even after having invented and refined the bicycle, which avoids all those troubles, we are now beaten by (prototypal) technology 9 to 1.

We have mentioned only males, so far, for a reason. The lower average body mass (~10 kg) in women decreases the load factor of cars less than the metabolic cost of running. Four 'standard' females

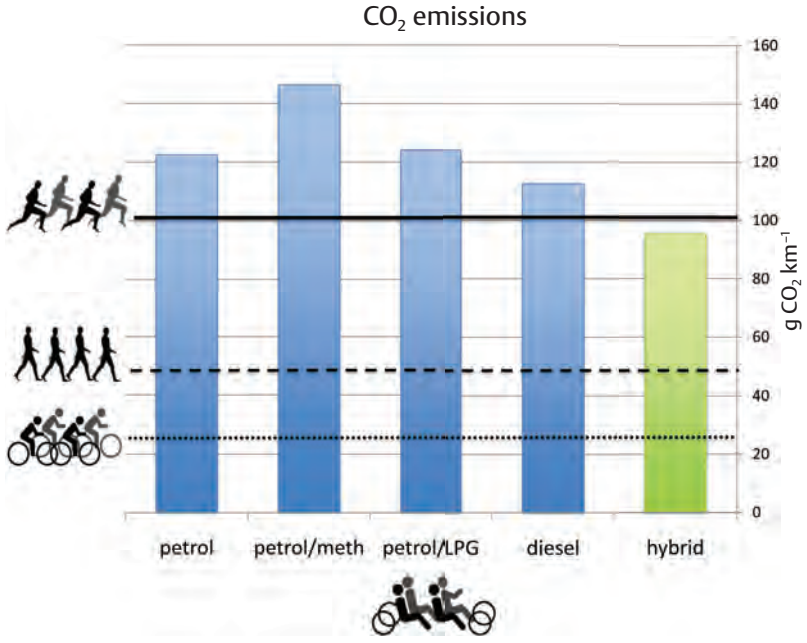

Figure 2. Bars represent CO_2 emission per unit distance of the most fuel-saving cars in each engine category, during urban use and full occupancy (4 passengers). Horizontal lines show the emission of 4 runners (at whichever aerobic speed), 4 walkers (at 5.5 km h^{-1}) and 4 cyclists on race bikes (at the constant speed of 30 km h^{-1}). 52 commercial vehicles have been included in the analysis.

still produce during running less CO_2 than a four-passenger car, independently of the automotive technology. However, the same 'green' attitude of four males in a hybrid car is obtained by extending the female group to five (passengers and runners).

Conclusions

Vehicle science is currently facing a turning point where automobiles, despite their large size, reaching a transport economy challenging our natural gaits (electric-only vehicles already beat ECO_2W, although probably due to the lower cost of producing electricity). Arguments against these considerations include air pollution and the non-renewable character of fossil fuels, when compared to foodstuff. However, the cost of processing and delivering foodstuff should also be considered: for the same amount of provided energy, olive oil, for example, ends up being much more expensive (200–300%) than automotive fuels.

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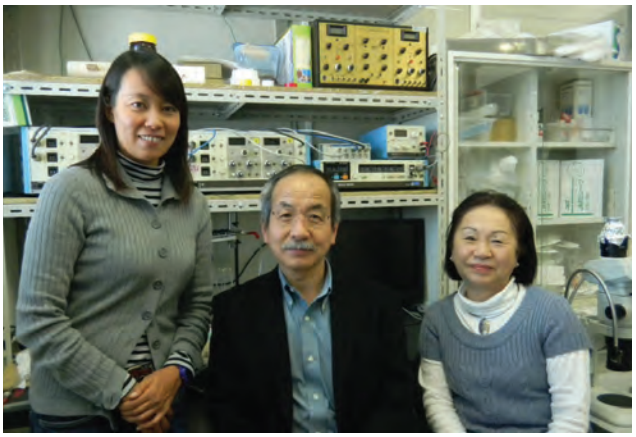
A novel feature of epithelial acetylcholine in intestinal luminal sensing

Textbooks describe acetylcholine (ACh) as a neurotransmitter that is released from cholinergic motor nerves in response to luminal stimuli to control gut motility and ion transport. In a new approach we demonstrated that ACh is synthesised in colonic epithelial cells and released on the basolateral side by luminal chemo-stimulation, which causes chloride secretion.



From left: Megumi Matsumoto, Takaji Yajima, Masako Yajima and Ryo Inoue.

Acetylcholine (ACh) is the best characterised neurotransmitter and is synthesised and stored in cholinergic neurons in the brain and gut wall. ACh synthesis is catalysed by choline acetyltransferase (ChAT) via the enzymatic conversion of acetyl-CoA and choline to ACh and CoA. Choline, the limiting substrate for ACh synthesis, is taken up by neurons by the high-affinity choline transporter 1 (CHT1). Synthesised ACh is stored in synaptic vesicles by the vesicular acetylcholine transporter, and is released from nerve terminals by membrane action potentials in response to physiological and pharmacological stimuli.

The gut wall is richly innervated with cholinergic neurons, and ACh is a predominant neurotransmitter of the enteric nervous system (Harrington *et al.* 2010). When the cholinergic motor neurons are activated via an enteric reflex by luminal stimuli (Fig. 1*A*), ACh is released at the neuro-enterocyte junction. Epithelial cells express many subtypes of muscarinic ACh receptors, which are involved in the reflex secretory response to mechanical and chemical signals from the luminal side of the intestine (Raybould *et al.* 2004). Stimulating muscarinic ACh receptors induces mucosal hydration due to chloride secretion from the crypt cells and inhibition of sodium absorption from the apical cells, and stimulates mucus secretion from the goblet cells. Thus, intestinal cholinergic regulation plays a role in providing surface lubrication to propel luminal contents aborally or for flushing out harmful microbes and toxins.

It is also noteworthy that ACh is synthesised and stored in a broad variety of non-neuronal cells, particularly in surface epithelia of the airway, bladder, placenta and skin of rodents and humans (Wessler & Kirkpatrick, 2008). Synthesis and storage of ACh in non-neuronal cells has been confirmed not only by positive anti-ChAT immunoreactivity but also by direct measurements with high performance liquid chromatography combined with a post column enzyme reactor and an electrochemical detector. ACh is not stored in vesicles of non-neuronal cells and its release

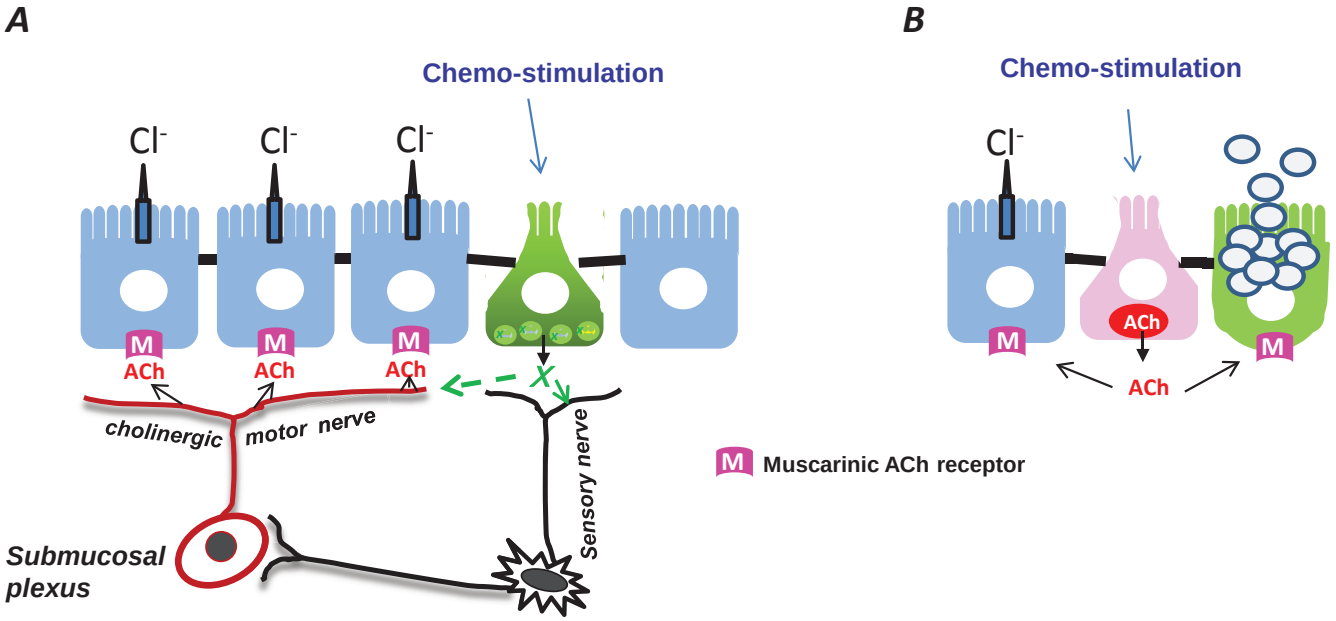


Figure 1. Schematic diagram showing the neuronal and non-neuronal mechanisms of the cholinergic control of chloride secretion. *A*, in the neuronal mechanism acetylcholine (ACh) is released from cholinergic motor nerve terminals projecting beneath epithelial cells in response to luminal chemical stimulation via mucosal sensing and a signal substance (*X*) from an endocrine cell followed by a neuronal enteric reflex. The *X*, otherwise, depolarises the nerve terminal and releases ACh. *B*, novel cholinergic control of chloride secretion is mediated by ACh release from epithelial cells in response to luminal chemical stimulation. ACh released at the basolateral side stimulates muscarinic receptors on neighbouring cells through a paracrine action of ACh.

is mediated by organic cation transporters (OCTs). Therefore, ACh release from non-neuronal cells is much slower than that from nerve terminals. Non-neuronal ACh also occurs in the epithelial cells of the small and large intestines of rats and humans (Klapproth *et al.* 1997). However, little progress has been made regarding the physiological significance of the synthesis and release of non-neuronal ACh into the intestine.

Previous studies have demonstrated that luminal short chain fatty acids (SCFAs) are major colonic microbial fermentation products and stimulate chloride secretion in the rat distal colon *in vitro* (Yajima, 1988). SCFA-stimulated chloride secretion is induced even under a nerve block by tetrodotoxin but is inhibited by atropine, a strong cholinergic receptor antagonist. Therefore, ACh may be released from cholinergic nerve terminals depolarised by an unknown signal substance (Fig. 1*A*) or released non-neuronally from colonic epithelial cells (Yajima, 1988; Hubel & Russ, 1993). In a recent study reported in *The Journal of Physiology*, we addressed the involvement of non-neuronal ACh in the secretory response to SCFA in rat colon (Yajima *et al.* 2011). Using epithelial sheets mounted on Ussing chambers that did not include the enteric nerve plexuses, we showed that the luminal stimulation by the SCFA, propionate, caused ACh release into the serosal side (Fig. 2). A significant linear relationship was observed between ACh release and chloride secretion, as indicated by

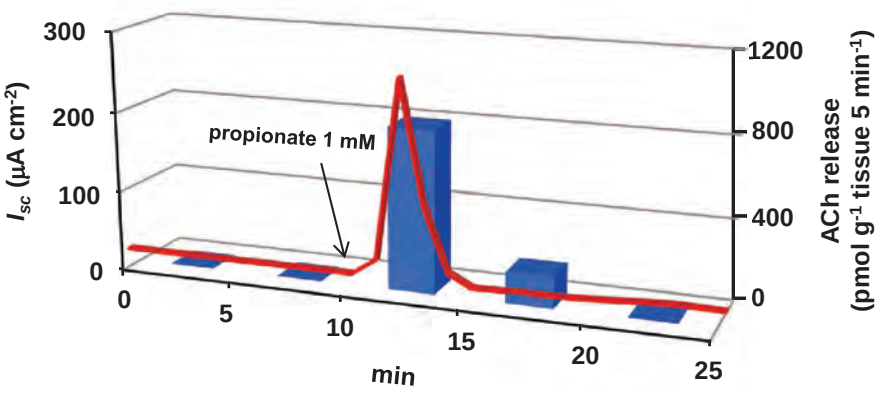


Figure 2. Acetylcholine (ACh) release induced by the luminal short chain fatty acid, propionate, in epithelial sheets, without the enteric nerves, mounted on Ussing chambers in the presence of tetrodotoxin and the acetylcholinesterase inhibitor, eserine. Red line shows the secretory response, which is shown by the changes in short-circuit current (I_{sc}) plotted as mean value per minute. Blue bars indicate ACh release into the serosal fluid of the Ussing chambers, which is plotted as mean value per 5 min. Propionate (1 mM) was added to the mucosal fluid 10 min after starting the experiment.

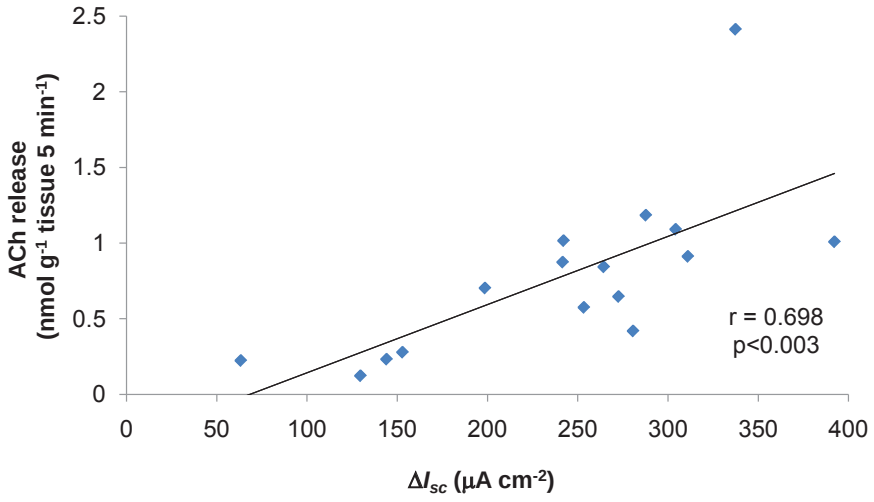


Figure 3. Relationship between acetylcholine (ACh) release and secretory response (indicated by changes in short-circuit current (I_{sc}) induced by luminal short-chain fatty acid, 1 mM propionate. A linear regression analysis was performed based on the difference between the amount of ACh released in 5 min and the maximal increase in I_{sc} induced by luminal propionate.

changes in short-circuit current (Fig. 3), suggesting that the release of ACh occurred non-neuronally and then caused chloride secretion by stimulating epithelial muscarinic receptors. These results were confirmed in an experiment showing that a muscarinic receptor antagonist and an inhibitor of chloride secretion inhibited chloride secretion but not ACh release.

To confirm the non-neuronal origin of ACh release in response to luminal propionate stimulation, we employed a method of crypt isolation from colonic segments to obtain neural components free of epithelial cells. We demonstrated the ChAT gene mRNA expression as well as the storage of a considerable amount of ACh compared to residual muscle tissues including the enteric nerves. Gene expression of OCTs but not CHT1 indicated that colonic epithelial cells use OCTs rather than CHT1 to take up choline. Although colonic epithelial cells store ACh, the amount is approximately three orders of magnitude less than that in neuronal tissues, rat brain and the myenteric plexus of the distal colon of guinea-pig.

ACh release from non-neuronal epithelial cells was reported for the first time in human placenta. Interestingly, the amount of ACh released from human placenta is comparable to that observed during propionate-induced ACh release from rat colonic epithelial cells. However, ACh release occurred spontaneously in the placenta epithelial cells without any stimulation, which was not the case in colonic epithelial cells. To address the physiological role of non-neuronal ACh release from epithelium, it is important to understand whether ACh is released into the luminal or serosal side. However, previous studies have not revealed the direction of ACh release from epithelial cells of the placenta or bladder. Our study clearly demonstrated that the ACh release coupled with luminal propionate stimulation occurred on the serosal side, but not the luminal side in the rat colon.

Non-neuronal ACh is expected to act as a local signalling or trophic molecule (Klapproth *et al.* 1997). Our study demonstrated that cholinergic colonic ion transport is regulated not only by neuronal but also by non-neuronal mechanisms. Non-neuronally released ACh in response to luminal stimulation acts as local signalling for the muscarinic receptors of neighbouring epithelial cells following chloride secretion as well as mucin secretion (Fig. 1*B*). However, many questions arise, such as what kind of cells store ACh, how is the chemo-signal received, how is ACh released from the stored cells, and what other physiological roles does non-neuronally released ACh play in addition to the secretory response? We expect future studies to answer these questions.

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Surviving life in the womb and the implications for vascular health in adulthood

The early environment of the fetus can shape its risk for cardiovascular disease in adulthood. Females, by and large, fare better than males following these early life insults. However, changes in the function of the uterine vessels may predispose to pregnancy complications and perpetuation of cardiovascular disease risk in the next generation.

The womb is not necessarily a cosy and nurturing environment, as one might expect. Although the fetus may be endowed with genes bestowing physiological fortitude and resilience, the environment in the womb can sculpt the physiological phenotype and ultimately influence the risk of disease. The early studies by McCance and Widdowson in the 1960s–1970s laid the scientific foundations that life *in utero* can influence, for better or for worse, the ability of the offspring to achieve its full genetic potential after birth (McCance & Widdowson, 1974; Bateson, 2001). In the 1980s the seminal work of Barker and colleagues linked slowed human fetal growth to an increased risk of cardiovascular and metabolic disease in adulthood (Barker, 1995), and this was the catalyst for an intensive research focus into what is now known as the 'developmental origins of health and disease' hypothesis.

From top left clockwise: Marianne Tare, Marc Mazzuca, Helena Parkington, Nicoleta Dragomir and Mary Wlodek.

Life in the womb can be a struggle, especially when faced with an inhospitable environment. In response to adverse conditions, the fetus is able to modify its development to optimise its survival in the womb. This developmental plasticity of the fetus enables preservation of life in the short term, but may have unfavourable consequences for long-term health in postnatal life. During fetal life, the organs, vasculature and body systems undergo 'critical periods' of development. Disturbances to the environment during these sensitive periods can induce permanent changes in the structure, function and physiological set-points of organs and body systems. External environmental cues sensed by the mother and transduced to the fetus via the placenta, such as quality and quantity of nutrition and oxygen, together with internal cues arising from the mother such as hormones, nutrients and the function of the placenta, can induce developmental and functional adaptations in the fetus. Myriad epidemiological and experimental studies indicate that events occurring around the time of conception, through to pregnancy and early postnatal life, can sculpt the phenotype of the offspring.

Cardiovascular disease is one of the leading causes of death worldwide. Apart from genetic predisposition and traditional risk factors such as obesity, high blood pressure and smoking, it is now recognised that the quality of very early life is an important factor that can also influence cardiovascular disease risk. Research into the developmental origins of health and disease has demonstrated that factors such as the quality and quantity of maternal nutrition, hormonal status of the mother, stress, placental function and oxygen delivery to the fetus have implications for vascular structure and function and blood pressure in the offspring. The wealth of data emerging from experimental studies indicates that, more often than not, there are sex differences in the expression and severity of vascular dysfunction and blood pressure (Grigore *et al*. 2008). Female offspring tend to fare better than males in response to an early life insult. Furthermore, the raised blood pressure in females seen in some models is resolved after puberty. Whether there are differences in the 'critical periods' of development *in utero* or the 'sensitivity' to the insult for males and females has yet to be elucidated. However, sex hormones appear to modulate the expression of the cardiovascular phenotype developed in response to early life insults.

Figure 1. Effect of late gestation uteroplacental insufficiency on offspring weight at postnatal day 1 (*A*) and mean arterial pressure (*B*) in male and female rat offspring. *A*, body weight (g) at postnatal day 1 in male (n = 9 per group) and female (n = 10 per group) control and restricted offspring. *B*, mean arterial pressure in 5-month-old males (n = 7 per group) and 18-month-old females (n = 5 per group). Values are mean ± SEM. $^*P < 0.05$ between control and restricted offspring.

Uteroplacental insufficiency rather than maternal undernutrition is a common cause of intrauterine growth restriction in Western societies. Endothelial and smooth muscle function and arterial mechanical properties are important regulators of vascular function. Humans of low birth weight tend to have impaired endothelial vasodilator function and increased stiffening of the arteries, risk factors for cardiovascular disease (Norman, 2008). In our recent study we used a model of late gestation uteroplacental insufficiency by bilateral uterine artery and vein ligation in rats. This reduces the supply of nutrients and oxygen to the fetus. This model results in fetal growth restriction of 10–15% (Fig. 1*A*) without the confounding influence of maternal hypertension. We examined the effect on blood pressure and vascular function in adult female offspring. Previous studies of this model in our laboratory demonstrated that adult male growth-restricted offspring had raised blood pressure, reduced nephron endowment, reduced vasodilator capacity and increased arterial stiffness. The adult growth-restricted females also had a reduced nephron endowment, but were found to be normotensive (Fig. 1*B*) and had normal vascular function in the major resistance beds. The low nephron number alone was not sufficient to cause an elevation in blood pressure. However, closer investigation revealed that there was dysfunction in the uterine artery of growth-restricted females. Endothelial vasodilator function mediated by endothelium-derived hyperpolarizing factor (EDHF) was impaired (Fig. 2) (Mazzuca *et al.* 2010). EDHF is an important vasodilator mechanism in small arteries and arterioles. The stiffness of the uterine artery wall was also increased in growth-restricted females and this was associated with an increased proportion of thick, less compliant collagen fibres (Fig. 3). Importantly, vascular dysfunction was localised to the uterine artery and was not evident in the major resistance beds as in the males, and this probably contributes to the lack of hypertension in the females.

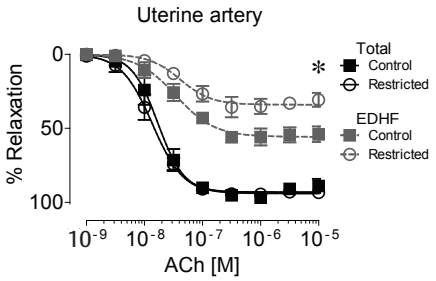

Figure 2. Dysfunction of endothelium-derived relaxing factor (EDHF)-mediated relaxation in uterine arteries of adult growth-restricted females. Total: endothelium-dependent relaxation evoked by acetylcholine (ACh) in the absence of blockers. The response to EDHF was revealed after blockade of nitric oxide and prostanoid production. Values are mean ± SEM, n = 5 per group. $^*P < 0.05$ between control and restricted offspring for maximal EDHF-mediated relaxation. (Taken from Mazzuca *et al.* 2010.)

Upregulation of endothelial vasodilator factors in the uterine artery, including EDHF, is part of the natural adaptation that takes place in this vascular bed in response to pregnancy, to ensure adequate perfusion of the fetus. Inappropriate adaptation of the uterine vasculature in pregnancy is associated with compromised uteroplacental

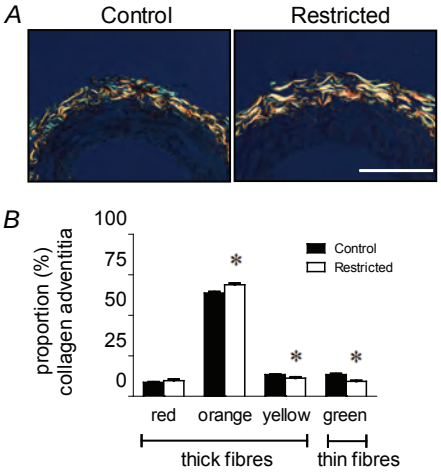

Figure 3. Thick and thin collagen fibres are more abundant in the adventitia of uterine arteries from adult growth-restricted females. *A*, sections of uterine artery stained with picrosirius red and viewed under cross-polarized light to reveal birefringent collagen fibre thickness. *B*, proportion of thick (red, orange, yellow) and thin (green) fibres in the adventitia of arteries from control and restricted female offspring. Values are mean ± SEM, n = 4 per group. $^*P < 0.05$ between control and restricted offspring. Scale bar, 100 μm. (Taken from Mazzuca *et al.* 2010.)

blood flow, growth restriction and pregnancy complications including maternal hypertension. Indeed, emerging evidence in the literature in humans and from our own laboratory studies in rats indicates that females born with low birth weights may have a higher risk of complications during pregnancy.

Research in the developmental origins of health and disease area has drawn attention to the critical role of early life environments in determining the cardiovascular and metabolic phenotype. It has also highlighted the complexity of the cardiovascular phenotype, and the reality that truly effective interventions in adults may need to be tailored according to the nature of the early life insult.

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Western blotting. Part III

In the last of this series, Patricia Leoni discusses the process of transferring electropheresed proteins to a membrane (the 'blot') and the use of antibodies to identify proteins of interest from the many that present on the membrane.

Patricia Leoni

The separation of proteins by electrophoresis on polyacrylamide gels does not provide any information on their identity. The proteins can be recovered from the gel for chemical characterization using a combination of liquid chromatography and mass spectrometry. However, this is a costly and laborious technique useful mostly when trying to identify proteins that are completely unknown or for accurate quantification.

The use of antibodies provides a very sensitive tool to identify the presence and relative proportion of specific proteins but the detection of proteins directly in the gel presents several problems due to diffusion. In order to overcome these, proteins can be electrophoretically transferred from the gel to a thin membrane, where they are immobilized. This procedure, known as Western blotting, has been adapted by Towbin *et al*. (1) from the technique developed by Southern (2) for the transfer of DNA (Table 1).

It should be noted that while for SDS-PAGE electrophoresis standard conditions recommended by equipment manufacturers are quite adequate for most protein extracts, Western blotting can require careful optimization. For proteins of medium to high abundance and not too low molecular weight, almost any condition will work, while proteins of low abundance and/or low or very high molecular weight require much more rigorous empirical testing.

Equipment

A wide variety of electroblotting equipment is commercially available, but there are only two basic designs. In both, the transfer occurs by applying an electric field perpendicular to the plane of the gel. The main difference between them is the cost of the equipment and the time required for the transfer.

Table 1

Steps involved in Western blotting	Conditions to be optimised
Polyacrylamide gel with separated proteins	
Equilibration of gel	Choice of transfer buffer, methanol and SDS content
Membrane staining	Choice of reversible stain
Electrophoretic transfer to membrane	Type of equipment and membrane, transfer conditions
Blocking of free binding sites	Choice of blocking agent, concentration of Tween-20
Incubation with primary antibody	Antibody dilution, time and temperature of binding
Washes	Number and length of washes
Incubation with secondary antibody	Manufacturer's recommendation
Washes	Number and length of washes
Detection	Choice of substrate

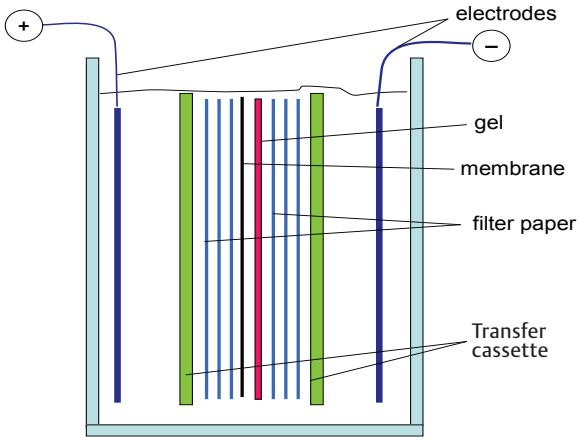

Figure 1. Diagram of a vertical buffer tank.

Vertical buffer tanks

The polyacrylamide gel containing the separated proteins is placed next to a membrane in a cassette and suspended in a tank containing buffer between two electrodes (Fig. 1).

As the electrodes are several centimetres apart the voltage gradient that can be applied should not exceed 5 V cm^{-1} to avoid overheating; refrigeration is needed when applying currents of 200 mA or more.

Semi-dry blotting systems

These use buffer-wetted filter paper instead of buffer, in close proximity with flat-plate electrodes (Fig. 2).

In this system, much higher field strengths can be achieved with lower current and cooling is not required. The maximum applied current is 0.8 mA cm^{-2} and the transfer takes 1–2 hours.

Membranes

The most commonly used membranes for protein transfer are nitrocellulose and polyvinylidene fluoride (PVDF). Both membranes are available with pore sizes of 0.2 and 0.45 µm. The larger pore size is used for most transfers, while the 0.2 µm pore is more efficient for low molecular weight proteins.

Proteins are thought to attach to the membranes by a combination of hydrophobic and electrostatic interaction. The binding capacity of nitrocellulose is 80–100 µg cm^2 while PVDF membranes can bind 100–200 µg cm^2 and have superior mechanical strength, chemical resistance and protein detection.

As a drawback, PDVF membranes are hydrophobic and will not wet-out in aqueous buffers. They must be wet first in a solution of 50% or more of ethanol, methanol or isopropanol, then rinsed in water and finally equilibrated with buffer.

Transfer buffers

Transfer buffers must be conductive and maintain the solubility of the proteins without interfering with the adsorption of the proteins to the membrane. Traditionally they consist of a buffer of pH higher than the isoelectric point of most of the proteins in the extract, and methanol. The most common formulations are: 25 mM Tris, 192 mM glycine, methanol 10–20% v/v, pH 8.3 and 48 mM Tris, 39 mM glycine, methanol 10–20% v/v, pH 9.2.

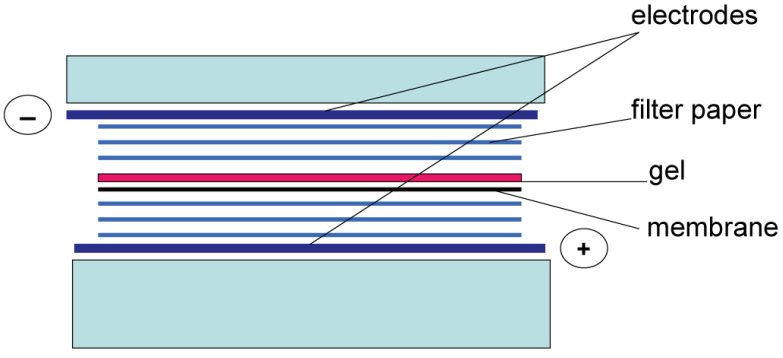

Figure 2. Diagram of a semi-dry system.

Methanol in a concentration of 10 to 20% stabilizes the dimension of the gel and strips SDS from the protein molecules.

Gels swell during the run and this could affect the resolution of the proteins. As swelling depends on polyacrylamide concentration, gradient gels expand unequally, acquiring a trapezoid shape.

On the other hand, proteins of high molecular weight can have limited solubility in the presence of methanol. If the protein of interest is more than 100 kD, the concentration of methanol should be lowered to 5% or even removed completely.

For routine blotting, excess SDS should be removed from the gel by equilibrating it with the transfer buffer for 5–10 minutes. SDS interferes with the ability of the protein to bind to the membrane; this affects particularly low molecular weight proteins. Also, SDS bound to the protein causes it to migrate faster through the membrane, not allowing enough contact time for the protein to adsorb to it. However, when trying to transfer very hydrophobic proteins, like protein from cell membranes, it is worth considering adding a very small amount of SDS (0.05%) as these proteins may precipitate in the gel in the absence of SDS.

Staining

Once the transfer has finished it is important to stain the gel with coomassie brilliant blue to make sure that the transfer has been complete. The fact that your pre-stained molecular weight markers have transferred to the membrane does not necessarily mean that all

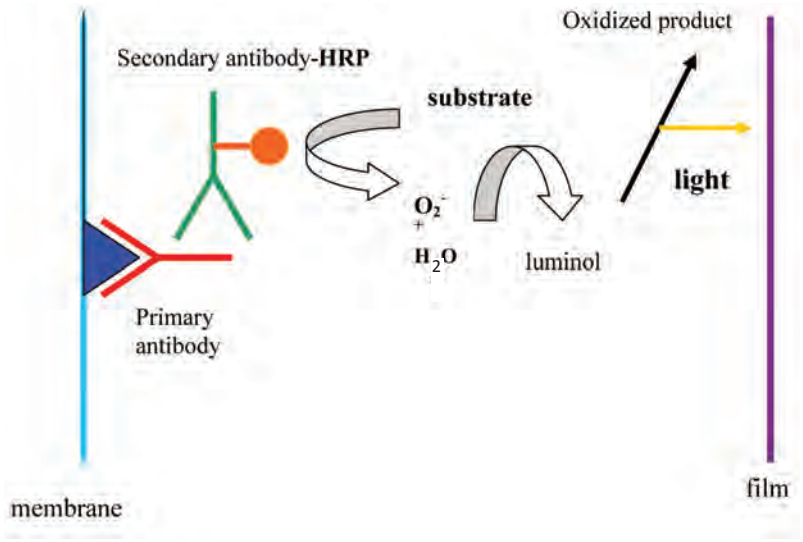

Figure 3. Mechanism of protein detection using chemoluminescence.

the proteins in your extract have, particularly the high molecular weight ones. In order to monitor the quality and efficiency of the transfer, the membrane should also be stained. There are several reversible stains that are compatible with immunodetection: Ponceau S red, Fast green FC and 3,4',4",4-copper phthalocyanine tetrasulfonic acid and tetrasodium salt (CPTS) are not very sensitive (1–5 mg protein per band) but are very fast and perfectly adequate for checking transfer homogeneity and loading. Spyro Ruby and Spyro Rose (Invitrogen) are highly sensitive fluorescent stains (1–2 ng per band). Reversible membrane protein staining kits of proprietary formulation are available from Thermo Scientific (Pierce) and Invitrogen

Blocking

After the transfer of proteins from the gel to the membrane it is essential to block the unoccupied surface in order to avoid unspecific binding of the antibodies used for protein detection. Blocking can be achieved using a variety of proteins and non-ionic detergent solutions, but no blocking agent is ideal for every occasion, so more than one should be tested to obtain optimum results. The most common blocking agents are bovine serum albumin, non-fat milk, casein, gelatin and dilute solutions of Tween-20 (0.05–0.1%). Buffers used to prepare blocking solutions should have ionic strength and pH as close as possible to physiological conditions; phosphate-buffered (PBS) or Tris-buffered (TBS) saline are the most commonly used.

Using the wrong blocking agent or wrong blocking conditions can obscure the protein of interest; some contain products that cause a high background. For instance, milk contains biotin and glycoproteins; as a consequence neither biotinylated nor lectin-bound antibodies can be used.

Blocking buffers of proprietary formulations are commercially available, like Casein blocking buffer (Sigma and Thermo Scientific), SuperBlock (Pierce), Pierce Protein-Free blocking buffer (Pierce) and ECL blocking agent (GE Healthcare). PhosphoBLOCKER was specifically developed for blocking membranes prior to detection of phosphoproteins by Cell Biolabs, to enhance phosphoprotein signal without increasing background. However, even manufactures show contradictory results when extolling the virtues of their blocking agents, so it is important to determine empirically which one is the best for the protein of interest.

Protein detection

Detection of a specific protein immobilized on a membrane with an antibody is called immunodetection. The antibody that binds to the protein of interest is the primary antibody. Once the primary antibody has been allowed to bind to the target protein, the membrane is washed and incubated with a secondary antibody conjugated to an enzyme that will pinpoint the location of the protein. The secondary antibody is raised against immunoglobulins of the animal species used to raise the primary antibody. Antibodies are diluted in blocking solution to avoid unspecific binding to the membrane. The presence of Tween-20 in the diluent prevents aggregation of the antibodies. It is important that the concentration of Tween-20 does not exceed 0.05% (v/v) as it has the potential to remove a proportion of the protein from the membrane.

The dilutions of primary and secondary antibody must be optimised for each target protein. A non-specific signal can be avoided with higher dilution of the primary antibody; high background can be minimized by higher dilution of the secondary antibody.

Washing of the membrane is necessary to remove unbound antibodies: too little washing will lead to a high background and too much washing can elute the antibodies. Washes are done in PBS or TBS containing 0.02–0.1% Tween-20. The right number and length of washes must be determined experimentally, starting with 3 washes of 5 minutes.

Detection

The most sensitive detection methods are based on an enzymatic reaction, using secondary antibodies bound to enzymes like horseradish peroxidise (HRP) or alkaline phosphatase (AP), β-galactosidase and glucose oxidase. The activity of these enzymes can be detected with chromogenic, chemoluminescent and fluorescent substrates.

In chromogenic detection the enzyme's reaction results in an insoluble coloured precipitate. WesterBreeze Chromogenic (Invitrogen), Amplified AP (Bio-Rad)

and Immun-Bolt BCIP/NBT (Bio-Rad) are some of the kits commercially available for chromogenic detection. However, this type of detection is not very sensitive, requiring 1–2 ng per protein per band.

In chemoluminescent detection (Fig. 3) the enzyme catalyses a reaction that results in the production of light which is detected on a film; the sensitivity is at least 10 times higher than the chromogenic method, making it the method of choice.

Several chemoluminescent detection products are available from Thermo Scientific, Bio Rad and GE Healthcare, all equally suitable. However, there are products that claim to detect proteins in the low femtogram level that are probably very effective, but tend to give high backgrounds and require laborious optimization in order to get the right noise-to-signal ratio. ECL and ECL plus from GE Healthcare give very good signals, adequate for most circumstances. The only drawback of this method is that the signal is not permanent and fades after a few minutes. The reaction can be repeated if needs be, but the background tends to increase.

Fluorescence detection uses a fluorogenic substrate that fluoresces at the site of the enzyme. This method is less sensitive than the chemoluminescent but it has the advantage that the signal is stable indefinitely so blots can be re-imaged. Invitrogen offers two products for fluorescent staining: DyeChrome which has two different fluorescent products, one yellow and one red, which allow the detection of two proteins at the same time and Ampex Gold, which produces bright yellow spots. They can be detected and recorded with a UV epi-illuminator.

Stripping

Once one of the proteins has been identified, the membrane can be stripped and re-probed with a different antibody. However, during stripping some of the proteins on the membrane can be partially removed so it is not always successful.

The most common methods for stripping are: 2% SDS, 100 mM β-mercaptoethanol, 50 mM Tris, pH 6.8. The membrane is incubated at 50ºC for 15–30 minutes and rinsed several times in TBS.

A milder stripping buffer is 0.1 M glycine HCl (pH 2.5–3.0). This buffer will dissociate most antibody–antigen interactions in less than 30 minutes at room temperature or 37ºC.

A simpler and very effective approach is to re-probe the membrane without stripping; two or three antibodies can be used sequentially, starting with the one that gives the cleanest background. Ideally, the proteins to be detected using this method should have relatively different molecular weights but there are instances where two proteins with very similar molecular weights can be detected sequentially providing one of them gives a stronger signal. For instance, connective tissue growth factor (CTGF, MW 38,000) and glyceraldehyde-3-phosphate dehydrogenase (MW 36,000) can be detected in this way as long as the antibody against CTGF, which gives a weaker signal, is used first.

Storage

Membranes can be stored for use at a later date. It is recommended that they are stored dry, between two sheets of Whatman 3MM, protected by cardboard and inside a plastic bag. They can be stored at 4ºC or –20ºC for up to 2 months or at –70ºC for long-term storage. I have successfully re-probed a membrane that had been stuck in my note book, wrapped in cling film for several months. Sometimes it is a matter of luck.

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Society Noticeboard

Scientific Meetings – 2011

Physiology 2011
The Physiological Society Main Meeting
University of Oxford, UK, 11–14 July

Epithelia & Membrane Transport Themed Meeting
University College London, UK
1–3 September
Abstract submission and registration open 23 June

Vascular & Smooth Muscle Physiology Themed Meeting
Edinburgh, UK, 6–8 December
Abstract submission and registration open 20 September

Scientific Meetings – 2012

Human & Exercise Physiology Themed Meeting, The Biomedical Basis of Elite Performance
The Queen Elizabeth II Conference Centre, London, UK, 19–21 March

Physiology 2012
Main Meeting, The Edinburgh International Conference Centre, UK.
3–5 July
Registration opens 1 January

The Journal of Physiology Symposia 2011

UCL Neuroscience Symposium
Institute of Education, London, 1 July 2011 Wednesday 13 July

Human hand function: the limitations of brain and brawn
Physiology 2011 Wednesday 13 July

Synaptic Mechanisms in the CNS - Symposium to honour Roger A. Nicoll
Silverado Resort, Napa Valley, CA, USA
18–20 September

Society-sponsored meetings – 2011

Advances in the cellular and molecular biology of angiogenesis
University of Birmingham, UK, 27–29 June

Motoneurons, their inputs and outputs
Warsaw, Poland, 5–7 September

Travel Grants

www.physoc.org/grants
www.physoc.org/international

Why the medieval Doctrine of Signatures will become the dominant biomedical research tool of the 21st century

As a scientist suffering from an externally imposed career hiatus (the bastards), I have begun to wonder "What's the point of an individual scientific technique, why do some become fashionable and others fall by the wayside?" Very often it's something of a historical mystery. Appearing transformational is easy for some newly emerging technologies – questions can be formulated around their potential and their limitations are not understood. Some scientists even manage to arrange entire careers by hopping from one fashionable technology to another but nobody ever seems to notice that they are often doing the same experiment again and again. One good career-enhancing move seems to be to deploy a given technology to answer a question, just when the technology has first appeared, just before everybody actually realises that the technology has been oversold and cannot answer every biomedical question on planet Earth.

In order to revive my career, I have decided that I need access to the next generation of transformational and dynamic biomedical technology, one that will keep me occupied until retirement. I thought about becoming a fake expert in the emerging technology of deep sequencing (but it's a crowded market and there are lots of them about). But due to stress, I have recently developed an irrational fear of rationalism, and as a consequence have developed a cunning plan. I plan to revive my career by resurrecting the ancient medieval medical technology of the Doctrine of Signatures.

The Doctrine of Signatures was an all-purpose medical technology developed by Paracelsus and Jakob Bohme in the 17th century. They suggested that God or the divine creator left signatures or marks on plants and animals that could be used to discern their use in medicine. A theological justification was made for this philosophy – "It was reasoned that the Almighty must have set his sign upon the various means of curing disease which he provided." The seeds of the skullcap plant, for example, resemble small skulls and were thought to be effective in the treatment of head injuries and there is an outrageously phallically shaped fungus, the stinkhorn (*Phallus impudicus*) that was believed to be effective for the treatment of impotence. The more I read about the Doctrine of Signatures the more I am convinced that it has a number of key advantages over a range of modern molecular biology techniques which I have summarised below.

Nine reasons why the medieval Doctrine of Signatures is a superior technology to modern molecular biology techniques.

(1) The Doctrine of Signatures is underpinned by destiny, sin and astral influences. These are likely to be far more reliable sources of research funding than the British government for the foreseeable future.

(2) Using *post hoc* attribution to discern the utility of a medicinal object is far less intellectually tiring than employing *a priori* reasoning and statistics.

(3) The philosophy behind the Doctrine makes far more sense to members of the general public than molecular biology. Try explaining deep sequencing technology to someone with a media studies degree, then explain how the shape and crinkled surface on walnuts resemble the brain and can therefore be used to treat attention deficit disorder. They will definitely agree to fund the work on walnuts.

(4) The medieval Doctrine of Signatures employs copious quantities of black bile, phlegm, blood and yellow bile – ordering these from Sigma will be far more fun than ordering monoclonal antibodies.

(5) Once the Doctrine of Signatures is fashionable it could lead to the revival of medieval medical terminology. Have you noticed how boring modern terminology is? Take psychiatry – there is a semantically turgid, disorder for every day of the week. Classifying a disorder as schizoaffective, narcissistic personality or bipolar is clinical and boring. These disorders need declassifying and renaming with

their medieval equivalents. I propose a return of "the vapours" as a term encompassing all forms of psychiatric and cognitive dysfunction. Consider the following modern ailments and their medieval equivalents – asthma induced by pollution *versus* "miasmas", cervical tuberculus lymphadenopathy *versus* "the scrofula" and the winter vomiting virus *versus* "the bloody flux" – what sounds more imposing and fun?

(6) Explaining the science behind molecular biology causes unnecessary distress to people who believe in homeopathy; empathetically explaining the science behind the Doctrine of Signatures to them will make them feel wanted, included and validated.

(7) Adopting the Doctrine of Signatures as a mainstream biomedical technology could encourage religious diversity by attracting pagans, wiccans and satanists to your lab.

(8) When preparing a CV writing in the key skills section "Extensive expertise in the medieval Doctrine of Signatures including the use of liverwort, snakeroot, lungwort, bloodroot, toothwort, wormwood and mandrake to modulate gene expression in transgenic mice" is likely to lead to interesting job interviews.

(9) And finally including "a proven ability to locate and extract mandrake roots from hallowed ground under a full moon" in the essential skills section of a job description is an excellent way of excluding uncommitted job applicants.

Dr Keith Cormorant

PS. The 17th century botanist and herbalist William Coles (1626–1662), author of *The Art of Simpling* and *Adam in Eden*, stated that walnuts were good for curing head ailments because in his opinion "they have the perfect signatures of the head".

More on Starling and Bayliss during the 1914–18 War

Ann Silver recently wrote in these pages (PN 82, p. 17) about these great physiologists at war, taking their stories up to 1917. This was before Bayliss's treatment for wound shock was tested in the field and before Starling demonstrated that he had stopped hating all things German. As she wrote, the choleric Starling was so outraged by the German invasion of neutral Belgium that he swore never to speak another word of German, even as lyrics in the Lieder he loved to sing, and announced that he would enlist as a rifleman. Friends convinced him that he would deal the Huns a harder blow in the Royal Army Medical Corps (RAMC). Bayliss, six years older, held the fort at UCL.

Captain Starling's first post was as a pathologist at the Herbert Hospital in Woolwich — presumably the brass feared that after years in the lab he was a danger to patients. After the German chlorine gas attack in April 1915 he was re-assigned to direct training of Kitchener's army in the use of the new protective helmet, breathing in through a chemically impregnated flannel bag covering the head and exhaling from the mouth through a rubber valve.

When the familiar box respirator was introduced in 1916 he was transferred to anti-gas duties in Salonica. The Allies had invaded that part of neutral Greece to use it as a base to keep the Central Powers from overwhelming their Serbian ally. They failed. As the Allies sat in their 110-km-long entrenchments in Greece, Starling's martial ardour cooled. He resigned his commission as a Lieutenant Colonel on the grounds that he was now more than 50 years old.

Back in London in August 1917 he chaired the first meeting of the MRC's Special Committee on Shock and Allied Conditions. Bright young army physicians were measuring blood pressures, which were seldom done at the time; few physicians kept a sphygmomanometer in their kit. Patients with wound shock had low pressures – 90 mmHg systolic or lower. If pressure fell further they died. No treatment was known: they were triaged and left to die. In the spring of 1916, Bayliss had reported to The Physiological Society that anaesthetized cats deprived of half their blood had low pressures and soon died, obeying Starling's law of the heart. Replacing the lost blood with saline raised blood pressure only transitorily, because the added salt and fluid were pushed into the interstitial fluid. A sustained rise in blood pressure was produced by an infusion of saline containing 5% gum acacia, a large molecule whose colloid osmotic pressure held fluid in the plasma. The cats survived, demonstrating the Starling mechanism. (Frank P. Knowlton had introduced gum acacia, obtained from certain plant exudates, as a substitute for plasma proteins in 1911, when he was at Cambridge on an astonishingly productive sabbatical from Syracuse University.)

Gum acacia solutions were finally tested on moribund soldiers in shock in November 1917. Blood pressure rose and was sustained. It was like watching Lazarus rising from the grave. By then Starling had resigned the chair and seldom attended; Bayliss took his place. Even after this dazzling success, the committee dragged its feet in getting gum solutions to the front. The eminent Harvard physiologist Walter B. Cannon, now a captain in the American Army, had discovered that the blood of shocked men was low in bicarbonate. He argued that acid caused shock and treated a handful of cases in early shock with bicarbonate. They survived. Ever the enthusiast, he persuaded the RAMC to stipulate that bicarbonate was to be stirred into tea provided for the wounded. In London,

William Van der Kloot with his Senegal parrot.

Cannon and Bayliss injected acid into several etherized stray cats; their blood pressure fell and they died. Therefore the gum must be dissolved in bicarbonate solution.

Cannon's prestige was such that weeks passed while they failed to get gum dissolved in sterile bicarbonate solutions. Finally the acid hypothesis was tested properly when Cannon, Bayliss and Henry H. Dale, the committee's secretary, injected acid into a healthy cat using a local anaesthetic. The subject played with Dale and strolled about panting for an hour or so. There were no other adverse effects. Dale had done little to push gum solution because he was sure that shock was produced by the release of a histamine-like molecule. Their goal should be to identify this molecule and find a drug that stopped it binding to its receptor.

Gum solutions finally were used during the last months of the war. Astonishing to us, no tally was kept of the number treated, their state when treated, or the results. Medics were not instructed about the volume to be infused, when it should be given, or the scientific rationale for the treatment. Blood was also transfused into shocked wounded. This was feasible after it was discovered that refrigerated blood could be stored without clotting by chelating calcium with citrate. Again no records were compiled. Post-war assessment of

these treatments was anecdotal and swayed by physicians' perceptions of blood as 'natural' while a solution made with a substance utilized in chewing gum and glue for postage stamps was obviously 'un-natural'. Some patients had been killed by dodgy gum solutions made in the field using tap water. There were no problems with solutions prepared in the RAMC's central laboratory.

Starling was doing less in the MRC committee because he was so busy chairing the Royal Society Food [War] Committee. On top of that he was sent briefly to Italy to start anti-gas training with the box respirator, accompanied by Captain Charles Lovatt Evans who later wrote a jolly, irreverent account of their mission. The Italians purchased the respirators because their own devices had failed abysmally when the Austro-German army broke through their front at Caporetto late in 1917. (The site of the gas attack there was selected by Captain Otto Hahn, the eminent chemist who later discovered uranium fission.)

Back home, Starling became the principal scientific advisor to the Ministry of Food, and then one of the two British representatives on the Inter-Allied Food Commission, which set daily rations in calories and projected future food needs for the Allies.

He wrote an article lambasting the lack of understanding of science that led to so many wartime gaffes by the establishment and prescribed educational reforms including less emphasis on dead languages and better salaries for teachers.

He was sent on a secret trip to Germany in June 1919 to evaluate their feeding – surely then he spoke German with old friends. He wrote a detailed paper about how the Germans fed during the war and in the months following the armistice during which the Allies kept blockading food and banned saltwater fishing. Many urban dwellers had starved: body weights were down 15–20%. There were only half of the predicted numbers of children in the age groups from 1–3 and from 10–15. He thought that the Germans might have had sufficient foodstuffs despite the blockade, but the shortfall was enhanced by inept rationing, failure to stop farmers from using feed to maintain livestock, and diversion into the black market. British food administrators had averted these problems. Starling's implication that the British had won the war by starving women and children was not popular.

Starling began the war hoping to kill as a warrior; he ended as a civilian helping to prevent starvation. Bayliss discovered an effective replacement for lost blood, but his role was largely forgotten. Gum was replaced by dextran, but whole blood or plasma was preferred. Now that we realize that infusing another's blood or plasma is a transplant that evokes unwelcome immunological responses, there is renewed interest in large molecules that can do the osmotic work of plasma proteins. Even in wartime the brothers-in-law showed what physiologists could accomplish.

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Hazards for the Committee

Talking to current members of The Society Council I get the impression that they may be missing some of the distractions that spiced up Committee service in the '70s and '80. In those days the Committee often met the day before a Society Meeting, ending with a Dinner, and overnight accommodation in the same place as the rest of the Members. This had the advantage, at least for the latter, that they could harangue the Committee about any contentious issues over breakfast. But it wasn't only Members who did the haranguing. I remember breakfasting in an Oxford College where each place was set with a bowl of cereal. The then Treasurer, a porridge-only Scot, pushed his cereal away but had it pushed back by a fierce waitress. Just after that contretemps, a late arrival sat down at the next table; this spurred the waitress into pushing him back on to our table with the words 'Who's in charge here?' Someone indicated the Meetings Secretary; for some reason this provoked her into saying 'I'm not joking.'

A more alarming episode concerned a winter Committee Meeting in Sheffield. We were dining in Buxton some 18 miles away and the coach took the 'scenic' route over the Pennines. It wasn't all that scenic because it was dark and we came to a halt on what seemed to be a foggy cliff top (but probably wasn't). Those lucky enough to have known the somewhat eccentric Pat Merton will probably agree that he was not the ideal man for the job but nevertheless he got out of the coach to direct the reversing driver. Later, in the safety of the restaurant, we compared notes on our thoughts during that manoeuvre. Those truly dedicated to The Society had comforted themselves with the thought that there were sufficient absentees to ensure a Committee could be reconstituted but the more pragmatic confessed to hoping their life insurance was up to date.

Ann Silver

Death of a diuretic

"Don't let the patient run to the bathroom frequently in the night. Give it sufficiently early". That was what the resident used to advise us, the interns. 'It' of course refers to 'mersalyl' the mercurial diuretic, much in vogue those days. That is nearly five decades ago. Soon it was replaced by the loop diuretic furosemide, and mersalyl just faded away. But in its day it was the drug of choice for patients with congestive heart failure to provide relief and a good night's sleep. The knowledge of its diuretic property I assumed came from patients with syphilis, who were treated with mercury in the pre-antibiotic era. Remember the old saying 'A moment with Venus and a life time with Mercury'. But it seems that an Irish man by the name of Stokes (of 'Stokes–Adams Syndrome' fame) had foreseen the diuretic properties of mercury much earlier (Ventura *et al*. 2001). Why was mersalyl given up then? Possibly because of toxicity of mercury salts and the fact that it was less effective when compared with the other diuretics. However, it is still around on the shelves of laboratories as a thiol binding agent. What is the site of action of mersalyl? For enlightenment on the subject, I looked for the 'classics' like '*Diuretics for Dummies*' and '*All you wanted to know about diuretics but were afraid to ask*'. Finding none on the shelves, I turned to two men of repute – Goodman and Gilman, for their text on pharmacology is considered the golden standard on the subject. However, there is no mention of mersalyl from the sixth edition onwards. Failing to locate the earlier editions, I turned to the net, the greatest source of information and misinformation.

The earliest study using the stop flow technique indicates that the proximal tubule is the site of action where mersalyl stops reabsorption of sodium and water (White & Rolf, 1963). Cafruny's review (1968) on the mercurials raises more questions than it answers, regarding the site of action. A later study (Burg & Green, 1973) mentions that at least part of the action might be due to the inhibition of chloride transport in the thick ascending limb of the loop of Henle. However, this inhibition may be a milder one since furosemide, known to act in a similar fashion, causes profuse diuresis. But all these reports belong to the pre-porin era.

The earliest publication (Whittembury *et al*. 1984) regarding the porins that I could locate, concluded that cell membranes of proximal convoluted tubules are pierced by aqueous pores that are reversibly shut by the mercurial diuretics. Agre, the Nobel awardee for aquaporins, himself used salts of mercury to inhibit water transport through these membranous pores (Jung *et al*. 1994). Curiously Benga (2009), much earlier in Romania, experimenting with erythrocytes, found water channel proteins that were closed by mercurial diuretics. Mersalyl seems to interact with the thiol groups of cysteine in the pore of the water channel and interfere with its function. Prolonged use of this diuretic was found to cause kidney failure due to mercury toxicity and signalled its death and deletion from clinical use but its resurgence as a tool of molecular biology seems to have begun. The question that daunts me is: if Stokes had the modern tools, would he have stumbled on the aquaporins. Yes, of course.

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Prize for Meetings report

With the introduction of focused scientific meetings, the Meetings Secretary no longer reads the minutes of the previous meeting at the next Society Dinner. Of course a formal record is kept of the numbers attending, the titles of the Symposia, Prize Lecturers and the like, but diners are not now entertained by the Meetings Secretary's account of highlights or lowlights – both scientific and not so scientific (such as the evacuation of the dining room at Cork in 2004 when the overcooked beef set off the fire alarm).

To continue the tradition that The Physiological Society has room for humour in its records, the History & Archives Committee, in conjunction with the Meetings Secretary, is inviting younger Members to submit a light-hearted report when they have attended a Society Meeting. If suitable, these may be published in *Physiology News* and/or online. An annual prize of £100 will be awarded for the best submission.

All entries should be submitted to Jill Berriman at jberriman@physoc.org **by the end of July 2011**.

100 years ago in *J Physiol*

The action of tri-valent ions on living cells and on colloidal systems: II. Simple and complex kations. George Ralph Mines (1911). *J Physiol* **42**, 309–331

This paper represents the first 'repeat appearance' in *From the Archives*, being the work of the brilliant but ill-fated George Ralph Mines (1886–1914). More on Mines' tragically short life, and his work, can be found in the Summer 2008 issue (PN71, p. 63). The current paper, only 3 years on, is a far more mature work. It shows the clear biophysical understanding, well ahead of its time, that was a feature of Mines' later work.

The paper is best read together with a report from the previous year that it follows up (Mines, 1910). The papers report the effects, mainly on frog heart, of micromolar concentrations of trivalent lanthanide cations, including La^{3+} and Gd^{3+}. Mines shows that doses as low as 1 μM produce complete inhibition of the heartbeat (Fig. 1)

Mines' interest in rare earth cations stemmed from his search for appropriate congeners to probe the effects of the physiological divalent cation magnesium. In the 1910 paper Mines explains:

"The study of the reactions of living tissues towards [non-physiological] inorganic salts... is of value for several reasons. We have a simplification of knowledge if we can state that some physiological property is characteristic of a group of substances, otherwise defined by their chemical or physical resemblances, rather than of one of its members. In such classification lies a method of attacking the problem of how the normal saline constituents of the tissue fluids exert their action... Particular interest attaches to any case where a clear parallel exists between the action of salts on a living organ and on a colloid system... [e.g. with] the alkali metals. Such discoveries help to bring the mechanism of the cell within range of the methods of physical chemistry."

This statement would have had obvious resonance for later investigators of the ionic basis of membrane excitability, and could still stand today.

Mines began with the divalent cation Be^{2+} and the trivalent Al^{3+}. However, both hydrolysed noticeably in solution, producing complex forms like $[Al(OH)]^{2+}$ and acidifying the saline. In another example of his clear logic, Mines discusses how the effects of these solutions might thus be mediated, in part, directly by H^+, whose inhibitory action on the heart had been famously described some 30 years before by Gaskell. Mines compared the actions of the "simple" (non-hydrolysed) Mg^{2+} and the hydrolysed Be^{2+}. This in turn led him to search for "simple" (non-hydrolysed) trivalent cations to compare with Al^{3+}, and thus to the rare earth elements.

Obtaining rare earth cations was not a trivial matter a century ago. Mines notes: "I have [previously] shown that salts of [several of] the rare earths... agree precisely in their action on the heart of the frog. Through the generosity of some of those chemists who have isolated them I have been able to experiment with several other of these precious substances."

The 1911 paper also makes use of complex trivalent cations such as $[Cr(NH_3)_6]^{3+}$, showing that they are far less potent at inhibiting the heart than the simple trivalent lanthanides. Mines then discusses the actions of the ions in terms of what they might imply about the binding sites of trivalent cations in the heart. Noting how slowly reversible the effects of the lanthanide cations are, he contrasts this with arrest of the heartbeat by elevated potassium concentrations, where "restoration of the beat takes place promptly on changing the perfusion fluid to Ringer without the excess of potassium". The most prescient passage of all in the paper is where Mines uses these facts to distinguish the potential mechanisms by which K^+ and the trivalent lanthanides could have their actions on the heartbeat:

"If... there arises at any stage in the cycle of chemical events concerned in a heart beat a difference in the concentration of diffusible electrolytes inside and outside the cells, this difference of concentration will lead to a difference of electric potential between the inside and the outside of the cells, or to a change in the previously existing potential difference. It is conceivable that such an alteration in potential difference might cause a change in the surface of the cells... modifying their shape.... it can hardly be doubted that the electrical change indicated must play some part in the mechanism of excitation or of contraction."

"Now the potential difference could be modified in two distinct ways by substances applied outside the cells, (1) by the addition to the surrounding fluid of some rapidly moving ion which, by diffusing, into the cells, would introduce its own electric charge, (2) by the addition to the fluid surrounding the cells of substances capable of altering the relative permeability of the surface of the cells to positive and to negative ions. The alteration of potential difference produced in the first way will quickly disappear when the excess of rapidly moving ion is removed from the environing fluid. ...it is to be expected that the alteration of potential difference induced by the second method will persist for a considerable time after removal from the surrounding fluid of the agent which induced the change."

Nowadays we know that the lanthanides bind tightly to, and block, voltage-gated Ca^{2+} channels. Given that Mines was writing some 40 years before the seminal work of Hodgkin, Huxley and their collaborators in the late 40s and early 50s predicting the existence of ion channels, the insight in the above passage is striking. It is worth considering how contemporary it would sound if one substituted the words "ion channels" for "surface of cells" and "gating" or "conformation" for "shape".

Gaskell WH (1880). On the tonicity of the heart and blood vessels. *J Physiol* **3**, 48–92.

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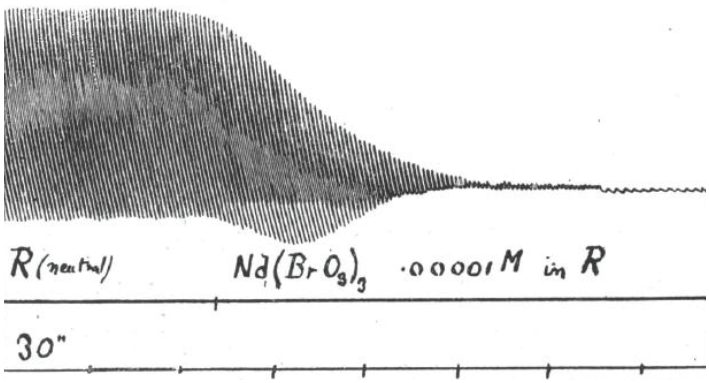


Figure 1 from Mines (1911), showing rapid inhibition of cardiac contraction by 10 μM Nd^{3+}.

Nerve and Muscle

By Richard D. Keynes, David J. Aidley and Christopher L.-H. Huang

Cambridge University Press, 4th edition: £24.99, 194 pages, paperback

ISBN-10: 0521737427
ISBN-13: 978-0521737425

First published in 1981, this book has evolved to emphasise key principles in electrophysiology, stressing concepts rather than facts to aid comprehension of most undergraduates undertaking related courses in biomedicine. In this edition, the authors have dedicated two extra chapters to cardiac and smooth muscle, compared to the previous editions where the topics were very briefly explored under the heading 'non-skeletal muscle'. The true essence of this book, however, is enmeshed within the review of classical electrophysiological experiments, which soared in the early 1950s to explain the fundamentals of neuroscience that we take for granted today. Nonetheless, recent findings are also included and, taken as a whole, provide a comprehensive overview of physiology in neurones and muscles.

The book begins with the basic morphology and properties of neural cells. The experimental evidence reviewed in this chapter is enough to provide a basic understanding of threshold, excitation, propagation and refractoriness of action potentials in myelinated and unmyelinated neurons. The discussion on the generation of resting potentials uses key examples to illustrate the implementation of the Nernst and Goldman–Hodgkin–Katz equations, and how different ionic distributions within and outside can be perceived as a single voltage across the cell membrane. The book has a section dedicated to cable theory and describes relevant equivalent circuits that mediate passive currents or action potentials. But most importantly, there is an in-depth analysis of how these properties, such as resistance and capacitance, influence generation of action potentials.

The chapters on synaptic transmission and neuromuscular junction review the evidence that established the role of acetylcholine as a neurotransmitter in frog skeletal muscle, and demonstrate the five criteria required for a compound to be labelled as a neurotransmitter, although the discovery of ATP and nitric oxide as putative neurotransmitters over the last 20 years has relaxed these criteria. The remainder of the book concerns muscle. Most textbooks in physiology fail to address the intimate relationship of a neuron with its muscle fibre and simply discuss muscles as an isolated system. Again using evidence, the authors compare and contrast the three major types of muscle fibre as well as the subtle differences involved in their signal transduction, from arrival of action potential to the linking of actin and myosin.

This updated version of a classic book should be required reading for all undergraduate Neuroscience students and is a wonderful model of a concise, clearly written textbook.

Angus Brown and Sina Tavakol

Portraits of the Mind

by Carl Schoonover

Harry N. Abrams, Inc., New York, £22.50, 240 pages, hardcover

ISBN-10: 0810990334
ISBN-13: 978-0810990333

This fascinating work presents powerful visualisations from the past, present and future directions of neuroscientific research. Written so that both general and knowledgeable readerships alike can take enjoyment from the content and appreciate the meaning behind the images, this book is wonderfully accessible. Schoonover and the various contributing authors exhibit a contagious enthusiasm through their words that is hard to resist, making for an inspiring and engaging read.

The content is divided into chapters entitled: 'Early history – from Galen to Golgi', 'The birth of modern neuroscience: Santiago Ramon Y Cajal', 'After Cajal: from black and white to colour', 'Breaking the diffraction barrier: from cells to molecules', 'Electricity in the brain', 'The brain as a circuit' and 'From brain structure to brain function'. Each chapter opens with an introduction written by an expert in the field, and provides an enlightening report of the relevant background information. The extraordinary images are all more than adequately captioned for the general reader and pleasingly so for the more knowledgeable reader, delivering detail on how the images were obtained and what they represent.

A particular strength of the book is that through its well-constructed framework it delivers succinct information on a variety of methodologies including microscopy, electrophysiology, staining and neuroimaging techniques, with attention paid to the development and applications of such techniques. The many references to integrated approaches accurately reflect the growing importance of such methods in current and future research.

One of the most exciting methods discussed in this publication is the rather elegant and recently developed 'Brainbow' staining technique. This particular technique is presented through some of the most striking and intricately worked images in the book. Another commendable quality of the writing is the relative ease with which challenging concepts underlying neuroimaging techniques are conveyed to the reader briefly and effectively but without oversimplification.

However, it is the images that linger, and the most memorable ones include: on pp. 84–85 axons emerging from selected retinal ganglion cells converge on the

optic disc then disappear like water down a plug hole, the scanning EM of a neurone on p. 124 looks like an octopus, on p. 210 the images of white matter tracts in the brain obtained by MRI resemble strips of coloured plasticine; and on p. 216 blood vessels penetrating deep into the brain parenchyma.

In short, *Portraits of the Mind* takes the reader on a marvellous journey of discovery you should not be disappointed with.

Angus Brown and Helen Smith

The Anatomy Colouring Book

By C.R. Constant, C. Brassett & M. Spear, with illustrations by J. Berrangé

New Holland Publishers, 2011, £12.99, pp. 240

ISBN-10: 1847738699
ISBN-13: 978-1847738691

It has always seemed to me that there is a fairly straightforward approach to learning about most issues in human biology: what it is, how it works, how it goes wrong. In other words anatomy, physiology, pathology. But while physiology and pathology have some powerful unifying principles which often allow you to work things out rather than simply remembering them, getting to grips with anatomy requires some pretty heavy lifting in the rote learning department, especially at the beginning. This is where any available help is welcome, and where this book scores highly. It fits directly into the educational research tradition that shows that when you are learning something new, multi-modality learning helps it go in better and makes it easier to retain. This nicely produced book, with judiciously chosen, uncoloured, clear and simple drawings on one side only of a double page, can thus be used as a supplement to entry level anatomy learning. When you have had enough of trying to memorise the lumbar plexus or the mediastinal relations of the lung from your standard anatomy text, you can have a break and colour them in using this book, not only getting the small thrill that all well-educated people will get from being allowed to deface a book, but reinforcing your anatomy learning at the same time. In fact, in order to maximise the effect, I recommend that you sing (or at least hum) the appropriate anatomy mnemonic while colouring (but not in the library of course, which is mainly useful for dozing through other people's whispered conversations). The preface gives some useful hints on colouring, so that if you want to you can make your finished products look like genuine anatomy text examples. On the other hand, if you are in colouring iconoclast mode, the authors encourage you to let rip – "there is no correct or incorrect colour coding". You want a blue liver or a green heart, you can have one. Unlike some similar books, each figure comes with brief but helpful words to explain what you are seeing, and comes with numerical labelling (numbers on the end of thin lines with a key below), ensuring that the diagrams will still be clearly labelled and useful even after you have finished with your crayons. I think that the figures have been well chosen for first time anatomy students and are neither too complicated nor too simple: they serve the purpose of the book well. Also important, the price is very reasonable. I heartily recommend this text to anyone embarking on a course in human anatomy. Not only will it help you learn, you may even discover some unexpected new skills.

John A. Lee

Betrayed Generation – Shattered Hopes and Disillusion in Post War Czechoslovakia

By Gerta Vrbová

Zuza Books, £9.99, paperback

ISBN-10: 1907890106
ISBN-13: 978-1907890109

Can you imagine yourself destitute, aged 19, arriving back at your childhood home in Slovakia after six perilous years of deprivation and enormous risk taking? Against all the odds you have survived the horrors of the Second World War but your immediate family has been killed and you have been deprived of an education since the age of 12, all for the crime of being born to a Jewish mother. Despite everything, there is a flame burning inside you – for Science. Me neither. But this is where Gerta Vrbová's latest autobiographical memoir begins.

In *Betrayed Generation,* Gerta describes her life first as a student, then as a medical doctor and finally as a research physiologist, wife, and mother of two in communist Czechoslovakia between 1945 and 1960. The immediacy of her story comes from the details and anecdotes of everyday life in the lab and at home, and her candid descriptions of tight-knit relationships with colleagues, friends, family and lovers. The naivety and idealism that drove her to embrace the communist ideal and ultimately to be let down by it seems incredible today.

Life in post-war Czechoslovakia was no party but it was infinitely better than the life she had experienced during the war. However, a glimpse of the West, plus falling head-over-heels in love with a British physiologist, led Gerta once again to risk her life and that of her children, this time to reach the UK. At this point new heroes enter her story, for her escape would not have been possible without the selfless help and personal risk-taking of colleagues within the international scientific community, many of whom are Physiological Society Members. British physiology, and indeed the British scientific community at large, owe an enormous debt to these and other unsung heroes. Who today would risk their career, let alone their life, for a colleague?

Gerta Vrbová's story is the triumph of unfailing optimism, hope and steely determination over experience. Read her book – it will put your own life into perspective.

Thelma Lovick

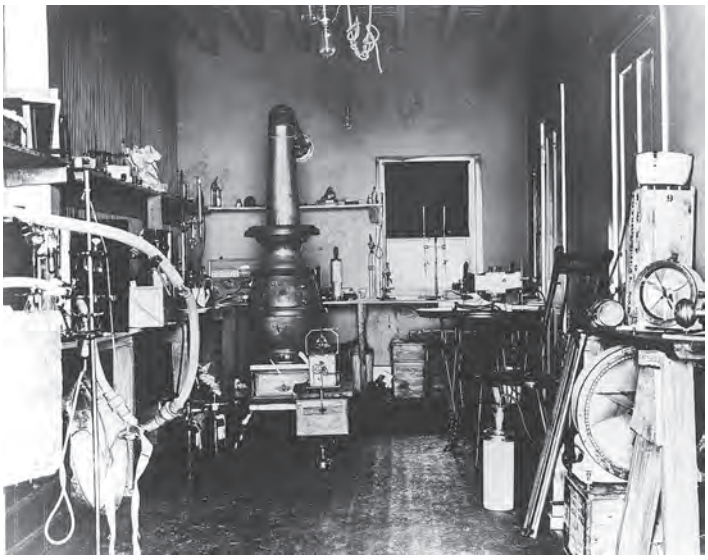
Paton Prize Bursary Photographic Competition

We are pleased to announce the winners of the photographic competition for equipment in use or within a laboratory setting. The two entries submitted were both awarded a prize: Piers Nye and Michael Gardner. Here are the winning photos plus an accompanying explanation of them.

Piers Nye

These two photographs are from Haldane's expedition to Pikes Peak, Colorado in 1911 – 100 years ago in July.

During their five week sojourn on the peak at 14,100 ft (4,300 m) Haldane, Schneider, Henderson and Douglas (left to right) gave the first good description of ventilatory and cardiovascular acclimatisation to high altitude while Mabel Fitzgerald (between Haldane and Schneider in the photograph) travelled around mining camps sampling alveolar gases and taking blood samples from fully acclimatised miners. The photograph was taken by Douglas using a shutter-release delay. In the background one can see part of the cog railway that carried their equipment to the summit. (The original of the photograph was bought from Douglas' effects by John Widdicombe.)



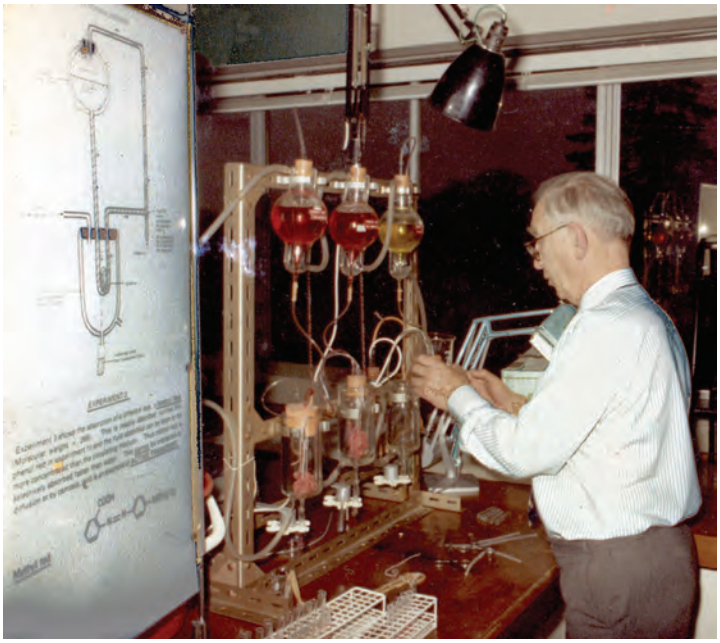
This photograph, which appears in the full publication of the expedition (1; Fig. 3 on p. 188, reproduced with permission of the Royal Society), shows the laboratory they set up for studying alveolar and blood gases. It is lit by an electric light (at 14,000 ft in 1911!) and warmed by the large wood-burning stove. Note the Douglas bag on the left with tubes that largely obscure three sets of Haldane alveolar gas analysis equipment. Behind the stove is a galvanometer and, to its right, is a circular Fuller's slide rule equivalent to a 25.3 m straight slide rule. Directly in front of the stove, sitting on a water bath heated from below by a Primus lamp, is a saturator for equilibrating blood with expired gas, required for Haldane's complicated method of measurement of arterial oxygen levels. In front of the window are measuring burettes and, at the right, a kymograph for recording events on smoked paper. On top of the box at the right is a reservoir that supplied controlled drips of water that displaced pure carbon monoxide from a cylinder. Beside and beneath this box are 1- and 10-litre gasometers to measure respiratory gas volumes. The tall white cylinder on the floor contains 'oxylith' (sodium peroxide, Na_2O_2), to which water was added for the generation of pure oxygen. (This photograph was given to me by Bob Torrance who may have acquired it at the same time as John Widdicombe.) I was given most of the above information when I took the original photograph to Brian Lloyd in 1999.

Reference

1. Douglas CG, Haldane JS, Henderson Y & Schneider EC (1913). Physiological observations made on Pike's Peak, Colorado, with special reference to adaptation to low barometric pressures. *Phil Trans R Soc Lond B* **203**, 185–318.

Michael Gardner

The late R.B. (David) Fisher beside a Fisher & Parsons intestinal perfusion apparatus being used to study copper transport across rat small intestine. The photograph was taken in about 1972 in Fisher's laboratory in the Department of Biochemistry, University of Edinburgh Medical School.



David Fisher setting up a demonstration on absorption across the small intestine at a meeting of the European Intestinal Transport Group in Southampton in 1980. The apparatus consists of 3 identical sets of Fisher & Parsons perfusion apparatus (modified from the original by removal of the serosal bathing fluid). The demonstration showed that: (1) when glucose was present in the luminal perfusate, fluid was absorbed but phenol red hardly crossed the intestine at all; (2) when glucose was omitted from the perfusate, there was no significant water absorption and intestinal barrier function soon failed; and (3) when methyl red was present in the perfusate, instead of phenol red, it readily crossed the intestine and appeared to be concentrated during transport, suggesting active transport against a concentration gradient.

The equipment shown on the left is a Fisher & Parsons apparatus for perfusion of animal small intestine, especially used for investigation of intestinal absorption (Fisher RB & Parsons DS (1949). A preparation of surviving rat small intestine for the study of absorption. *J Physiol* **110**, 36–46). This, using bespoke organ chambers and other components made originally by Harold Vincent, Oxford's 'ace' glassblower, was the first successful method for studying intestinal absorption *in vitro*; a key feature was that the animal's circulation was maintained under anaesthesia during the setting-up procedure until perfusion with well-oxygenated media was established, since brief anoxia would seriously compromise tissue viability. It provided the earliest evidence that glucose absorption was by carrier-mediated active transport, and that glucose absorption was normally essential for fluid to be absorbed. Arising from the success of this methodology and the underlying principles, a number of other approaches – other perfusion methods including vascular perfusion, everted sacs, tissue accumulation slices, mucosal sheets, isolated cells, etc. – were developed which duly established the basic mechanisms of intestinal transport and *inter alia* enabled investigation of effects of drugs on intestinal function. Fisher strongly warned that maintenance of physiological viability was *de rigeur* and that experiments *in vitro* needed to account quantitatively for rates and activities seen *in vivo*.

Fisher (1907–1986), originally a student of Sir Rudolph Peters in Oxford, was a University Demonstrator in Oxford (1933–1959) before taking the chair of biochemistry in the University of Edinburgh in 1959. Though his appointment in Edinburgh was in biochemistry, he was primarily a physiologist or physiological biochemist, with interests predominantly in whole-organ function. After retirement in 1976, he returned to Oxford where he continued to perform research on intestinal absorption, including demonstrating active transport of salicylates and zenobiotics. Horace Davenport credited Fisher for having steered him in the direction of elucidating the mechanism of gastric acid secretion when he had spent a Rhodes Scholarship in Fisher's laboratory in 1937. Fisher's other work included creatine metabolism, mechanisms of action of insulin, enzyme kinetics, analytical paper chromatography, and many aspects of intestinal absorption. He loved inventing (and using) laboratory equipment and gadgets, and his statistical and numerical abilities were well renowned. His outstanding analytical and statistical skills led to him being seconded to the RE8 (Research & Experimental) Group of Bomber Command during World War II. His personal tutorials were noteworthy, and many of his postgraduate and postdoctoral workers have achieved academic distinctions; most would attribute their scientific successes to his strong influence and intellectual drive.

Ask a physiologist!

How many times do you blink in an hour? Why do we blink? (Alex, age11)

Dr Patricia de Winter of University College London replies:

This is a very good question but it does not have a simple answer because blink rate in humans varies tremendously – from 2 to 50 blinks per minute depending on the circumstances. For example, if you are concentrating very hard on reading something, such as this answer, you will probably blink less frequently than if you were sitting chatting with your friends (not to be recommended in class). There are three types of blink: spontaneous, reflex and voluntary.

Spontaneous blinks are those that we don't notice (unless we start thinking about it) and serve to keep the eyeball clean and moist. However, it has been noted that the spontaneous blink rate is more than that needed to simply prevent the eyeball from drying out – so why do we blink more than required? One answer is that spontaneous blinking is affected by a substance called dopamine in the brain which affects particular nerve cells – some studies have shown that giving drugs that affect dopamine levels changes the blink rate, although how exactly this works is yet to be explained. It is also known that people with Parkinson's disease, who have lower dopamaine levels in the brain, blink less frequently.

Reflex blinks are a response to an unexpected event, for example, if an object approaches your eye (such as when someone tries to put eye drops into your eye when you have an infection). Loud noises or any other startling event can also cause reflexive blinking. This type of blink lasts a fraction of a second longer than spontaneous blinks and is a protective mechanism to prevent damage to the eyeball from potential threats.

Finally, voluntary blinks are those which you do consciously (deliberately). These also last fractionally longer than spontaneous blinks.

Why do men have beards but women do not? (Lewis, age 11) **Why do we have armpit hair? (**Chris, age 14) **Why do men have facial hair? (**Oliver, age 14)

Dr Kirsten Poore, University of Southampton, replies:

Men and women do sometimes look quite different don't they? The first answer to this question is about the development of 'girls and boys' into 'women and men'. And it can be explained by what goes on around the time of puberty, in the early 'teen' years. In both boys and girls, a part of the brain called the hypothalamus sends more signals to a gland underneath the brain called the pituitary. This gland releases signals that are able to 'kick into action' the ovaries (in girls) or testes (in boys). The ovaries make the hormone oestrogen and the testes make the hormone testosterone. These hormones are what make children start to look more like adults after puberty and help us to tell adult males and females apart. The features that develop are called the secondary sex characteristics. For girls, these include the development of breasts and the appearance of hair under the arms – but not usually on the face. In boys, there is a deepening of the voice, growth in height and strength and the appearance of hair on the face, as well as on other parts of the body. Interestingly though, testosterone is also responsible for slowing and even stopping hair growth in older men, leading to baldness.

The second answer is actually another question. We could also ask why is it that males and females appear so different. Why do men have beards, for example, at all? Perhaps beards help to protect against the cold? But this can't be necessary for survival since women don't need beards. In other animals, when there are big differences in how adult males and females look, it is usually to make one of them more attractive to the other sex, or more powerful to potential rivals. Think about the manes of male lions or the tail feathers in male peacocks. This is known as 'sexual selection'. Not that many centuries ago, most men still had beards, but these days most men shave off their beards. Has our idea of what is attractive changed?

PS. Beards grow faster than any other hairs. Without shaving, a man's beard might reach 30 feet in his old age.

In most Biology text books it states that a moist surface is required for efficient gas exchange at respiratory surfaces yet I seem to remember an examiner stating that this is not the case and that diffusion would take place more rapidly without a layer of moisture. Could you, please, clarify? (Lesley Thompson, Teacher, Spalding High School)

Although we usually answer questions posed by pupils, we thought this was quite a good one, so asked Dr Glenn Baggott of Birkbeck, University of London to reply:

They are both wrong and right!

First, it depends on what is meant by efficient gas exchange. For example, if there were not a moist surface on the alveolus of the lung, by whatever definition you use the word efficient, it would be bad. The thin layer of water is needed to contain the surfactant which ensures adequate inflation and deflation of the alveolus. Without that, gas exchange will indeed be inefficient.

On the other hand, the proposition that diffusion would be perfect without the moisture depends on the gas species. The permeation of a liquid by oxygen is slower than carbon dioxide. The term permeation here refers to the rate of transfer of oxygen through the layer. This is not the same thing as diffusion, which depends on diffusion properties of the gas species, the driving force for the diffusion and the solubility of the gas species in liquid. Carbon dioxide in the liquid layer is much less of a barrier. For example, in the chicken egg the ability to lose carbon dioxide to the atmosphere is always greater than the uptake of oxygen in early development, but as the membranes inside the shell dry, as water is removed from them by osmosis (during the first week of incubation) the ability of oxygen to permeate the shell and membranes, so reaching the circulation, increases substantially. In consequence of this, the permeability of the shell and its membranes reaches a maximum so allowing the chick embryo to grow.

Meet the Physiologists

In partnership with the Manchester Museum of Science and Industry (MOSI), The Physiological Society hosted a 'Meet the Physiologists' day at the museum on Saturday 6 November 2010.

The day formed part of MOSI's monthly series of events at which members of the public can meet scientists and engineers. The events are free and provide a range of exciting hands-on activities and demonstrations for families and people of all ages to engage with. For more information, please visit www.mosi.org.uk

The Society funded three exhibits at 'Meet the Physiologists'. Each was hosted by Members based at Manchester Metropolitan University or the University of Manchester and was funded through our Outreach Grant scheme.

The event attracted over 1500 visitors and was thoroughly enjoyed by all those involved. The following are summaries of reports written by the organisers of the exhibits; full reports can be found on our website at www.physoc.org/schools

Report 1. Manchester meets its physiologists

A group of us from the Faculty of Life Sciences and Cardiovascular Research Group at the University of Manchester hosted an event to introduce the field of cardiorespiratory physiology to the public, with a particular focus on the heart, kidney, circulation, blood and lungs. The whole day was very interactive, with hands-on activities designed to suit a wide range of age groups.

The event was held in the Power Hall - a large and popular area of the museum that attracted over 1500 visitors throughout the day. Quiz sheets were handed out at the entrance to encourage older children and parents to visit each station, gather information and find out answers to questions such as "how many chambers does the heart have?"

Posters and plastic models of organs were placed around the stations, providing further information; however, heart and kidney from sheep and pig provided a more realistic demonstration and certainly provoked a variety of interesting reactions from the crowd. The younger children enjoyed the play area, which included a scientist dress-up box, interactive posters, physiological pictures for colouring in and clay for constructing models.

The event provided us with an opportunity to interact with many different people and to hear their views on science. In fact, many people we spoke with expressed the necessity for science and commented on how important research is, which was very gratifying to hear. The event has shown how vital it is to communicate science to the local community and to make the public aware of some of the cutting-edge research that is being carried out in their neighbourhood.

A young physiologist!

The organisers (from left to right): Fiona Lynch, Tristan Pocock, Paolo Tammaro, Liz Sheader, Tracy Speake and Alison Gurney.

Rebecca Brookfield

Comments from visitors on the day:

"I thought it was really interesting and I learnt a lot in a fun way!" Child, age 11

"A great learning opportunity. The experiments were great for engaging the kids." Parents

Report 2. What do you need to make you move?

Members of the School of Healthcare Science at Manchester Metropolitan University developed a group of interactive demonstrations entitled 'What do you need to make you move?,' which had been designed to illustrate how different parts of the body contribute to our ability to move.

Activities included: ultrasound imaging, which enabled visitors to see the muscles in their arm; a wireless EMG electrode, held over biceps and linked to a speaker, allowing participants to hear the activity in their muscle when it was activated; and the role of vision in motor control was also demonstrated with a ball catching challenge in which visitors wore stereoscopic glasses to affect their perception of depth. In an additional activity, we asked whether people really stand 'still', by inviting volunteers to stand on a pressure mat (kindly loaned from Biosense Medical Ltd) to show that they actually sway and that the amount of sway is affected by whether eyes are open or closed. Younger visitors were encouraged to collect stickers from each stand they visited in order to complete a printed sheet we provided.

The museum received a large number of visitors and it seemed as though we did not stop talking to people from when the doors opened until the end of the day. We were amazed by the range of people who visited and the wonder that was created by showing them simple aspects of their body using tools that have become part of our everyday work routines. The majority of our visitors were families with young children and our hands-on approach really encouraged participation. The parents seemed as impressed as their children and we hope they left with the impression that visiting similar events in the future could be engaging and educational for the whole family. We also found the day rewarding and left tired, but with great plans for future events.

Emma Hodson-Tole

Linda Tersteeg, a PhD student, preparing to reveal the muscles in the forearm of a young visitor using B-mode ultrasound imaging.

Report 3. A heart to heart talk

The event was very busy, with over 1500 visitors of all ages. Our objective was to enthuse people about science, and offer them a glimpse of research and clinical practice related to the heart in particular. We presented posters, plastic models, PowerPoint shows, and The Physiological Society's DVD on the use of animals in physiological research – quite a few visitors wanted to know more about this subject and the DVD helped. The highlight of our exhibit was an oximeter activity where visitors measured their heart rates. We also had related quizzes, handouts and giveaways.

We learnt a lot about how to make an exhibit look appealing and exciting to young people. We found that hands-on activities like the oximeter were better appreciated than passive presentations and posters, but the posters provided information for the more inquisitive visitors about ongoing research in our research group. We noticed that the most curious visitors were aged 5–11 years.

Support from The Physiological Society was critical in our successful delivery of this exhibit, which has given us our first experience of preparing, organising and delivering an outreach event. Staff at the museum were also very helpful.

Sanjay Kharche

Outreach Grants

The Outreach Grant scheme is open to all Ordinary Members, Affiliates and Associates of The Society who would like to communicate the excitement of physiology to young scientists and the wider community. For more information, please visit our website www.physoc.org/grants or email outreach@physoc.org

Undergraduate Vacation Studentship Scheme

Established in 1994, the Vacation Studentship Scheme provides funds for undergraduates wishing to gain experience of laboratory-based research. Projects are carried out over a period of eight weeks during the summer, under the supervision of an Ordinary Member of The Society.

In 2010, The Society awarded Vacation Studentships to thirty-seven undergraduates who were asked to provide a summary of their project and overall experience afterwards. Of these reports, the Education and Outreach Committee was particularly impressed by one undergraduate, Laura Bella, who worked under the direction of Dr Ita O'Kelly at the University of Southampton (see Vacation Studentship Report on next page).

Laura describes how her placement changed her perceptions of research and plans for the future:

"While I found the initial training period quite difficult and demanding, once I was able to perform the various techniques required for the studentship, I began to enjoy my experience more. I learned about the importance of factors such as team work, attention to detail, organisation and perseverance. I experienced the fascination this job offers and the frustration of not obtaining the expected results. I could suddenly apply what I had learned in the books for the past two years, and learned to efficiently read scientific papers. This placement allowed me to realise that this career path was exactly what I had been looking for."

Ita describes her experience as a supervisor:

"Having Laura in the lab through The Physiological Society Vacation Studentship was a really positive experience for both the lab and Laura. It has given Laura the opportunity to experience a research environment and has really spurred her on to aspire to a career in research."

Feedback from other students and their supervisors was also positive:

"I am immensely grateful to The Physiological Society for funding this studentship. Will has been a real asset to my laboratory and I sincerely believe that this funding and the opportunity it allowed will have a long-term influence on Will."

Dr James Fisher, Will's supervisor at the University of Birmingham

"During many moments of the placement, I was fairly sure that a career in research couldn't possibly be worth the annoyance of inexplicably dropping glass micropipettes like they're going out of fashion, or software crashing again. But as I write this, in between playing with my Excel spreadsheets of results and drafting my abstract, those frustrations seem fairly insignificant and suddenly research doesn't seem so bad at all. In fact, all the data in the spreadsheets are mine. I obtained them. And that's pretty cool, actually."

James Selvey, student at Cardiff University

We are extremely pleased to receive such excellent feedback this year and plan to continue running the Vacation Studentship Scheme for the foreseeable future. We wish all the awardees the best of luck in their studies and future career.

For more information about the Undergraduate Vacation Studentship Scheme, please visit

www.physoc.org/vs

or contact education@physoc.org

Live-Cell Imaging

University of East Anglia, 14–16 June 2011

The Physiological Society is supporting a three-day, hands-on workshop for Members and non-members to learn a range of imaging techniques. The workshop has been extended to include a day of image analysis software demonstrations.

For more information, visit www.physoc.org/education or contact education@physoc.org

This course is organised by Dr Paul Thomas.

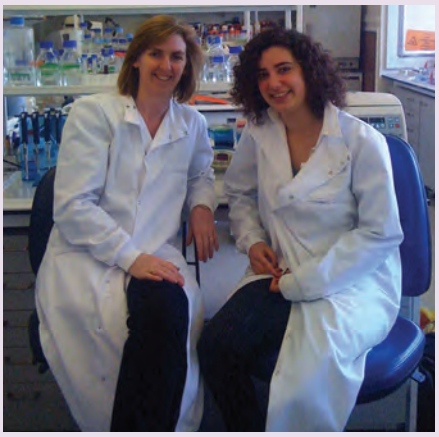
Vacation Studentship Report

Laura Bella was awarded a Vacation Studentship in 2010 to undertake a lab-based project at the University of Southampton under the supervision of Dr Ita O'Kelly. Here is her report:

Investigation on the internalisation and recycling mechanisms of the K2P channel subunits

The aim of this project was to investigate the endocytic pathway(s) of the two-pore domain potassium channel, K2P3.1 (TASK1). K2P3.1 shows widespread tissue distribution and is expressed in neuronal, cardiac, pulmonary, genitourinary and gastrointestinal tissues (Duprat *et al*. 2007). When K2P3.1 is on the plasma membrane, it is active and allows potassium efflux, which changes the electrochemical potential of cells to a more negative state and so regulates cell function. As, therefore, the protein is a regulator of the cell's function, it is of paramount importance to understand the regulation of the channel itself, in particular the control of surface expression. Surface expression is maintained by balancing the forward transport of membrane proteins with their retrieval from the cell surface (endocytosis). The team I joined have previously identified mechanisms of forward transport for the protein (O'Kelly *et al*. 2002) and so my aim of identifying mechanisms of internalisation would provide a more extensive knowledge regarding the regulation of surface expression of this protein.

There are numerous protein internalisation pathways, including clathrin-mediated or caveolae-dependent endocytosis. By disrupting proteins that function specifically in these different pathways, the mechanism of internalisation can be deduced. To determine the rate of turnover of the channel at the cell surface, I transfected cells (HEK293) with a tagged channel (tagged with green fluorescent protein on the N-terminus and an external haemaglutinin tag). Using flow cytometry (FACS) and immunocytochemistry (ICC), I monitored the cell surface expression and cell localisation of the channel. In this experiment, cells were treated with Brefeldin A (BFA) for different time periods. BFA prevents protein transport from the endoplasmic reticulum and so prevents forward transport of the channel, thus allowing us to determine the rate of channel retrieval from the cell surface. This revealed that K2P3.1 had a turnover rate in excess of 5 hours.



Dr Ita O'Kelly (left) and Laura Bella.

The role of phosphorylation in channel internalisation was also investigated with the tagged channel. HEK293 cells expressing the channel were treated with protein kinase A (PKA) activator (8 Br cAMP) or protein kinase C (PKC) inhibitor (Gö6976). Cell surface expression of the channel was examined by ICC and FACS. In both cases, the amount of channel on the cell surface (detected by an antibody to the external tag) was compared to the amount of total channel (detected by green fluorescence protein) in the control and treated cells. I found that activation of PKA or inhibition of PKC resulted in increased surface expression of the channel. This will require further studies but suggests that inhibition of PKC stops channel retrieval from the cell surface as less channel was visualised in the endosomes.

Finally, the role of dynamin in channel internalisation was examined. Dynamin is critical to enable clathrin-mediated endocytosis and caveosome internalisation, and I found cells incubated with dynamin inhibitor (dynasore) showed more channel on the surface of the cell (by FACS and ICC). This indicates that it is likely that K2P3.1 is internalised via a dynamin-dependent pathway.

Duprat F *et al*. (2007). The TASK background K2P channel: chemo- and nutrient sensors. *Trends Neurosci* 30, 573–580.

O'Kelly I *et al*. (2002). Forward transport. 14-3-3 binding overcomes retention in endoplasmic reticulum by dibasic signals. *Cell* **111**, 577–588.

Annual General Meeting 2011

The AGM of The Physiological Society will be held at the University of Oxford on Wednesday 13 July at 12.30 pm.

All Members have the right to attend but only Ordinary and Honorary Members may vote at the AGM. Please note that you do not have to register for Physiology 2011 to attend the AGM.

Matters for discussion

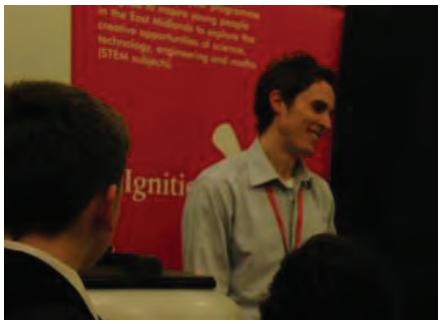
As well as the formal business, time has been allowed for discussion of items raised by Members. Anyone wishing to raise any substantive matter are invited to indicate this in advance. Note that this is not a requirement. Members remain free to raise any matter they wish during the AGM, although items that have been pre-notified will receive preference if time is short.

If you wish to submit a question in advance please contact Orly Burgess (oburgess@physoc.org).

Lab_13

Ignition* is a programme of creative approaches to STEM (science, technology, engineering, mathematics) subjects in the East Midlands, involving teachers, students, parents, artists, scientists, inventors, designers, engineers, film-makers, writers and journalists. There are six integrated programmes to promote creativity in the teaching and learning of these subjects, one of which is Lab_13.

Lab_13 is an exploratory space within a school or college, run by young people for young people, with the guidance of a scientist, engineer or inventor in residence. One scientist-in-residence shares his typical day...



As a Lab_13 Scientist-in-Residence, I hold a unique and privileged position within our school. My role is to facilitate STEM-based subjects and to provide opportunities for our students to learn through discovery.

My day begins by supporting a Life Skills class where students are learning about sustainability and the environment. My aim is to provide a scientific investigation to reinforce the learning objectives of the lesson. Today we are assessing the energy consumption of household gadgets and students have to conduct an energy survey using plug-in energy monitors. The students were surprised to find that the humble kettle consumed more than 40 times the energy of a standard light bulb. We manage to draw on some numeracy skills as the students calculate the cost of the energy consumed by each item.

I then head over to the maths department where we are trialling a project to emphasise the importance of maths in various professions. The students have to program a robot to perform a number of challenges. These navigational skills will no doubt be valuable when careers in space exploration take off in the future.

After a busy morning I am back in my lab where I am greeted by a group of eager students who have given up their lunch hour to perform their own research. The students are keen to identify which of their favourite energy drinks is the best and have persuaded me to run on the treadmill after consuming one of their *horrible* drinks. This is actually the third test in a trial of three where I have been asked to run to fatigue to settle the debate. The students were disappointed to find that the flavoured water worked best.

As a Scientist-in-Residence I am able to act as a mediator between STEM subjects which can only dream of cross-collaboration within the school timetable and I am able to provide an opportunity for students to be creative and to explore science on their own terms. It is common for students to want to blow things up and set fire to things but after this desire to test the limits of science has passed it is clear that what they really want is a safe place to do it. I believe that from this will grow the scientists of the future.

Tom Carter

Tom is Scientist-in-Residence at Crown Hills Community College in Leicester. The college has also taken part in other strands of the Ignition* programme, including Come Alive With Science where students and teachers worked with an architect and a media professional to develop creative STEM activities during National Science and Engineering Week in order to engage the wider school and feeder primaries. As part of the project they designed and constructed an installation in which students were able to showcase various forms of media to the rest of the school and visiting primaries. The students took leading roles in the design of the installation, as well as in the script writing and film-making process.

The project at Crown Hills Community College was one of over 30 creative STEM collaborations, involving schools, artists and scientists, taking place across the East Midlands as part of Come Alive with Science.

Ignition* is delivered by Ignite! in collaboration with The Mighty Creatives, and funded by the East Midlands Development Agency (EMDA).

For further details see: www.ignitefutures.org.uk

Lab_13 does Genoa. Crown Hills Lab_13, along with Tom Carter, their Scientist-in-Residence, headed off to Genoa for the Genoa Science Festival, to show how creative science should be done.

Lab Profiles: Welcome to the Muscle Cellular and Molecular Physiology Research Group

Recently I was talking with colleagues and marvelling at the incredible range of research questions that are being asked by physiology researchers and the equally large variety of techniques and approaches that are being used to answer them. We thought it would be interesting to profile some labs from around the world in *Physiology News* to highlight the range of exciting research that Phys Soc Members are involved in. So here I am to get the ball rolling by introducing our lab and the research we are undertaking to further understand muscle physiology.

Since March last year I have had the great pleasure of working in the Muscle Cellular and Molecular Physiology Research Group (MCMPRG) led by Mark Lewis. The research group is part of the Institute of Sports and Physical Activity Research (ISPAR) Bedford at the University of Bedfordshire and since its arrival here in September 2009 it has been growing and developing at an amazing rate. I am pleased to be able to write this profile of the lab for many reasons, but particularly because it has changed so much since I came here and now, 1 year on, it is pleasing to look back and see how much we've all progressed since I arrived.

As the name suggests, the group is interested in muscle research and uses a variety of physiology, cell biology and molecular techniques to study muscle physiology and the response of muscle to exercise, ageing, changing environmental conditions and in different disease states.

Due to our convenient location within the Department of Sport and Exercise Science, we have access to a range of human physiology

Sam Passey

expertise and equipment, including an environmental chamber that allows for controlled changes in environmental conditions such as temperature and humidity during exercise, and other physiological testing equipment to look at exhaled gases, blood lactate and body composition.

However, the major focus of the MCMPRG is on studying cellular and molecular physiology, and this is where our research group really comes into its own. Before moving to Bedford and forming the MCMPRG in its current guise, Prof Lewis spent many years with colleagues and collaborators developing a 3-dimensional *in vitro* model of skeletal muscle and it is this 3D model that is the main tool used by current MCMPRG researchers.

In vivo, skeletal muscle consists of bundles of aligned contractile myofibres surrounded by extra-cellular matrix, and these 3D culture models aim to produce a muscle construct that mimics the *in vivo* structure and behaviour of skeletal muscle.

The 3D collagen model involves seeding cells within a collagen gel that is tethered at opposite ends by attachment to mesh floatation bars. This tethering provides the tensional cues required by the skeletal muscle cells in order to properly align with each other, fuse and differentiate to form myotubes. Once formed, these 3D constructs show many structural and molecular similarities with *in vivo* skeletal muscle (Mudera *et al*. 2010).

The tension generated by the muscle constructs can be measured using a culture force monitor (CFM) that incorporates a force transducer, and the constructs can also be 'exercised' using a piece of bespoke equipment called the tensioning culture force monitor (tCFM) to stretch the constructs according to pre-programmed regimes (Cheema *et al*. 2005). The associated molecular and structural changes can be measured following this 'exercise' using confocal microscopy, and Q-PCR.

In collaboration with Keith Baar at UC Davis in California we have recently started using an alternative *in vitro* model where the muscle constructs develop in a fibrin matrix (Huang *et al*. 2005). Cells are seeded on a fibrin gel layer that is anchored in place using small pins and suture material. As the cells grow and differentiate, the tension formed from the anchor points causes them to roll up around the anchors and form a cigar-shaped muscle construct containing myotubes aligned along the lines of tension. These muscle constructs can be electrically stimulated to contract and the force they produce can be measured using a sensitive force transducer, allowing us to probe muscle structure, function and molecular makeup in response to different treatments and conditions.

These are the tools we use in our lab – so what are we doing with them? Well, there are a number of exciting ongoing projects that range from exercise physiology to neuroscience. PhD students Darren Player and Neil Martin are investigating the molecular and cellular adaptive responses of the *in vitro* muscle constructs to mechanical stimulation, aiming to model both endurance exercise and resistance training using the 3D models. David Hughes, who is in the first year of his PhD, is undertaking a detailed study on the mechanism of action of testosterone in promoting muscle hypertrophy.

In addition to the exercise physiology projects that are going on in the lab that have clear

relevance to sports performance, other projects aim to investigate health-related issues that are prominent in this country today. In particular, the lab is interested in the muscular changes that accompany ageing, and research by postdoc Adam Sharples is attempting to unravel the complex effects of ageing on the muscle function and adaptation, and the ability of skeletal muscle to repair and regenerate as we age.

There are also interesting projects underway that lie at the interface between *in vitro* and *in vivo* exercise physiology. For example, Paul Davies, a second year PhD student, is using ultrasound scanning to investigate changes in core muscle activation with ageing, and combining this with molecular studies of ageing muscle in the 3D models. Similarly, James Tuttle is combining traditional *in vivo* exercise physiology studies with molecular studies in the cellular models to investigate the involvement of heat shock proteins in muscle damage following downhill running and heat stress for his PhD project.

The research I have been doing since moving to the MCMPRG is slightly different to many other current lab projects. I am working jointly with Alec Smith, a final year PhD student based at the Institute of Neurology at UCL. Our work is aimed at engineering a neuromuscular junction by co-culturing skeletal muscle cells and primary motoneurons in the *in vitro* models – very challenging. Alec has put in a lot of work during his PhD to optimise the co-culture of the primary muscle cells and motoneurons, and the project is producing some promising results although there is still much to do.

The lab has grown and developed dramatically since I joined in March last year, both in terms of the people working within the research group and also the lab itself which has gone from an empty room to a bustling hive of activity and excitement. We have maintained and developed numerous collaborative projects and working relationships with researchers at other institutions including UCL Institute of Orthopaedics and Musculoskeletal Science at Stanmore, the UCL Institute of Neurology, Loughborough University, Cranfield University and UC Davis in California. So as I enter the second year of my research in the MCMPRG, I look forward to more exciting developments, revelations and new collaborations in all of our research projects, and am thrilled to be able to contribute to the growth and development of muscle research here in Bedford.

Samantha Passey

Affiliate Representative for The Physiological Society and Postdoctoral Researcher at ISPAR, University of Bedfordshire, Bedford

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Cheema U *et al*. (2005). Mechanical signals and IGF-I gene splicing *in vitro* in relation to development of skeletal muscle. *J Cell Physiol* **202**, 67–75.

Huang YC *et al*. (2005). Rapid formation of functional muscle in vitro using fibrin gels. *J Appl Physiol* **98**, 706–713.

Mudera V *et al*. (2010). The effect of cell density on the maturation and contractile ability of muscle derived cells in a 3D tissue-engineered skeletal muscle model and determination of the cellular and mechanical stimuli required for the synthesis of a postural phenotype. *J Cell Physiol* **225**, 646–653.

New Society staff members

Orly Burgess

I was born in Jerusalem and moved to the UK in 2004 after I finished studying for my first degree in History at the Hebrew University. I spent five years working at the Israeli Embassy while studying at Birkbeck College, completing my Masters Degree in Medieval History in 2009. I joined The Physiological Society in January 2011 as PA to the Chief Executive and Office Administrator.

I live in the East End of London with my English husband, who I married in 2009 – it was a very busy year for me!

Living and working in London is important as I love going to lectures and exhibitions throughout the year and enjoy the history and culture that the UK has to offer.

Clare Kingston

I joined The Society as Head of Media Communications for 12 months, providing maternity cover for Mary Arbuthnot. In addition to maintaining The Society's current activities, I will be conducting a review of media and communications – producing a new strategy for The Society. Previously, I have worked as Press Secretary for the Science Minister and Government Chief Scientific Adviser, as senior press officer at the Royal Society and have most recently been making science documentaries with the BBC series, *Horizon*.

The Journal of Physiology

New Journal Editors 2011

Simon Gandevia

I am an NHMRC Senior Principal Research Fellow and Deputy Director of Neuroscience Research Australia. I began as a medical student in 1972 and subsequently received a number of Bachelor and post-doctoral degrees from the University of New South Wales (BSc Med, MBBS, PhD, MD, DSc). My passion for medical research developed during my medical course when I stopped to complete a PhD in physiology.

After my medical training, I subsequently conducted research in clinical neurophysiology at the Prince Henry Hospital. My research continues to focus on the way in which the human brain controls movement, with emphasis on three major areas: the neural mechanisms underlying proprioception; the sensory and motor mechanisms involved in the control of human movement and posture; and the central control of human breathing.

With my colleagues, David Burke, Ian McCloskey and Erica Potter, I established an independent medical research institute which was named the 'Prince of Wales Medical Research Institute', and later renamed 'Neuroscience Research Australia'. I am the only founder still working at the Institute.

My work has provided insights into pathophysiological mechanisms in several branches of medicine including neurology, rehabilitation and cardiorespiratory medicine. One hallmark of this approach has been to use incisive human experiments to expose underlying neurophysiological principles. As one example, I underwent complete paralysis of the body with curare while awake to determine the neural mechanisms which generate the feeling of breathlessness when carbon dioxide levels rise (Gandevia *et al*. 1993, *J Physiol* **470**, 85–107).

I serve on a number of editorial boards. Of note was my initial role on the board of *The Journal of Physiology* (1993–2000). I was one of the first Australians to contribute in this way. I have published more than 80 papers in *The Journal* on a wide-range of topics in human physiology, including the behaviour of human muscle spindles, the operation of the muscle–tendon unit, proprioceptive sensations, maximal muscle performance and central fatigue, neural control of the hand, and human respiratory muscle performance. I have also helped develop concepts about the ethics of experimental studies in humans. I was elected a Fellow of the Australian Academy of Science in 1998.

Andrew McCulloch

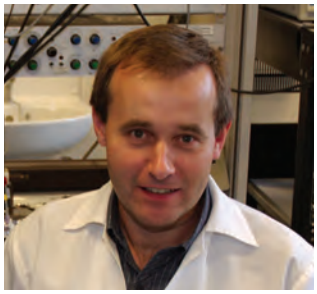
I am Professor of Bioengineering and Medicine and Jacobs School Distinguished Scholar at the University of California San Diego (UCSD), where I joined the faculty in 1987. I am a member of the UCSD Institute for Engineering in Medicine, the California Institute for Telecommunications and Information Technology, a Senior Fellow of the San Diego Supercomputer Center, and a member of the UCSD Center for Research on Biological Systems. I am a Principal Investigator of the National Biomedical Computation Resource and Co-Director of the Cardiac Biomedical Science and Engineering Center at UCSD. I served as Vice Chair of the Bioengineering Department from 2002 to 2005 and Chair from 2005 to 2008. I am the Director of the HHMI-NIBIB Interfaces Graduate Training Program and the accompanying UCSD Interdisciplinary PhD Specialization in Multi-Scale Biology.

I was educated at the University of Auckland, New Zealand in Engineering Science and Physiology receiving my PhD in 1986. I was an NSF Presidential Young Investigator and am a Fellow of the American Institute for Medical and Biological Engineering. I served on the Board of Directors of the Bio-Medical Engineering Society, and am currently Associate Editor of *Medical and Biological Engineering and Computing* and *PLoS Computational Biology* and co-Editor-in-Chief of *Drug Discovery Today: Disease Models*. I am on the editorial boards of the *American Journal of Physiology: Heart and Circulatory Physiology*, *Computer Methods in Biomechanics and Biomedical Engineering* and *Cellular and Molecular Bioengineering*. I gave the Konrad Witzig Memorial Lecture and the Donald Wassenberg Memorial Lecture. Recently, I was elected a Fellow of the Cardiovascular Section of the American Physiological Society. I also chair the Physiome and Systems Biology Committee of the International Union of Physiological Sciences.

My lab uses experimental and computational models to investigate the relationships between the cellular and extracellular structure of cardiac muscle and the electrical and mechanical function of the whole heart during ventricular remodelling, heart failure and arrhythmia. Current interests include developing multi-scale models of myocyte excitation–contraction coupling mechanisms and their regulation by PKA and CaMKII. My group has also scaled cellular level models of these processes up to the tissue and organ scales to investigate mechanisms of arrhythmias and ventricular

dysfunction associated with targeted gene defects and congestive heart failure. Genetically engineered mice are an important model system for developing and validating these computational models. Important phenotyping techniques in the mouse include optical electrical mapping, isolated muscle mechanics testing and magnetic resonance imaging. A second major area of research in the lab has been the role of cytoskeletal and membrane proteins in cardiac myocyte mechanotransduction mechanisms and how defects in costameric and z-disk protein complexes can alter mechanotransduction and lead to dilated cardiomyopathy. The effects of stretch on myocyte membrane configuration and electrical conduction are also under investigation. Finally, we have been using *Drosophila* as a model system to explore hypoxia tolerance and susceptibility mechanisms in conjunction with metabolomics and metabolic network modeling.

Julian Paton



I was educated at Taunton School, Somerset, and obtained my first degree at the University of Birmingham in 1984 in Biological Sciences (Physiology). In 1987 I was awarded my PhD from the University of London. My post-doctoral training included periods at the Department of Physiology, Royal Free Hospital, London (1987–1989), as a Visiting Scientist at E.I. DuPont de Nemours Inc., Wilmington, Delaware, USA (1989–1992) and as an Alexander von Humboldt Fellow at the University of Göttingen, Germany (1992–1994). Subsequently, I was awarded a British Heart Foundation Lectureship (1994–2004) and obtained a Chair of Physiology at the University of Bristol in 2001. I have published more than 170 original research papers and 45 reviews/book chapters. My main research interests are in neurogenic hypertension and interactions with respiratory and immune systems in animal models, and more recently, humans. I won the Sharpey–Schafer Prize of The Physiological Society (1998) and delivered the Carl Ludwig Prize Lecture of the American Physiological Society (2005). I hold a Royal Society Wolfson Research Merit Award. In 2002, I was appointed Honorary Professor of the University of Belgrade, Serbia. In 2005 I presented the inaugural Public Lecture of The Physiological Society on hypertension. I have sat on the British Heart Foundation Project grant committee and am the current Deputy Editor-in-Chief for *Experimental Physiology* (2001–2011), and will remain on their Board *ex-officio*. I served as an Editor for *Neuropharmacology* (2002–2007), *Autonomic Neuroscience: Basic to Clinical* (since 1997) and *Archives Italiennes de Biologie* (since 2009). I am Vice-President of the International Society for Autonomic Neuroscience (ISAN) and Chair the International Programming Committee for the inaugural joint meeting between ISAN and the American Autonomic Society (Buzios, Brazil, 2011). I sit on the International Programming Committee for the International Union of Physiological Sciences meeting (Birmingham, UK, 2012) and the International Society for Hypertension (Sydney, Australia, 2012).

Kenneth W. Spitzer

I obtained a BA degree in Biology (1966) from Virginia Military Institute, an MS degree in Zoology (1968) from Virginia Polytechnic Institute, and a PhD in Physiology (1977) from the School of Medicine, State University of New York (Buffalo). I am currently a Professor of Physiology at the University of Utah School of Medicine and Director of the Nora Eccles Harrison Cardiovascular Research and Training Institute (http://www.cvrti.utah.edu/) at the same institution. My research focuses on heart cells to include intracellular pH regulation, excitation–contraction coupling and electrophysiology. I have served on the editorial board of the *American Journal of Physiology: Heart and Circulatory Physiology* and currently serve on the editorial board of the *Journal of Molecular and Cellular Cardiology*.

Irving Zucker

I am the Theodore F. Hubbard Professor of Cardiovascular Research and Chairman of the Department of Cellular and Integrative Physiology at the University of Nebraska Medical Center in Omaha, Nebraska, USA. I have been Chairman since 1989. I received my PhD in physiology from New York Medical College in 1972. I continued my post-doctoral training at the University of Nebraska Medical Center where I became a faculty member in 1973. My research involves studies related to the neural regulation of cardiovascular function. My studies have revolved around cardiovascular reflex control of sympathetic nerve activity in animal models of chronic heart failure. These investigations focus on the role of central mediators of sympathetic nerve activity such as angiotensin II, nitric oxide and reactive oxidant stress. More recently, I have carried out experiments designed

to understand the regulation of angiotensin II receptors in the brain. I have published over 200 papers in this field and this work has been continuously funded by the National Institutes of Health and the American Heart Association. I am the Principle Investigator of a Program Project currently in its 12th year of funding. I have been the recipient of a MERIT Award from the NHLBI, an Established Investigatorship from the American Heart Association and a recipient of the Wiggers Award from the Cardiovascular Section of the American Physiological Society. I currently serve on the editorial boards of 10 journals. In addition to my research, I am active in administrative activities for the American Physiological Society and the American Heart Association. I was a member of the National Research Committee of the American Heart Association. I am currently an Associate Editor of the *American Journal of Physiology*. I am a past-President of the Association of Chairs of Departments of Physiology and a past-President of the American Physiological Society. I currently sit on the Finance Committee of the American Physiological Society. In my spare time, I enjoy travelling, cooking and photography. I am happy to be a member of the editorial team at *The Journal of Physiology*.

Experimental Biology 2011

Washington, DC, USA
10 April 2011

The Journal of Physiology sponsored a research symposium entitled *'Molecular mechanisms underlying neurovascular protection in stroke'* at the Experimental Biology meeting held in Washington DC, USA on 10 April 2011. The symposium was organised by Giovanni Mann (Department of Physiology, King's College London) on behalf of *The Journal of Physiology*.

Stroke is associated with one of the highest rates of mortality, with ~150,000 cases each year in the UK. The failure of bench-to-bedside translation highlights the importance of understanding the mechanisms underlying damage to the brain and the processes involved in its repair. Tissue plasminogen activator (tPA) is the only approved treatment for stroke but its beneficial thrombolytic actions are counterbalanced by the limited window of efficacy following the onset of symptoms and its neurotoxicity. Ischaemia–reperfusion (I/R) injury after stroke leads to disruption of the blood–brain barrier and potentially fatal cerebral oedema and an increased incidence of haemorrhage. The initial rapid loss of viable brain tissue in the ischaemic core region is often followed by subsequent damage to the surrounding penumbra. Rescuing the penumbra is a key objective for stroke research, and targeting endogenous defence mechanisms by which the brain protects itself and recovers from ischaemic damage may provide novel insights for effective treatment strategies. Although ischaemic preconditioning ('tolerance to ischaemia') has been employed to improve endogenous antioxidant brain defences, the cellular mechanisms underlying protection of the neurovascular unit remain to be elucidated.

David Paterson looking through *The Journal of Physiology*, his 'new' journal, at EB. Note the special JP- and EP-branded bottles of water on the Wiley-Blackwell stand. All 1,100+ bottles of water were taken during the meeting.

The Symposium provided an up-to-date overview of basic and clinical research focused on characterising the cellular mechanisms underlying protection of the neurovascular unit in stroke and featured lectures by Constantino Iadecola (Division of Neurobiology, Department of Neurology and Neuroscience, Weill Cornell Medical College, USA) on 'Neurovascular protection by ischemic tolerance: roles of nitric oxide and reactive oxygen species', Alastair Buchan (Experimental Medicine Division, John Radcliffe Hospital, University of Oxford, UK) on 'Cerebral blood flow alteration in neuroprotection following cerebral ischaemia', Giovanni E. Mann (Cardiovascular Division, BHF Centre of Research Excellence, School of Medicine, King's College London, UK) on 'Improving outcome after stroke by targeting the Nrf2-Keap1 defence pathway for neurovascular protection' and Ulrich Dirnagl (Department of Experimental Neurology, Center for Stroke Research Berlin, Charité – Universitätsmedizin Berlin, Germany) on 'Pre-, per-, post- and remote conditioning of the brain: expressway or roadblock?'.

The Symposium review articles will be published in a special Neuroscience issue of *The Journal of Physiology* and should be of interest to physiologists and researchers in the field of neuroscience and vascular biology.

Giovanni E. Mann
King's College London

Experimental **Physiology**
Translation and Integration
A publication of The Physiological Society

Paul McLoughlin, the new Editor-in-Chief for *Experimental Physiology*

The Physiological Society is pleased to announce the appointment of Paul McLoughlin as new Editor-in-Chief of Experimental Physiology.

Following a long association with the journal and having previously served on the Editorial Board from 2002 to 2010, Paul took up his post on 10 April 2011.

Paul is currently Professor of Physiology and Head of Biomedical Sciences at the School of Medicine and Medical Sciences, University College Dublin. He obtained his degree in medicine from University College Dublin in 1983 and undertook his doctoral studies at St Thomas's Hospital London, gaining his PhD from the University of London in 1993.

Paul's special interest is the vascular biology of the lung. His research focuses on the key mechanisms in the development and progression of lung diseases such as adult respiratory distress syndrome, chronic obstructive pulmonary diseases and cystic fibrosis. His group are working to identify novel therapies through the exploration of these mechanisms, and their work on the pathogenesis of pulmonary hypertension is recognised internationally.

Paul commented on his appointment: 'It is a great honour to take up the position of Editor-in-Chief of *Experimental Physiology*. The journal has made great advances under the editorship of David Paterson and it is ideally poised to become a leading journal in the field of integrative and translational physiological research. I'm really looking forward to developing the journal over the coming years.'

Under his five-year tenure, Paul plans to maintain the journal's focus on original, integrative physiological research and reviews, whilst developing new areas such as genomics, bioinformatics and imaging – which offer novel insights into physiological mechanisms.

He commented: "It is a particularly exciting time for the journal. Whole genome sequencing, rapid developments in bioinformatics, advances in *in vivo* genetic manipulation and powerful novel imaging technologies mark a new era in physiological and pathophysiological research. *Experimental Physiology* is extremely well placed to publish research from this 'post-genomic' era and really advance the field of integrative physiology.'

Early Career Author's Prize 2010 Winners

The winner of the 2010 *Experimental Physiology* Early Career Author's Prize is Thomas D. O'Brien, for the paper entitled 'In vivo *measurements of muscle specific tension in adults and children*'.

Thomas D. O'Brien, Neil D. Reeves, Vasilios Baltzopoulos, David A. Jones & Constantinos N. Maganaris (*Exp Physiol* (2010) **95**, 202–210).

The runner-up is Thomas Seifert for their paper '*Glycopyrrolate abolishes the exercise-induced increase in cerebral perfusion in humans*'

Thomas Seifert, James P. Fisher, Colin N. Young, Doreen Hartwich, Shigehiko Ogoh, Peter B. Raven, Paul J. Fadel & Niels H. Secher (*Exp Physiol* (2010) **95**, 1016–1025).

Both these papers are available free online: http://ep.physoc.org/

The Prizes will be presented on Tuesday 12 July at Physiology 2011 in Oxford UK.

For details of the 2011 prize and entry requirements, see Early Career Author's Prize on the website.

New cover for *Experimental Physiology* from June 2011

Erratum

It has been brought to our attention that the article by Richard Boyd (*Physiology News* **82**, 24) contained a couple of errors:

Richard Keynes was born in 1919 and died art the age of 90 (not 93).

Also, Alan Hodgkin lived from 1914 to 1998. Thus, he was 5 years older than Richard Keynes and therefore the senior author of the cited articles.

Alison Brading

1939–2011

Alison Brading (photo supplied by Anant Parekh, source unknown).

Alison Brading, who died on 7 January 2011, was a well-known and much loved figure in The Physiological Society who will be sorely missed. To those who worked with her she was a source of inspiration, a vast fund of enthusiasm and scientific knowledge, and a loyal and supportive friend. Her services to The Society were extensive. She was elected in1970, at a time when membership was strictly limited, peer-reviewed in competition with others, and largely male. She served on Council, was an editor of *The Journal of Physiology*, and Chairman of the editorial board of *Physiological News* between 1990 and 1992. She was elected to Honorary Membership in 2008. Although stricken with polio in her late teens, she was fiercely independent and determined to lead a normal academic life. She showed indomitable spirit in dealing with her disability. Her early training was in Zoology at Bristol University. She came to the Department of Pharmacology, Oxford, to work with Professor Edith Bülbring of whom she became a life-long friend, establishing a trust to support young scientists when Edith died. She was a familiar and respected personality at Physiological Society Meetings, Lady Margaret Hall and the Pharmacology Department. Throughout her life, she made substantial contributions to our understanding of smooth muscle function.

Alison was born in 1939. Her father was an army officer and so, as was common in such cases, she attended boarding school while her father was posted abroad. As well as being academically gifted at school, she was also a superb athlete, winning the *Victor Ludorum* at Maynard School, Exeter, for her athletic achievements. During the long vacations she would visit her parents in Nigeria. She made three such trips, usually with her brother Roy but tragically on the last she contracted polio, which caused severe disease. She was hospitalised in an iron lung, first in Africa and then, after an emergency flight home, in England. She was fortunate to recover, but suffered permanent and considerable loss of motor function. She was unable to walk unaided and had restricted use of her respiratory muscles, which caused her particular discomfort with bronchial infections and even the common cold. All this was a considerable blow to someone who, until then, had been outstandingly fit and able. There followed a prolonged period of convalescence, which involved operations to improve the motor function of her hands.

Before Alison's last trip to Africa she had applied for, and been accepted, to read medicine at Oxford. However, after two years of convalescence, she was informed that she would not be permitted to enter the medical course, because of her disability. Her subsequent life then involved an ongoing fight to remain independent and mobile. In her early years, with considerable will-power, she was able to walk distances using crutches (her 'sticks', as she called them) which placed considerable demand on her forearms and elbows. As she got older, however, walking became increasingly difficult, and she was forced to resort more to a wheel-chair. Nevertheless she was supreme captain of this transport, issuing firm and precise commands to whosoever was pushing at the time. Alison was also undeterred by the prospect of international travel. She frequently went abroad, whenever possible with friends or relatives. Thus, she was able to visit most international scientific meetings to which she was invited. In 1976, for example, she travelled to Leningrad, Moscow and Kiev with Edith Bülbring and Tom Bolton, visiting palaces and places of local interest, and finally contributing to the Smooth Muscle Symposium in Kiev organised by Mykhailo Shuba. Alison also travelled extensively in Europe, the United States and the Far East, including Taiwan and Japan, where she had good friends and contacts. Even in the final year before her death she travelled to a scientific meeting in Japan.

Alison Brading (right) with Edith Bülbring (photo supplied by Anant Parekh, source unknown).

Not discouraged by her early rejection from Oxford, Alison applied to read for a BSc in Zoology at Bristol and was accepted. She obtained a first-class degree and then chose to study for a PhD with Peter Caldwell, an expert membrane biophysicist, investigating the somatic muscles of the nematode roundworm *Ascaris lubricoides*. She qualified in 1965, but still held Oxford in her sights. It succumbed when she joined Professor Edith Bülbring at the Oxford Department of Pharmacology, as a post-doctoral research assistant, a position which continued until 1971 when she was appointed as a Departmental

Demonstrator, although she retained a close association with Edith. She was elected Fellow and Tutor in Physiology at Lady Margaret Hall in 1967 and University Lecturer in 1972; she became Professor of Pharmacology in 1996 and Emeritus Fellow at Lady Margaret Hall in 2005.

Although Alison's PhD training was on nematode somatic muscle, all her subsequent work was in the field of mammalian smooth muscle. While in Edith Bülbring's research group, she began to elucidate mechanisms that generate ionic asymmetry across the cell membrane. In smooth muscle this was a largely unexplored and open field. Although the Bülbring research group was internationally pre-eminent for the study of smooth muscle receptors and contractility, and for the first electrophysiological studies of smooth muscle using sharp microelectrodes, little was known about carrier-mediated ion transport. Alison's work initially involved using radioactive tracers and flame photometry. The task proved difficult as smooth muscle cells are generally long and thin with transverse dimensions not greatly different in size from those of the local extracellular space; thus it can be difficult to identify ion fluxes specifically across the plasmalemma, especially when ion diffusion is slowed by binding to extracellular sites. Measurements of intracellular ion concentrations also required elaborate correction for the extracellular space.

Alison's meticulous experimental approach set the scene for a more rigorous application, particularly of the radiotracer technique. Although initially part of the Bülbring group, she first published her work independently with Tadao Tomita from Nagoya, Johan Setekleiv from Oslo, and Alan Jones from Philadelphia who were visiting workers at the time. Edith did not have the now popular habit (and funding necessity) of group leaders adding their name to publications from individual members. As a result, Alison published only a couple of original papers with Edith although she did co-edit a book, '*Smooth Muscle*', a comprehensive summary of smooth muscle research, with Edith (and T. Tomita and A. W. Jones) in 1970 and a later edition of this book in 1981. Alison's early pioneering flux work was summarised in her paper, presented in a symposium at the Royal Society (Brading, 1973). Another brief publication from this period was with Tadao Tomita. They reported, at a meeting of The Physiological Society and published as a refereed abstract, that smooth muscle can generate action potentials in low sodium solution; indeed the rate of rise and overshoot was *greater* when extracellular Na^+ concentration was reduced (Fig. 1). This was a crucial step in the discovery that smooth muscles generally have calcium-based rather than sodium-based action potentials. A full paper followed (Brading *et al*. 1969). The report was thus the forerunner of a major branch of smooth muscle physiology and pharmacology, including the clinical use of calcium antagonist drugs that influence smooth muscle contraction.

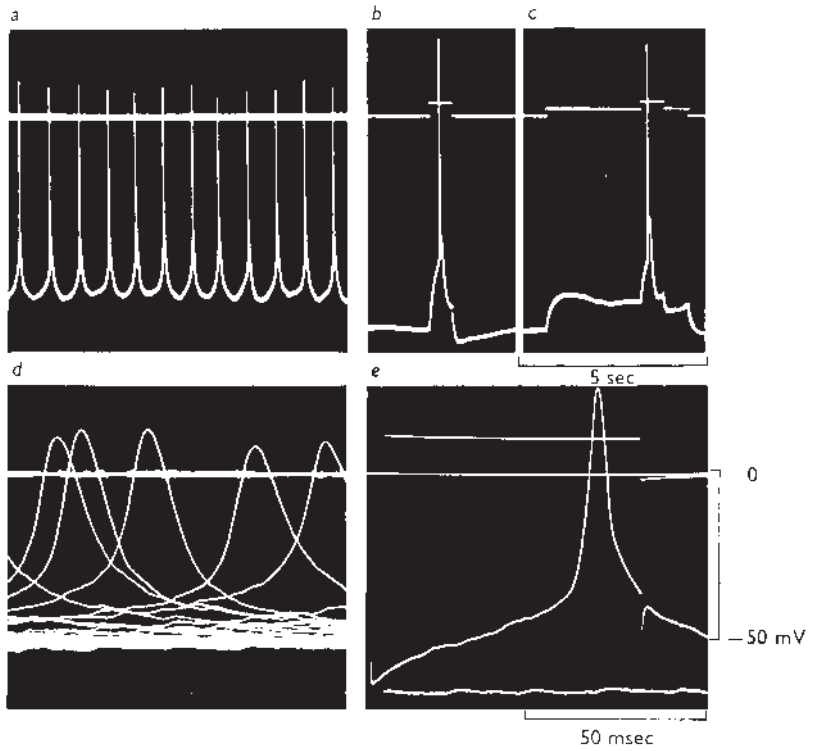


Figure 1. *a* and *d*, action potentials, spontaneous and evoked, in normal $[Na^+]_o$ (Krebs solution) and *b*, *c* and *e*, in 10 mM $[Na^+]_o$ (sucrose substitution). Note the increase in rate of rise and overshoot in low $[Na^+]_o$ indicating that the action potential is calcium based. Taken from Brading *et al*. (1969).

The analysis of ion fluxes and intracellular concentration continued to be a principl focus when Alison established her own laboratory in the Pharmacology Department, following her tenured appointment. She was joined by her first PhD student, Jonathan Widdicombe, and together they studied Na^+-dependent ion transport across the plasma membrane. These included Na^+/Na^+, Na^+/K^+ and Na^+/Ca^{2+} transport mechanisms.

The problems associated with radiotracer and flame photometric techniques continued to dog the smooth muscle field. Another technique, however, for recording local ion concentration was coming on-stream through the work of Roger Thomas in Bristol. He had developed the use of ion-selective microelectrodes (ISMs) for monitoring intracellular Na^+, Cl^- and pH directly inside snail neurones. Alison was joined for a period by a post-doctoral assistant, Richard Vaughan-Jones, from the Thomas laboratory. Vaughan-Jones

started to use ISMs initially to measure intracellular Cl^- and H^+ ion concentrations (with Tom Bolton) in skeletal muscle, and later in cardiac muscle. Alison was joined a few years later by a further post-doctoral assistant, Claire Aickin, again from the Thomas stable. Claire extended the ISM technique to include mammalian smooth muscle. Applying the technique was difficult enough in tissues with relatively large cells, such as those of skeletal muscle and cardiac Purkinje fibres. Adapting the technique to the small contractile cells of smooth muscle required true perseverance, and not inconsiderable serendipity. But with Alison's enthusiasm and support, and Claire's expertise, it was achieved. This led to a major series of studies on the membrane transport of Cl^-, H^+ and Na^+ ions, including the first description in smooth muscle of the Cl^-/HCO_3^- exchanger and its participation in the process of intracellular pH regulation. Further ISM recordings of intracellular Na^+ led to important functional work on the Na^+/Ca^{2+} exchanger (Fig. 2; Brading *et al*. 1987), a transporter whose expression in smooth muscle had previously been questioned. This ISM work in the 1980s (and reviewed in Brading & Aickin, 1990), produced several milestone publications on the fundamental physiology of smooth muscles.

Alison's work on Na^+/Ca^{2+} exchange was paralleled by her studies of smooth muscle contractile activity, particularly the role of the sarcoplasmic reticulum as a receptor-operated store of releasable Ca^{2+}. Indeed, she was among the first to moot for smooth muscle the possibility of capacitative entry of extracellular Ca^{2+} directly into intracellular stores as a means of rapid refilling, a phenomenon that to the present day is still being researched and debated. Indeed, a later doctoral student, Anant Parekh, now Professor of Physiology at Oxford, cut his teeth in Alison's laboratory on studies of Ca^{2+} regulation, and has gone on to specialise in the study, in various cell types, of the I_{CRAC} (capacitative entry) channel.

The 1980s saw Alison being joined by many visiting scientists from abroad, and her interest moved increasingly from taenia coli to other smooth muscles such as vas deferens and ureter. She formed collaborations with surgeons and clinical urologists, such as Jacek Mostwin, Gary Sibley and Mark Speakman, and later with others at the John Radcliffe Hospital in Oxford. This shift into more clinically oriented research began with investigations of the overactive bladder and the resulting incontinence it causes. The quest, an excellent example of a successful translational research axis for a basic scientist (and long before it was fashionable or politically encouraged) would occupy Alison for the next twenty-five years. Her research involved examination of bladder innervation and the effects of potentially useful drugs to relax the detrusor muscle. In addition, with the help of her clinical colleagues she studied the effects of partial outflow obstruction on the pig bladder.

While collaborating on her clinical studies, Alison also investigated the fundamental cellular properties of detrusor muscles, thus helping to fuse more effectively both basic and clinical science. This work involved electrophysiological investigations by doctoral, postdoctoral and visiting academics such as Inoue, Burdyga, Teramoto, Nakayama, Parekh, Bramich and many others. Investigations were also extended to smooth muscles from a variety of other organs, and from a widening range of species, including pigs and even humans.

In addition to her research on the bladder, Alison in later years studied the properties and responses to drugs of anorectal smooth muscles. As with much of her earlier work, this was a largely unexplored area, ripe for development. Her interest focused on two areas: to what

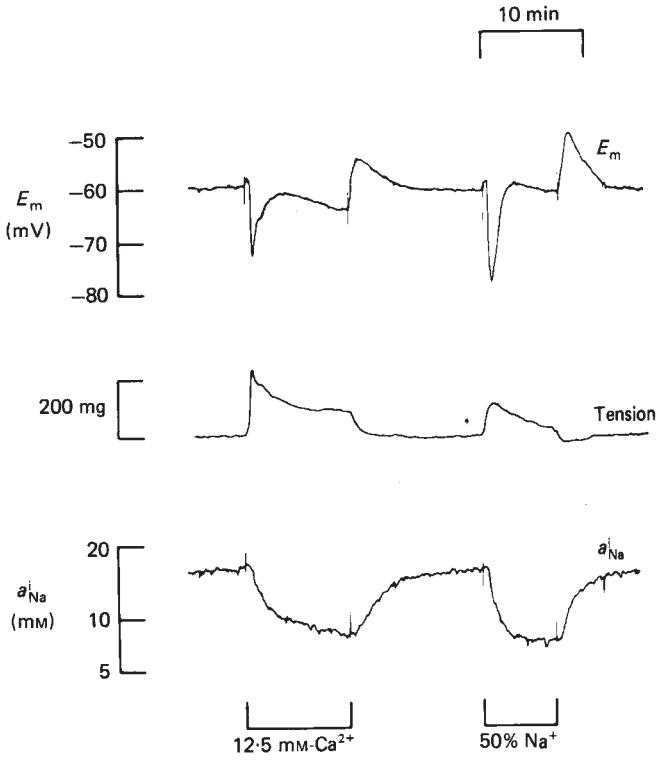


Figure 2. Sodium–calcium exchange in ureter of guinea-pig. Raising $[Ca^{2+}]_o$ hyperpolarizes the membrane, causes contraction due to the increase in calcium entry, and a reciprocal reduction in $[Na^+]_i$. Lowering $[Na^+]_o$ allows the Na^+/Ca^{2+} exchanger to hyperpolarize the membrane, while the reduction in calcium extrusion results in contraction (Fig. 1 from Brading *et al*. 1987).

extent derangement of bladder (detrusor) function was associated with alterations in anorectal smooth muscle function, and whether diseases such as irritable bowel syndrome were associated with changes in the properties of anorectal smooth muscle. Many of her studies were again collaborative, notably with Mortensen, a consultant gastro-intestinal surgeon at the John Radcliffe Hospital. Most recently Alison studied the innervation and function of the anal sphincter and rectum in collaboration with Radomirov and Ivancheva of the Institute of Neurobiology, Bulgarian Academy of Sciences.

In her determination to be mobile after her convalescence from polio, Alison learned to drive a car in which the controls were modified. Although this could sometimes be rather scary for an unwary passenger, it enabled Alison to lead an essentially independent life, eventually commuting between her Department in Oxford and her cosy cottage in the outlying village of Thrupp. In later years, her relatives moved in next door, so that family members were gathered around her. The house bordered the Oxford canal, and so she bought a narrow-boat and enjoyed trips in the company of friends and students. Just as she was captain of her wheel-chair, she was captain of her boat, issuing firm commands from the wheel-house, with crew members dispatched regularly onto the towpath to operate lock-gates and wave her through. For those involved, the trips were immensely sociable, and always memorable.

The job of a Tutorial Fellow at Oxford requires maintaining a research group, but also admitting, teaching and mentoring undergraduate students, in Alison's case in Medicine and Physiological Sciences at Lady Margaret Hall. Her dedication to her research group was matched by her commitment to her College and her undergraduate charges. She was an extremely gifted and vigorous

Alison Brading at the tiller of her narrow boat (photo courtesy of Roger Thomas).

teacher, who talked with, rather than at, her students. She did not tolerate fools gladly, but she was genuinely caring about students' welfare and academic development. And she was passionate about enthusing students with an understanding and respect for science. That was also true of her approach to her own scientific learning. In her early years in the Department of Pharmacology at Oxford it was not uncommon for Alison to appear suddenly on her 'sticks' in one's office, enthusing about a marvellous paper she had just read in the most recent edition of *The Journal of Physiology*, often in an area far outside her own

Photo kindly supplied by Karen Brading.

expertise. For her, it was a lifelong love affair with science, and with the teaching of science.

In the end, Alison was defeated physically by her disability. She contracted pneumonia and, with her respiratory difficulties, this was hard to fight for a prolonged period. But Alison's spirit, even during this last illness, was never dimmed. In all, she should be celebrated for her tenacity in the face of early misfortune, for her energy and indomitable enthusiasm, for her loyalty and support of colleagues and friends, and for her broad and far reaching scientific and academic achievements. There is no doubt The Physiological Society has been the better for her.

Tom Bolton[1] and Richard Vaughan-Jones[2]

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[2]Burdon Sanderson Cardiac Science Centre, Department of Physiology, Anatomy & Genetics, University Laboratory of Physiology, Parks Road, Oxford, UK

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James (Jim) Edward Pascoe

1924–2011

Jim Pascoe was a Reader in Physiology at University College London. He was born in Falmouth and at the age of 12 moved to Penzance. Thence at age 16 he worked at his mother's behest in an apprenticeship at Boots, transferring to Plymouth College to start his studies for the Diploma in Pharmacy which he completed at the School of Pharmacy, London where he then became a Junior Lecturer and where he met Margaret, a pharmaceutical chemist who became his wife. He studied Physiology whilst working nights in Boots, Piccadilly and so came to UCL at the invitation of G.L. Brown, the head of department, and then on to the staff where he was known for his absolute integrity and devotion to the subject. In 1954 he was elected a Member of The Physiological Society.

In the '60s and '70s the Annual General Meeting of The Physiological Society was always at UCL. There would be Jim Pascoe listening to the Communications in the lecture theatre, often alongside G.L. Brown, and clearly a close friend. Sometimes I thought they appeared very similar except that Jim was thinner. He had that intent questioning look that did not allow you to get away with any nonsense. At the same time he was wonderfully jolly and positive if he judged you to be serious about advancing the subject. At that time I did not know him at all well – he was to me a figure in The Society. Always something of a stirrer, he enjoyed tweaking the tail of The Physiological Society Committee. After joining the Committee in 1979, he remained the blunt-speaking champion of many causes.

He quite naturally, perhaps inevitably, had something of a falling out with *The Journal of Physiology* where the editors rejected a paper on the grounds, so he was told, that there was too much pharmacology in it. He was of course in good company since the great Nobel Prize winner Eccles had a paper rejected. Jim's reaction in the demotic argot was

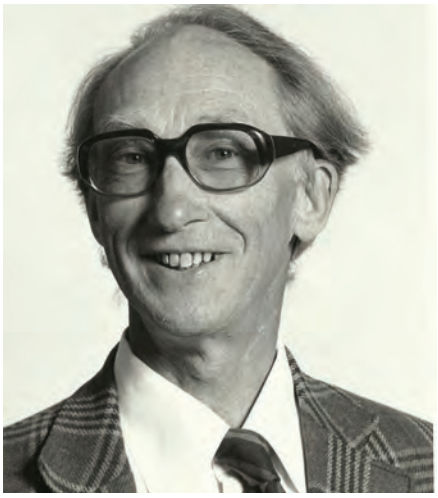


Photo with kind permission of Alice Pascoe.

'sod them', and he never really got round to publishing much after that rejection. Such is the untoward power of editors.

He was a sailor, and Ann Silver describes one of his trips:

"He sailed his boat along the south coast and round to East Anglia, then up the River Deben to Woodbridge. This involved his successful negotiation of the tricky Horse shoal at the mouth of the river. I was very impressed by this but what impressed me even more was that when I drove over from Cambridge to show him some of north Suffolk, he made sandwiches for us without benefit of breadboard, slicing the loaf held vertically between his knees". Furthermore she describes on this trip how "We visited Orford Castle and several splendid churches. Jim couldn't resist the urge, left over from his choirboy years, to test the acoustics by bursting forth into hymns and psalms – occasionally startling some poor lady engrossed in arranging the flowers."

I came to know him well after I went to work at UCL. He was a marvellous ally who would drift in to see me from time to time to discuss the department and check out how I was getting along running it. We would talk about all manner of problems and he was always absolutely frank with me, telling me if he thought I was wrong about some problem and also if I was correct. Such straightforward advice from someone of absolute integrity was invaluable to me.

We also did some experiments together and he wrote the software for an early laboratory computer, the Z80. He would stand in the lab dictating the machine code while Michael Duchen and I would listen in awe and write it down. And the program worked, enabling us to measure changes in sensitivity of primary afferent nerve terminals in the spinal cord in real time.

He had a great fund of stories about the previous heads of department he had known including G.L. Brown, Andrew Huxley and Doug Wilkie – stories always told with great good humour and affection. He had the greatest respect for the College and its secular traditions, and for the Department of Physiology with a long history going back to the foundation of the College in the 1820s.

We worked most closely during the 1980s when there was considerable activity in the Palace of Westminster over the issue of animal experiments. The anti-vivisectionists were very active at this time and increasingly violent, for example, attempting to set fire to the house of a Nobel Laureate and attaching a bomb which fell off and detonated under the car of a Bristol physiologist. The Act of Parliament under which we all did experiments supervised by the Home Office Inspectorate had been passed in 1876. It was clear that with the changes in biological science there was a need to update the Act. Thus we became involved with the political process in The Lords and the House of Commons. There was a Private Member's Bill in the Lords and in the Commons, more seriously, there was a Private Member's Bill and a Government Bill succeeding it. There were a number of MPs who were positive supporters – Sir Nigel Fisher, Mr Ray Mawby and some others, but the most outstanding one was Tam Dalyell. As ever he was brave, articulate and hugely supportive of the scientific endeavour.

During discussion of the Private Member's Bill in the Committee Stage there was an accusation that experiments were being done involving cutting off the feet of rabbits, and this procedure should

be stopped. It turned out that there were no such experiments in the UK and if there were such things done in any country they were on anaesthetised mice. Tam Dalyell suggested that Jim and I bring to the House a rabbit and a mouse which he could then produce during the debate to show to our opponents the difference between these species. This we did and were, all four of us, smuggled into the Palace of Westminster through a back door by Tam Dalyell and so to the Committee room. Naturally the display of these animals caused embarrassment to the other side and even some mirth. The Chairman of the Committee, Mr Ted Leadbitter, did not demur. Not long after this the Bill was withdrawn. There followed the long period of argument and debate over the Government Bill in which Tam Dalyell was steadfast and Jim was continually involved along with a small group of other physiologists including Ann Silver, Cecil Kidd, Tony Angel and David Whitteridge. Robert Comline the Treasurer of The Society insured that whatever funds were needed were available.

Through this long period, perhaps 4 or 5 years, Jim retained his sense of humour and moral support and

A cartoon of Jim, drawn by Ralph Sallon (with kind permission of Alice Pascoe).

without him we would all have been much less effective.

He retired from UCL Physiology and then, as he said, was a bag or case carrier for his beloved wife Margaret who had become an authority on 'blackwork embroidery'. He took the photographs of embroideries and materials that she needed for her book, a technology that he had learned as a physiologist and in which he was expert. They travelled abroad with Jim as the significant other, a reversal of earlier roles which he greatly enjoyed. Such was his expertise that he then went on to photograph the work of others in the Embroiderers Guild as well as the historical collection of the Guild housed at Hampton Court Palace.

After Margaret died in 1995 he stayed on in Molesey and eventually in 2004/5 went to live in Chacewater in Cornwall and latterly in Truro. He was cremated at the Penmount Crematorium a place where my father used to take services and was also cremated, a touch of continuity that Jim would have greeted with a merry laugh. He also laughed heartily when long ago I told him that my father had made an insurance claim for his cassock which caught fire while he was waiting to take a service at the same crematorium.

He was a great colleague and a dear friend to many of us; he lived life and was much loved.

Tim Biscoe

An aside from Ann Silver

The oldest Members of The Society will have started their animal experiments under legislation enacted in 1876. Since this was long out-of-date by the middle of the 20th Century, it is understandable that from about 1965 onwards several moves were afoot to modernise the legislation. The Physiological Society and the Research Defence Society (now 'Understanding Animal Research') were at the forefront of those keen to weed out any new, ill-conceived regulations that could hinder research with no proportionate benefit to animals. Tim Biscoe indicates above that Jim was particularly active in these efforts. As well as priming Tam Dalyell at Parliamentary Subcommittees, he spent many a Saturday in Leeds with Cecil Kidd, Bernard Ginsborg and me, discussing the latest government proposals and formulating The Society's response. In addition, he once came to Cambridge for a memorable meeting in St Catharine's College at the invitation of Robert Comline, then the Society's Treasurer. Although productive, it was memorable for the wrong reasons. A cold, overcast day, Robert took us into Hall for a self-service lunch before we began our discussion. The lighting was rather poor and the most recognisable offerings were the salads but Robert insisted that we needed something hot. He hustled us along the counter to a rather tempting steak and kidney pudding with a thick suet crust; to this Cecil and I added a good helping of cabbage. Jim, invoking his choirboy days, smugly said that as it was Friday he would have the fish. As soon as Cecil and I started to eat we realised our mistake – our pudding was not full of steak and kidney but sweet, stewed, plums that went none too well with the cabbage. To add to the problem, I had a stone – plum not kidney – and not wanting to disturb Robert, I stored it, hamster like, in my cheek. Had he been aware of our dilemma, the ever-hospitable Robert would have insisted Cecil and I started again. When Jim and I went to fetch the coffee he said 'What the hell is the matter with you two?' Inevitably there was much scarcely suppressed mirth whenever we caught each other's eyes during our afternoon deliberations. A year or so later, The Committee met (I think in Leeds) ready for the Scientific Meeting beginning the following afternoon. When it came to the question of the pre-Meeting lunch, Robert said, indicating Jim, Cecil and me, 'You can't trust this lot not to disgrace us.' Apparently our failure to identify puddings had been revealed to Robert, during a convivial gathering the previous evening, after I had gone off to bed.

Sidney Montague Hilton

17 March 1921–28 January 2011

Sidney Montague Hilton who died recently had been Bowman Professor and Head of the Department of Physiology at the Medical School in Birmingham from 1965 to 1983. He was a distinguished physiologist who made leading contributions to understanding brain control of cardiovascular changes accompanying behavioural reactions associated with sudden emergencies like the fear–flight–fight reaction. These have relevance to psychosomatic disease and to chronic hypertension. Hilton was in the vanguard of thinkers who provided experimental evidence against the long-held view that there is a single brain centre controlling blood pressure. He was an excellent lecturer and travelled widely.

Sidney Hilton was educated at St Paul's School, London from where he won a place to study medicine at Cambridge having gained an exhibition at Jesus College. During the second World War he was allowed time to take the Part II Honours Tripos course in Physiology following which he did his clinical training at Guy's Hospital, London where he subsequently held several house appointments. He then was required to do National Service and for this he served in the RAF at the Institute of Aviation Medicine, Farnborough. Here he assisted in developmental work on the design of flying equipment and with Professor J Beattie at the Royal College of Surgeons carried out studies on ways to accurately measure oxygen levels in the blood, non-invasively. On leaving the RAF in 1950, Sidney returned to Cambridge to the lab of Professor Adrian (a Nobel Laureate, later to become Lord) who had done ground-breaking work on the generation of vasomotor nerve activity by the brain, a key element in the control of blood pressure. It was here that he decided on a career in physiological research and possibly the seeds were sown for his later major contributions. He then joined the scientific staff of the Medical Research Council

Sidney Hilton on his arrival in Birmingham in 1963.

(MRC) at Mill Hill, London, where he worked in the division led by another great figure in biomedical science, Professor Feldberg, who had made major discoveries concerning chemical transmission between nerves. Hilton's major interest at the time was the local mechanical and chemical control of blood vessels. He found that the widespread dilatation in the large arteries supplying skeletal muscles during contraction depended on transmission by smooth muscle conducting elements in the walls of the arteries. This conducting system turned out to be functionally important for ensuring that a local stimulus caused a widespread opening up of the arteries supplying an organ. This was probably the earliest evidence pointing to a chemical mediator that we now know is nitric oxide, a major dilator in all vascular beds although Hilton concluded at the time it was a mechanically driven mechanism. There followed studies on blood flow and secretion in salivary glands which in collaboration with Dr Graham Lewis provided the first evidence that the increase in blood flow essential for secretion was not solely dependent on nerve-mediated release of acetylcholine but also on the simultaneous release of polypeptides. Subsequently the polypeptides became recognized by others as important agents for ensuring blood flow in organs such as the heart and other vascular beds, and were later targeted in clinical treatments for cardiovascular disease. At the end of the 1950s his attention began to move to the regulation of the heart and vascular system by the brain, and it was here that arguably he made his biggest contribution. He had become fascinated by the hypothesis of Cannon concerning the fright–flight–fight response (defence response) so fundamental to survival in wild animals but when over-expressed in humans can lead to chronic hypertension. At the time little was known as to how the brain initiated the defence response. Hilton realized that a key feature of this response is a selective vasodilatation in the skeletal muscle vascular bed and this could possibly be used as a marker to locate relevant brain regions in experimental animals. To study this he needed to gain experience of behavioural methods and for this there was no better place than the laboratory of Professor Kornoski (a former pupil of Pavlov) at the renowned Nencki Institute of Experimental Biology of the Polish Academy of Sciences in Warsaw. Thus, he returned to the land of his grandfather, a Polish Jew who had emigrated to London from Lodz in the early part of the 20th century. It was at the Institute in Warsaw that he was fortunate to team up with Andrzej Zbrożyna, a brilliant young behavioural physiologist who later joined Hilton at the MRC Institute in England. The two were then joined by Viv Abrahams and together they used the muscle vasodilatation in anaesthetized animals as an identifying feature when electrically stimulating at sites throughout the brain. Subsequently repeating these experiments in conscious animals it was shown these were the same regions that evoked defence responses. These studies provided the first description of brain regions that are essential for initiating the typical pattern of cardiovascular, respiratory and skeletal muscle changes that comprise defensive behaviour. More recently modern brain imaging techniques and deep brain stimulation, which is primarily used to treat a variety of brain-based disorders, has confirmed that similar brain regions are involved in aggressive behaviour in humans. From these studies it was clear to

Hilton that no longer could a single localized area in the hind brain be considered responsible for blood pressure regulation, a concept that had stood for 50 years and appeared in every textbook. It was now necessary to define a model based around discrete reflex control of parallel pattern generators for the cardiovascular responses accompanying various types of behaviour. These studies which were started at the MRC Research Institute, continued with Andrzej Zbrożyna, who moved with Hilton on his appointment to the Bowman Chair of Physiology in Birmingham, a university where his younger brother Peter, a highly distinguished mathematician, had recently occupied the chair of mathematics. Interestingly Dr Zbrożyna had also played a significant part in helping Hilton's future second wife Gerta Vbová and her two children to accomplish a remarkable and harrowing epic escape from Soviet-occupied Czechoslovakia described in Professor Vbová's recent book '*Betrayed Generation*' (for a review of this book, see p. 41).

Hilton was a complex, perhaps egotistic, person whose measure of mischief in scientific and administrative confrontations often led to acrimony and this perhaps resulted in his not receiving the full credit for his scientific contribution. However, the move to Birmingham provided an opportunity to build up a department that had lost most of its staff. Hilton built up its teaching and its research to a state where it became not only one of the strongest departments in the University but also in the UK, whilst its research quality became recognized internationally. It was also a happy department and during this time it nurtured many 'prima donnas' providing three Heads of Departments at other universities as well as a Director of the Army Personnel Research Establishment in Farnborough. During this time Hilton also helped to establish the Physiology Department in the new Medical School in Salisbury (Harare), Southern Rhodesia (now Zimbabwe) that had been founded in 1963 with a special relationship to the University of Birmingham. The early days of Hilton's time in Birmingham were very happy ones for budding physiologists who not only enjoyed the intense 24/7 research activity but also the social events of parties in Gerta Vbová and Hilton's home as well as other social occasions. During this time Hilton served as Secretary of The Physiological Society where his irreverent sense of humour was not always enjoyed although his contributions to scholarly debate at scientific meetings of The Society were often a highlight and much missed on his retirement. In 2004 he was awarded the distinction, given to only a few, of being elected as an Honorary Member of The Society.

He retired in 1984 and moved with his third wife Mary to what up to then had been her family holiday home, a lovely rural but somewhat remote setting on a mountain side, above Lanelltyd near Dolgellau in West Wales. Despite being quite cut off from the scholarly environment he appeared to be very happy, much enjoying the attention of Mary's children and grandchildren. Recently he had renal failure but was looking forward to reaching his 90th birthday in March of this year but this sadly was not to be. He is survived by his third wife Mary and a number of children from previous marriages.

John Coote and Mike Spyer

Will Redfern (former postdoc of SMH) continues:

Sidney Hilton was the external examiner for my PhD in 1982, and after the viva he invited me to join his group as a postdoc. I was honoured to be asked, and am still proud to have been a part of the last research group he had before he retired. I was funded jointly by the MRC and ICI, and worked with him from 1982 to 1985 in the Department of Physiology at Birmingham. The bulk of my project involved a comparison of electrical stimulation of brain stem 'defence areas' (hypothalamus and periaqueductal grey) in the rat with pharmacological stimulation using an excitatory amino acid. Back then, everything was analogue, cardiovascular and respiratory recordings were collected on a multi-channel chart recorder, all analysis was manual, manuscripts were first written out in long-hand, and there was no PubMed. He had an office adjoining our lab, and would pop in enthusiastically, puffing on a cigar, inadvertently triggering a nasopharyngeal reflex in the anaesthetised rat with the smoke.

I had read most of his published work on central cardiovascular control during the course of my PhD, and it had captured my imagination. His writing style reflected his personality, particularly in his reviews: in person he was engaging and witty, with a formidable intellect. Where most physiologists grappled with 'single issue' topics, he quite happily took on multi-functional, integrated responses that were fundamental to survival of the organism. He was very territorial in 'owning' this area, and like a gamekeeper apprehending a poacher, would tackle head-on anyone who wasn't going about it correctly! Before I joined his group I saw him doing this on more than one occasion at scientific meetings – it wasn't just what he said, but the subtly disparaging inflections in his voice – which later left me apprehensive ahead of my PhD viva, and always somewhat in awe of him. Fortunately I avoided ever being on the receiving end of this; on the contrary, he was always very encouraging and supportive. Sidney operated in a golden age of 'holistic' systems physiology (not to be confused with the current trend for 'systems biology') in the UK. He and his numerous collaborators have left a lasting legacy in our understanding of centrally co-ordinated patterns of responses to internal and external challenges and perturbations. I joined the pharmaceutical industry after my postdoc at Birmingham, and eventually moved to AstraZeneca, Alderley Park (formerly ICI), which I suppose could be considered a return on their investment. I last saw Sidney at his 80th birthday 'do' in Birmingham in 2001, where he was in fine form, and I'm so sorry he didn't make it to his 90th. It wasn't like Sid to miss a party.

John Archibald Browne Gray FRS

1918–2011

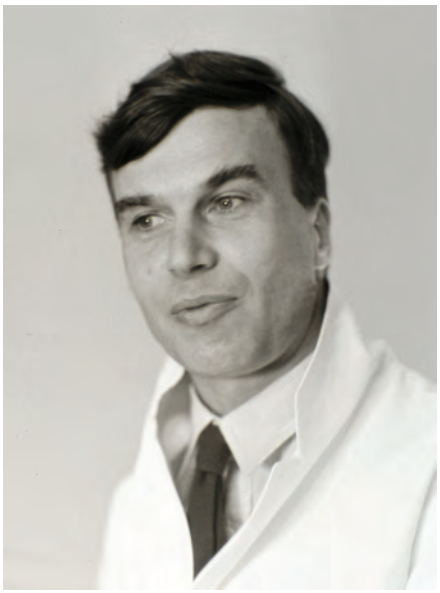
Died Jan 4th 2011, age 92

From Pacinian corpuscles to the MRC

John Gray was one of an amazing generation of physiologists who contributed so much to our subject in the years immediately following the Second World War.

After studying at Clare College, Cambridge and UCL Medical School, John Gray graduated in medicine in 1942. He then did wartime physiological research, much of it at sea with the Pacific Fleet. Post war he worked at the MRC's National Institute for Medical Research (NIMR) at Mill Hill with J.L. Malcolm and G.L. Brown (always known as G.L.).

The topic was sensory transduction of touch and pressure and whether this involved direct mechano-electrical transduction or occurred via an intermediate chemical step. The parallel was with the then great controversy about synaptic transmission – chemical or electrical? G.L. was firmly in the chemical camp on synaptic transmission, but one of the main achievements of John Gray was to show that certainly for mammalian mechanoreceptors, chemical intermediates were not involved. When G.L. moved to head Physiology at UCL he soon recruited John Gray as a Reader. John Gray started his work on Pacinian corpuscles at Mill Hill and continued it at UCL. In a series of papers from 1950 to 1957 in *The Journal of Physiology* he established the rapidly adapting nature of this mechanoreceptor, demonstrated its remarkable sensitivity to rapid movements (threshold less than 1 μm) and established the importance of ion gradients for the receptor potential. The experiments were mostly *in vitro* and were carried out with a series of students and postdocs including Peter Matthews, David Inman, Jack Diamond and notably M. Sato. The Gray and Sato paper in 1953 (*J Physiol* **122**, 610–636) was a technical tour-de-force and significantly pushed forward our understanding of mechanoreception.



John Gray from his days at the NIMR, Mill Hill.

John Gray played an active part in planning and delivering the teaching programme at UCL. A big project was trying to get more integration across departments within the biomedical degree programmes. He also insisted that all physiology students get serious university-level teaching in physics and chemistry. His Inaugural Lecture (he became a professor in 1959) was entitled *The Place of Physiology in University Education* and he argued for the value of physiology as a broad-based education in life sciences. John Gray became Dean of the Science Faculty at UCL in the early 1960s and found himself much distracted by academic administration.

In 1966 he was offered the post of second secretary at the MRC, with the prospect of soon taking over as Secretary (in practice Chief Executive). In the end he decided to desert research and ran the MRC until 1977. He explained part of his thinking to me as follows: "If I am to become an administrator, better to do this in an organisation that understands administration".

When he finished as MRC Secretary he arranged a deal that ensured his employment for some years as a member of the MRC External Staff. This enabled him to move to his home area in Devon and base himself at the Marine Biological Association Labs in Plymouth. There he worked with his old friend Eric Denton on the physiology of the lateral line in fish, publishing two papers in *Nature* in 1979 and 1982, as well as a number of other papers through to 1991, mostly in the *Journal of the Marine Biological Association*.

For me, John Gray was an inspirational teacher and supervisor. As mentioned at the start, he belonged to the post-war cohort whose memorial is surely that their research provided the foundation that fostered the development of physiological science over the last 70 years.

Bruce Lynn

The Society also notes with regret the death of Francis (Frank) Beswick, who died peacefully on 11th January 2011 and was elected a Member in 1960, and Carlos Chagas Filho, who was elected a Member in 1952

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(For 2011 Symposia see Noticeboard on p. 34)

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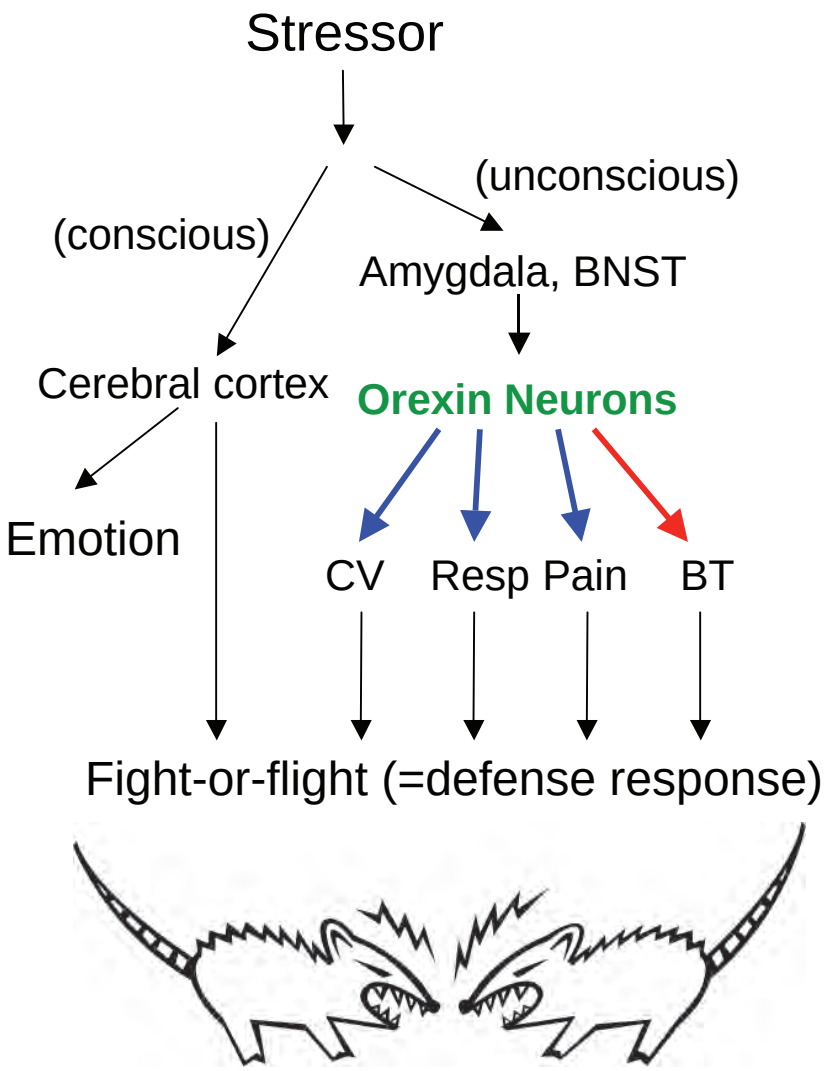

Hypothetical diagram showing the key role of orexin neurons in the fight-or-flight response (p. 15).

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