PHYSIOLOGY NEWS

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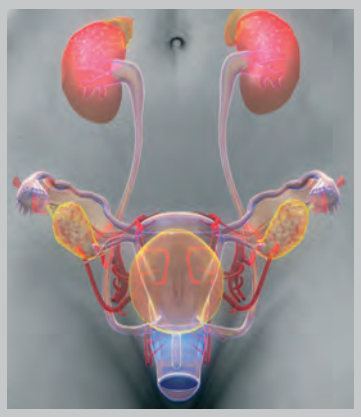

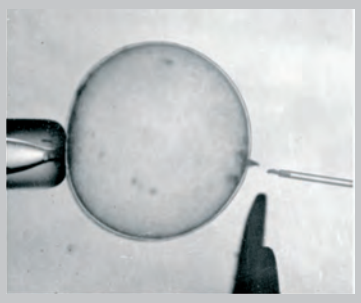

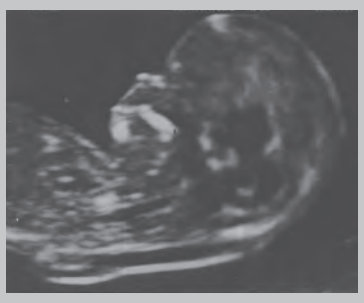

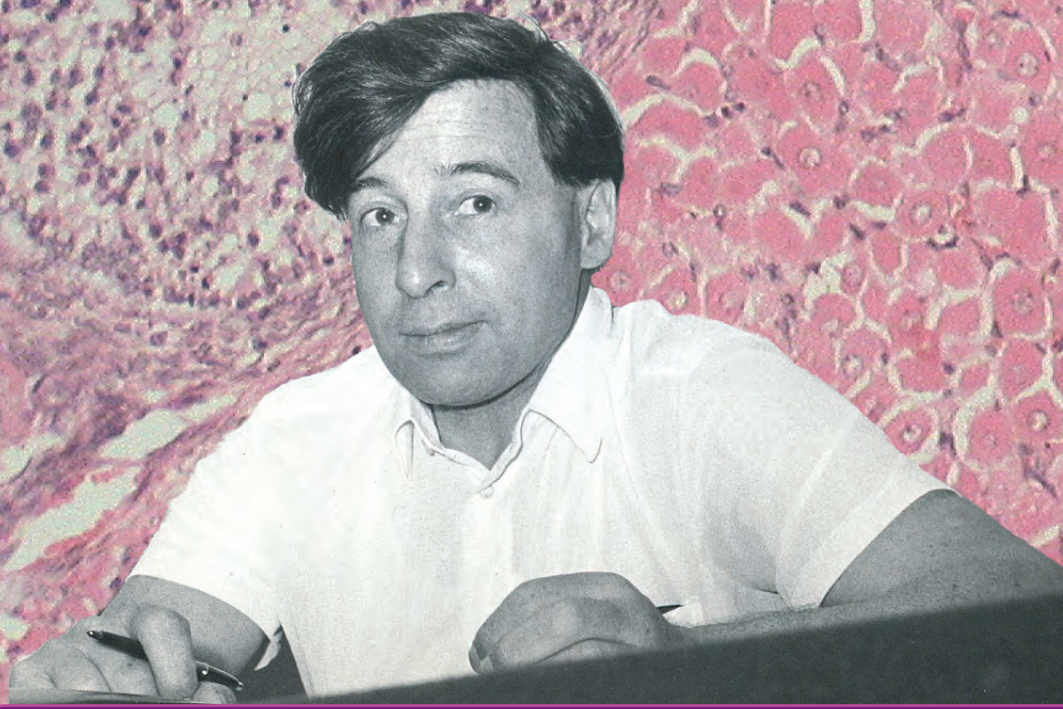

Bob Edwards' pioneering research into fertility

The cost of breathing in heart failure

A tribute to Richard Keynes' legacy

What makes some axons more excitable?

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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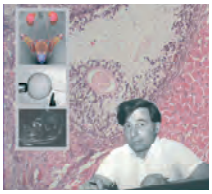
The Physiological Society

Advancing the science of life



Cover image: background, guinea pig corpus luteum (from Dr Dhia Mukhtar, University of Southampton; large photo of Bob Edwards (courtesy of Ruth Edwards): top to bottom of 3 images on left: female reproductive system (from SPL); rabbit blastocyst; and a 12 week fetus. p. 18.

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Grants
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Membership applications
Applications for membership of The Physiological Society are considered on a rolling basis, and a decision is normally made within 15 working days. For full information, please visit: www.physoc.org/membership

Is your membership information correct?
Please check and update your details at www.physoc.org, under 'My Physoc Profile'.

Physiology News

Deadlines
Letters and articles and all other contributions for inclusion in the Summer 2011 issue, No. 83, should reach the Publications Office (magazine@physoc.org) by **7 April 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).

Physiology News online
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 Spring 2011 *Physiology News*.

The centrepiece of this issue is obvious – Sir Richard Gardner's riveting personal account of Bob Edward's Nobel Prize winning work (p. 18), which Richard himself was a part of in the 1960s and 70s. With some wonderful hitherto-unseen contemporary photographs, we hope it is a fitting tribute to Bob Edwards, a real visionary and a worthy (and long overdue) Nobel Laureate.

In some ways this Spring 2011 issue is a kind of 'serendipitous' special one for the history of late 20th century physiology. Thus, Richard Boyd comments on the lasting legacy of another Cambridge physiologist, Richard Keynes (p. 24), while John Coote gives us his ten key papers on the brain–heart connection (p. 10), which span the years 1960–2001. Meanwhile, nerves and muscles – including heart – also form a (wholly spontaneous) theme in our Science News and Views articles.

In Society matters, we welcome David Paterson as the new Editor in Chief of *The Journal of Physiology* (p. 51), and interview his predecessor, William Large, now departed for retirement in the sunnier climes of Southern California. Though sadly we don't find out if William is going to be surfing.

Finally, even though 2011 is not long underway, meeting organisers are already gearing up for this Summer's meetings (pp. 4–5) and indeed for some a couple of years beyond that (p. 8). Start planning now ... given the fairly grim news on UK science funding (see Editorial), I dare say we will be needing a get-together and a bit of mutual support.

Austin Elliott
Editor

All change please... government funding terminates here

The result of the votes on higher education (HE) funding and student finance in the House of Commons on 9th December 2010 will change the face of English higher education entirely. Anyone hoping that the House of Lords would rescind the coalition government's victory was sadly disappointed, as the Lords' votes on 14th December supported the proposals by a clearer majority than the earlier 21-vote margin in the Lower House (Table).

Had the eight Liberal Democrat MPs who either abstained or were absent voted against the motions to increase tuition fees, the government would have still won. Had the 27 Liberal Democrats who voted with the government rebelled, and stuck to their pre-election promise not to raise tuition fees, the government would have been defeated. Amongst the Lords who voted for the motions to increase fees were academics Baroness Blackstone, former Master of Birkbeck, University of London, and currently Vice-Chancellor of the University of Greenwich, and Lord Krebs, son of Hans and currently Principal of Jesus College, Oxford. Surprisingly, the vast majority of Contents (>160) were Labour Peers, and most Not-Contents Conservative. Such is politics. The changes to tuition fees will little affect those who actually voted, except perhaps for the 27 'turn-coat' Lib-Dems – who may find their electorate sadly disillusioned (and ready to exact penalty) at the next trip to the polling booth. The changes resulting from the politicians' actions, however, will fundamentally alter HE when they take effect on 1st September 2012.

The issue runs deeper than simply the fees students will pay, but I will start there. The government's argument is that nobody will have to pay fees up front, a compelling point. Furthermore, they need not pay back a penny until they earn over £21,000, and then only at a rate of 9% of earnings above this amount, so for example, a graduate earning £25,000 will repay their loan at a rate of £6.92 per week. Finally, after 30 years, any outstanding debt is written off. This does not sound too onerous, on the face of it, but the devil, as ever, is in the detail. The weekly repayment rises to £34.61 per week for those earning £41,000, for example. And the £21,000 earnings threshold refers to earning some years in the future; comparisons to current earnings suggest most graduates will start loan repayments as soon as they get their first full-time job. Overall, the minimum debt a student will incur via fees is £18,000, to which the cost of student loans for living and other expenses will need to be added. For the fees loan, those earning above the repayment threshold will be charged interest at a variable rate reaching a maximum of the retail price index (RPI) plus 3%. In November 2010 the RPI was 4.7%[1], which means that someone earning £41,000 would be charged a minimum of £1386 interest per annum. Clearly it will be advantageous to pay your debt off as quickly as possible, if you earn enough to do so.

Whilst the media focus on loan repayments, more disturbing to academics is the change to university funding that accompanies the new structure. Currently the money an English university receives from the public purse for teaching is ring-fenced for that purpose. The charging of fees, to the disappointment of academics, will replace government funding rather than complement it (with the exception of clinical and laboratory science, which will have government funding drastically reduced rather than abolished). This means, however, that income derived from fees need no longer be spent exclusively on teaching. With reductions in research funding, it may be tempting for many universities to divert fee income away from pedagogy.

Although the charging of fees is now effectively written in stone, it remains unclear to universities exactly how much income they can expect from 2012–13. According to UCL Provost, Malcolm Grant, in his Newsletter of 13th December 2010: "There are still several critical decisions to be taken before universities can plan properly for the future, including: 1) the amount of funding that will remain for laboratory and clinical education; 2) what level of funding might continue for postgraduate taught programmes, where there is no underpinning loans package; 3) the conditions under which a university may be permitted to charge a fee higher than £6,000; and 4) whether the government will maintain a cap on student numbers at each university". Professor Grant is quite correct – the proposals, and votes, have clearly been rushed through for political reasons, and do not really seem to have been thought through.

Quite apart from the funding rearrangements, the new structure turns university applicants and students very much into consumers; 'power in the hands of the student', as the government proudly proclaims. Student choices, we are told, will shape higher education. Be very afraid. What undergraduate students generally want – maximum outcome for minimum effort – is not conducive to producing a fit-for-purpose graduate, that is, one equipped with the critical thinking skills for lifelong learning, and educated to a level that is beneficial to subsequent employers.

It is not difficult to understand the government's reasoning, of course. If you wish to educate more than the top, say, 15–20% of the population to degree level, the pre-1998 system of tax-payer-funded HE in the form of grants is not financially viable. So how can England (university funding in Scotland and Wales is independent of the English vote and that of Northern Ireland is as yet undecided) retain a competitive edge in the global market? Give it a decade or two, many commentators pedict, and we will probably go back to restricting, not widening, participation. After all: what goes around, comes around.

Patricia de Winter

1. http://www.statistics.gov.uk/

Table. How they voted – the results from the Lower and Upper Houses as recorded in the Business Papers for each House

Motion	House	Ayes/Contents	Noes/Not-contents
Basic amount increase to £6k	Commons	323	302
Higher amount increase to £9k	Commons	323	302
Basic amount increase to £6k	Lords	283	215
Higher amount increase to £9k	Lords	273	200

Physiology 2011 – University of Oxford, UK

11–14 July 2011

The dreaming spires of Oxford will play host to the largest physiology meeting in the world this summer. From 11 to 14 July, the Department of Physiology, Anatomy and Genetics at the oldest university in the UK will be the place for global physiologists to meet, learn, collaborate and drive forward scientific breakthrough. Over 1000 people came to the Main Meeting last year in Manchester, and The Society expects that we will see record numbers of delegates at Physiology 2011.

What can you expect? Highlights for last year's Meeting attendees include 'Listening to state-of-art lectures from world-renowned scientists and making new friends and starting to build relationships with other researchers', 'I enjoyed all of it, from oral communications to socials, a must for physiology graduates' and 'I had such a wonderful experience and met so many excellent scientists in my field'. We also listened to your advice on how we could improve the meetings so this year we are providing all abstracts on a USB stick and are allowing more time for moving between sessions. We have also made more strategic scheduling to avoid clashes with symposia.

Twenty symposia around The Society's six core Themes – Cardiac & Respiratory Physiology, Cellular & Integrative Neuroscience, Epithelia & Membrane Transport, Human & Exercise Physiology, Metabolism & Endocrinology and Vascular & Smooth Muscle Physiology – give you the opportunity to explore the advances in your field as well as learning from others. The Society is proud that we offer researchers of all levels the opportunity to share their work as one of 108 short oral communications that we have scheduled.

At the heart of the Meeting will be a 1000 m^2 marquee (one of the largest in the country) which will be the home to over 250 poster communications and a 30-strong trade exhibition from key companies supplying the scientific community. All catering will be here, and we expect to serve 5000 cups of coffee, nearly 5000 sandwiches and 1000 bottles of beer! For further refreshment, take a trip to one of the city's many historic pubs. *The Bear* is the oldest pub in Oxford, founded in 1492, and probably has the lowest ceilings of any pub in the city, the *Eagle and Child* was the frequent haunt of the Inklings, a group of Oxford literary dons that included CS Lewis and JRR Tolkien, and the *Turf Tavern*, an ancient pub (a favourite with *Inspector Morse*), is an unmissable Oxford institution that many consider to be the best pub in the city.

The *Sheldonian Theatre,* which was Sir Christopher Wren's first major architectural commission, and the *Oxford Museum of Natural History* are just two of the historic venues in the University that we are using for the Meeting. However, Oxford has so much to offer, and key highlights include the *Bodleian Library,* which is one of the oldest libraries in Europe, and the *Radcliffe Camera,* which functions as a reading room for Oxford students. It is well worth viewing for its grand exterior. Other notable highlights include *Hertford Bridge* or the *Bridge of Sighs,* and *Balliol*, *University* and *Merton Colleges,* which each claim to be the 'oldest' in the University, with founding dates in the 13th century. All are examples of the collegiate Gothic architecture for which Oxford is renowned. These are all a stone's throw away from the Meeting, so taking a break from the science is easy and rewarding.

Your Physiology 2011 Oxford to-do list should definitely include a visit to at least one museum, visit at least one college and – if possible – hear one of the world-class college chapel choirs. We might also suggest a pint or two in a traditional pub with friends and colleagues, old and new. The local Scientific Organisers from the University of Oxford – Andrew Parker, David Paterson and Richard Vaughan-Jones – and all at The Society look forward to seeing you in July.

For more information visit the website at:

www.physiology2011.org

Prize Lectures 2011

The Physiological Society is delighted to announce that the following Prize Lectures will be delivered in 2011.

Lecture	Lecturer	Venue
Annual Public	Russell Foster (University of Oxford)	Physiology 2011, Oxford, July
Annual Review	Carla Shatz (Stanford University)	Physiology 2011, Oxford, July
GL Brown	Lucilla Poston (King's College London)	Various across UK & RoI
Hodgkin–Huxley–Katz	Roger Nicoll (University of California – San Francisco)	King's College London, April 2011
Michael de Burgh Daly	Tobias Wang (Aarhus University)	Physiology 2011, Oxford, July
Paton	John West (University of California – San Diego)	Physiology 2011, Oxford, July
Sharpey–Schafer	Walter Marcotti (University of Sheffield)	Physiology 2011, Oxford, July


Epithelia &
Membrane
Transport
The Physiological Society
Royal Free Campus,
University College London, UK
1–3 September 2011
Themed Meeting of
The Physiological Society
including a focused symposium:
Ion Transport: Insights from
disease and animal models
Abstract submission
& registration open
23 June 2011
www.physoc.org/em2011

Cross-Themed meeting

Durham University, UK
15–17 December 2010

Well ... there are benefits of holding a meeting in the week before Christmas! Being a southerner, it's not often that I see snow like this. As I look out of the window on the train home from Durham, I look out at the blizzard and reflect on The Physiological Society's Cross-Themed meeting.

In the days approaching the conference, the North–East had received some of the heaviest snowfall for years with Durham having around a metre: some of the effects were still apparent as piles of ploughed snow over two metres high remained scattered around the city. Despite this, the attendance was very good from the outset. The first day of the Meeting was compelling; we were introduced to the complex field around acid-sensing ion channels (ASICs) and were posed with a question: is a mechano-sensitive channel the same as a stretch-sensitive channel? In the first poster session that followed, we were able to browse some of the research that has recently been occurring in the field and discuss our opinions on this question that had been put to us. A group of us retired to a local pub next to Durham prison where the drink of choice was Gaols (pronounced Jails) Ghouls. Conversation turned from mechano-sensitive channels to our experiences of research in our respective fields, the effect of the dreaded comprehensive spending review and finally, the broad incomprehensible Durham accent from some inebriated locals.

Throughout the week, further weather warnings had been issued for heavy snow: I would say that on Thursday morning, it delivered. As I walked in at 09.00, I was able to take some beautiful snowy pictures of Durham Cathedral. I was glad to finally make it to the venue where we were treated to some fantastic

Left to right: Meera Senthilingam, Henning Franzel, Gary Lewin, Stefan Lechner, Ewan Smith and Kate Poole.

talks: my personal favourite was the oral communication given by Angus Wann (Queen Mary, University of London) as he presented a new technique for applying forces to primary cilia in cartilage. In addition to the high profile speakers, I really felt that this conference attracted exceptionally interesting research from early-career scientists displaying a range of novel techniques to provide insight into a small field. In the evening I attended The Society Dinner at St Hild & St Bede College. We were treated to a culinarily unique meal (fillet of beef with a coffee gravy) followed by entertainment from a local group playing the Northumbrian pipes, a violin and a harp – The Border Minstrels. We might not have known the songs (except for Pip Garner, University of Leeds, who informed me that one of them was used in a BirdsEye fish advert) but they were beautiful nonetheless.

Left to right: Esther Lopez, Pedro Redondo Liberal and Natalia Dionisio.

The final day took us through mechano-transduction, where we covered a range of subjects including primary- and stereo-cilia. Here, I was privileged to present an oral communication of my work which I felt was well received considering the conversation it stimulated afterwards! At lunch time, I was still receiving suggestions for further experiments that I could try: a testament to the benefit of Themed Physiological Meetings. Bringing together academics working in closely related fields allows greater understanding, the chance to share ideas and knowledge, and therefore benefits all.

The final comments of the Meeting returned to our initial question which we were set – is a mechano-sensitive channel the same as a stretch-sensitive channel? Personally, I feel it all depends on the experiment. When we truly start applying forces to channels and not just the near vicinity, I think we can start to fully appreciate the need for clarity. My final comment must turn to Stuart Wilson (University of Dundee) who was returning to Durham where he studied as an undergraduate. Throughout the Meeting, I learned that Stuart really appreciates Durham as a beautiful city, and it is. But Stuart, I'm sure Durham will appreciate you in your new University of Durham top that you were wearing so proudly on the final day. Fantastic! Now, I just hope this snow doesn't get any worse.

Stephen Kocher
University of Bristol, UK

The GL Brown Prize Lecture reunites with his descendants

Sir Lindor Brown, GL, our father, father-in-law and grandfather, was a great man. He died relatively young at just 70 years almost 40 years ago. At that time we, his offspring, were busy with our own lives. I only became aware this year that The Physiological Society continued to honour him by arranging the GL Brown Prize Lecture after my son discovered them on the internet. In recent years family and professional responsibilities have made travel somewhat difficult for me and this year was the first time I was able to attend a lecture.

Graham McGeown, the recipient of the 2010 GL Brown Prize, presented here by Lucy Donaldson from Bristol University.

The postponement of the second half of the lecture series (given by Graham McGeown, Queen's University Belfast, UK) until the end of last year due to the impact of the eruption of Eyjafjallajökull on air travel was fortuitous and meant that a number of us might attend one of the sessions. Telephone wires hummed and emails crossed the Atlantic in rapid succession to enable us to make the pilgrimage. We took the opportunity for a family reunion and managed to gather one daughter of GL, two grand-daughters (all in the medical profession), two sons and two in-laws.

The Canadian contingent was the first to arrive, a few days before the lecture. We spent time acquainting ourselves with the city and finding the location of the lecture hall, and the hills of Bristol gave our legs a fantastic challenge. Other members trickled in from Dublin, Ipswich and Leighton Buzzard. We gathered together to exchange memories and memorabilia and contemplate the huge amount of work GL managed to accomplish. We shared stories of his artistic and poetic achievements, his sense of humour and his great capacity to find the right word at the right time. Many of his phrases are passing down the generations!

On the day of the lecture we gathered six strong to present ourselves at the lecture theatre. We, the middle aged and white-haired elderly, sitting in one row, initially felt somewhat out of place in the audience of today's students. I held my breath when Professor McGeown started with a biography of GL... fortunately we had no quarrels with it and enjoyed the descriptions of his work and personality. We all appreciated the lecture; even those of us with no medical or scientific background were pleasantly surprised at how much they enjoyed it. The most fascinating part was realising how far the investigative sciences have evolved in the past decades. Now one works on the minutiae of a single arterial muscle cell with the aid of exceedingly accurate and expensive measuring equipment. Seventy years ago they had to smoke their own drums to record the contraction of the whole muscle. How things have changed.

We found it a memorable occasion, one that GL would have appreciated. He continued throughout his life to have a keen interest in teaching and eagerly encouraged those who came after him. The day was rounded off by The Society's invitation to dinner. We enjoyed the opportunity to have conversations with Professor McGeown and to meet the members of the University of Bristol's Department of Physiology. It was interesting to learn of some of the issues in physiology today and a pleasure to share in an evening of good food, wine and humour that GL himself would have enjoyed.

I feel that it has been a great privilege for us to celebrate our father's life and work almost forty years after his death. I would like to thank The Physiological Society very much for the opportunity to attend this Lecture and celebrate GL's legacy once again.

Helen Brown, MB BS FRCP(c)
Ottawa, Canada

On behalf of:

Christopher and Wendy Brown, Humphrey Brown, Katherine Brown, David Hartshorne, and Jane Uygur.

Measurement of gene expression using real-time quantitative PCR

27–28 April 2011, King's College London

Affiliates and Members can register for free to attend this workshop, which will provide an opportunity to gain both practical and theoretical experience of gene expression analysis using real-time RT-qPCR.

To register, please email education@physoc.org

Course organised by David Sugden and Patricia de Winter

The run-up to IUPS – Thelma Lovick talks to Bridget Lumb, Chairman of the Organising Committee

What goes on behind the scenes to organise a big meeting like the International Union of Physiological Sciences (IUPS)? The next IUPS congress will be held in Birmingham in 2013 so the organisation is now well under way. Thelma Lovick talked to Bridget Lumb, Chair of the IUPS Organising Committee.

Thelma Lovick (TL). Most of us go to large international meetings without giving a thought to what goes on behind the scenes to put them on. We've got IUPS coming to the UK in 2013. Can you tell me a bit about how that decision was made?

Bridget Lumb (BL). IUPS organises a scientific meeting every 4 years for which it invites tenders from constituent Societies. The Physiological Society put in a bid about 5 years ago during the IUPS meeting in San Diego in 2005. We were in competition with three other bids. Two were from within Europe – Austria and the Czech Republic – and the third was from China.

TL. But surely the thinking behind the bid must have started well before that?

BL. Oh yes, we'd already done a lot of background work to decide on the venue. We looked at various places in the UK before we decided on Birmingham. What really turned us on to Birmingham was the conference centre right in the city centre. It's such an attractive venue on the canal side with lots of bars and restaurants very close by. The other really very good thing there is the capacity to accommodate everyone within walking distance of the conference centre. Birmingham is also incredibly accessible. It has an international airport very close to the city centre – it's just the perfect venue.

Geographically IUPS moves round the globe – it's not only the science, it's a question of getting the geographical location right too. You've got to remember that people come to meetings not only for the science, but also for a cultural experience – and Birmingham offers fantastic potential as a base for exploring the UK. We had to appeal scientifically and economically.

Thelma Lovick (left) and Bridget Lumb

TL. How was the Organising Committee set up – did you choose its makeup?

BL. No, that was determined by the Executive of The Society. People were approached – usually members of various subcommittees or Executive – according to their particular experience and expertise. It's more than just the obvious things like sorting out the programme and arranging the venue. For example, there's also a lot of outreach activity, hosting events for the public, talks about physiology in schools and things like that.

TL. How did you get to be Chair of the Organising Committee?

BL. At the time the committee was set up I was Meetings Secretary of the Physiological Society, so I brought experience of organising national and international meetings. So I suppose that made me the natural choice.

Birmingham's floral trail.

TL. How many people make up the Organising Committee?

BL. We have five key people, including a representative from FEPS, who remain on the committee throughout the 10 years or so that it actually takes to organise the meeting. This is to maintain continuity. Then there are seven people who are representatives from Phys Soc Exec at any point in time. What is critical is maintaining that core representation.

TL. Do you also have local people on the ground?

BL. Yes, of course. It's important to have people with local knowledge; so we have a local organising committee as well, with representation on the main Organising Committee.

TL. How much other help do you get? Do you hire professional conference organisers?

BL. We're very lucky – we've got our own. The Phys Soc office – led by Nick Boross-Toby – offers us experienced support; they advise and lead the academic committee in many ways. There's also David Bennett, who is the International Affairs and Events Manager. They bring with them experience in hosting national meetings on behalf of The Society. Our annual meeting in the summer now attracts around a thousand delegates and covers a diverse scientific remit, so the Phys Soc team is pretty experienced.

Birmingham Town Hall.

TL. So essentially the summer meeting is a dress rehearsal for IUPS?

BL. Yes, I guess you could say that.

TL. Will Phys Soc be putting on its annual meeting as well as IUPS in 2013?

BL. No. Phys Soc won't host a main meeting that year, nor will FEPS or the Scandinavian societies. They are all going to designate IUPS as their meeting that year.

TL. What about satellites – who organises them?

BL. Interesting question. Satellites are still quite a lively topic of debate! There will be the opportunity for satellites in the UK and Europe but their exact format hasn't been finalised. What I can say at this stage is that a call will be going out.

TL. How are these meetings funded?

BL. They're underwritten by the host country's physiological society. But the IUPS also raises funds, particularly to support young scientists to attend, and people from developing countries – those who would find it very difficult to raise the money to come.

TL. How do you decide on the registration fee? International meetings are getting very expensive to go to these days

BL. The fee is a realistic amount that covers the cost of each delegate, plus a proportion of the cost of funding young physiologists and delegates from developing countries.

TL. Even so, it's likely to be a lot compared to going to The Society's annual meeting.

BL. What you've got to remember is that our national meeting is massively subsidised by The Society. What you are asked to pay is nowhere near the real cost. So we in the UK sometimes have a rather unrealistic view of what these events cost to put on.

TL. How many people are you expecting to come?

BL. 3000–4000 based on previous experience. Anything above this will be a bonus.

TL. Everything is getting so specialised these days – don't you think the days of the big general meeting are over?

BL. No, I don't think so – IUPS will be something that cuts across the boundaries of different scientific disciplines.

TL. It all sounds a bit like organising the Olympics

BL. Very similar!

The Sea Life centre.

For further information on the IUPS meeting visit The Society website at www.iups2013.org

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My 10 key papers

John Coote selects the ten papers which he considers had a major influence on the understanding of heart–brain interactions

A topic which is rapidly gaining interest from clinicians is the brain control of the heart and vascular system. This area of research some 40 years ago, when I became involved, would have seemed of purely academic interest. Now the importance of the brain in cardiovascular diseases such as hypertension, stroke, cardiac failure and arrhythmias is well recognized and a strong focus of research.

Interestingly the link between brain and heart has been topical since the time of Aristotle (384–322BC) although then the emotions and feelings now known to emanate from brain structures were thought to be in the heart. It is a huge topic and I wondered whether I could pick out just 10 papers from the 100,000 or so that might be of current interest to readers of *Physiology News*. I decided to take on the rather daunting task and the chosen papers are ones that had particular impact on my own studies but I believe have played an important part in the main developments in thinking on the heart–brain connections. Inevitably this is a personal choice and has meant leaving out many important contributions.

My involvement with the brain–heart connection started when I was a student at the Royal Free Hospital School of Medicine in the early 1960s as more and more scientists worldwide began to question the idea of a vasomotor centre in the medulla. Studies were initially with Charles Downman who was then Professor of Physiology at the Royal Free. The field has expanded considerably since then with the advent of modern anatomical tracing methods and the application of new neurophysiological and pharmacological techniques. Instead of the old concept of a brainstem centre containing neurones responsible for cardiac or vascular control, current models of autonomic control are based around discrete reflex control of sympathetic and parasympathetic

John, giving a lecture in China in 2010.

outflows mediated via different brain regions in the medulla, mid-brain, hypothalamus and cerebral cortex which act via parallel pathways to parasympathetic and sympathetic preganglionic neurones in medulla and spinal cord.

The following papers are my personal choice of studies that I consider underpinned the subsequent explosion of knowledge which daunts many young researchers at the present time and may be studies of which they are unaware.

1. The brain elicits patterns of autonomic response

Abrahams VC, Hilton SM & Zbrozyna AW (1960). Active muscle vasodilatation produced by stimulation of the brain stem: its significance in the defence reaction. *J Physiol* **154**, 491–513.

Autonomic responses rarely appear in isolation, and are usually seen as part of a pattern of change involving a number of organs and systems that participate in a behavioural response. This was the central idea put forward by Sidney Hilton first at the MRC centre in Mill Hill, London and then at the University of Birmingham. The paper from Hilton's group showed that sites in the brain that elicited a sympathetic cholinergic vasodilatation in muscles in the cat whilst increasing sympathetic activity to other organs were the same as those that elicited the defence reaction. It was the first study showing that stimulation of the perifornical region of the hypothalamus or of the periaqueductal region of the mid-brain areas which integrate alerting, aggressive display and flight are also responsible for the autonomic responses accompanying the defence reaction. These facts alone imply complex interactions within the central nervous system. This and subsequent studies changed ideas about a vasomotor centre in the brainstem. The hypothesis stimulated much of my own work on patterns of autonomic response. We now know that other cardiovascular response patterns such as those accompanying changes in environmental temperature, feeding, water submersion, sleep and exercise are similarly organized and involve different brain regions. The studies of the defence reaction also laid the foundation for numerous studies suggesting a preservation of an intense defence response in some individuals could be causally related to hypertension.

2. Rostral ventrolateral medulla and the vasomotor centre

Amendt K, Czachurski J, Dembowsky K & Seller H (1979). Bulbospinal projections to the intermediolateral cell column: a neuroanatomical study. *J Auton Nerv Syst* **1**, 103–107.

Up to the early 1970s pressor and depressor regions of the medulla, which were thought to constitute a vasomotor centre, had been localised to the dorsal and medial reticular formation. A dramatic change in thinking occurred when Feldberg & Guerzenstein (1972, *J Physiol* **224**, 83–103) and Guerzenstein & Silver (1974, *J Physiol* **242**, 489–503) found that dramatic changes in blood pressure occurred when drugs were topically applied to the ventral surface of the rostral medulla just caudal to the trapezoid bodies. This was not the first such observation since almost 20 years previously Loeschcke & Koepchen (1958, *Pfluegers Arch* **266**, 628–641) reported that the ventral surface of the medulla was a site from which respiratory and circulatory effects

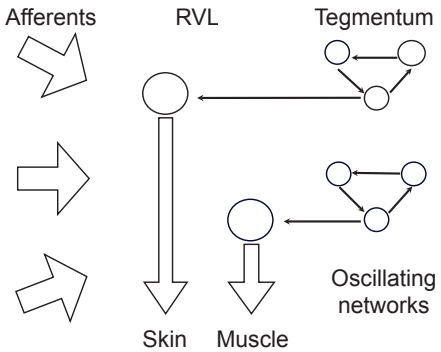
were obtained by topically applied drugs. The cardiovascular effects obtained from this region in the latter experiments largely went unnoticed because the authors had been most concerned with respiration and chemosensitivity. The significance of Feldberg and Guerzenstein's results was much debated because it was argued by some that the applied drugs could have diffused as far as the pressor region in dorsal–medial areas of the medulla. The question was resolved by the Amendt *et al*. study (1979) which used retrograde transport of horseradish peroxidase (HRP) to demonstrate that a group of neurones located close to the brain surface in the rostral ventrolateral medulla, projected to the sympathetic region of the grey matter of the third thoracic segment. This was the beginning of numerous investigations on the characteristics and organization of pre-sympathetic neurones, which contribute to vasomotor tone and that can be identified by antidromic activation from the spinal cord. For many it appeared that all that had happened was that the vasomotor centre had been moved from the dorsal medulla to the rostral ventrolateral medulla. However, numerous studies now show that there are parallel pathways from other regions of the brain that contribute to vasomotor tone. Interestingly, the study of Amendt *et al.* also showed there are neurones in the nucleus of the solitary tract that send axons which directly end in the sympathetic intermediolateral cell column at T3. The latter finding has been largely ignored but the neurones may well represent inhibitory neurones that studies in my lab have shown to cause a hyperpolarisation of pre-sympathetic interneurones at T3 of rat (Lewis & Coote, 2008, *Neurosci* **152**, 534–546).

My personal opinion is that the main role of the ventral medullary pre-sympathetic neurones is in cardiovascular–respiratory coupling and thus they play a key role in matching cardiac output to metabolism. As a consequence, the region has two-way connections to other cardiovascular regions of the brain and can be affected by peripheral afferent receptors whose primary role is to influence higher regions of the brain as is evident in blood volume regulation and more complex behaviour.

3. Origin of vasomotor tone

Gebber GL & Barman SM (1985). Lateral tegmental field neurons of cat medulla: a potential source of basal sympathetic nerve discharge. *J Neurophysiol* **54**, 1498–1512.

It has long been known that various rhythms originating in central nervous structures, including respiratory rhythm, are reflected in autonomic outflows. The degree of neural activity in sympathetic nerves to blood vessels and the heart is fundamental to the operation of efficient cardiovascular control and allows the brain to direct cardiac output to organs that are essential in a particular behaviour. A major quest in cardiovascular research has been to identify how tonic activity originates. This paper is just one of 12 papers from Geri Gebber's lab that by focusing on the basic rhythms of vasomotor activity provide a very plausible explanation of tone generation. It had been thought that a population of neurones in the brain, previously thought to be in the vasomotor centre of the dorso-medial medulla but subsequently in the bulbospinal neurones of the ventrolateral medulla, randomly generated discharges which were synchronized by powerful inputs from cardiovascular and respiratory sources. Gebber's group showed that the oscillations could be uncoupled from these inputs and they still persisted after removal of the inputs. The paper by Gebber & Barman (1985) was something of a tour de force, carrying out single cell recording of brainstem neurones at two sites and also recording peripheral sympathetic nerve activity. It was the first to provide evidence that assemblies of interconnected neurones acted as multiple oscillating networks, the output of which is synaptically coupled to bulbospinal vasomotor neurones which are suitably phase related to bursts of activity in postganglionic sympathetic nerves to various organs. This accords with results showing that brainstem pre-sympathetic neurones (Lovick, 1987, *J Physiol* **389**, 23–35) as well spinal preganglionic neurones (Boczek-Funcke *et al*. 1993, *J Auton Nerv Syst* **43**, 189–200) are functionally dedicated. The significance of the latter data and studies by Gebber's group seem to have been largely ignored. I believe the multiple oscillator hypothesis better explains the data and will prove to be a major landmark in understanding the origin of vasomotor tone. I suspect that because Gebber used mathematical analyses, such as time series and spectral plots to rigorously show significant relationships, many who were less skilled with these techniques were somewhat suspicious about the conclusions. I should refer to another school of thought which was that spontaneous activity in vasomotor nerves arises from the intrinsic activity of pacemaker neurones amongst the ventrolateral bulbospinal neurones. So far there is no strong evidence for this although it may be that some neurones are conditioned to be pacemakers by specific synaptic inputs.



Hypothesis of vasomotor tone generation. RVL, rostral ventrolateral medulla.

4. Multiple oscillators in autonomic control

Staras K, Chang HS & Gilbey MP (2001). Resetting of sympathetic rhythm by somatic afferents causes post-reflex coordination of sympathetic activity in rat. *J Physiol* **533**, 537–545.

Recordings from sympathetic postganglionic nerves to cardiovascular end organs reveals that they display a rhythmic bursting activity which has several periodicities in the firing pattern;

2–6 Hz, 10 Hz and frequencies related to the cardiac and respiratory cycles. Gebber *et al*. (1994, *Am J Physiol* **267**, R387–R399), provided persuasive evidence that the spontaneous activity of vasomotor nerves was dependent on network oscillators as I referred to above. In fact, more specifically, Gebber *et al*. found there is a variability in the rhythmic patterns of activity measured in the nerves supplying vascular systems of different organs suggesting there are multiple oscillators. The studies by Gilbey's group on the innervation of the tail vasculature support this interpretation since it reveals that the sympathetic nerves display a characteristic rhythm of discharge, the T rhythm, that does not appear in sympathetic nerves to the kidney. They are involved with the vascular responses to body temperature changes. Gebber's group showed that the fundamental rhythms can be entrained by afferent inputs, providing the frequency of the input is close to the uncoupled frequency of the discharge, thus supporting the idea of oscillating networks. The paper from Gilbey's group, reported that stimulation of afferent fibres in a somatic nerve, the radial nerve of the fore leg in rats or pinching the paw, can reset the oscillatory network responsible for the T rhythm in thermoregulatory sympathetic vascular nerves of the tail and transiently synchronize neurone firing. I consider this study important because it provided a robust test of the multiple oscillatory network hypothesis and the result lends it further support.

5. Central catecholamine pathways and cardiovascular control

Dahlström A & Fuxe K (1965). Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol Scand* **232**,1–55.

This paper describes the location of discrete groups of catecholamine- and 5-hydroxytryptamine-containing neurones in brain sections that had been freeze dried and exposed to formaldehyde vapour, and then visualized under fluorescence microscopy. The method was based on that devised by Hillarp and Falk and described by Falk (1962), *Acta Physiol Scand* **56**, 1–25. Forty-five years later it is difficult to convey the importance of this and the later paper by Fuxe (1965, *Acta Physiol Scand* **64**, 37–85) and the excitement created. Now for the first time the brain could be visualized like words on the pages of a book instead of a seemingly tangled web of various shapes of neurones so meticulously described by the earlier neuroanatomists. Furthermore, it suggested that catecholamines that are chemical neurotransmitters in the peripheral nervous system might also be involved in transmission between neurones in the brain and in particular might have a role to play in blood pressure regulation. This stimulated numerous studies and has benefitted understanding of mechanisms involved in behaviour, endocrine reactions, arousal and sleep to name but a few. It was soon realized that sympathetic preganglionic neurones in the spinal cord were densely contacted by catecholamine terminals and the search began, in many groups worldwide, to determine their functional role in blood pressure regulation, and is still ongoing. In particular, there is evidence that some spinally projecting catecholamine neurones are located in the ventrolateral medullary vasomotor region and synthesize adrenaline since they are immunohistochemically positive for the enzyme PNMT. This led to strong opinions that adrenaline was a neurotransmitter mediating tonic vasomotor drive from the brainstem. However, my own studies and those of others have shown that the concentration of adrenaline in the spinal cord of cat and rat is so low it is hardly measureable, whereas both noradrenaline and dopamine are present in significant amounts. The mystery deepens because no evidence so far has shown a contribution of catecholamine neurones or their terminals in the spinal cord to tonic activity in postganglionic cardiovascular nerves or blood pressure regulation. At present, the function of brain catecholamine neurones in cardiovascular regulation is unclear even though much is known of the electrophysiology and cellular actions of each of the amines on sympathetic neurones in brain and spinal cord.

6. Extrahypothalamic pathways to spinal sympathetic neurones

Sawchenko PE & Swanson LW (1982). Immunohistochemical identification of neurons in the paraventricular nucleus of the hypothalamus that project to the medulla or to the spinal cord in the rat. *J Comp Neurol* **205**, 260–272.

Prior to this paper studies from several groups had shown that there are direct neural projections from the paraventricular nucleus (PVN) to the spinal cord. The importance of the work by Sawchenko and Swanson was that it characterized the phenotype of some of the neurones in the PVN that projected to the intermediolateral cell column of the upper thoracic cord. By using a method that allowed the concurrent localization of retrogradely transported fluorescent dye and an antigen it was shown that a high proportion of neurones contained either vasopressin or oxytocin. This was a remarkable discovery since up to this time these peptides had only been thought of as intrahypothalamic hypophyseal hormones. Here was evidence for the first time, surprisingly indicating that the peptide precursors can be transported long distances in axons projecting from the forebrain to distant regions of the spinal cord. Subsequent studies by several groups including my own showed that these peptides are also expressed in nerve terminals surrounding sympathetic preganglionic neurones. One study using electronmicrosopy is particularly outstanding in showing oxytocin terminal synapses on at least one population of these sympathetic neurones (Hosoya *et al.* 1995, *Exp Brain Res* **107**, 9–16) so providing a strong indication the peptides could be involved in synaptic transmission. These neuroanatomical discoveries soon

led to physiological experiments by a number of groups, initially spearheaded by Mike Brody's lab in Iowa but sadly cut short by his untimely fatal heart attack whilst at a conference in Australia. Data from Quentin Pittman's group in Canada and my own have documented a predominant excitatory action of the peptides and a clear function of their role in combating increases and decreases in blood pressure and changes in plasma volume. Furthermore, there is increasing evidence that there is a functional impairment of the action of the extrahypothalamic peptidergic system in heart failure (Patel, 2000, *Heart Fail Rev* **5**, 73–86) and salt-sensitive cardiovascular disease (Toney & Stocker, 2010, *J Physiol* **588**, 3375–3384).

7. Reflex effects of cardiac atrial receptors

Karim F, Kidd C, Malpus CM & Penna PE (1972). The effects of stimulation of the left atrial receptors on sympathetic efferent nerve activity. *J Physiol* **227**, 243–260.

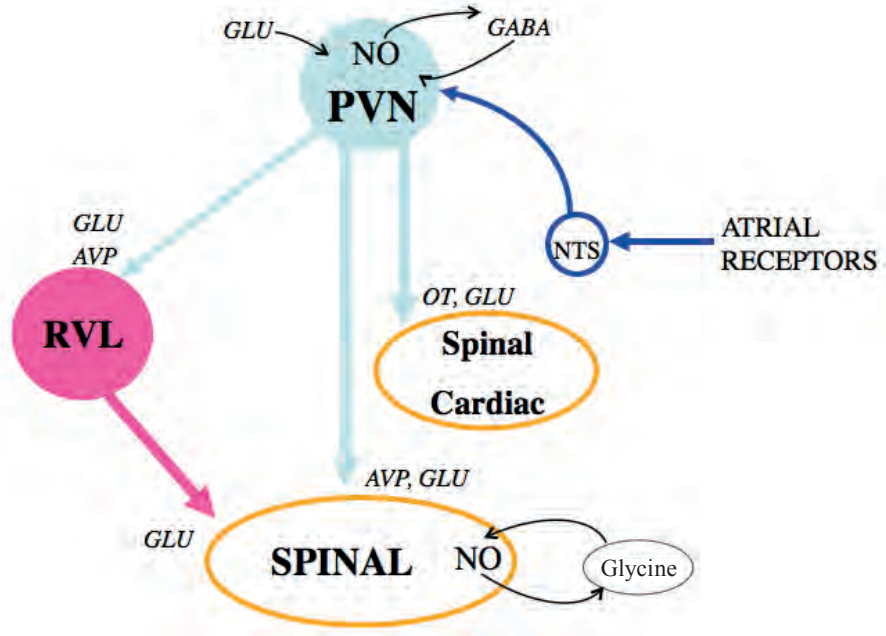
I chose this paper because it is an excellent example of the basic principle of the response of sympathetic cardiovascular nerves to signals from a specific group of receptors in that each evokes a unique pattern of autonomic activation. The cardiac atrial receptors play a significant role in moment-by-moment plasma volume regulation. The experiments on anaesthetized dogs conclusively demonstrated that activation of atrial receptors by distension of small balloons at the pulmonary vein–atrial junctions causes an increase in cardiac sympathetic nerve activity and simultaneously a decrease in renal sympathetic nerve activity. This indicated that vagal afferent receptors situated at a site ideal for detecting changes in venous return, reflexly initiate a selective pattern of sympathetic nerve response that quickly corrects for alterations in fluid status. Importantly, the atrial reflex threshold becomes higher in pregnant rats but returns to normal after parturition (Deng & Kaufman, 1995, *Am J Physiol* **269**, R552–R556). Interestingly the reflex is impaired in heart failure and is possibly one reason for sympathetic activity continuing to increase in this condition despite greater distension of the caval–atrial region with the inability of the heart to respond adequately to venous return. At the present time, this is a subject of intense research by a number of groups. Historically, knowledge of the atrial reflex effects on sympathetic nerves started with Bainbridge's report (1915, *J Physiol* **50**, 65–84) of an increase in heart rate with increased venous filling, which was largely forgotten until revived by Ron Linden and his group in Leeds of which Karim *et al*. were a part. The studies reported from this group were the centre of many acrimonious discussions at Physiological Society meetings in the '70s, the main protagonists being Linden on the one side and Hilton on the other. Sadly we see few such eloquently argued view points at the meetings these days. There is now much evidence that the atrial-sympathetic reflex is pivotal to plasma volume regulation and my own lab has shown that it is mediated via the paraventricular nucleus and involves the extra-hypothalamic vasopressin and oxytocin pathways.

8. Cerebral cortex and cardio-vascular control

Oppenheimer SM & Cechetto DF (1990). Cardiac chronotropic organization of the rat insular cortex. *Brain Res* **533**, 66–72.

This paper was highly significant since it demonstrated that cerebral control of cardiovascular organs may be highly selective. It describes a study that was the first, of several from these authors, to show that stimulation of a specific region of the cerebral cortex can affect changes in heart rate and no other autonomic parameter. The 20th century saw several studies indicating that autonomic functions are considerably influenced by areas of the cerebral cortex linked to the forebrain. There was a problem in interpreting the results of these studies since the methods were not sufficiently precise to conclude that the cardiac or vascular effects were primary or secondary to other events.

This was overcome in the studies initiated by Cliff Saper in USA and David Smith in Oxford, which provided clear evidence that cerebral control of one parameter may be accomplished not just by connections to cardiovascular regions of the brainstem (Yasui *et al*.



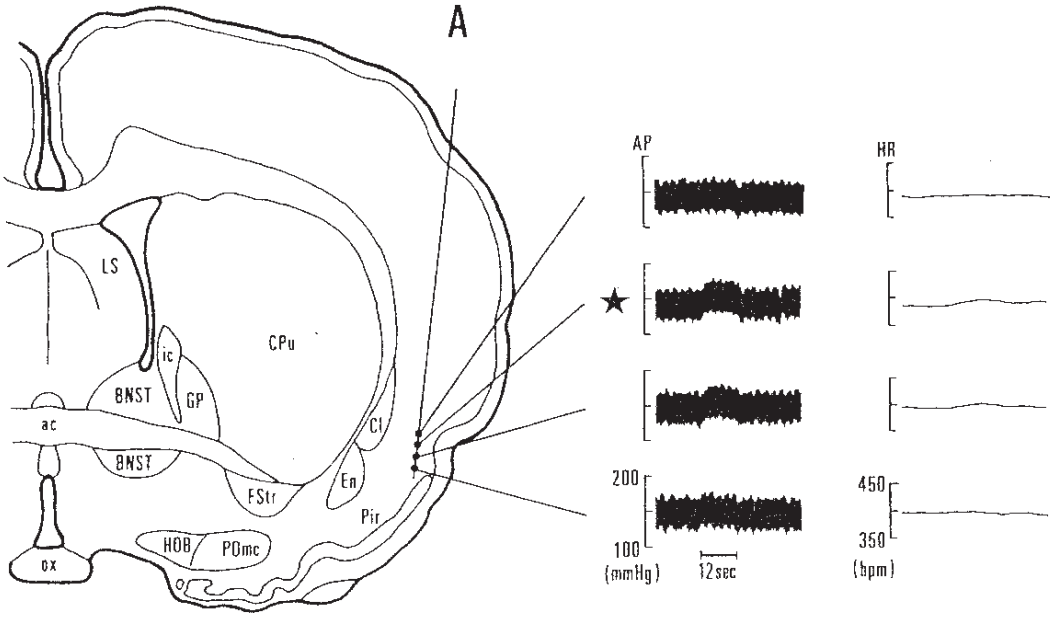
Summary of current knowledge of brain circuits involved in atrial receptor feedback from the heart. RVL, rostral ventrolateral medulla; PVN, paraventricular nucleus; AVP, arginine vasopressin; OT, oxytocin; NTS, nucleus of the solitary tract.

1991, *J Comp Neurol* **303**, 355–374) but also via direct connections to spinal sympathetic neurones (Bacon & Smith, 1993, *Neuroscience* **54**, 719–728). Oppenheimer & Cechetto (1990), went a step further by devising a technique of phasic cortical microstimulation synchronized with the R wave of the ECG that enabled identification of regions in the insular cortex that elicited only heart rate effects. Subsequent studies showed that there is a selective lateralization of cardiac vagus *versus* cardiac sympathetic control between the right and left posterior insular cortices and the neurones receive baroreceptor input. Oppenheimer went on to confirm that a similar organization was present in primates, including humans, the latter by taking advantage of deep brain stimulation in patients treated for epilepsy (Oppenheimer *et al*. 1992, *Neurology* **42**, 1727–1732). These studies have been the forerunners of many others that are leading to new understanding of myocardial injury in patients with stroke and induction of arrhythmias and may well help to direct treatment for patients.

9. Spinal neurones and sympathetic tone generation

Dembowsky K, Czachurski J & Seller H (1985). An intracellular study of the synaptic input to sympathetic preganglionic neurones in the third thoracic segment of the cat. *J Auton Nerv Syst* **13**, 201–244.

The pattern of tonic neural activity to heart and blood vessels plays a key part in optimizing cardiac output and blood supply to every organ. A leading idea that I have highlighted earlier, is that input to ventral medullary vasomotor neurones arises from an oscillatory network in the brainstem tegmentum and is entrained by baroreceptor and respiratory inputs which then drive sympathetic preganglionic neurones in the spinal cord, resulting in synchronized bursts of activity observed in postganglionic nerves recorded in rodents, cats, dogs and humans. However, this is only one of many synaptic inputs since studies have shown that sympathetic preganglionic neurones are synaptically contacted by several types of chemically coded supraspinal axons forming parallel pathways that originate from different cell groups in the brainstem, hypothalamus and cerebral cortex. In the absence of intracellular recordings, speculation surrounded the possibility that a single source of synaptic input gave rise to slow membrane depolarisations in sympathetic preganglionic neurones rather similar to those that were displayed by spinal respiratory neurones. Additionally, a much argued question related to inhibitory inputs concerning whether they are direct or mediated via interneurones or only mediated by an action on supraspinal neurones. Two earlier studies by others had only achieved short time intracellular recordings and so the answer was still uncertain. The importance of this paper by Dembowsky *et al*. is that it describes for the first time, in a beautiful series of remarkably stable intracellular recordings obtained in anaesthetised cats, ongoing synaptic activity consisting of EPSPs and IPSPS some of which occurred monosynaptically following stimulation of axons (probably of supraspinal afferents) in the dorsolateral funiculus. It was also apparent that there were no slow membrane potential oscillations and that synaptic activity was far greater than inferred from extracellular recordings, thus establishing that the preganglionic neurones were acting as integrators rather than relay neurones. More recently much of the findings have been confirmed for the rat (Lewis & Coote, 2008, *Neuroscience* **152**, 534–546). These *in vivo* studies and a host of others using *in vitro* preparations show that sympathetic preganglionic neurones have a number of characteristics such as increases and decreases in firing, gain enhancement,



Coronal section through cerebrum of rat showing sites in the insular cortex from which blood pressure and heart rate increases are elicited by electrical stimulation with a microelectrode (This material is reproduced with permission of John Wiley & Sons, Inc. from Yasui *et al.* 1991, *J Comp Neurol* **303**, 355–374).

John with a group of Chinese colleagues at Nakai University, Tianjin, China.

prolongation of action and burst activity. These properties are probably due to modulation of intrinsic membrane conductances by the variety of chemically coded inputs acting either directly or via spinal interneurones. Therefore there is great versatility in the control of tonic vasomotor activity as a consequence of integration at the spinal cord level, a feature that needs to be accounted for when considering the causes of cardiovascular diseases such as hypertension or cardiac arrhythmias.

10. The reflex nature of the cardiovascular response associated with exercise

Lind AR, Taylor SH, Humphreys PW, Kenelly BM & Donald KW (1964). The circulatory effects of sustained voluntary contraction. *Clin Sci* **27**, 229–244.

Up to the time this paper was published, the nature of the stimulus giving rise to the cardiovascular response to muscular exercise had not been identified. It was originally postulated that it was due to a direct action of the cerebral motor cortex on circulatory centres in the brainstem but little conclusive evidence was forthcoming. An alternative hypothesis was that it was a reflex initiated by afferent fibres in the contracting muscles. Considerable strength for this idea came from studies by Alam & Smirk (1937, *J Physiol* **89**, 372–383; 1938, *J Physiol* **92**, 167–177) and Asmusssen *et al*. (1943, *Acta Physiol Scand* **6**, 168–175) using dynamic exercise of forearms or legs showing that a pressor or heart rate increase was proportional to work done and this was potentiated by prior occlusion of the circulation to the limbs. The paper by Lind *et al.* (1964), took a slightly different approach by using sustained (isometric) contractions of muscles that had the advantage of enabling easy quantification of the stimulus as well as the use of different groups of muscles including those controlling hand grip or even one finger. The study showed that the pressor and tachycardia responses were proportional to the percentage of maximal voluntary contraction and were enhanced by occluding the blood supply to prevent any possible chemical mediator leaving the contracting muscles. Though this work and that of others on human subjects provided suggestive indirect evidence, direct experimental proof of the possible reflex nature of the cardiovascular effect of muscle contraction was still awaited. The importance of this paper was that it indicated a way of testing whether the exercise pressor response was centrally derived or a reflex in experimental animals. I turned my attention to this response in my own lab because I had wondered what possibly could be the physiological function of the huge population of small myelinated and unmyelinated afferent fibres in muscle that increased sympathetic activity and raised blood pressure and heart rate (Coote & Perez-Gonzalez, 1970, *J Physiol* **208**, 261–278). From the knowledge that isometric contractions were a feasible method to mimic exercise it was a small step to evoke these by stimulation of the motor fibres in ventral roots of anaesthetized or decerebrate animals (Coote *et al.* 1971, *J Physiol* **215**, 789–804) and, as it transpired later, others in Oxford had also realized the advantage of this approach (McCloskey & Mitchell, 1972, *J Physiol* **224**, 173–186). Studies in this area on experimental animals and humans subsequently expanded enormously and continues to this day, and some very clever experiments on human subjects have shown that the cardiovascular response to exercise is both a reflex and a central command-induced response. This has led to an understanding of how the arterial baroreceptors function in exercise and how cardiac vagal and sympathetic activity is influenced. Furthermore, we now have an idea of the nature of the stimuli within contracting muscle and some insight into the role of the afferent fibres in heart failure as illustrated by a paper in a recent issue of *J Physiol* (Wang *et al*. 2010, **588**, 5033–5047). Also the significance of regular exercise in treatment of heart failure and hypertension is now better understood.

Concluding remarks

This series of papers provides a brief history of one part of my life in medical research, showing the startling increase in knowledge and understanding of brain–heart control that has occurred over the last 40 years. Many of the early papers may have been forgotten or not even known about by younger scientists. Interestingly, all of the studies I quote are based on whole experimental animals or humans. Modern genetic or molecular approaches have yet to provide much new fundamental information although I expect/hope this will happen in the future. However, it will be necessary for the modern scientist skilled with the newer techniques, to read the older literature to get a better idea of what the important questions are and even to apply them in the whole animal.

John Coote

Professor Emeritus, School of Clinical and Experimental Medicine, College of Medical and Dental Sciences, University of Birmingham, UK

Peter Wilmshurst, a Consultant Cardiologist at the Royal Shrewsbury Hospital, gives his personal experience of the effect of the English libel laws on scientific debate

NMT Medical is an American company, which makes the STARFlex device for closing holes in the heart. It is suing me for both libel and slander in the High Court in London after I spoke in October 2007 at the Transcatheter Cardiovascular Therapeutics (TCT) conference in Washington DC. TCT is the World's largest interventional cardiology conference with over 10,000 delegates. My comments to a Canadian journalist were put on an American website. The website and the journalist are not being sued. I am not being sued in the USA.

The law suit arises out of my work as co-principal investigator and principal cardiologist in the MIST (Migraine Intervention with STARFlex Technology) Trial. This was a multicentre UK trial sponsored by NMT to investigate whether closing a type of hole in the heart that allows a right-to-left shunt of blood (called a patent foramen ovale or PFO) using a STARFlex device would cure migraine with aura.

I was invited to speak at TCT 2007 about PFO closure. My lecture included comments about the MIST Trial, which had ended in early 2006. Some data had been presented at scientific meetings, but journal publication was awaited.

The other co-principal investigator in the MIST Trial was Dr Andrew Dowson, who is a headache specialist. When I arrived at TCT in October 2007, I discovered that Dr Dowson was also speaking at the meeting about the MIST Trial. This was contrary to the agreement that I would present MIST data at cardiac meetings and that Dr Dowson would present at headache meetings. Shelley Wood, a journalist for the online cardiology magazine called *Heartwire*, heard both presentations and realised that there were disparities. She spoke separately to Dr Dowson and to me about these.

Peter Wilmshurst

She also spoke to executives of NMT and wrote an article. I made it clear that I believed that information being presented was both inaccurate and incomplete. Shelley Wood's article pointed out my concerns. She also presented and discussed the comments of Dr Dowson and NMT executives.

On my return to the UK after TCT, I found that Dr Dowson had sent me a copy of a paper about the trial, which he said had been accepted for *Circulation*. *Circulation* is the cardiology journal with the highest Impact Factor and is the main journal of the American Heart Association. I wrote back to Dr Dowson saying that I was unwilling to be an author because I could not vouch for the integrity of the article because NMT had refused to allow the steering committee to see all the data – but that even without all the data it was clear to me that the article contained errors. I also officially informed the independent Clinical Research Organisation (CRO) and the National Research Ethics Service that I intended to ask the GMC to investigate the conduct of Dr Dowson. One other doctor on the steering committee (Dr Simon Nightingale) also refused to be a co-author for the same reasons.

The next day, NMT's lawyers wrote threatening legal action, enclosing copies of the email correspondence I had had the previous day with the CRO. In the course of the proceedings it became clear that they were alleging that I was in effect the author of virtually the entire 2800 word article by Shelley Wood and not just the 79 words in the article that she attributed to me in quotes.

Shelley Wood attributed quotes to NMT that I believe most people would consider defamed me more than what I had said about them. They told Shelley Wood that I had never been a co-principal investigator in the trial, but she also pointed out that she had found numerous press releases on NMT's website that said I was a co-principal investigator. In addition, in her article Shelley Wood stated that NMT executives had said that I had been removed from the trial because I had committed protocol violations. She also wrote that NMT had refused to tell her what the violations were. In fact, if I had committed any protocol violations the CRO should have officially informed me of them and they have not.

The MIST paper was published in *Circulation* in March 2008. I immediately contacted the editor about the inaccuracies and omissions. I supplied hundreds of pages of documents. Eighteen months later, in September 2009, *Circulation* published a 700 word correction, a 4 page data supplement and a new version of the paper. Despite that vindication of my view that the paper was inaccurate and incomplete, NMT has not withdrawn its action. Indeed, in October 2010 they sent me a further letter before action threatening a new writ for comments that I made on the BBC Radio 4 *Today* Programme in November 2009 when I spoke about NMT's libel action against me. That threat was made against me not the BBC.

The libel case has cost me a lot of money and an enormous amount of time. I felt that NMT was bullying me, and life has been made very difficult for my family and me. For

example, a letter was emailed to me with a Claim Form sealed by the Court 'by way of notice rather than service'. It was sent to me as a non-lawyer, without explanation, at 17.09 h on the last working day before Christmas 2007. Because it was sent just after lawyers' offices closed for Christmas, I was unable to get legal advice for 2 weeks. For 2 weeks, I had no idea that it had no legal standing until served. In fact, NMT had 4 months to serve it. They served it at the last possible moment in April 2008 – nearly 4 months of worrying from Christmas to Easter. How did issuing a Claim Form but not serving it help NMT's reputation?

Since then the case has dragged on very slowly, but it has involved an enormous amount of paper work. For example, my Defence to NMT's Claim is 95 pages. NMT's Reply is 55 pages. Sorting out the thousands of pages of supporting documents has consumed every weekend and nearly every day of annual leave in the last 3 years. I took 2 weeks annual leave just to re-check the 95 pages of my Defence. The latest hearing in December 2010 was for 'security for costs', to ensure that NMT pays money into the court so my costs can be paid when I win (as I am sure I should). Otherwise, I might find that I win and have difficulty getting the company, whose share price has fallen as low as 18 cents, to pay my costs. At that hearing NMT was ordered to pay £200,000 security for costs as a first tranche (the time for payment was extended as a result of NMT's application for more time, and they have paid £61,000 of the £200,000 so far) or the case would be struck out with the verdict in my favour. This £200,000 is just the first instalment, because my costs will be much higher by the time the case gets to trial.

The case cost me £100,000 before my solicitor (Mark Lewis) and barrister (Alastair Wilson QC) kindly agreed to take my case on a Conditional Fee Agreement (CFA – no win, no fee). Since then the costs have escalated to over £250,000. The independent estimate is that should this go to trial, my costs are likely to exceed £2 million. There is no guarantee that I will recover my costs even if I win.

The English defamation laws are weighted in favour of the Claimant, and some lawyers act for Claimants in a libel case on a CFA, getting up to double the actual costs. For lawyers to act for a Defendant in a libel case on a CFA is very rare, because of the high risk of losing and ending up with no money after a lot of work. I am therefore very grateful to my lawyers, who are acting for me because they consider the issues here so important for medicine and science. If they had not agreed to act on a CFA, I risked being bankrupted or fighting the case on my own as a litigant in person.

I believe I have no option but to continue to fight the case, because there is a moral imperative that medical researchers ensure that the data are accurately and fully published. The reasons are: (1) The ethical agreement between doctors and patients who are subjects in the research involves an assurance that the data would be accurately reported; and (2) If there is inaccurate or incomplete reporting, patients entering future trials based on our reported findings may be put at risk. My Defences are 'justification' (that what I said is true) and 'qualified privilege' (that what I said was to people who were entitled to know).

In addition to fighting the case, I had to spend considerable time getting *Circulation* to publish a major correction and data supplement. I therefore face a costly libel action for fulfilling my ethical responsibilities, as a doctor and scientist, to ensure that published research is accurate.

From my experience, I believe that scientists and doctors are under threat of a gagging libel action whenever they question the messages that device and drug manufacturers want put across. Medical and scientific advances will be delayed, and the public and patients will be put at risk while the defamation laws are used to stop scientists speaking out. Doctors and scientists need the protection of a cheap and easy 'public interest defence' so they do not have to make the choice between their wealth and their patients' health.

Peter Wilmshurst
Consultant Cardiologist, Royal Shrewsbury Hospital, Senior Lecturer in Medicine, University of Keele

E H Starling and W M Bayliss: the 1914–18 War

The first Bayliss–Starling Lecture, given by Charles Lovatt Evans in 1963, has already provided the odd item for *Physiology News* (**76**, p. 14). Here is another:

Lovatt Evans mentioned how the 1914–18 War affected physiology at University College London. The foreign visitors went home almost instantly, among them G. V. Anrep and B. P. Babkin from Russia who, thanks to Starling, were able to come back after the war as refugees. The war was a great shock to Starling who had German sympathies but, according to Lovatt Evans, he functioned on the 'all-or-nothing principle'. Not only did he wish to attack the Germans but he spurned their Lieder and language. Instead he learnt French which he 'masticated teutonically.'

Bayliss stayed on running the UCL lab and working on wound shock but Starling was put in charge of the Anti-gas Department at the Royal Army Medical College in London; Lovatt Evans worked with him there. Starling's advice that β-β-dichloroethyl sulphide (mustard gas) could be used as an offensive agent was rejected. When, later on, the Germans used it, he protested vigorously. In response he was promoted 'and sent to Salonika as Army Chemical Adviser, with nothing particular to do. He did this very well...'.

Ann Silver

Bob Edwards – 2010 Nobel Laureate in Physiology or Medicine

Sir Richard Gardner gives a personal account of Bob Edwards' pioneering research into fertility which culminated in the establishment of human IVF

Pre-Cambridge years

Born in Batley in Yorkshire in 1925, Bob Edwards completed his schooling in Manchester before embarking on a degree in Agriculture at the University College of North Wales in Bangor. Following a four year interruption in his studies for military service, his interests shifted towards more basic biology which was reflected in his enrolling in Conrad Waddington's laboratory at Edinburgh University after completing his degree to do a Diploma in Genetics and then a PhD. After five very productive years during which he both collaborated with, and then married Ruth Fowler, one of Ernest Rutherford's grand-daughters, Bob obtained support to spend a year at the California Institute of Technology in Pasadena. Here he developed a friendship with the late Albert Tyler who inspired his enduring interest in reproductive immunology. It was for pursuing research in this area that Bob gained a five year contract to work in the Division of Experimental Biology at the National Institute for Medical Research at Mill Hill. At the end of this stint, Bob accepted an appointment in Glasgow University's Department of Biochemistry where, together with the late Drs Paul and Cole, he made the first attempts to derive cell lines from pre-implantation mouse and rabbit embryos. This, of course, later became a major preoccupation of many laboratories in the drive to extend the bounds of regenerative medicine.

After just one year in Glasgow, Bob was recruited to Cambridge in 1963 by his former head of laboratory at Mill Hill, Alan Parkes, through the offer of a Ford Foundation Fellowship in Reproductive Physiology. Parkes, who had been appointed three years earlier to the newly established Marshall Chair of Reproductive Physiology, occupied laboratories on the top floor of the Physiology building. This was the start of Bob's association with Cambridge that was to endure for the remainder of his research career.

Richard Gardner

Bob as mentor

I embarked on Part II Physiology in the third and final year of the Natural Sciences Tripos at Cambridge with the aim of doing research either in biophysics or neurophysiology, providing my degree was good enough to allow me to secure the necessary funding. These were the two areas in which the Department had particular research strengths in the 1960s, and which therefore dominated the Part II course. This course, irreverently referred to as 'blood and guts and biophysics', included various additional topics that reflected the research interests of other members of staff. We were thus offered a series of lectures and practical classes on reproductive physiology given primarily by Bob. His treatment of the subject was impressively wide-ranging, extending from endocrinology to the initial stages of mammalian development, a subject that was just starting to progress from an almost purely descriptive to an experimental phase. Both I and a fellow Part II student, Martin Johnson, were completely captivated by Bob Edwards' extraordinary energy and enthusiasm, and most especially by his treating us like intellectual equals in numerous discussions we had with him both during his practical classes and thereafter. In consequence, and in defiance of advice from some traditional physiologists, we elected to become his first research students which was made possible through our both completing Part II with Firsts. Thus, we joined the Marshall Laboratory in the early Autumn of 1966, with me sharing Bob's small laboratory and Martin occupying another room nearby. This was for both of us the start of an endlessly stimulating and rewarding

Bob in lecturing mode in the 1960s (courtesy of Ruth Edwards).

Bob's small working area within the Marshall Laboratory. Left to right: Barry Bavister who, together with the late Patrick Steptoe, co-authored the 1969 *Nature* paper on achieving human fertilization *in vitro*, the author, Bob and Alan Henderson, one of his collaborators (from Getty Images).

relationship with one of the most notable and independently minded scientists we could ever have hoped to encounter.

Bob's style of supervision suited us both extremely well, and I am sure it enabled us to operate independently in research rather sooner than might otherwise have been the case. Thus, having initially discussed with each of us an impressive range of subject areas we might wish to consider, Bob then assigned us the task of choosing a project through acquainting ourselves with the relevant literature. On coming back with our choice, we were then challenged by him to defend it in terms of both feasibility and the likelihood of generating findings that were novel, interesting, and substantial enough for a thesis. Once this was settled, we were very much left to our own devices, but on the clear understanding that it was up to us to seek him out if we needed further advice or guidance. This was not a problem since he was invariably very approachable, but proved an exceedingly tough critic where necessary. He could also be infuriatingly robust in championing a viewpoint that seemed utterly untenable, simply to sharpen our critical faculties. Often, the three of us would repair to the nearby Fountain pub for discussions over lunch, which typically consisted of a couple of pernicious cheese and onion rolls washed down with a pint or two of Tartan bitter.

Proof of principle of pre-implantation genetic diagnosis

While I was still engaged in developing microsurgical techniques for transplanting cells and tissues between pre-implantation embryos, Bob invited me to collaborate on determining the feasibility of undertaking genetic diagnosis on such stages. We chose the rabbit for this purpose because its blastocyst attains a diameter of 5 mm before implantation. The aim was simply to see if we could sex the embryos without compromising their subsequent development. Our assay was the presence or absence in cell nuclei of the sex chromatin body, which represents the one of the two X chromosomes in females that is genetically inactivated to equalize the dosage of genes on this chromosome between the two sexes. Initially, we attempted this on intact blastocysts using a fluorescent dye that bound specifically to DNA, but this proved incompatible with further development of the sexed embryos. Hence, we then resorted to biopsy and staining of the excised tissue which did allow continued normal development so long as the operated embryos were well expanded within a minimally damaged investing coat on transfer to the uteri of recipient does. In this way we were able to demonstrate that the sex of all resulting term offspring had been correctly determined at the blastocyst stage. This proof of principle of pre-implantation genetic diagnosis, which was first successfully applied in man somewhat over two decades later, caused a considerable stir at the time. We presented this work as an alternative to post-implantation diagnosis and abortion for avoiding the birth of males to women who were carriers of serious X-chromosome-linked genetic diseases like Duchenne-type muscular dystrophy. But, so far as the media and public were concerned, the possibility of being able to choose the sex of one's offspring and the various issues this might raise were foremost in mind. Of course, without any means of accessing pre-implantation human embryos at that time, this approach was not a realistic prospect clinically, a situation that was to change dramatically within the next decade.

The origins of IVF

By the time Martin and I joined him in the Marshall Laboratory, Bob was already thinking along the lines of bringing the emerging technologies of induced ovulation, and *in vitro* fertilization and embryo culture in laboratory and domestic mammals to bear on the problem of human infertility. In fact, one of his precious human oocytes was among the earliest specimens we examined together. However, since a first degree in agriculture and a PhD in mouse genetics are hardly apposite qualifications for carrying out novel procedures on women, a critical task for Bob was to identify a clinician who was sympathetic

to his aims. This proved quite a challenge since even those in the UK who were prepared to provide him with ovarian tissue often failed to appreciate the need for it to arrive from the operating theatre in a fresh rather than fixed condition. In fact, he had to cross the Atlantic to find the first obstetricians who were really attuned to his ideas, namely Howard and Gorgeanna Jones, who were then at Johns Hopkins University in Baltimore. Back in the UK Bob's search took longer but was eventually richly rewarded by his encountering Patrick Steptoe whose particular skills in laparoscopic surgery were vitally relevant for retrieving human oocytes. Thus began a long and close collaboration between Bob in Cambridge and Patrick in Oldham. Despite the invasiveness of the procedure for obtaining pre-ovulatory oocytes, which required general anaesthesia, inflating the abdomen with CO_2, and then probing it with instruments inserted though the navel, there seemed to be no shortage of volunteers. This was despite these unsung heroines being clearly given to understand that the chances of alleviation of their own infertility were slim. For me, the collaboration meant the inconvenience of repeatedly being denied the services of a wonderful technician, Jean Purdy, who I shared with Bob, as the pair of them disappeared, often at short notice and for very variable intervals of time, travelling back and forth between Cambridge and Oldham with their mobile laboratory.

While, with hindsight, Martin and I recognize that we were extraordinarily privileged to witness the origins of IVF from ring-side seats, our view of this endeavour was rather different at the time. Bob felt it was vitally important to inform and engage the public about what he was trying to achieve. To this end, he seized every opportunity to write popular accounts of his work in both broadsheet and tabloid newspapers. This had a number of consequences. First, it encouraged the view among the mainstream physiologists in the building that the Marshall Laboratory was inhabited by noisy self-publicists of dubious scientific merit. Second, it meant that when Bob was away from the laboratory, Martin and I had to deal with nosey and often extremely devious reporters. Third, it fuelled widespread criticism and condemnation of Bob and, perhaps surprisingly, extraordinary hostility from the medical profession, most notably the British Medical Association. This organization branded Bob 'Dr Frankenstein', thereby prompting him to sue successfully for libel on several occasions. Given the present almost universal acceptance of assisted conception *in vitro* by both professionals and public, it is very difficult to convey the extraordinarily poisoned atmosphere Bob and Patrick had to endure during the early years of their work. To a humble research student, it was quite an 'eye-opener' to see how visceral rather than cerebral the reactions were of many for whom objectivity was supposed to be the stock in trade. Even today, I find people refer to Bob as 'controversial', which misses the point that he could never have achieved what he did in the climate that then prevailed without being so.

Bob with Patrick Steptoe (courtesy of Ruth Edwards).

Prominent scientists were among the more vociferous critics of IVF, including two Nobel Laureates, JD Watson and MF Perutz. Rather than being cowed by such eminent persons, Bob always rose to the challenge, and almost delighted in delivering robust responses. This is illustrated particularly clearly by the following exchange in which I was the unwitting instrument in providing Bob with critical ammunition. Publication on 15 February 1969 of the paper in *Nature* in which Bob and his colleagues reported having obtained the early stages of human fertilization *in vitro* provoked a very prompt response from Victor Rothschild in the House of Lords. Rothschild, who had earlier worked on fertilization in sea urchins, questioned in rather patronizing terms whether Edwards *et al*. really had demonstrated the early stages of fertilization *in vitro*. Entirely coincidentally, at precisely that time, I had purchased a copy of Rothschild's book entitled '*Fertilization*' from Heffer's Bookshop for 21 shillings. Though devoted entirely to studies on this phenomenon in the sea urchin, it unaccountably had a mouse egg and associated sperm as its frontispiece. The nub of the exchange that appeared in the correspondence section of the March 8 1969 issue of *Nature* under the title of '*Did fertilization occur?*' read as follows:

Rothschild

..."Without wishing to engage in semantic hair-splitting, one must observe that the 'early stages' of fertilization may be and, in the note by Edwards *et al*., are so early as to raise the question whether fertilization, if the word is to have any meaning, occurred at all."

Edwards, Bavister & Steptoe

...."It should also be noted that conclusions in many papers reporting fertilization in progress have been based on the evidence of pronuclear stages ... Indeed, Rothschild is hoist with his own petard, for the frontispiece of his book on fertilization shows a mouse egg with a spermatozoan in the perivitelline space and labelled ""A live fertilized mouse egg showing the whole spermatozoan in the cytoplasm"". This illustration shows fertilization in the same early stage as in our figure 4B which presents a human egg with a perivitelline sperm."

In the early days Martin and I could not understand why a highly original creative scientist of Bob's stature was allowing himself to be diverted from fascinating and challenging basic scientific problems in the field of mammalian reproduction and development by what we then regarded as a rather pedestrian piece of applied research. To obtain reproducible *in vitro* fertilization and pre-implantation development in the mouse was the result of years of work in many laboratories, requiring the use of literally millions of oocytes and embryos. To achieve this in man, where the numbers of oocytes would preclude systematic, controlled, exploration of the myriad of potentially relevant variables seemed to us an intellectually unrewarding and utterly daunting exercise. Nonetheless, despite numerous reverses and disappointments, Bob and Patrick pursued this goal doggedly for nine years following publication of their 1969 *Nature* paper before they were finally rewarded by the birth at 11.47 pm on July 25 1978 of a baby

Bob in discussion with the distinguished geneticist Mary Lyon, who spent a sabbatical year in the Marshall laboratory (courtesy of Ruth Edwards).

weighing 5lb 12oz. The fact that Louise Brown was a normal healthy girl caused much of the hostility towards their work to evaporate almost overnight, and some of their harshest critics within the medical profession wasted little time in jumping on the bandwagon and developing careers within the field of assisted conception *in vitro*. This, however, was not the end of difficult times for Bob because Patrick's retirement from Oldham meant that they had to find a base in Cambridge in order to capitalize on their success. What the University had to offer proved entirely inadequate, so that their work was suspended for three years while they sought private funding to establish Bourn Hall clinic. During this time, the ever energetic Bob wrote a massive tome entitled '*Conception in the Human Female*', which was described by one of his former scientific critics as the best book on obstetrics published in the 20th Century.

Once Bourn Hall was established, work soon progressed rapidly with more consistent success in IVF being achieved so that the birth of the first 1000 babies was celebrated in 1987, sadly, the year when Patrick died. Most interestingly, medical opinion in the early years that IVF would only be of value in treating infertility due to blockage of the fallopian tubes proved to be entirely wrong. Most of the successfully treated cases are 'idiopathic' and, contrary to the view persisting from Tudor times that problems typically lay with the female partner, male factor infertility has emerged as a major consideration.

Interestingly, particularly early in their long collaboration, Steptoe's name almost invariably preceded that of Edwards whenever they were referred to by the media. However, Patrick always openly acknowledged that Bob provided the intellectual impetus for their work. This was certainly clear to all the staff at Bourn Hall by whom they were known as 'Steppy' and the 'Boss'.

Other interests

Bob was throughout these years not only engaged in other research, but also very active in the international arena, notably in the founding of the European Society for Human Reproduction and Embryology and the editing of its journals. He disengaged from such editorial work on failing to persuade colleagues to embrace state-of-art publication practices which he then adopted in a new journal he founded called *Reproductive Medicine Online.* This quickly gained success for its speed of publication and emphasis on promoting lively debate of contentious matters. An issue of this journal is currently being prepared to celebrate Bob's editing of it for the first decade of its existence

and, after the project was started, also the excellent news of his being awarded the Nobel Prize. It contains selected editorials, articles and other contributions he made to the Journal during that period, prefaced by commentaries prepared by me together with its new editor, Martin Johnson. What the selection serves to show is the extraordinary breadth of Bob's interests and his deep knowledge of the literature within the very diverse fields he chose to explore. Of particular note is his very early engagement with the ethical implications of his work which featured in a seminal paper published in 1971. Nowadays, with the existence of countless committees, chairs and, indeed, entire university departments devoted to bioethics, it is difficult to recall that the subject hardly existed at that time.

I ceased to have regular contact with Bob following my migration to Oxford in 1973, but we continued to meet at conferences at which, particularly in latter years, he was an avid attendee. Before his recent illness we often chatted on the phone about common research interest and other matters. I greatly miss these conversations, which he often began with "How are you old Bean?", and were invariably spliced with his infectious enthusiasm and wonderful sense of humour.

I feel very privileged to have had such an original and visionary scientist as my mentor, collaborator and friend. Bob not only revolutionized the treatment of infertility, thereby making the early stages of our own embryonic development accessible to study, but also introduced the concepts of pre-implantation genetic diagnosis and use of surplus IVF embryos for regenerative medicine. For this astounding legacy, he richly deserved to be honoured with the Nobel Prize in Physiology or Medicine. The only shame is that the award was not made earlier when he was in good enough health to be able to collect it in person. A clue as to why it was so long coming might be gleaned from the Vatican's bioethics spokesman's response to the announcement as "totally out of order".

Sir Richard Gardner, KB, FSB, Hon FIAT, FRS

Formerly Edward Penley Abraham Research Professor of the Royal Society in the University of Oxford

Professor John Conrad Waterlow FRS remembered – with fondness

I read with sadness obituaries to Professor Waterlow in the press and listened with equal sadness to the tribute on Radio 4's *Last Word*. Both marked his passing on October 19 2010 aged 94.

Some twenty five years or so ago at a Society scientific meeting, I was failing in my attempts to withstand some aggressive questioning about a presentation I had just made. I thought the questioning was harsh in content but in particular, in tone. I did not have the experience to mount an effective counter. Just before the vote – remember those? – with some difficulty, a frail figure stood up and with immaculate diction, proceeded to provide much needed and welcome support. My communiqué was accepted.

Afterwards, I went and thanked my supporter whose act of kindness, modesty and graciousness created and left a lasting impression. I was informed later that my advocate was Professor Waterlow.

It seems that what he did was entirely characteristic and my memory reflects that of those who had the privilege to work with him. I take this opportunity to add my tribute.

Edward M. Winter

Winner of the *Physiology News* annual prize

Every year, *Physiology News* awards a prize of £200 for the best article written or co-writen by either an Affiliate Member of The Society or someone within two years of having obtained their PhD.

The 2010 prize has been awared to Peter Bayguinov for the article '*Generation of complex neuronal behaviour in a mammalian nervous system*', which was published in *Physiology News* **80**, pp. 29–32.

Get involved and write an article for *Physiology News*

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Email us for more information or to discuss ideas at magazine@physoc.org

A letter from Portland, Oregon

Shortly after the US economy had taken a nose-dive and when Obama was in the early months of his presidency, I made my big move to the US. I was thrilled to be starting my post-doc position at the Oregon Health & Sciences University (OHSU). I had spent very little time in America but growing up in Australia I was served the standard dose of Americana, so felt I understood the basics – I would just need to apply adjectives such as 'bigger' and 'louder' to most things. But Portland, I can report, is a special case.

Portland is a town where riding a unicycle carries more caché than driving a Hummer. In fact, it is rare to spot the huge gas-guzzlers amongst the sensibly sized hybrids. Thanks to some thoughtful civic planning, including light rail and bike lanes, I haven't needed to buy a car which is remarkable for a small US city of around 500,000 people. The Portlanders I've met also do not fit my American stereotype. Their ambitions are not for fame and plastic surgery, rather they aspire to secure a plot in the community garden, brew their own beer and be on a first-name basis with the local chicken farmer. Living more simply and sustainably in Portland, as far as I can tell, is not a reaction to recent environmental doomsday-speak. Rather, it seems a combination of the ideals from the hippies that moved North from San Francisco once the 60s petered out, and a genuine reverence for the astonishing physical beauty of the region.

'Green' does not just describe the attitude of the inhabitants, it is also the dominant colour here. This is not the hazy grey–green of the dry Australian landscape, but a crisp, deep-green produced by steady drizzle and rich soil. I'm lucky enough to work at the OHSU West Campus which is on the site of an old conifer forest and I still whip out my camera when I spot a deer, squirrel, hummingbird or other foreign critter on my walk to the train station.

Rebecca and her husband along the Columbia River Gorge.

I have become more of an outdoorsy person since moving here. People assume that as an Australian – and one from the country no less – I must already be an avid bushwalker, or rather 'hiker'. Not so. In Australia only those with a disposition for enduring long hours in the heat while being pestered by flys ever get involved in hiking. In Portland everyone is a hiker. Even pale nerds pull on a polo-fleece and can be seen walking along the Columbia River Gorge on weekends (see photo). There are many places to explore. Mount Hood stands to the East of the city and to the North looms Mount St Helens, which is close enough to have dusted volcanic ash over Portland during its eruption in 1980. I have peered into the gaping wound left by that massive side-ways blow-out, and the rising steam hints she still has more to come.

I am also exploring a new language. I have found there is an efficiency and directness in American English – qualities that would benefit my scientific writing. Try ordering a jug of beer and you will be met with a chuckle, 'did you mean a pitcher of beer?' A jug is a tiny version of a pitcher – who knew? You can't expect to find beer or wine in a liquor store, there are distilled liquids only. Also, you don't hire a car, you would hire a person to drive you about in a car that you have rented. I can generally find the appropriate American terminology: lift instead of elevator, trash can instead of rubbish bin etc., so watching years of American television does have some educational value. But this is not a reciprocal relationship, choose the wrong word and confusion results: 'A "stationary" cupboard? When did the office supplies cabinet ever move?'

Studying postural control in Professor Fay Horak's lab has been great. Fay is a trail-blazer who has forged an impressive career in the field of balance disorders through her 'can do' attitude, strong collaborations and stellar grant writing skills. I have been busy investigating how deep brain stimulation affects balance in people with Parkinson's disease. It is still poorly understood why delivering high-frequency current to parts of the basal ganglia should improve tremor and muscle stiffness, but the improvements are remarkable. Unfortunately, I am finding that balance control is not as responsive. In fact, in some cases balance may be worse after deep brain stimulation surgery.

As a post-doc I am just beginning the daunting task of applying for grants. In America there are numerous opportunities to apply for federal funding throughout the year, while non-profit and private funding is on a scale that doesn't exist in Australia. It is possible to be perpetually writing grant applications! But there is also intense competition for funds and I see the familiar shake of the head and shrug of the shoulders in the lunchroom when people discuss their futures. The struggle remains much the same.

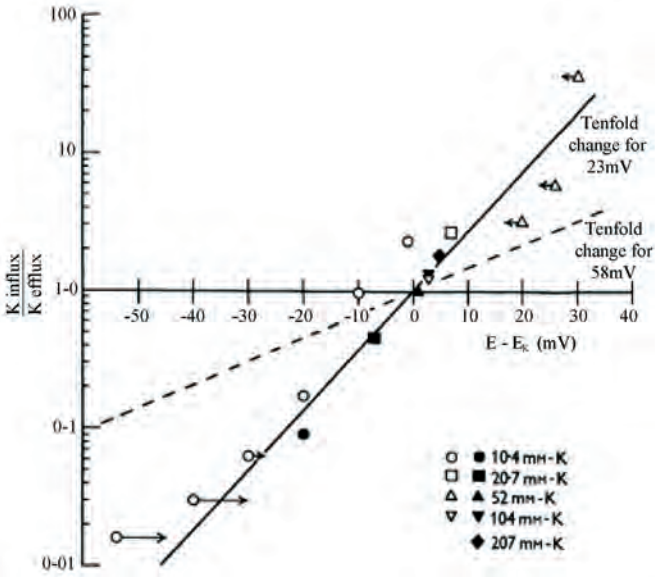
I am very fortunate to have become colleagues with the emeritus Russian Professor, Victor Gurfinkel. The man is a treasure chest full of tales about his seminal scientific discoveries and varied life experiences. At 89 he remains active in the lab with many ideas yet to test. He stresses that accepted knowledge is often based on poor experimentation and a narrow point of view. The same could be said of my assumptions about America, which have been challenged by living in this vibrant pocket of the country.

Rebecca St George

R.D. Keynes (1913–2010): the legacy from two papers

R.D. Keynes, who died at the age of 93 in June last year, was a distinguished, indeed central figure in British physiology of the second half of the 20th century. In adding to what has already been written, including the delightful piece by Christopher Huang (*Physiology News* **80**, 51) and will doubtless yet be written (more systematically) concerning his scientific contributions, here I want simply to draw attention to two seminal experimental papers that Keynes wrote early in his career. These contributions, published (jointly and significantly with A.L. Hodgkin) in *The Journal of Physiology* in 1955, led rather directly to, indeed could be seen to under-pin, no less than two different Nobel prizes, awarded to other very distinguished scientists some half-century later.

It is widely appreciated that the concept of the 'ion channel' arose from work carried out by Hodgkin and his colleagues both in Plymouth (at the Marine Biological Laboratory) and in Cambridge (at the Physiological Laboratory) during the decade 1945–55. This central idea arose from the functional characterization of ionic flow (measured both as current and by radiochemical fluxes) across the plasma membrane of the giant axon of squid. The 2003 Nobel Prize for Physiology or Medicine was awarded to R. MacKinnon for his group's spectacular structural work that laid open the exquisite atomic detail that underpinned the functional properties of ion channels, specifically of potassium channels. But what, for such potassium channels, were these properties? They include not only the specificity of ion translocation (e.g. of K^+ but not Na^+); the high velocity of ion translocation across the membrane (near-diffusion limitation in aqueous solution); and the gating of translocation (e.g. by voltage). And for potassium channels they also include another distinct functional characteristic, the 'long-pore effect' of Hodgkin & Keynes. How was this property discovered? Figure 1 from the relevant 1955 Hodgkin & Keynes paper shows the key experimental finding, and the attached equation what their interpretation of this was.



$$\frac{\text{Influx}}{\text{Efflux}} = \left(\frac{[\text{K}]_O}{[\text{K}]_\text{f}} e^{EF/RT} \right)^{n'}$$

Figure 1. Passive potassium ion movements across the axon membrane observed by Hodgkin and Keynes showing the relationship between the measured driving force, $E_m - E_K$ (abscissa) and the ratio observed for potassium fluxes (influx/efflux) (ordinate). Each data point is from an individual determination. Note that the slope of the (continuous) line fitted to the data is steeper than that predicted (dashed line) by a 'simple' Nernst relationship. The equation shown below the data is from the same paper, and suggests an explanation for this discrepancy, namely that the relationship observed is that predicted were potassium ions to flow through a 'long pore' containing on average more than a single ion, where n' describes the mean number of potassium ions present ('the occupancy') in these channels. From their data, n' has a value of about 2.5.

The X-ray crystallographic observations made 50 years later (see Fig. 2) spectacularly prove these postulates. As MacKinnon wrote in his Nobel lecture: 'As early as 1955 experimental evidence for channel mediated ion flow was obtained when Hodgkin and Keynes measured the directional flow of K^+ ions across axon membranes using the isotope ^{42}K (Hodgkin and Keynes, 1955). They observed that K^+ flow in one direction across the membrane depends on flow in the opposite direction, and suggested that 'the ions should be constrained to move in single file and that there should, on average, be several ions in a channel at any moment'... After ... our crystals improved dramatically, and we were able to solve an initial structure at a resolution of 3.2 Å (Doyle *et al.*, 1998). We could not clearly see K^+ in the pore at this resolution, but my years of work on K^+ channel function told me that Rb^+ and Cs^+ should be valuable electron dense substitutes for K^+, and they were. Rubidium and Cs^+ difference Fourier maps showed these ions lined up in the pore, as Hodgkin and Keynes might have imagined in 1955'.

It is also appreciated, though perhaps less widely, that in a quite separate study also published in 1955, Hodgkin & Keynes provided the evidence that showed how in nerve the process of action potential generation (by ionic current flow through gated channels) was experimentally distinguishable from the subsequent reactions that restored (by ion pumping) the pre-existing ion gradients ('recovery'). For example, in this

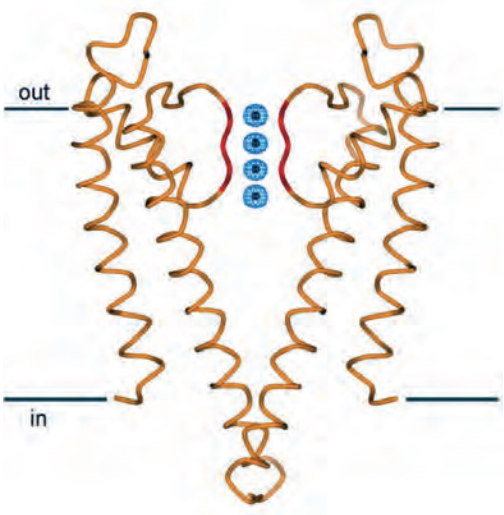


Figure 2. The atomic structure of a potassium channel with ion-binding sites in the permeation pathway (of the bacterial potassium channel KcsA). Two diagonally opposed subunits of the tetrameric channel are shown. The selectivity filter is coloured in red. K^+ binding sites along the permeation pathway are shown as spheres with the water molecules surrounding them shown as a blue mesh (modified from Zhou & MacKinnon, 2003).

paper it was shown that these two processes differed in: their rates of ion flow (rapid for the action potential; slow for recovery); their dependence (using dinitrophenol) on metabolism (absolute for recovery; not required for the action potential 'spike'); their temperature dependence (high Q_{10} for recovery; low Q_{10} for the action potential). In this seminal paper (Fig. 3) they showed that the 'recovery' events causing the restoration of the ionic gradients themselves had characteristic properties including: mutual interdependence ('coupling') of Na and K fluxes; metabolic energy requirement; and the (relative) voltage independence of these fluxes. An additional feature was that the size of the (pumped) Na efflux was slightly but significantly larger than that of the (pumped) K influx (as indicated in the figure).

All this preceded the discovery of the Na^++K^+ ATPase (Skou, 1957); its stoichiometry (Sen & Post, 1964); and its electrogenicity (see Thomas, 1972). It was for his 1957 discovery of the Na^++K^+ ATPase that much later Skou was awarded his Nobel prize. In his Nobel lecture Skou acknowledges the critical importance of this Hodgkin & Keynes (1955), publication: 'I had little knowledge about the active transport of Na or K ... the closest that I came across [in the literature] was Hodgkin & Keynes 1955 who had shown that poisoning giant axons with DNP, cyanide or azide decreased the active transport of sodium suggesting that high energy phosphate esters are the substrate, and as ATP is a high energy phosphate ester I thought that it could be the substrate [for my enzyme]'.

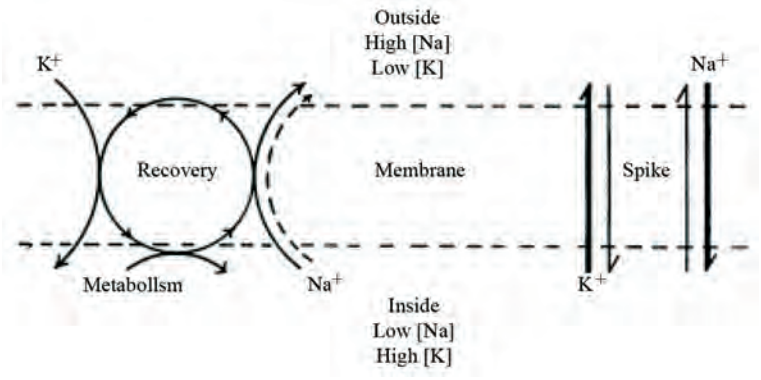


Figure 3. The Hodgkin and Keynes scheme for ion transport in the axon membrane, distinguishing events involved in action potential generation from those in recovery.

Both of the Hodgkin & Keynes, 1955, papers are beautifully written studies which draw attention to intrinsic experimental limitations. There is balance in consideration of competing plausible theoretical underpinnings, then the incisive use of quantitative reasoning and finally a set of unambiguous conclusions. Out of this arose insights that are very much alive and kicking 60 years later. What, in our experimental discipline, could be better examples than these two papers, for any undergraduate or graduate to look at, to read and to study today if they are to find what is meant by deep rather than ephemeral science?

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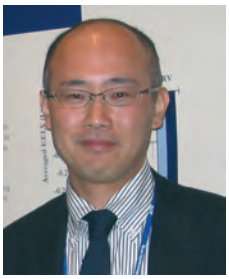
Two arms as one: position and movement sense in an elbow-matching task

Masahiko Izumizaki and Uwe Proske have found that when subjects match the position of one unseen arm by placement of the other, sensory information coming from both arms is used to achieve the observed matching accuracy.

This story is about the kinaesthetic senses. They are part of a group of senses, the proprioceptive senses, concerned with signalling the body's own actions. The kinaesthetic senses comprise the sense of limb position and the sense of limb movement (Proske & Gandevia, 2009). Other proprioceptive senses include the senses of effort, tension and balance. The proprioceptive senses are somewhat mysterious because we are largely unaware of them. When we look at an object or touch it we experience a specific, identifiable sensation. By contrast, we know precisely where our limbs are when we are not looking at them, but that awareness is not accompanied by any identifiable sensation. It presumably means that the sensory information concerned with signalling the position and movement of our limbs is processed largely unconsciously.

The present day view is that the principal kinaesthetic sensors are the muscle spindles. At some joints, particularly the finger joints, skin and joint receptors also contribute to the senses of position and movement. At the elbow joint skin stretch receptors are likely to supplement the main kinaesthetic signal coming from spindles in elbow muscles. The primary endings of muscle spindles are thought to contribute to both the sense of limb position and the sense of limb movement while secondary endings of spindles contribute to the sense of position (McCloskey, 1973).

We have been studying position sense at the forearm in a simple matching task. One arm, designated the reference arm, is placed in a particular position and the blindfolded subject is asked to match it with their other, indicator arm. Subjects are normally quite accurate



Masahiko Izumizaki (left) and Uwe Proske

and are able to match positions with errors of 2 deg or less. If, instead of using a matching process, subjects are asked to indicate the position of one arm with a pointer, they are less accurate. It has led us to conclude that arm matching is a cooperative process in which both arms play a contributory role. In order to further explore this idea we have done some simple experimental manipulations of one arm to see what effect it had on matching accuracy (Izumizaki *et al*. 2010). We carried out these matches under two conditions, one where the reference arm lay relaxed on a support and the second, when its elbow flexor muscles were vibrated at 80 Hz. Muscle vibration

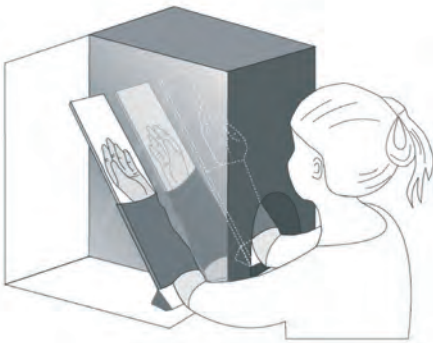


Figure 1. Subjects sat in front of a two-compartment box. The partition between the compartments was a mirror that showed an image of the left, indicator arm. Position of the image was arranged to coincide with the position of the right, reference arm. The mirror could be covered up. Subjects' arms were taped to paddles which hinged at a point close to the elbow joint. Joint angle was measured with potentiometers coincident with the elbow joint.

is a largely selective stimulus for the primary endings of muscle spindles. The extra impulses generated by the vibration lead to an illusion of limb movement and displaced position (Goodwin *et al*. 1972). We wanted to know whether altering the conditions of the indicator arm affected matching accuracy when the reference arm lay relaxed and when it was being vibrated, that is, in the presence of a large, false proprioceptive signal coming from the reference arm.

The manipulations of the indicator arm were of four different kinds. In all trials the reference arm lay hidden from the subject's view. In the first series of matches the experiment was done in the traditional way, with the subject blindfolded so that they could see neither arm. In the second series subjects could see their indicator arm; in the third the indicator was placed out of view and subjects were required to match the perceived position of the reference arm by placement of a dummy arm. Finally, a mirror was slipped into the partition between the two arms (Fig. 1). The position of the mirror was arranged so that the reflected image of the indicator arm appeared to occupy a position coincident with that of the reference arm. Here subjects were instructed to look only at the mirror image and not at the actual indicator. Example records of matching trials are shown in Fig. 2 and the group results are shown in Fig. 3.

The first result was that for the matching series where the reference arm was not being vibrated, subjects managed to achieve comparable matching accuracy no matter what the condition of the indicator. Errors were similar (Fig. 3) when subjects were blindfolded and could not see either arm, or they could see the

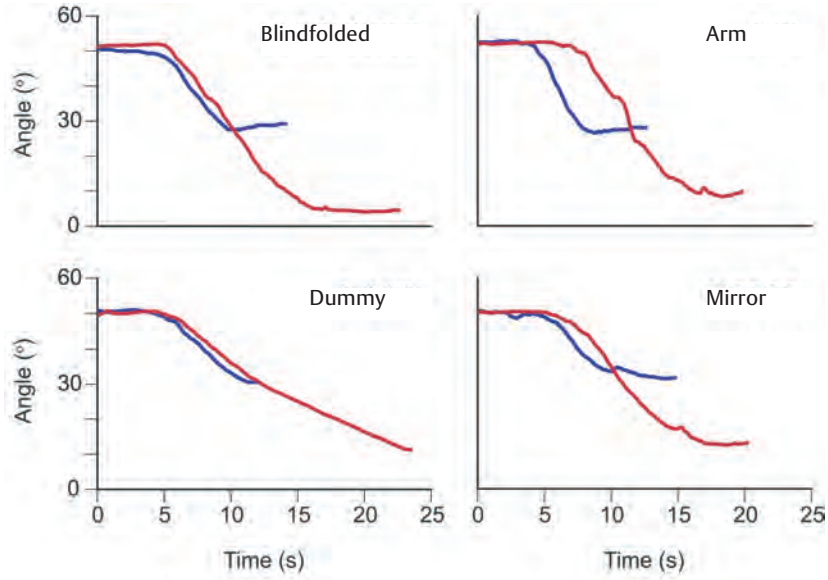


Figure 2. Forearm position matching. Each panel represents two matching trials. The reference arm, which was always hidden from view, was placed at 30 deg to the horizontal and several seconds later the subject matched its position. The blue trace is the position of the indicator during matching without vibration, the red trace during vibration of the reference arm. The end of each trace was the point where the subject declared they had achieved a satisfactory match. The four conditions were matching blindfolded, matching while watching the indicator arm, using a dummy indicator arm for matching and looking at the mirror image of the indicator during matching. Notice during vibration that matching angles are small, that is, the indicator is more extended than the reference.

indicator or its mirror image, or they used the dummy to match positions. This last result is interesting in that it implies subjects were equally able to match position using proprioceptive information only (blindfolded) or visual information only (dummy). However, significant differences between conditions emerged when the reference arm was vibrated.

Vibration of the reference arm elicited a strong illusion that the arm was moving into extension and that its position was more extended than really was the case (Figs 2 and 3). The brain was falsely interpreting the muscle spindle signal from vibration as an elongating muscle, that is, an extending forearm. The size of the measured illusion was largest when the subject was blindfolded and they relied entirely on proprioceptive signals for matching. Here, presumably, the subject was trying to match the spindle signal coming from the non-vibrated arm with the much larger vibration-evoked signal coming from the reference arm. They did this by adopting an extended position with their indicator, in the process stretching its flexor muscles and raising the flexor spindle signal to a level somewhere near that coming from the reference arm. The observed error was 12 deg (Fig. 3).

Interestingly the error was only slightly smaller, 11 deg, when subjects matched while being able to see their indicator arm (Fig. 3). However, the errors were significantly smaller, 8.6 deg, when subjects used a dummy to match and 8.4 deg when they looked at a mirror image of their indicator arm during matching. With the dummy, subjects were using a visual cue to match the position of a proprioceptively perceived position.

In a simple interpretation, based on experience, vision of a particular arm posture is calibrated in terms of the normally expected proprioceptive signal. The discrepancy between the expected position indicated by sight of the dummy and that indicated by the proprioceptive signals coming from the reference arm led to a reduction in the position errors

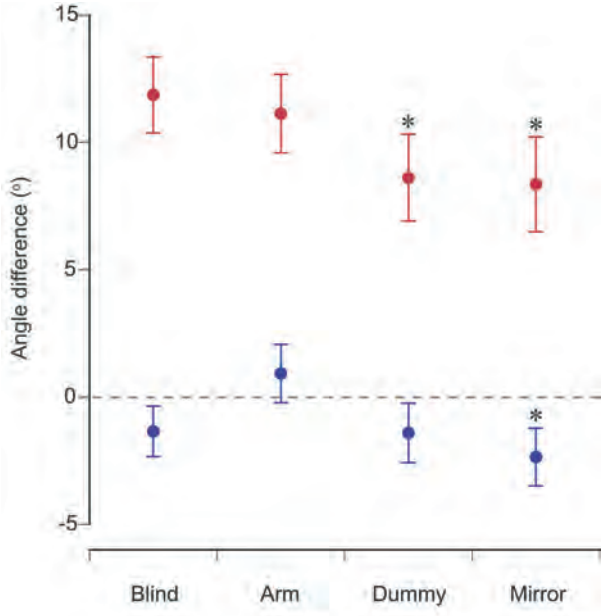


Figure 3. Position matching errors for the group. Matching angle differences for the group of 15 subjects shown as means (± S.E.M.), without vibration (blue) and with vibration of the reference arm (red). The four conditions were as in Fig. 2. Angle difference was calculated as the position of the reference arm minus the position of the indicator arm. 0 deg represented a perfect match. Positive errors were where the indicator arm had adopted a more extended position relative to the reference; negative errors were where the indicator had adopted a more flexed position. Asterisks indicate significant differences ($P < 0.05$) from the blindfolded condition for the vibration trials and from the condition where the subject could see their indicator arm for the non-vibration trials.

produced by the vibration. We think something similar was occurring when subjects looked at the mirror image of the indicator arm. Here an additional factor was likely to be that subjects were unsure of which arm they were looking at. In the trials where subjects simply looked at the indicator arm during matching, vibration errors were only slightly smaller than in the blindfolded condition as here both visual and proprioceptive signals coming from the indicator would be available and, very probably, the proprioceptive signal would be prioritised.

The main conclusion from this first set of experiments was that the condition of the indicator arm clearly played an important role in determining the accuracy of forearm position matching. Under normal circumstances, vision and proprioception can be used interchangeably during matching. However, if a false proprioceptive signal is introduced in one arm with vibration, this creates a conflict between the information provided by the proprioceptive and visual inputs from the two arms to reduce the effect of the vibration.

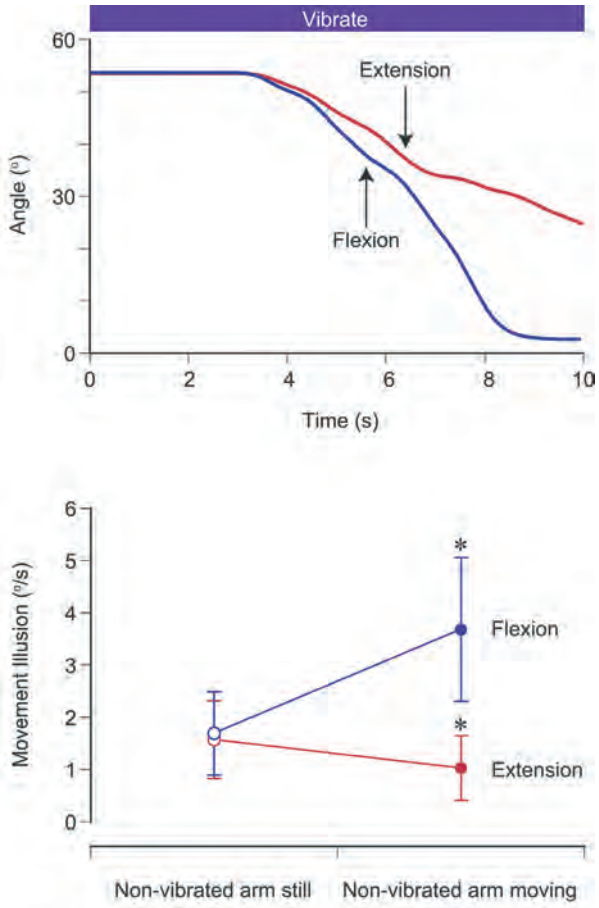


Figure 4. Movement sensations in a vibrated arm during movement of the other arm. Upper panel, perceived movement of the vibrated arm with the other arm stationary and then moving in the direction of extension (red trace) or the direction of flexion (blue trace). The arrows indicate the point at which the non-vibrated arm began to move. The bar at the top of the figure indicates the period of vibration. Notice the illusion of forearm extension in the vibrated arm took about 3 s to develop and that in the two trials shown its speed before the other arm was moved was not quite the same. However, as soon as the non-vibrated arm was moved into extension, perceived movement speed of the vibrated arm slowed and when the non-vibrated arm was moved into flexion it sped up. Lower panel, the pooled data from seven subjects. Mean (± S.E.M.) values for the movement illusion speed (deg s^{-1}) when the non-vibrated arm was held still, when it was moved in the direction of extension (red) and when it was moved in the direction of flexion (blue). Asterisks indicate significant differences in speed between when the arm was still and when it was moving (both $P < 0.05$).

All of the observations described so far were concerned with the sense of forearm position. What about the sense of movement? The way we explored this was as follows: a vibration illusion was generated in one arm as before, but this time subjects were asked to track the perceived movement with the vibrated arm itself. So now during vibration the arm is slowly extending according to what the subject felt. We measured the speed of the movement on a computer. Throughout this time the other arm was held still at a particular angle. Then the experimenter began to move the non-vibrated arm in either the direction of extension or flexion. When the movement was in the direction of extension, subjects were seen to slow the speed of their tracking movement with the vibrated arm. When the movement was into flexion, movement of the vibrated arm speeded up (Fig. 4). Remarkably, when subjects moved their non-vibrated arm up and down in a continuous motion, they reported a 'flutter' sensation of slowing and speeding-up of their vibrated arm, even when this was not moving at all. There can be no more dramatic example of an interaction between proprioceptive signals coming from the two arms than this change in the quality of the sensation evoked by vibration in one arm that depended entirely on what the other arm was doing.

Our interpretation of this result is that what is felt during vibration is based, at least in part, on a difference signal derived from the inputs from the two arms. When the non-vibrated arm was moving into extension its elbow flexor muscles would be stretched and flexor spindles would therefore raise their level of discharge, thereby reducing the difference in proprioceptive signal between it and the vibrated arm. It led to slowing of the tracking movement by the vibrated arm. Conversely, if the non-vibrated arm was flexed, its spindles would be slackened by the muscle shortening and their discharge would be slowed, increasing the difference in signal

between it and the vibrated arm. That led to an increase in speed of the tracking movement.

The present study has shown that both the sense of limb position and the sense of limb movement of one arm are dependent to some extent on the information coming from the other arm and that this information can be either visual or proprioceptive. Such a conclusion should be kept in mind in experiments on proprioception allegedly involving only one arm. Input from the other arm may not be entirely irrelevant. When the two arms are involved in a motor task where they are required to act cooperatively, for example, fine manipulations that require both hands close together, the central comparator is influenced by information coming from both arms, be it proprioceptive, visual or both. At the same time the identity of each arm is preserved. So in some circumstances the two arms can be considered to be acting as one, a single instrument, in the execution of certain skilled tasks.

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A novel way to test human motoneurone behaviour during muscle fatigue

Descending voluntary drive during a sustained contraction makes it difficult to test the true effect of fatigue on the excitability of spinal motoneurones. Transient, artificially induced interruption to voluntary drive enables assessment of motoneurone behaviour during muscle fatigue. This technique reveals a profound reduction in motoneurone responsiveness not seen during intact voluntary drive.

Fatigue is one of the most common symptoms reported to general practitioners. Although it may be difficult to separate general malaise from muscle fatigue in some circumstances, muscle fatigue is an unavoidable consequence of life. This is true across the full spectrum of activity, from simple daily tasks such as standing or climbing stairs to voluntary exercise or training. A commonly accepted definition of muscle fatigue is 'any exercise-induced reduction in the ability to exert muscle force or power regardless of whether or not the task can be sustained' (Bigland-Ritchie & Woods, 1984). It is well known that muscle fatigue has both central and peripheral components which occur in the central nervous system and working muscle, respectively. Changes within the muscle usually have the greater contribution to muscle fatigue and as a consequence much more research has been devoted to peripheral rather than central fatigue. However, it has been estimated that as much as 25% of force loss can be attributed to nervous system changes during a sustained 2 min maximal voluntary contraction and this proportion is even greater for weaker sustained

From top left clockwise: Janet Taylor, Chris McNeil, Simon Gandevia and Peter Martin

contractions (Taylor & Gandevia, 2008). Despite this significant contribution, the proportion of central fatigue which resides in the motor cortex *versus* spinal cord remains unclear.

One method to test for fatigue-related changes at the level of the spinal cord in humans is to stimulate the corticospinal tract non-invasively at the cervicomedullary junction. This form of stimulation is usually accomplished by fixing electrodes behind the ears to the mastoid processes and passing an electrical current pulse between the electrodes. The stimulus produces a short-latency cervicomedullary

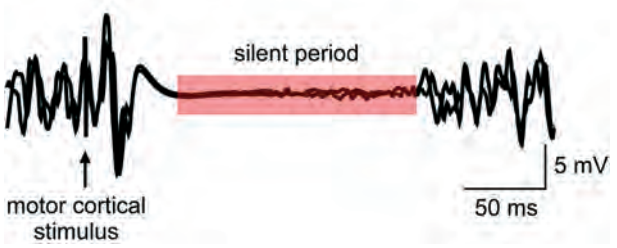

Figure 1. Silent period induced by high-intensity transcranial magnetic stimulation. The responses obtained during two brief (2–3 s) maximal efforts are overlaid. The shaded area identifies the period of electrical silence which follows the motor cortical stimulus. Note, in this study and many others, the onset of the silent period is actually measured from the time of the motor cortical stimulus.

motor-evoked potential (CMEP) in the electrical signal recorded from the target muscle. As the stimulus is delivered below the level of the motor cortex, a CMEP can be used to assess motoneurone excitability without the need to consider cortical changes induced by fatigue.

Using this method of stimulation, we have demonstrated that CMEP size decreases during the last 30 s of a sustained 2 min maximal voluntary contraction due to a reduced excitability of the motoneurone pool (Butler *et al.* 2003). What mechanisms are responsible for this fatigue-related reduction in excitability of the motoneurones during a sustained maximal effort? We proposed two candidates: (1) a change to intrinsic motoneuronal properties caused by their repetitive discharge during the sustained maximal effort; and (2) disfacilitation of the motoneurones caused by a fatigue-related reduction of excitatory input from muscle afferents. However, the question of mechanism is difficult to address because motoneurone responsiveness is strongly affected by the presence and magnitude of descending voluntary drive from the brain and it is uncertain if the level of this drive that reaches the motoneurones increases, decreases or has no net change with fatigue. Hence, we wanted to assess motoneurone excitability without, or with minimal influence from, the confounding factor of an unknown level of descending voluntary drive. We recently used a paired-stimulus protocol in an attempt to accomplish this (McNeil *et al.* 2009).

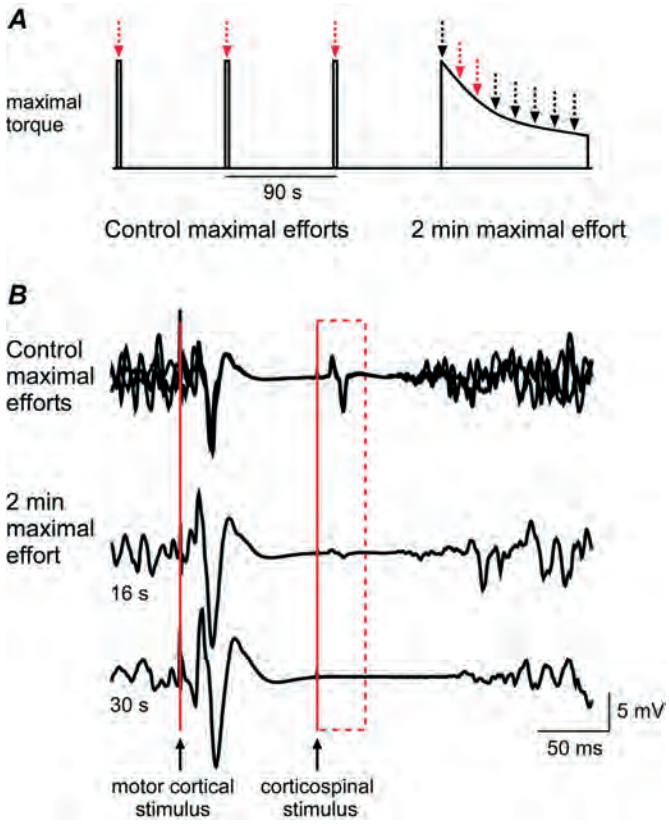

Figure 2. Individual traces of biceps electrical activity recorded from a single subject in brief control maximal efforts and a sustained 2 min maximal effort. *A*, schematic diagram of maximal contractions performed and the timing of paired motor cortical–corticospinal stimuli. Red arrows identify the paired stimuli for which electrical responses are shown in panel *B*. *B*, electrical responses obtained after paired motor cortical–corticospinal stimuli during three brief control maximal efforts are overlaid in the top trace. The bottom traces are two of the responses recorded during the sustained 2 min maximal effort. The dashed box surrounds the CMEPs evoked in the silent period following the conditioning motor cortical stimulus. In this subject, the CMEP in the silent period is abolished within 30 s of contraction. Modified from McNeil *et al*. 2009.

Transcranial magnetic stimulation is a non-invasive means to activate neurones within the motor cortex. A stimulating coil that produces a magnetic field is held against the appropriate region of the scalp. When a strong stimulus is delivered during a voluntary contraction, the discharge of the coil affects cortical output cells and transiently interrupts descending voluntary drive. This stops output from the motoneurones and causes a period of silence in the electrical activity recorded at the target muscle (Fig. 1). If a corticospinal stimulus were delivered in this *silent period*, motoneuronal properties could be tested during the development of fatigue without the complication of unknown levels of descending drive. Thus, we paired a corticospinal stimulus with a strong transcranial magnetic stimulus delivered 100 ms earlier. This pair of stimuli was given at regular intervals throughout a sustained 2 min maximal effort to assess the effect of fatigue on motoneurone excitability during the silent period (Fig. 2*A*). Raw traces showing CMEPs during the silent period are displayed for a single subject in Fig. 2*B*.

In the absence of descending voluntary drive from the brain, there is a rapid and profound decrease of motoneurone excitability during a sustained maximal effort (Fig. 3, filled symbols). This result is in stark contrast to the previous and current finding that motoneurone excitability (normalised to the increase of the peripheral potential; Fig. 3, red line) shows no change for the first 90 s of maximal effort when assessed in the presence of descending voluntary drive (Fig. 3, open symbols). A comparison of fatigue-related changes to CMEPs recorded during the silent period to CMEPs recorded during ongoing voluntary drive (Fig. 3, filled and open symbols) demonstrates the potency of descending voluntary input to the motoneurones. Namely, the facilitatory effects of this input

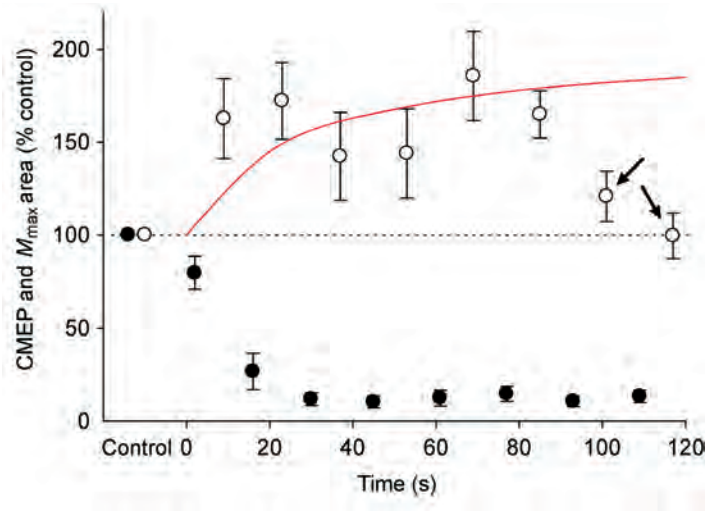

Figure 3. Normalised CMEP responses during a sustained 2 min maximal effort. *N* = 8. Data are mean values (± S.E.M.) for CMEPs in the presence (○) and absence (●) of descending voluntary drive and are expressed as a percentage of values obtained during brief control maximal efforts. The red line represents an approximation of the typical growth of the compound muscle action potential (M_{max}) during a 2 min maximal effort, which is a consequence of changes in the muscle fibre membrane. The area of CMEPs in the presence of voluntary drive increases during the first 90 s of contraction but this is largely the result of an increase in the area of M_{max}. The decrease in the area of these CMEPs during the final 20 s of contraction (data points denoted by arrows) represents a reduction in excitability of the motoneurones. In contrast, the area of CMEPs in the absence of voluntary drive decreases rapidly and reaches a nadir within 30 s of contraction. Modified from McNeil *et al*. (2009).

delay *any* manifestation of reduced motoneurone responsiveness until 90 s of maximal effort have elapsed. Further, the decrease in responsiveness seen after 90 s is still markedly attenuated compared to the reduced excitability seen in the silent period less than 20 s into a sustained maximal contraction.

Despite the ability to provide novel insight into the effects of fatigue on motoneurone excitability, the combined use of transcranial magnetic stimulation and corticospinal tract stimulation is necessarily non-invasive and hence it remains difficult to identify the mechanisms responsible for the marked fatigue-related reduction of motoneuronal excitability. Recall that our earlier experiment with intact descending voluntary drive (Butler *et al.* 2003) led to two proposed mechanisms, a change to intrinsic motoneuronal properties and a reduction of excitatory input from muscle afferents on to the motoneurones. It is unacceptably invasive to obtain a direct recording of intrinsic motoneurone properties in a human, but intracellular recordings from animals show that motoneurones adapt to a constant-current input and discharge at a reduced rate due to altered conductances and a lower input–output gain of the motoneurone pool (see Powers & Binder, 2001 for review). Such processes, and no doubt others, are at the heart of the generic term *changes to intrinsic properties of motoneurones*. The limits to probing these changes in humans prompted us to examine the role of the reduced excitatory input from muscle afferents. We tweaked our previous protocol (seen in Fig. 2*A*) to include intermittent tendon vibration to increase muscle afferent input to the motoneurones during the silent period. Tendon vibration strongly excites muscle spindle afferents which should provide excitatory input to the motoneurones innervating the muscle in which the afferents are located. Preliminary findings indicate that vibration during a sustained maximal contraction does not change CMEP size. A possible interpretation is that reduced muscle spindle input has only a limited contribution to the profound fatigue-related decrease of motoneurone excitability.

In summary, a test of motoneurone responsiveness after interruption of descending voluntary drive shows that the facilitatory effect of this input to the motoneurones masks a profound and rapid decrease of motoneurone excitability during a sustained maximal effort. We believe that a stimulus to the corticospinal tract paired with a preceding strong stimulus to the motor cortex is a promising technique to explore spinal contributions to central fatigue in humans.

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Physiology News

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What makes some axons more excitable than others?

Have you ever wondered why some motor axons may be activated before all others in a muscle as an electrical stimulus is increased? It could just be that the low threshold represents a quirk of anatomy and current flow, but it could also be that the excitability of the axon is greater than that of other axons innervating the muscle. The latter seems to be the case and a key factor seems to be the hyperpolarisation-activated current (I_h)

The excitability properties of human axons can be studied non-invasively by tracking the changes in the strength of the electrical stimulus required to generate an action potential. This technique is known as threshold tracking.

We teach that the largest nerve fibres not only conduct faster but are also easier to excite electrically than smaller axons. Small axons arising from small motoneurones are recruited first by voluntary effort or by reflex inputs (according to Henneman's size principle; Henneman *et al*. 1965). On the other hand, sensory axons in human peripheral nerve are excited electrically at lower threshold than motor axons due, in part, to a difference in sodium channels.

Now a study using the threshold tracking of human motor axons has shown that the first motor axons to be excited electrically are those with greater activity of the hyperpolarization-activated current, I_h. I_h is a depolarizing current that plays a pacemaker role in the heart and CNS and is due to hyperpolarization-activated cyclic nucleotide-gated (HCN) channels.

This previously unsuspected role of HCN channels in controlling the resting excitability of axons may be linked to their role in the hyperexcitability of the nociceptive afferents responsible for neuropathic pain.

Sensory and motor axons

Differences in membrane conductances between sensory and motor axons have been demonstrated in human peripheral nerves. There is a significant difference in the accommodation to hyperpolarization between sensory and motor axons (shown as changes in excitability in Fig. 1).

Left to right: James Howells, Susan Tomlinson, David Burke, Louise Trevillion and Hugh Bostock.

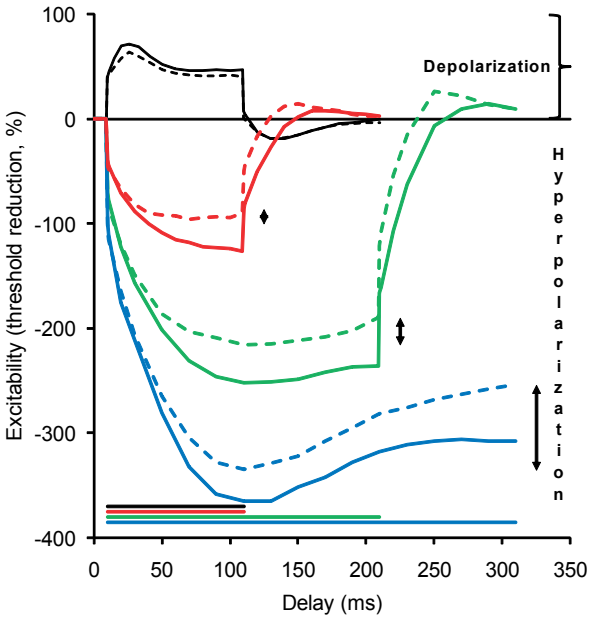

Figure 1. Excitability changes in sensory and motor axons of the median nerve in response to subthreshold polarizing currents (unpublished mean data for 3 subjects). Threshold electrotonus was recorded by tracking compound sensory nerve action potentials (dashed lines; recorded from digit 2) and compound muscle action potentials (continuous lines; recorded from abductor pollicis brevis). The injected depolarizing current was set to 40% of the 1 ms threshold for the target potential and was 100 ms in duration (black). The injected hyperpolarizing current was set to –40% (red), –70% (green) and –100% (blue) of the threshold for the target potential and was 100 ms, 200 ms or 300 ms in duration, respectively (duration of conditioning current indicated by horizontal lines just above the *X*-axis). Note that the threshold changes induced by hyperpolarization were less for sensory axons than motor axons, reflecting greater activity of I_h in sensory axons. Note also that the threshold overshoot when the polarizing current was switched off (110–175 ms, red traces; 210–300 ms, green traces) was greater for sensory axons. The accommodation has a relatively slow onset and, as indicated for sensory axons by arrowed lines, it increases with the degree of hyperpolarization.

Sensory axons appear to have a greater activity of I_h (Fig. 1; Bostock *et al*. 1994). It is believed that the role of the current is to limit activity-dependent hyperpolarization following the conduction of trains of impulses. Sensory axons also have a greater persistent Na^+ conductance (Bostock & Rothwell, 1997), reflected in a longer strength–duration time constant (Panizza *et al*. 1992). This Na^+ conductance is active at threshold and contributes to resting membrane potential. These differences have been suggested to render sensory axons more susceptible to ectopic activity than motor axons, while making them less susceptible to conduction block.

In addition to differences between sensory and motor axons, there are differences within an axon type. It is well documented that motor units of a muscle differ in their motoneurone properties and the mechanical and histochemical properties of the innervated muscle fibres. Motor units are remarkably consistent in their recruitment thresholds, whether they be in voluntary or reflex contractions on the one hand, or in response to electrical stimululation on the other. All things being equal, voluntary effort should recruit motoneurones with smaller, more slowly conducting axons first (Henneman *et al*. 1965), while electrical stimulation should activate larger axons first. There are, however, a number of factors which need to be taken into account. In studies in human subjects, stimulation is percutaneous, at some distance from the closest nodes of Ranvier. Do proximity to the stimulus and anatomical factors determine which axons are first recruited from any one site rather than variations in intrinsic excitability due to differences in their biophysical properties? If low threshold is a reflection of increased excitability, what are the determinants?

Nerve excitability and threshold tracking

The excitability properties of human axons can be studied non-invasively by tracking the changes in the strength of an externally applied electrical stimulus required to generate an action potential. (Typically adhesive ECG electrodes are used with the cathode positioned over the nerve and the anode placed remotely on electrically less excitable tissue nearby.) The stimulus is submaximal, and is adjusted by computer to activate a preset submaximal nerve volley (usually 40–50% of maximum). The nerve volley generates a compound muscle action potential (CMAP), recorded using surface electrodes placed over the belly of the innervated muscle, and a compound sensory nerve action potential (CSNAP or, more simply, SNAP) recorded with ring electrodes placed around the innervated digit. If axons become depolarized (for example, during ischaemia induced by inflation of a pressure cuff), they are more excitable and a smaller current will be required to produce the target potential. If axons become hyperpolarized (for example, after conducting trains of impulses during a sustained muscle contraction), they are less excitable and more current is required. This technique of threshold tracking provides measures of excitability which are determined by the electrical properties of the axonal membrane at the point of stimulation. The procedure differs from nerve conduction studies in which a supramaximal stimulus is used to ensure that all axons within the nerve are excited.

The field of nerve excitability owes a debt to Joseph Bergmans who, in 1970, documented the excitability properties of single human motor axons. He found that single motor axons could be selectively activated over a large range of stimulation strengths and that the same motor units could be activated by threshold stimuli on repeated occasions when the stimulating electrodes were placed in the same location. He defined threshold as the lowest stimulation strength that would elicit three to five consecutive motor unit potentials (a motor unit potential is the potential generated by the muscle fibres innervated by a single motor axon) and then painstakingly adjusted stimulus voltage by hand to track changes in the threshold of the axon. He made meticulous and methodical recordings from single motor units in order to study recovery from single and repetitive activation and the influence of ischaemia, electrical polarization and temperature on these processes. From these non-invasive, *in vivo* studies, Bergmans was able to describe the mechanisms responsible for the different phases of post-tetanic hyperpolarization.

The development of an automated technique for tracking the threshold of a compound sensory or muscle action potential (Bostock *et al*. 1998) gave researchers a remarkable tool that allowed them to rapidly assess axonal excitability. In a matter of minutes, the stimulus–response relationship, strength–duration properties, accommodation to subthreshold changes in membrane potential and recovery after activation can be measured, providing information about both nodal and internodal conductances. The measurements are made non-invasively and in real time, allowing the time course of interventions such as drug administration and renal dialysis to be followed.

Many studies have been conducted using this tool and there is now a considerable body of literature describing findings in normal nerves and in numerous pathophysiological conditions ranging from nerve disorders, such as channelopathies and demyelinating conditions, to metabolic disorders, such as renal failure and diabetes. Further, differences between the excitability properties of axons in the upper and lower limbs and also between the proximal and distal segments within the same nerves have been found. The technique has also been successfully applied to the study of animal nerves (and single axons), giving rise to a body of literature on the changes in axonal excitability with maturation.

Excitability properties of single motor axons

Automated threshold tracking has recently been applied to investigate the excitability of selectively activated single motor units (a motor unit being the motoneurone, its motor axon and the muscle fibres it innervates) to determine whether the reason that they have a low threshold to electrical recruitment is due to intrinsic properties (Trevillion *et al*. 2010). Threshold tracking enabled comparisons to be made between the excitability properties of the single motor axons with those of higher threshold motor axons recruited in compound potentials recorded in the same experimental session.

Based on earlier published findings on the ionic determinants of threshold (Bostock & Rothwell, 1997), it was anticipated that a difference in the persistent Na^+ conductance between the low- and high-threshold axons would be revealed. However, this proved not to be the case. The key point of differentiation between the axons was found to be in the accommodation to hyperpolarizing currents (Fig. 2). Low-threshold axons were found to have greater accommodation to hyperpolarizing changes in membrane potential, presumably due to a greater hyperpolarization-activated inwardly rectifying conductance, I_h. This conductance has been shown to have a pacemaker role in spontaneously firing neurones and the sinoatrial node. HCN channels mediate the inwardly rectifying conductance and are known to exist in four isoforms. All have been found in the mammalian nervous system, although channel expression in the human peripheral nervous system is yet to be characterised. The findings for single motor axons suggest that HCN channels provide a depolarizing current at rest and therefore play a role in setting membrane potential.

Physiological implications

The above findings suggest that the motor axons that are less readily recruited by voluntary activation may have a greater activity of I_h and may, therefore, be more secure against the development of conduction block in disease processes. The flipside is that the increase in excitability makes these axons more prone to ectopic activity, such as fasciculation, and perhaps to motoneurone loss in amyotrophic lateral sclerosis. A corollary of this is that axons from smaller motoneurones, recruited more readily into voluntary contractions, may be more likely to develop conduction block when hyperpolarized during activity. This has implications for conditions such as multiple sclerosis, chronic inflammatory demyelinating polyneuropathy and multifocal motor neuropathy, where these conduction abnormalities occur.

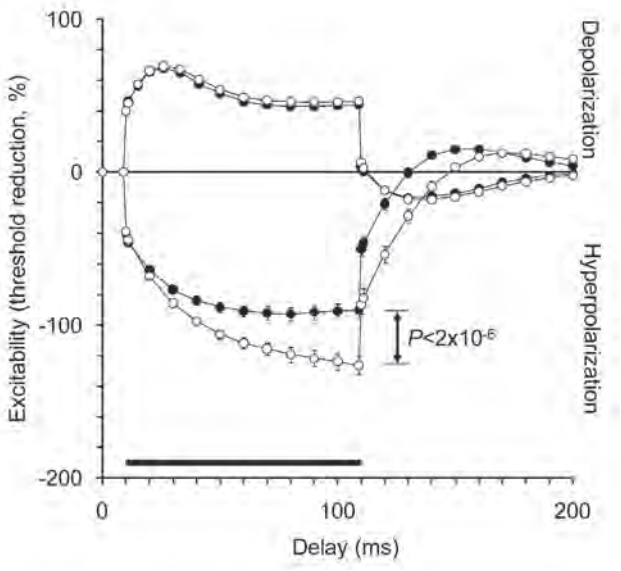

Figure 2. Threshold changes in motor axons during threshold electrotonus. Subthreshold currents, both depolarizing (40% threshold for the target potential) and hyperpolarizing (–40% threshold for the target potential), were applied for 100 ms (as indicated by bar just above the *X*-axis) and the threshold of the single motor units (filled circles) and compound potentials (40% of maximal amplitude; open circles) were tracked before, during and after the currents. Note the divergence in the threshold changes to hyperpolarization between the low-threshold axons of the single motor units and the high-threshold axons recruited in the compound potential. Hyperpolarization produces a lesser threshold change for the single motor units, much as for sensory axons shown in Fig. 1 (see the responses to 100 ms hyperpolarization). This and other findings support the conclusion that there is greater inward rectification, due to greater I_h, on the low-threshold single motor axons. Data presented as mean ± S.E.M. (n = 22).

The stimulus–response curve for motor axons is relatively steep, reflecting minimal variation in threshold. However, the above findings demonstrate a significant variation in the intrinsic properties of different motor axons. This may have implications for some motor unit number estimation (MUNE) techniques that are based on the 'mean' motor unit measured using surface electromyography.

Spontaneous discharges from injured peripheral nerves are believed to be responsible for the induction and maintenance of neuropathic pain syndromes. Upregulation of I_h in injured nerves is believed to be a factor in the spontaneous action potentials (Chaplan *et al*. 2003), making HCN channels the targets for pharmacological treatments for neuropathic pain. Just as I_h expression varies between motor axons, resulting in threshold variation, it may be that I_h expression varies considerably between sensory axons rendering some more prone to generating the spontaneous activity seen in neuropathic pain syndromes.

Computer-controlled threshold tracking provides a reliable method for assessing I_h expression in human motor and sensory axons, non-invasively by tracking compound action potentials (Tomlinson *et al*. 2010). In fact, threshold tracking

could be used to assess the efficacy of HCN channel blockers in animal models of neuropathic pain and to monitor the effects of these agents on axonal function.

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Hugh Bostock and colleagues run occasional 3-day, hands-on nerve excitability workshops, which provide a detailed introduction to the theory and practice of nerve excitability testing. Those interested are invited to email h.bostock@ion.ucl.ac.uk for further information.

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Paton Prize Bursary Photographic Competition

The Paton Prize fund promotes interest in an historical perspective on physiological research. Under the auspices of the History & Archives Committee, a £100 prize is offered for the best photograph showing equipment in use or within a laboratory setting.

Suitable photographs should show equipment with modern or historical significance, either by dint of association with renowned experimentalists or as items that are fascinating technically. Where relevant, multiple images or a brief movie are acceptable. A photograph that is itself historic would be welcome, but one that shows experimental equipment and its users could be ideal.

An early ECG recording system

(Reproduced from the Ministry for Social Policy, Health, the Elderly and Community Care, Malta)

The best examples offered, as well as the prize-winning entry, will appear in *Physiology News* and on The Society's website.

Submissions: Please provide a jpg file or equivalent. (Where the photograph itself is a venerable object, please arrange for scanning and enter it as an electronic file.)

Photographs need to be supported by a brief statement to explain the equipment and to highlight its claim to fame.

Address your entry to Simon Kellas: skellas@physoc.org

NOS1, *S*-nitrosylation and cardiac contraction

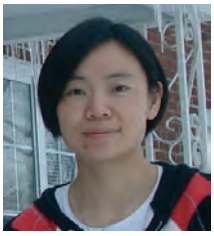
Nitric oxide (NO) is a major regulator of cardiac contraction in both healthy and diseased hearts. NO is produced via enzymes termed nitric oxide synthase (NOS). The neuronal NOS (NOS1) isoform alters key cardiac proteins via post-translational modifications resulting in enhanced contractile function. Therefore, it provides a potential therapeutic target for clinic treatment and new drug development.

Contraction of cardiac myocytes occurs by a process termed excitation–contraction coupling (ECC) (Bers, 2002). The initiating event is the opening of L-type Ca^{2+} channels (I_{Ca}), which leads to a release of Ca^{2+} from the sarcoplasmic reticulum (SR) via the SR Ca^{2+} release channel (ryanodine receptor, RyR2) (i.e. Ca^{2+} transient). Upon RyR2 opening, the large efflux of Ca^{2+} will diffuse and then activate the myofilaments to contract. During relaxation, the majority of Ca^{2+} is resequestered into the SR by the SR Ca^{2+}-ATPase–phospholamban (SERCA–PLB) complex while the remaining Ca^{2+} is removed from the cell by the Na^{+}–Ca^{2+} exchanger.

Nitric oxide and cardiac function

Within cardiac myoctes, nitric oxide (NO) is endogenously produced via two constitutively expressed enzymes: endothelial NO synthase (NOS3) and neuronal NO synthase (NOS1). Another isoform (inducible NO synthase, NOS2) is not constitutively expressed, but its expression is induced during an inflammatory response. NO has been shown to play an important role in both physiological and pathological states. Depending on its source of production, NO may be beneficial or detrimental to cardiac function and remodelling. Although NO is a highly diffusible gas, signalling via the two constitutively expressed isozymes (NOS1 and NOS3) is compartmentalized and each isoform modulates cardiac function differently (Barouch *et al.* 2002; Ziolo *et al.* 2008).

NOS3 is localized to the plasmalemmal caveolae. It has been shown that NOS3 signalling decreases the β-adrenergic receptor (AR)-stimulated I_{Ca} via activation of the cGMP-dependent protein kinase.



Honglan Wang and Mark Ziolo

NOS3 signalling ultimately decreases the functional response to β-AR stimulation and protects against arrhythmias (Champion *et al.* 2004; Massion *et al.* 2004; Wang *et al.* 2008*b*).

Unlike NOS3, NOS1 is localized to the SR along with RyR2. NOS1 signalling in the myocyte leads to enhanced contraction and accelerated relaxation. Another difference compared to NOS3 is that NOS1 targets RyR2 (Gonzalez *et al.* 2007; Wang *et al.* 2010) and PLB (Wang *et al.* 2008*a*) through a cGMP-independent pathway.

RyR2 *S*-nitrosylation and activity in NOS1 knockout hearts

S-nitrosylation has emerged as an important protein post-translational modification related to cellular signal transduction (Ziolo, 2008). RyR2 contains sulfhydryl residues which can be modified by NO through *S*-nitrosylation (Xu *et al.* 1998). Our recent study demonstrated that there were decreased RyR2 *S*-nitrosylation levels in NOS1 knockout ($NOS1^{-/-}$) hearts (Wang *et al.* 2010) (Fig. 1). Along with the deceased RyR2 *S*-nitrosylation levels, $NOS1^{-/-}$ myocytes also displayed reduced RyR2 activity. We employed various techniques to measure RyR2 activity ranging from direct single RyR2 protein activity to indirect measurements in intact cardiac myocytes.

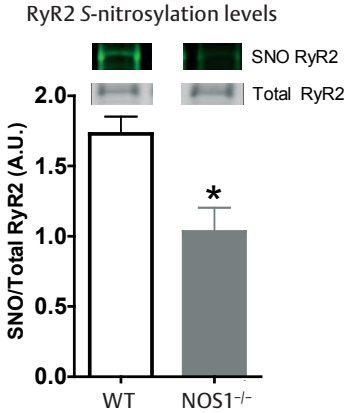


Figure 1. RyR2 from NOS1 knockout hearts ($NOS1^{-/-}$) have decreased RyR2 *S*-nitrosylation levels measured by the DyLight Switch method. Representative blots show *S*-nitrothiols (SNO) (top) and total RyR2 (bottom). $^{*}P < 0.05$ *vs* WT.

Data from single RyR2 channel recordings showed that the knockout of NOS1 resulted in a 3-fold reduction in RyR2 channel open probability (P_o) compared to RyR2 channels from wild-type (WT) hearts (Fig. 2*A*). In the physiological Ca^{2+} range (pCa 7 to pCa 3), NOS1 knockout resulted in decreased [^{3}H] ryanodine binding to RyR2 (Fig. 2*B*) in SR vesicle preparations, indicative of decreased P_o. In intact mouse myocytes, NOS1 knockout or acute inhibition significantly reduced Ca^{2+} spark frequency and amplitude (Fig. 2*C*). Finally, in a more physiologically relevant approach, $NOS1^{-/-}$ myocytes and WT myocytes with acute NOS1 inhibition (SMLT) had a significant rightward shift in the SR Ca^{2+} leak–load relationship compared to control WT myocytes (Fig. 2*D*). Thus, in four distinct methods to measure RyR2 activity, we consistently found impaired RyR2 activity when NOS1 was absent or acutely inhibited.

$NOS1^{-/-}$ hearts and myocytes exhibit a blunted force–frequency response and decreased contraction (Barouch *et al.* 2002; Wang *et al.* 2008*a*).

We suggest that decreased RyR2 activity via reduced *S*-nitrosylation levels observed in NOS1$^{-/-}$ hearts contribute to the depressed contraction. If this concept is correct, then addition of a NO donor (or *S*-nitrosylating agent) should be able to reverse the reduced RyR2 activity by increasing *S*-nitrosylation leading to increased myocyte contraction.

Can a NO donor increase NOS1$^{-/-}$ RyR2 activity?

SNAP (*S*-nitroso-*N*-acetyl-DL-penicillamine), a NO donor and *S*-nitrosylating agent, was used to test our hypothesis. In NOS1$^{-/-}$ mouse hearts, SNAP was able to increase RyR2 activity in our distinctive methods. That is, SNAP significantly increased RyR2 P_o, enhanced both Ca^{2+} spark frequency and amplitude, and caused a leftward shift in the SR Ca^{2+} leak–load relationship. There was little effect of SNAP in WT hearts. More importantly, SNAP now normalized RyR2 activity in the NOS1$^{-/-}$ hearts to WT levels. Taken together, SNAP is able to reverse the blunted RyR2 activity.

Can the increased RyR2 activity by SNAP reverse the depressed contraction observed in NOS1$^{-/-}$ myocytes?

Contraction in myocytes was determined by simultaneous measurement of Ca^{2+} transients and cell shortening. SNAP resulted in an increase in Ca^{2+} transient and cell shortening amplitudes in WT myocytes, NOS1$^{-/-}$ myocytes and WT myocytes with acute NOS1 inhibition (Fig. 3). However, SNAP had a much larger effect in myocytes with NOS1 knockout or acute inhibition. More importantly, SNAP now normalized the Ca^{2+} transient and cell shortening amplitudes in these myocytes. Thus, SNAP is indeed able to reverse the depressed contraction in NOS1$^{-/-}$ myocytes.

Is RyR2 *S*-nitrosylation involved in the effects mediated by SNAP?

In NOS1$^{-/-}$ mouse hearts, SNAP increased RyR2 *S*-nitrosylation levels.

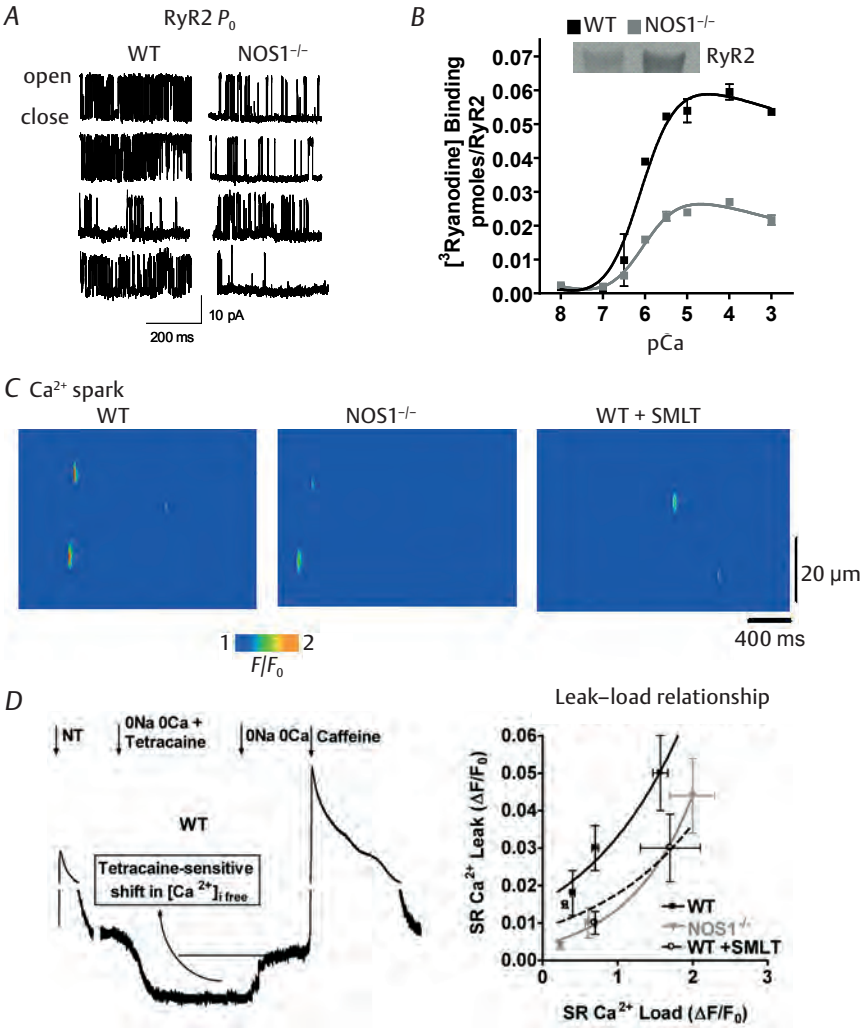


Figure 2. Decreased RyR2 activity with NOS1 knockout or acute inhibition. *A*, representative traces from WT (left) and NOS1$^{-/-}$ (right) single RyR2 channels showing decreased open probabillity (P_o). *B*, WT and NOS1$^{-/-}$ [^{3}H]ryanodine binding at different Ca^{2+} concentrations. *C*, representative images of Ca^{2+} sparks from WT, NOS1$^{-/-}$ and WT+SMLT (NOS1 selective inhibitor) myocytes. *D*, left, representative trace of the SR Ca^{2+} leak–load protocol from a WT myocyte. Right, NOS1 knockout or acute inhibition results in a significant rightward shift.

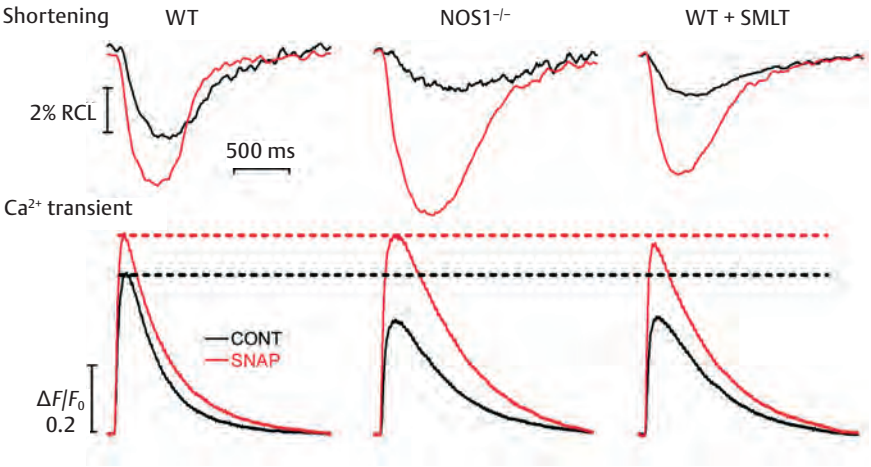


Figure 3. The NO donor SNAP reversed the depressed cardiac function in NOS1$^{-/-}$ and SMLT (NOS1 selective inhibitor)-treated myocytes. Representative shortening (top) and Ca^{2+} transient (bottom) traces. Dotted black line highlights decreased Ca^{2+} transient amplitudes with NOS1 knockout or acute inhibition. Dotted red line highlights normalized Ca^{2+} transient amplitudes after treatment with SNAP.

As with contraction, SNAP had a much larger effect on NOS1$^{-/-}$ RyR2 *S*-nitrosylation compared to WT RyR2. Further, in the presence of SNAP, RyR2 *S*-nitrosylation levels were now normalized. These data suggest that the effect of SNAP to reverse the contraction is, in part, via increased RyR2 *S*-nitrosylation.

In conclusion, NOS1 signalling results in *S*-nitrosylation of RyR2, increasing its activity that ultimately leads to enhanced contraction. Therefore, the NOS1-mediated post-translational modification of RyR2 is an important modulator of cardiac myocyte function and may be a useful target for clinical treatment of cardiomyopathies.

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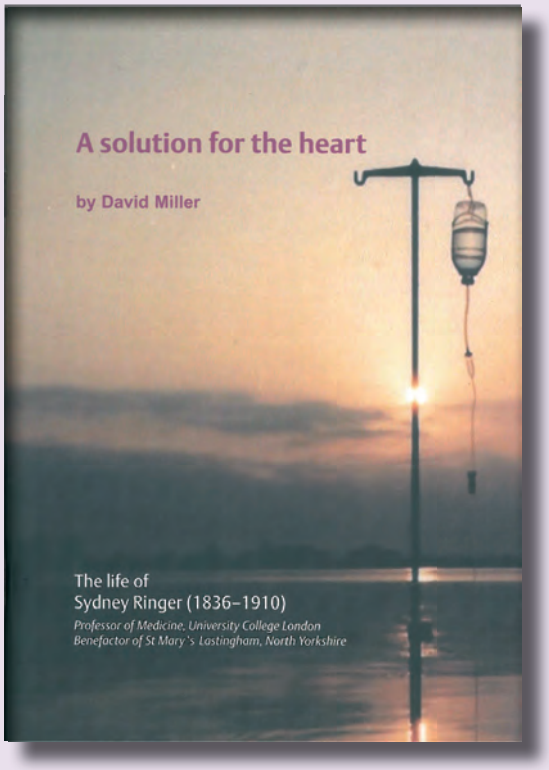
Paton Prize Bursary

The Paton Prize should not be confused with the Paton Lecture. The Prize was established in 1994 and takes the form of a bursary of up to £1000 to cover travel and incidental expenses. It is open to all Members and Affiliates of The Society, as well as established scientists.

Professor Sir William Paton originated the Prize to encourage the historical study of major ideas and concepts that have shaped modern physiology. The History & Archives Committee administers the Prize and would be delighted to receive applications describing a proposed piece of historical research which could lead to a published paper, a booklet or an article for *Physiology News*.

The Paton Prize was awarded in 2007 to David Miller for research into the life and work of Sydney Ringer.



A solution for the heart: the life of Sydney Ringer (1836–1910) David Miller (2007) (available on The Society website at: www.physoc.org/site/cms/contentviewarticle.asp?article=759)

Applications, in the form of an outline of the proposed work on one side of A4, should be submitted to Simon Kellas (skellas@physoc.org) by the last day of March 2011 for consideration by the committee in April. Anyone wanting more details or to talk through an idea is invited to contact the committee chair, Dafydd Walters (dwalters@sgul.ac.uk).

NO is pivotal in motor pathologies, but is it harmful or protective?

Dysregulation of the expression of nitric oxide synthase (NOS) is a hallmark of many neuropathological conditions. Using a model of peripheral motor neuropathy, we demonstrated that NOS up-regulation delays nerve repair and evokes many of the changes that occur in damaged motoneurons. Therefore, NOS might be a pivotal target for the development of therapeutic tools for diverse neuropathies.

Traumatic injury of a peripheral motor nerve evokes physiopathological changes in the damaged motoneurons that are common to a broad array of neuropathological states, including dysregulation of protein expression, function and/or aggregation. One of the proteins involved is nitric oxide synthase (NOS), which synthesizes nitric oxide (NO), a highly reactive gas. First identified in biological systems as the endothelial-derived relaxing factor (EDRF), NO is an intercellular messenger that freely crosses plasma membrane, with multiple functions in the cardiovascular, immunological and nervous systems. The relative ubiquity of NO actions suggests that this molecule is important for physiological and pathological states and confers upon NOS important therapeutic possibilities for a variety of neuropathies.

There are three major isoforms of NOS, coded by different genes and differing in localization, regulation, catalytic properties and inhibitor sensitivity (Alderton *et al*. 2001). The isoform predominantly found in the neuronal tissue (nNOS) was the first to be purified and cloned. The inducible isoform (iNOS) occurs in a wide range of cells and tissues, including activated macrophages, and in pathological states of the central nervous system, in astroglia and microglia. Finally, the primary isoform found in vascular endothelial cells is known as eNOS. At the low tissue concentrations of NO generated by nNOS and eNOS, NO acts as a molecular messenger in multiple physiological transduction pathways; at high concentrations, such as those produced by iNOS, NO is cytotoxic. Remarkably, over-expression of eNOS or nNOS in pathological conditions can also yield toxic NO levels.

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It is also noteworthy that NOS expression is up-regulated in motoneurons and reactive astrocytes in amyotrophic lateral sclerosis (ALS) and in neurons and/or glial cells in multiple sclerosis, HIV dementia, and Alzheimer's, Parkinson's and Huntington's diseases, to cite the most prevalent neurological pathologies. Up-regulation of this enzyme also occurs after peripheral and central traumatic lesions of the nervous system. As a result, unmasking the role of NOS imbalance in neuron physiopathology is central to the development of feasible therapeutic tools. Over the past decade, our team has focused on investigating the role of NO in nerve repair and motoneuron pathology after the traumatic lesion of a nerve. We selected the hypoglossal motor system of the adult rat as an easily accessible system and crushing as the method of nerve injury.

Physical injury to a nerve is the most frequent cause of acquired peripheral neuropathy, which is responsible for loss of motor, sensory and/or autonomic functions. Injured axons in the peripheral nervous system maintain their capacity to regenerate in adult mammals. Therefore, the identification of molecules that regulate degenerative and regenerative processes is indispensable in developing therapeutic tools to accelerate and improve functional recovery following nerve injury.

This type of injury up-regulates the three major isoforms of NOS in the affected nerve: nNOS accumulates in the growing motor axons, eNOS is over-expressed in vasa nervorum in the distal stump and around the injury site, and iNOS is expressed by the recruited macrophages and phagocytic Schwann cells (Fig. 1).

Although the pivotal role of NO is evident, whether it is harmful or protective is less clear. In nNOS or iNOS knockout mice, peripheral recovery is impaired due to delayed Wallerian degeneration, axon breakdown and Schwann cell reaction after nerve injury (Zochodne & Levy, 2005); this supports a beneficial role of NO. Paradoxically, chronic systemic inhibition of NOS accelerates the onset of functional muscle reinnervation, indicating a harmful impact on nerve repair because NO slows down axonal regrowth (Sunico *et al.* 2008). This apparent controversy was resolved by the finding that chronic, systemic, specific inhibition of eNOS – but not specific inhibition of n/iNOS – accelerated neuromuscular reconnection. To avoid unwanted side-effects due to the systemic administration of drugs and to improve specificity, an adenoviral vector was used to locally express a dominant negative of eNOS within the injured nerve. A single injection of this adenovirus on the day of nerve lesion increased axonal regeneration and significantly accelerated functional recovery of neuromuscular junction (Sunico *et al.* 2008). This suggests that NO of endothelial origin slows down muscle reinnervation by detrimental actions on axonal regeneration after peripheral nerve injury. These experiments identified eNOS as a potential therapeutic target for treatment of traumatic nerve

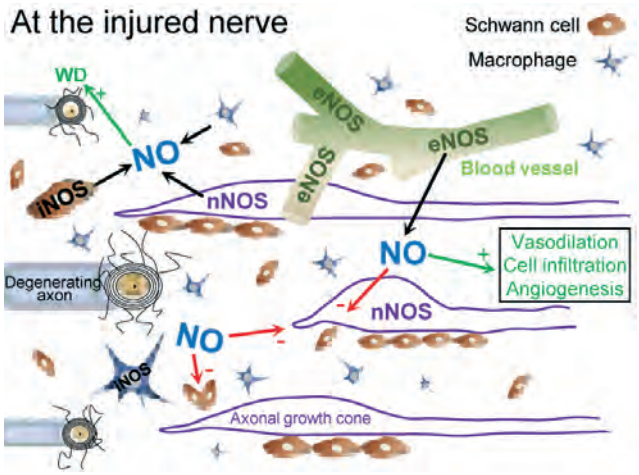

Figure 1. Diagram at the nerve level of the sources and actions of NO after motor nerve injury. The three isoforms of NOS are up-regulated: eNOS in vasa nervorum, nNOS in fibres and iNOS in infiltrated macrophages and reactivated Schwann cells. NO from different origins mediates pro- (+) and anti- (–) regenerative processes. NO favours vasodilatation, inflammatory cell infiltration, angiogenesis, Wallerian degeneration (WD), myelin breakdown and clearance, all of which are expected to be beneficial for axonal regeneration. On the other hand, NO could exert anti-proliferative and toxic effects on Schwann and endothelial cells, as well as axonal growth cone collapse. Figure modified from Moreno-López, 2010, with permission from Wiley-Blackwell (for a more detailed description, see that paper).

injuries, highlighting the potential value of gene therapy using viral vectors to suppress the activity of eNOS.

In a recent study reported in *The Journal of Physiology*, we analysed the role of induced NO in motoneuron pathology (Montero *et al.* 2010). To up-regulate nNOS in the hypoglossal motoneurons (HMNs), we used two different approaches. Crushing of the XIIth (hypoglossal) nerve provokes axonal transection of motoneurons. The alternative procedure was to transduce motoneurons by injecting an adenoviral vector, directing the expression of nNOS, into the hypoglossal nucleus (Fig. 2). The XIIth nerve crushing induces *de novo* nNOS expression in the perikarya of motoneurons, which normally lack this enzyme.

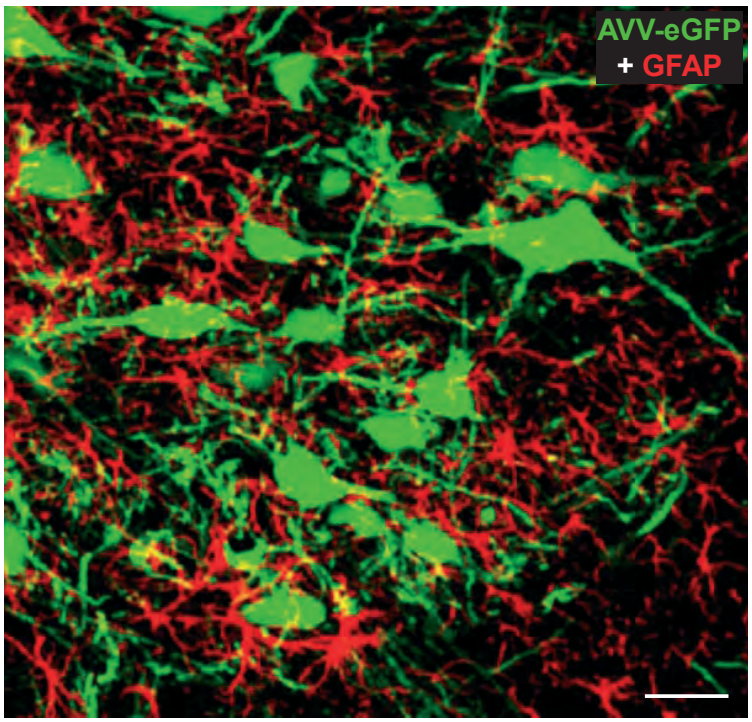

Figure 2. Specificity of viral transfection. Confocal photomicrograph of a coronal section, obtained 7 days after adenoviral (AVV) administration in the hypoglossal nucleus, at 300 µm rostral to the injection site, showing a broad number of eGFP-expressing hypoglossal neurons (green). Immunohistochemistry against the glial fibrillary acidic protein (GFAP) illustrates the absence of infected astroglia (red). Scale bar, 40 µm.

At the central level as well, NO has both harmful and beneficial roles for motoneuron survival after nerve injury. The gas is a pro-apoptotic molecule affecting motoneuron survival after motor nerve transection: nNOS deficiency or inhibition effectively reduced motoneuronal apoptosis induced by avulsion of the sciatic nerve in adult mice. Motoneuron apoptosis mediated by NO was accompanied by an increase in the NO subproduct peroxynitrite ($ONOO^-$), a potent oxidant, and in protein nitration (Martin *et al.* 2005) (Fig. 3). In addition, chronic NO synthesis strongly increases intrinsic motoneuron excitability by inhibiting background potassium currents, suggesting that induced nNOS sensitizes injured motoneurons to apoptosis by mediating the hyperexcitability experienced by the motoneuron following axonal injury (Fig. 3). This NO-mediated action may sensitize motoneurons to excitotoxic damage (Gonzalez-Forero *et al.* 2007), with detrimental effects on motoneuron viability after axonal damage. On the contrary, however, nNOS up-regulation mediates the loss of excitatory synaptic inputs on injured HMNs (Fig. 3). This appears to be an NO-mediated protective action against excitotoxic stimuli. The activation of regrowth programs in adult motoneurons following axotomy may require a reversion to an immature electrical phenotype. Whether these apparently opposing impacts of NO on motoneuron survival form part of a motoneuron dedifferentiation process is a topic that merits attention.

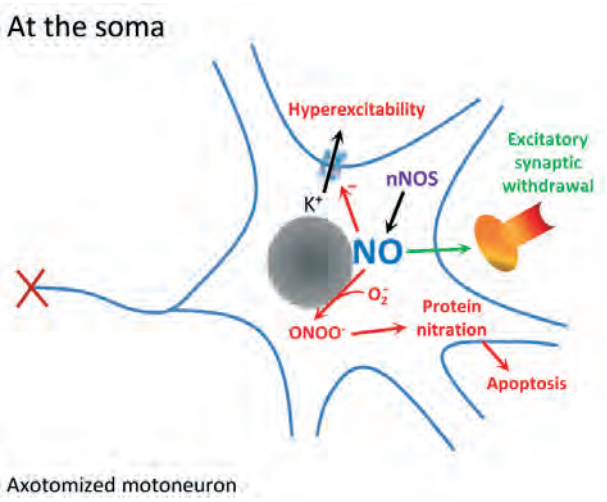

Figure 3. Diagram at the soma level of the sources and actions of NO after motor nerve injury. After nerve injury, nNOS appears *de novo* at the motoneuron. The nNOS-derived NO reacts with different targets, compromising motoneuron survival. NO can react with the anion superoxide (O_2^-) to form the potent oxidant peroxynitrite ($ONOO^-$), with the subsequent increase in protein nitration that precedes motoneuron apoptosis. Furthermore, through inhibition of background potassium currents, NO increases excitability. This might sensitize motoneurons to excitotoxic damage. In contrast, NO mediates a protective mechanism against excitotoxic stimuli such as excitatory synaptic withdrawal from injured motoneurons. Figure modified from Moreno-López, 2010, with permission from Wiley-Blackwell.

Disruption of the trophic communication between motoneurons and their target myocytes causes a well-characterized range of functional and synaptic impairments in insulted motoneurons. The NO-mediated disturbances involve changes in intrinsic membrane properties and anatomical synaptic deterioration that suggest a major pathological role of nNOS (Sunico *et al.* 2005, 2010; Gonzalez-Forero *et al.* 2007). However, nNOS is only one of the numerous proteins dysregulated after nerve damage. Thus, the actual role for nNOS is less clear within the complex synergistic and/or antagonistic actions of multiple dysregulated proteins. We further scrutinized the effects of virally induced *de novo* expression of nNOS in motoneurons, together with complementary attempts to down-regulate nNOS expression in damaged hypoglossal motoneurons using virally mediated gene knock-down (Montero *et al.* 2010). Our results demonstrate that nNOS over-expression in motoneurons is a sufficient signal to evoke many of the electrophysiological and anatomical changes associated with axonal damage.

These results point to nNOS as a pivotal target for the development of therapeutic tools for the treatment of peripheral neuropathies and neurodegenerative disorders characteristically accompanied by nNOS up-regulation. This finding also opens a line of research to establish a method for studying the role of dysregulated proteins in the neuronal impairment that occurs during the course of so many neuropathological conditions.

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Acknowledgements

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The 'cost' of breathing in heart failure

Heart failure patients develop a number of pulmonary abnormalities which increase the work and oxygen cost of breathing. Demands of the respiratory muscles are further accentuated during activities of daily living that require moderate exercise. In the face of limited cardiac reserve, heavily recruited respiratory muscles preferentially 'steal' blood flow from the working skeletal muscles. This phenomenon may be one mechanism that contributes to the enhanced perception of locomotor muscle fatigue commonly encountered in patients with heart failure.

The two most common symptoms heart failure (HF) patients suffer from include shortness of breath (dyspnoea) and fatigue. As such, exercise intolerance is a hallmark of symptomatic HF. Initially, investigators sought to determine which measure of cardiac function (left ventricular ejection fraction, cardiac output, filling volumes, etc.) best related to an individual's exercise tolerance. Unfortunately, these studies failed to find a clear link between resting measures of cardiac function and exercise tolerance in patients with HF.

While altered cardiac function clearly plays an initiating role, HF becomes a systemic illness which impacts on multiple organ systems. One system particularly influenced is the pulmonary system. This system is intimately linked with the cardiovascular system both anatomically and haemodynamically in that the lungs lie in series with the heart, share a common surface area within the thoracic cavity, and are exposed to similar intrathoracic pressure changes. Heart failure patients often develop mild pulmonary function impairments including restrictive and to a lesser extent obstructive changes, reduced lung diffusing capacity (surface area for gas exchange), and reduced respiratory muscle strength. They also require greater levels of ventilation for a given metabolic demand, due to a more rapid–shallow breathing pattern, altered matching of ventilation to perfusion, and chronic, mild hyperventilation. The end result of these pulmonary manifestations of HF is an elevated work and cost of breathing, particularly during activities of daily living that require moderate exercise intensities (Olson *et al.* 2006). This increase in the work and cost of breathing during activity

Thomas Olson (left) and Bruce Johnson

is particularly concerning when coupled with a severely blunted ability to increase cardiac output due to the disease pathology.

Exercise elicits an increased need for blood flow to both the respiratory and working skeletal muscles. Under normal circumstances, during exercise in healthy individuals, a combination of localized vasoconstriction (renal, splanchnic, etc.) and an increase in cardiac output combine to meet the demands of both the respiratory and locomotor muscles. This appears to be the case up to approximately 75–80% of maximal intensities. However, at higher intensities, the respiratory muscles begin to preferentially recruit blood flow at the expense of the working skeletal muscle. In fact, Harms and colleagues suggested, during maximal cycle exercise in healthy individuals, reducing respiratory muscle work by ~60-65% resulted in ~4–5% increase in blood flow to the legs whereas increasing respiratory muscle work by ~25–30% led to ~7% reduction of blood flow to the legs (Harms *et al.* 1997). From these findings, the authors concluded that the work of breathing exhibited during heavy exercise results in a reflex vasoconstriction of the locomotor muscle vascular beds contributing to reduced leg blood flow.

As noted previously, due to a number of pulmonary system manifestations from the failing heart, the work and oxygen cost of breathing for a given level of oxygen consumption will be accentuated in HF and requires greater relative blood flow than that seen in healthy individuals. This increase becomes particularly exacerbated during increasing intensities of exercise; however, it is possible that even low levels of physical activity in HF patients (e.g. representing typical activities of daily living) may result in a redistribution of blood flow away from the working skeletal muscles to the respiratory muscles in an effort to compensate for the respiratory muscle work in the setting of limited cardiac reserve. If true, a respiratory muscle blood flow 'steal' from the locomotor muscles may be one mechanism which contributes to heightened perceptions of fatigue that are prevalent in this population. Consistent with this premise, previous work in animal models of HF have suggested that the diaphragm (a primary muscle of respiration) will take blood flow from working skeletal muscles during physical activity. In addition, O'Donnell and colleagues have shown that providing human HF patients with inspiratory pressure support using a ventilator resulted in longer submaximal steady-state exercise duration and reduced leg discomfort (O'Donnell *et al.* 1999). Further, Borghi-Silva *et al.* (2008) have demonstrated that HF patients undergoing constant load exercise at 70–80% of peak work while using a proportional assist ventilator increased skeletal muscle oxygenation and an estimate of leg blood volume.

Accordingly, we sought to determine the relationship between respiratory muscle work and leg blood flow during moderate-intensity exercise in patients with HF (Olson *et al.*

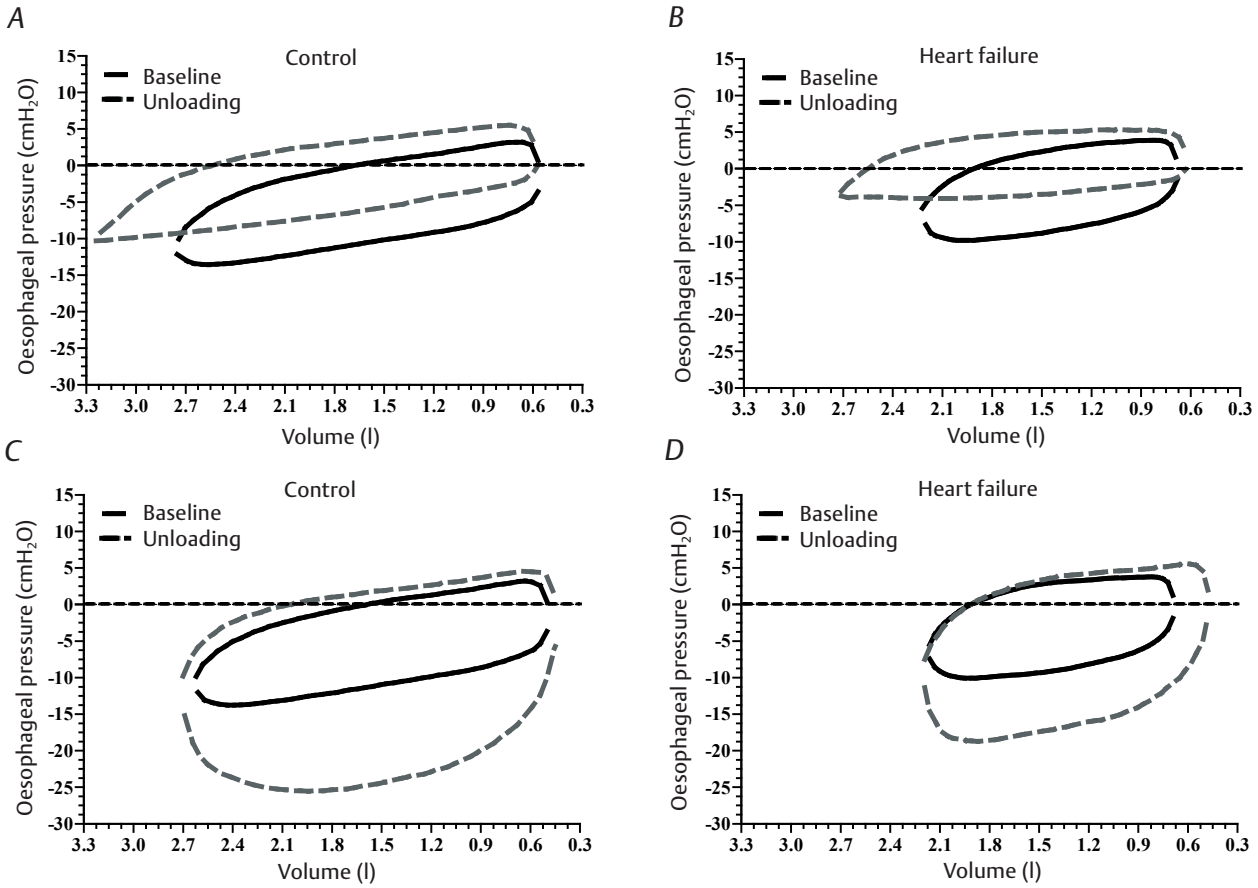

Figure 1. Influence of inspiratory pressure unloading and inspiratory pressure resistance on the pleural pressure–volume response during steady-state exercise. *A*, inspiratory unloading in healthy control participants. *B*, Inspiratory unloading in heart failure patients. *C*, inspiratory resistance in control participants. *D*, inspiratory resistance in heart failure patients.

2010). During this study, HF patients exercised at a constant submaximal rate of 60% peak work while breathing room air, with inspiratory muscle unloading via a ventilator, or with inspiratory muscle resistance (Fig. 1). During these exercise bouts we measured the work of breathing using an oesophageal balloon to quantify intrathoracic pressure (a measure of work) as well as leg blood flow by thermodilution using catheters in the femoral vein. We observed that reducing the normal inspiratory muscle work via inspiratory pressure support during exercise resulted in a significant increase in leg blood flow in HF patients, but not in healthy control participants (Fig. 2). In contrast, increasing the work of breathing by adding inspiratory resistance did not alter leg blood flow or cardiac output in HF patients, but increased cardiac output in the healthy controls. These results suggest that the HF patients were able to redistribute blood flow to the locomotor muscles when respiratory muscle work was reduced, but were unable to reduce locomotor blood flow beyond that observed during normal breathing when inspiratory muscle work was increased. This was most probably due to the lack of cardiac reserve and a maximal level of vasoconstriction in the active locomotor muscles.

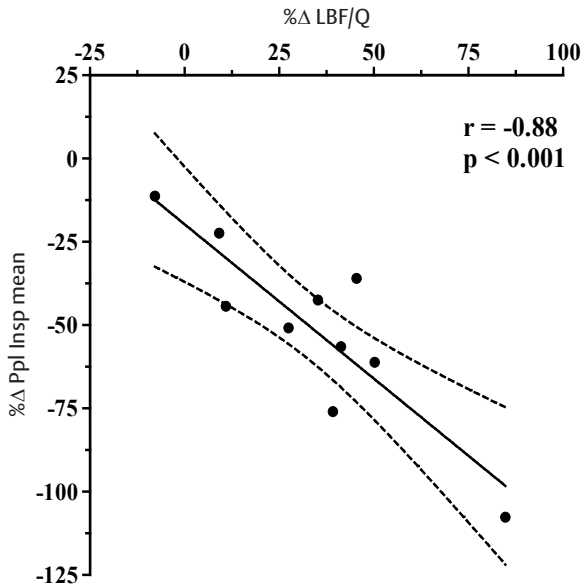

Figure 2. Relationship between the per cent change in mean inspiratory pleural pressure (%ΔPpl Insp mean) and the per cent change in the leg blood flow/cardiac output ratio (%ΔLBF/Q) during the transition from normal room air breathing during exercise to inspiratory assistance in heart failure patients. This relationship demonstrates the impact of a reduction in the work of breathing on leg blood flow during exercise in patients with heart failure.

In summary, our research in HF is consistent with that observed in healthy athletic adults and is an example of the intricate balance between the demands for blood flow relative to the available reserves. For the HF patient, very modest amounts of activity appear to challenge the ability to deliver blood flow to both the respiratory muscles and locomotor muscles, while in the athlete this only occurs near maximal levels of exercise. However, in both cases, the respiratory muscles appear to preferentially 'steal' blood flow at the expense of the locomotor muscles. This 'steal' phenomenon may result in a mismatch in oxygen supply and demand and be one mechanism which contributes to the enhanced perception of fatigue commonly encountered by patients living with HF.

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Western blotting. Part II

In the last issue, Patricia Leoni reviewed protein electrophoresis. In this article she examines the different methods for preparing protein extracts from cells, tissues or fluid media. Selecting the method that is suited to your sample is important as it will provide more efficient protein extraction and better results.

Sample preparation for protein electrophoresis

The majority of parameters regarding equipment, gel preparation and running conditions have been optimised by commercial companies so that there is no longer a need to investigate the right conditions in the laboratory. What is still left to the individual researcher is the preparation and loading of the sample which is a crucial step for a successful separation. The most important considerations are: complete solubilisation, accurate measurements and lack of modification of the proteins through proteolysis and de-phosphorylation during preparation. These are crucial to get reproducible results.

Preparation of protein extracts from cells in culture

The preparation of cell extracts depends on the relative amount of the protein of interest and location within the cell. For a protein that is relatively abundant, a whole-cell extract will be adequate. Cells must be rinsed quickly with cold phosphate-buffered saline (PBS) in the monolayer or after centrifugation if grown in suspension. It is important that excess PBS is removed completely using a suction pump. Cells should be lysed with a detergent-containing buffer which does not interfere with the protein's biological or immuno reactivity such as RIPA. A typical formulation for this buffer is: 150 mM NaCl, 1.0% NP-40, 0.5% sodium deoxycholate, 0.1% SDS and 50 mM Tris, pH 8.0. Other formulations exclude SDS. Protease inhibitors must be added to the lysis buffer. If the protein sample is to be analysed for phosphoproteins, the buffer should also contain phosphatase inhibitors. Inhibitors can be added individually but, as more than one type are necessary,

Patricia Leoni

cocktails of protease inhibitors containing different combinations and phosphatase inhibitors containing combinations that inhibit most proteases and phosphatases are commercially available. The most common protease inhibitors contain different combinations of antipain, bestatin, chymostatin leupeptin, phosphoramidon, EDTA, aprotinin, pepstatin, E-64 and 4-(2-aminoethyl) benzenesulfonil fluoride. Phosphatase inhibitor cocktails include sodium orthovanadate, sodium molybdate, sodium tartrate, sodium fluoride, imidazole, okadaic acid and calyculin A. Protein concentration can be measured at this point.

Proteins of low abundance need to be enriched; in order to achieve this, as many irrelevant proteins as possible need to be removed from the extract. Nuclear proteins or proteins that are embedded in the plasma membrane are relatively easy to isolate, eliminating the majority of soluble proteins and proteins contained in other sub-cellular structures. Nuclei can be isolated by centrifugation for 1 minute in a refrigerated bench top centrifuge after lysing the cells in a hypotonic buffer, and membranes can be isolated using linear sucrose gradients and centrifugation at high speed (26,000 revs min^{-1}, average of ~90,000 *g*) for 3 hours in a swing-out rotor. Sucrose gradients allow the recovery of cytosolic proteins and proteins from all subcellular structures.

There are also commercially available protein extraction kits that allow enrichment if not purification of the different compartments. For example, cytoplasmic/nuclear preparations, membrane and hydrophobic protein preparations and cytosolic/membrane/nuclear/cytoskeletal preparations can be achieved using products such as the ReadyPrep range made by Bio Rad and the ProteoExtract range from Calbiochem/Merck. These greatly reduce labour, although they are expensive.

Extraction of proteins from tissue

Fresh tissue can be dissociated using a buffer containing a collagenase preparation suitable for the tissue of interest. This will result in a suspension of all the cell types contained in the tissue. Alternatively tissue should be homogenised in a detergent-containing buffer like RIPA or T-Per tissue protein extraction reagent from PIERCE, in the presence of protease and phosphatase inhibitors.

Homogenisation can be done manually in a Dounce homogenizer, or automatically in a bead-mill homogeniser such as the Tissuelyser (Qiagen) or GentleMACS (Miltenyi biotec). Frozen tissue can be pulverised in a mortar and pestle, adding liquid nitrogen during the grinding. The resulting powder can be extracted with an appropriate buffer.

Proteins from plasma, serum or conditioned medium

Extraction of proteins from liquid sources presents two problems: the proteins of interest might be too dilute and need to be concentrated and the presence of other proteins that are present in large quantities (albumin, immunoglobulins). Such highly abundant proteins may need to be eliminated prior to the run, as they would otherwise constitute the greater part of the sample, thereby reducing the sensitivity for detecting proteins present in low quantities, as the total amount of protein that can be loaded onto a gel is limitied.

The classic method to eliminate albumin from serum is to use affinity binding to the dye cibachron blue, in the right conditions of ionic strength and pH. Resins bound to the dye are available from several companies (Pierce blue from Pierce, HiTrap blue from GE Healthcare). This dye also binds other proteins, so it can only be used if these proteins are not being investigated. Also, albumin-associated proteins will be removed from the extract. A more selective method to remove actin is immunoprecipitation using a monoclonal antibody. Immunoglobulins can be effectively removed using a mixture of protein A and protein G sepharose.

Concentration of diluted samples

If the sample is too dilute for direct analysis it will require concentration as the total volume of sample is limited by the capacity of the wells. There are several methods to concentrate protein extracts. If the volume of the sample is large, an easy and fast method is by filtration against dry or a concentrated solution of polyethylene glycol (PEG; Carbowax M-20). The sample is placed in a large-diameter dialysis tube and this is submerged in a concentrated solution of PEG or covered with dry PEG. The classic rotatory vacuum evaporator like Buchi, is a fast and gentle method to concentrate samples of any volume. Ultrafiltration membranes are disposable, centrifugal devices with membranes that filter out water and ions (Amicon from Millipore, Pierce protein concentrators from Pierce). They are very effective and fast, but there is some protein loss during the process.

Precipitation

Most protein will aggregate in the presence of high salt concentation. Because of its high solubility $(NH_4)_2SO_4$ is commonly used to increase ionic strength. Different proteins precipitate at different ammonium sulfate concentrations. This could be used to selectively precipitate the proteins of interest. The proteins are recovered in a pellet after centrifugation at 35,000 *g* for 30 minutes, but they need to be dialysed in order to eliminate excess ammonium sulfate before electrophoresis. Protein pellets can be dissolved in 30 mM Tris-HCl pH 7.4–8.0. Any insoluble residue should be removed by centrifugation. Protein concentration should be measured at this stage.

Trichloroacetic acid

The negatively charged trichloroacetate (TCA) triggers protein unfolding by disrupting the electrostatic interactions that stabilise the native conformation of proteins. Partial unfolding of proteins results in coalescence of protein molecules leading to their precipitation. Proteins are precipitated in ice-cold 10% TCA solution for 2–3 hours. After centrifugation for 30 min at 15,000 *g* at 4°C, the pellet is washed two or three times with absolute ethanol to remove the remaining TCA, as remaining acidity would impair solubilisation. The pellet can be dissolved in 0.1–0.2 N NaOH, removing any insoluble residue by centrifugation. Protein concentration should be measured, prior to the addition of Laemmli buffer.

Ethanol and acetone precipitation

Upon dehydration by organic solvents, protein molecules will attract each other by van der Waal's forces strongly enough to become insoluble in the ethanol–water and acetone–water mixtures. Ethanol precipitation (66% final concentration), and acetone precipitation (85%) require several hours, typically overnight, at –20°C. Acetone precipitation is particularly useful when retrieving proteins from interfaces after nucleic acid extraction. After precipitation the proteins are recovered by centrifugation for 30 minutes at 15,000 *g* at 4°C. The pellet can be dissolved in a suitable buffer, like PBS. Any insoluble residue should be removed by centrifugation, prior to the measurement of protein concentration.

Sample loading

Full solubilization and accurate measurement of protein concentration are essential for high-quality informative electrophoretic runs. The amount of protein loaded on the gel varies according to the thickness of the gel, the volume of the well and the detection method. Though 50–200 mg is usually sufficient in the most common models, when stained with Coomassie blue, it is better to determine this empirically by running different quantities of extract and staining the bands. Prior to the electrophoretic run, sample buffer (2–8x) should be added according to the dilution of the sample. DNA in the sample distorts the run and can be eliminated either by incubating the sample with DNAse (5 units ml^{-1}) or by shearing it using a Hamilton syringe, taking care not to introduce air bubbles into the sample. If the buffer contains a reducing agent and/or SDS, the sample should be boiled for 5 minutes in order to achieve complete binding of SDS and disulfide bond disruption. The most commonly used sample buffer was described by Laemmli in 1970 (1); it contains 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, 0.002% bromo phenol blue and 0.065 M Tris–HCl buffer pH 6.9 in its final concentration. Glycerol can be replaced by solid sucrose in order to achieve up to 8x concentration; this is often useful to avoid diluting the protein extract too much. After heating, it is advisable to centrifuge the sample for 5 minutes to remove any insoluble proteins, as this would cause streaky bands. Other factors affecting band pattern and separation are protein overloading, uneven gel polymerization, temperature gradients within the gel, uneven well surface and the presence of small air bubbles in the gel.

Gel staining

The most common method to visualise proteins is staining. The soluble stain Coomassie brilliant blue binds directly to the protein in the gel and has a detection sensitivity of approximately 1 µg. With stains containing metal salts, usually silver chloride, the grains of metal precipitate on the protein. The detection sensitivity is in the nanogram order of magnitude. Staining with copper or zinc chloride is a fast and sensitive method to detect proteins; they show as clear bands in an opaque background. This method takes only a few minutes, does not affect the proteins and can be removed quickly by rinsing. After photographing the gel, the stain can be rinsed and the proteins can be electro-eluted or blotted. This is not possible with the other stains because the proteins are fixed on the gel during staining. Zinc or copper staining can detect 10–100 ng of protein in a band.

Fluorescent staining of proteins

Fluorescent dyes provide a fast and very sensitive method to stain proteins in acrylamide gels. These dyes fluoresce when bound to proteins and are compatible with UV base imagers. Some can be visualised with a 302 UV box and photographed with a Polaroid black and white film. The most commonly used are Sypro orange, Flamingo orange, Oriole and Sypro ruby from Bio Rad and FITC and fluorescein dyes from Pierce.

These dyes are relatively photo stable and can be photographed several times; they are also compatible with electro-eluting and enzyme digestion prior to mass spectrometry. This method can detect 1–10 ng of protein in a band.

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1. Laemmli UK (1970). Nature **227**, 680–685.

In the third and final article in this series of Techniques articles, the process of transferring proteins to a membrane and the use of antibodies to detect specific proteins will be discussed.

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The Physiological Society Main Meeting
University of Oxford, UK, 11–14 July
Abstract submission 1–31 March, early registration now open, ends 1 June

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, Edinburgh, UK, 3–5 July

The Journal of Physiology Symposia 2011

Physiology, pharmacology, and pathology of tissue fluid exchange
Satellite Symposium at the 31st International Symposium on Intensive Care and Emergency Medicine
Brussels, Belgium, Tuesday 22 March

Molecular mechanisms underlying neurovascular protection in stroke
Experimental Biology 2011,
Washington, DC, USA, Sunday 10 April

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

Non-Society meetings

YLS 2011, Autonomic control in health and disease
University of Birmingham, 26 May 2011

Travel Grants

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

How I learned to love my aura (not really – it cost me £60)

I realised that I might be suffering from a personality disorder or at the very least be missing a chunk of my frontal cortex when I became erotically obsessed with Sarah Palin. During daylight hours I couldn't stop thinking about her and every night she would stalk my sub-conscious mind. I don't really know what it is about that woman, but I have the same vivid dream every night. I see her slowly running across the Alaskan wilderness wearing a white negligee accompanied by stirring Wagnerian music and carrying one of those really neat military assault rifles. It's the way she caresses the rifle that makes the dream seem so vivid and when she finally points the rifle at a critically endangered but unbelievably cute looking piece of Alaskan wildlife and shoots, I wake up feeling exhausted and drenched in sweat.

When I casually mentioned this to a psychologist, he suggested I seek professional help. He said I might be suffering from a form of obsessive compulsive disorder with the strangely alluring Ms Palin as its disturbed focus. It was at this point I decided to act like a responsible adult and so I looked up treatment options for obsessive disorders on the internet. There weren't many, mainly cognitive behavioural therapy or drugs that would interfere with my serotonin system.

So I obsessed for several days and then decided to take a course of action that would change my life. I decided to have my aura read! Although I have a PhD in neuroscience, I have always been interested in the quirkier side of physiology, neuroscience and the mind: spontaneous human combustion (I tried it once on a neighbour – the fire went out and they just smouldered a bit – I think it was the rain), the use of colonic irrigation to influence an animal's physiological state (I tried it on a fruit fly, it died) and above all the mysteries of the aura.

How does the unique physiology of an individual organism interact with the universe to transmit an energy signature that can be received by the 'sensitive'? What are the hidden physiological processes, the biomedical benefits and the possible applications in famine-stricken countries that exploring the mysteries of the aura might uncover?

Actually, its bullshit! I am being narcissistic, the real reason I am interested in auras is not to provide me with any unique insights into physiology, but because a close relative claims he can see them. He is an emotionally balanced man – he thought for 20 years or so that L Ron Hubbard was the Messiah and that a recurrent shoulder injury was caused by the trauma of being struck by an axe during one of his previous lives. So when he claims that auras can be used to divine a wide range of physiological dysfunctions and diseases I believe him. Another sensitive relative (who unfortunately is a physiotherapist and so allowed near patients in a hospital) then paid £50 to have her aura read. Dr Aura told her she was a caring person and a healer. Being an insensitive misanthrope I do remember saying 'You have just given him £50 for 15 minutes work – he's not going to call you a bastard is he?'. (N.B. this particular paragraph is entirely accurate).

So I made an appointment with an aura consultant (best not say who, or where in case he decides to sue, we have really strange libel laws in England). He was a friendly man and when I told him I was a social worker (as I thought it might be the type of profession that was sensitive to electromagnetic fields), he conducted a detailed clinical analysis: 'What path are you choosing in life?' he asked, followed by 'what would you describe as your basic health problem?'. This might possibly have activated my mischievous streak so I explained 'I have a range of problems. I can't sleep or concentrate, I am drinking too much and I think that I might be suffering from a range of

inappropriate obsessions. I don't have a biomedical background, so you will have to explain this simply. How are auras generated by living things?'

What I discovered was fascinating. 'Auras are the electromagnetic fields that surround living things, in your case the field will vibrate at different frequencies depending on your state of mind'. 'Scientists (you evil scum) are unable to measure such subtle physiological effects'. 'Most of this knowledge is suppressed as it is cheap and it would affect drug company profits' and this little gem 'the technology behind this stretches all the way back to Atlantis'.

'What is the primary focus of your obsessions?' he then asked almost as an afterthought. 'Mainly an erotic interest in Sarah Palin' I replied. He didn't react much, but put my hand on a plate and produced a picture of my hand surrounded by an energy field. It looked quite pretty and he suggested that a number of things were wrong, that my life lacked balance and that I might be suffering from stress. I asked him about my obsessions but he was a bit evasive but did suggest I was a caring person and a potential healer. Eventually after an hour, I paid him his £60 fee and left.

After paying £60 and having my energy fields probed by a technology developed in Atlantis, am I any the wiser? Not really! But I have discovered I am a caring person and a healer (it's news to me) and I have developed an extreme sensitivity to electromagnetic fields. Now when I dream about Sarah Palin walking across the Alaskan wilderness she is surrounded by an aura.

Dr Keith Cormorant

PS Some parts of this article are true.

You are what you hear – How music and territory make us who we are

By Harry Witchell

Algora Publishing, 2010,

£14.30, 232 pages, paperback

ISBN-10: 0875868045

ISBN-13: 978-0875868042

Music is organised sound, created to elicit responses in the listeners. So why is music so different from noise or even talking? Harry Witchell's thesis is that humans have music to establish and reinforce social territory.

By the author's own admission, there are not as much controlled experimental data to support the hypothesis as one would normally like to see, as an academic. However, Harry Witchel has read widely. He provides examples ranging from the behaviour of songbirds and the Third Reich to modern day shopkeepers, to illustrate how music is used to manipulate the behaviour of populations.

The 11 chapters each pose a question related to a different aspect of music. Why does music make sex better? Can music cure? Does violent music lead to violent behaviour? Although packed full of interesting facts and anecdotes related to music, the thesis of music and territory does seem to get overlooked. In Chapter 9, for example, on whether music makes the brain grow larger, there is an intriguing story about the fate of Haydn's brain, which relates how, after being removed from his coffin by bodysnatching phrenologists, his skull passed through the circles of Viennese society for the next 145 years before finally being reunited with the rest of his body. There's another fascinating story about the fate of Einstein's brain. The mathematical genius was also an accomplished violinist.

The book aims to be accessible to a wide audience. It is an easy read and source material, together with explanations of technical procedures, like fMRI, are referenced at the bottom of each page. I quite like this format because it is interesting to know where all the diverse and obscure facts were gleaned from, without having to keep turning to a bibliography at the end.

I read this book by dipping into it a chapter at a time, sometimes accompanied by music but often in silence in my 'safe' home territory. I also read it on trains and buses to the accompaniment of noise. Did the music make any difference? I tried Chapter 1 to the Eagles and then a Malian band called Tamikrest. I tried some later chapters with Vivaldi or a bit of Mozart. I don't think any of them made a difference. Did I feel safer or more territorial with the music on? Not really. But I did quite enjoy reading the book.

Thelma Lovick

The root of thought

By Andrew Koob

Financial Times/ Prentice Hall, 2009

£17.99, 192 pages, hardback

ISBN-10: 0137151713

ISBN-13: 978-0137151714

In his first book 'The root of thought', author Andrew Koob aims to enlighten the reader about the under-appreciated glial cells that comprise 90% of the cells in our brains, cells that were previously thought to be simply the glue that holds neurones together.

Koob opens with a brief overview of exactly why we should pay attention to glia, including such compelling facts as 'the ratio of glia to neurons increases with our definition of intelligence', and that glia can communicate through networks via increases in intracellular calcium levels. The reader is then taken on a journey though the history of neuroscience and neurotransmission before being introduced to glial cells, and learning how they communicate, about their potential roles in development and memory, and how Einstein's brain possessed many more glial cells compared to 'normal' people in the left parietal cortex, a region believed to be involved in language, mathematics and spatial learning. In the last few chapters of the book, Koob postulates the various roles astrocytes could play in dreaming, affective disorders, drug abuse as well as degenerative disorders, trauma and cancer.

The main thesis, repeated numerous times throughout the text, is that glia play vital roles in various neurophysiological functions; in particular, they serve a role in thought processing, suggesting that glia rather than neurons are the substrate of the root of thought. At points Koob even goes so far as to argue that 'Neurons have no reason to exist except to support astrocytes', acting simply as the 'roads' to the 'glial cities' but offers insufficient evidence to support this, or many of his other statements throughout the text. However, whilst much of the content is merely speculative, the evidence detailed is very interesting and does illustrate the many complex functions of glial cells. Although it is tempting to believe that astrocytes could be involved in intelligence, the reader is advised to take the information in this book with a pinch of salt.

A further criticism of the book is that the level that the book has been pitched at seems to be oversimplified for the neuroscience aficionado yet too complex to be appreciated by a general readership.

Despite the fact that this book consists largely of metaphors, intertwined with biased personal opinion and numerous references to popular culture, it does present an interesting hypothesis as well as highlighting the error of considering glial cells as uninteresting, but which are in fact complex, multifunctional cells, in a similar way that nucleic acids were initially overlooked as the material of heredity due to their simple structure.

Angus Brown and Lucy Brooks

The physiological action of β-iminazolylethylamine

H.H. Dale and P.P. Laidlaw (1910)

J Physiol (December 31) **41**, 318–344

There is a natural tendency to picture eminent scientists as their 'mature' selves. This is obvious in the choice of photographs to accompany valedictory or memorial articles, images which in turn tend to become the 'received' version of the person. Thus it is with Sir Henry Hallett Dale OBE (1875–1968), Nobel Laureate, President of the Royal Society, Chairman of the Wellcome Trust, scientific adviser to Churchill's War Cabinet, Member of the Order of Merit, and first director of the MRC's National Institute of Medical Research (NIMR). If any image of Dale is familiar to readers, it is almost certainly that of the eminent elder that accompanied his obituaries.

One has to stop a moment to recall that many of the physiological greats of Dale's generation did their pioneering experimental work comparatively young, at the stages we now call PhD student, postdoc/research fellow, and junior lecturer. It is for this reason that it seems appropriate to include the other picture of a much younger Henry Dale, taken in 1904 when he was around 30. This was the time when Dale took up his first substantive post at the Wellcome Physiological Research Laboratories (WPRL), then situated in a large house (since disappeared) in South East London's Brockwell Park.

The paper is from this period of Dale's career, part of a series in 1910–11 in which Dale and his co-workers George Barger and Patrick Laidlaw describe the identification in ergot fungus, and the biological actions of β-iminazolylethylamine – or histamine, as it shortly afterwards came to be known.

Barger & Dale (1910) had reported some months earlier that histamine was a component of ergot fungus. Dale and Laidlaw describe the actions of histamine injections in intact animals and in a range of perfused and/or isolated organs. *Inter alia*, they show intravenous histamine causes a profound fall in blood pressure. This effect is independent of actions on the heart, and reflects dilatation in the vascular beds of many organs.

Perhaps most striking, they note that *in vivo* histamine injection causes "acute obstruction of air-entry ... this must be attributed primarily to bronchial spasm."

They continue:

"It may be noted ... that the symptoms and post-mortem condition in the

Wellcome Images

Sir Henry Dale

guinea-pig correspond in a suggestive manner with those described ... as the effects of poisoning in that animal by ... serum or other protein in the sensitised guinea-pig ("anaphylactic shock")."

Several years later, a chance observation relating to animal sensitization to serum (which was produced by the Wellcome Laboratories and tested at the WPRL) was to lead Dale to return to sensitized smooth muscle (especially uterine muscle) and the spasm produced by antigen re-challenge. By simple and elegant experiments Dale showed that the antigen must be localized in the cells and tissues, rather than circulating in the blood (Dale, 1913).

Together, this body of work amounted to a key pioneering contribution to understanding anaphylaxis. It is described in depth in a fascinating article by science historian and Physiological Society archivist Tilli Tansey (Tansey, 2003). Tansey, who has written extensively about Dale, his work and his milieu, relates that Dale was always keen to credit both serendipity, and the work and insights of colleagues, as key to his own discoveries. Of Dale's arrival at the WPRL she tells us:

"[Dale] had ... no immediate research project of his own in mind, and very readily followed Henry Wellcome's personal suggestion that he investigate the pharmacology of ergot, a fungus known to contaminate cereal crops such as rye ... [and] often used as an abortificant ... Ergot has been described as 'a treasure house for drugs', and many of those treasures were discovered by Dale and his colleagues at the WPRL. Indeed, much of [Dale's] research career stems directly from his investigations of ergot and its constituents."

Dale always said his major motivation in taking the Wellcome post was the desire to do research unencumbered by other commitments. At the time, though, the move was unprecedented. Dale's later colleague and obituarist Wilhelm Feldberg noted: "[Dale took the job] against the advice of nearly all his scientific advisers, who told him that he would be selling his medical and scientific birthright for a mess of pottage".

Utimately, the presence at Wellcome of organic chemists, and commercial production facilities, played a key role in the WPRL's success. Indeed, the WPRL offers an early exemplar of many of the things that would characterise later successful pharmaceutical industry drug discovery programmes. Thus, Dale's operation was well-resourced, with trained scientific personnel already within the company, and easy access to analytical and synthetic chemists and to the company's chemical and biological materials. Apart from drawing on the expertise of his Wellcome colleagues, Dale could and did remain in close contact with the academic labs elsewhere in London and in Cambridge, as well as further afield.

George Barger (1878–1939) and Patrick Laidlaw (1881–1940), Dale's co-workers, both became eminent FRSes. The Anglo-Dutch Barger was a chemist, a Cambridge acquaintance of Dale's who was already at Wellcome when Dale arrived. He went on to be Professor of Medical Chemistry in Edinburgh. Patrick Laidlaw was some years younger than Dale, and had been taught by Dale when a 16-year-old pupil at Dale's old school in Cambridge – Dale, like many an impoverished student, was tutoring to supplement his allowance. Laidlaw became a distinguished pathologist and virologist, later re-joining Dale at the MRC and becoming Deputy NIMR Director to Dale's Director. He is remembered particularly for his work on isolating the influenza virus, and on developing a canine distemper vaccine. Though Laidlaw's role in the histamine story is less well-known, Dale saw it as critical, writing in Laidlaw's *British Medical Journal* obituary that "The first detailed studies on histamine were his work as much as mine."

Though Dale remains best known for his Nobel-winning work on acetylcholine, several earlier nominations for the Prize specifically mention his histamine work. He retained a life-long interest in histamine and anaphylaxis, publishing and giving lectures on the topic, and even being elected President for Life of the Histamine Club formed in the late 1940s.

Austin Elliott

Barger G & Dale HH (1910). *Proc Physiol Soc* **40** (Suppl), xxxviii–xl (abstract).

Dale HH (1913). *J Pharmacol Exp Ther* **4**, 167–223.

Tansey EM (2003). *Stud Hist Phil Biol & Biomed Sci* **34**, 455–472.

Ask a physiologist!

I know that our brain tells our body what to do, but how does the brain know? (George, age 11)

Austin Elliott, University of Manchester, replies:

There are lots of answers to this question!

First of all, many of the nerves in your body outside your brain carry information about what is going on *back* to the brain. There are your 'special senses' (vision, hearing etc.); there is sensing of things like touch, pressure, temperature and balance; and in a typical nerve in the body that runs to one of your muscles, as many as 80% of the nerve fibres (axons) are bringing information *back* to the brain about how hard the muscle is contracting, the length of the muscle, its position, the position of the limb, and so on. Finally, the body has many, many other 'internal sensors' to tell it about what is going on inside it – like how much oxygen is in your blood, for instance.

The next answer is that all this information is 'received' by centres in the brain (mostly) and decisions then made – sometimes by special cells, sometimes by 'networks' of many cells working together – about what signals to send out to control the body. A lot of these are automatic, like controlling your heartbeat. Some things you do are voluntary – like standing up, if you decide to! – but need the brain to 'automatically' make a lot of different things happen. You choose to stand up, but the brain's processing then tells all the various muscles that help you stand up to coordinate their actions to make it happen.

Some things require thinking at first, but as you do them over and over again get progressively more automatic – like learning to stand and walk as a baby, for instance, or learning to catch a ball. Catching a ball involves your eyes tracking the ball, your brain predicting its flight, and your body, arm and hand moving to catch it, but this may all happen so fast – think a catch in the slips in cricket – that much of it is semi-automatic without you having to consciously think 'Must catch ball – thinks – must move arm.' This is because all the practise has 're-wired' your brain's circuitry to allow the relevant bits – eyes, tracking, prediction, move to catch ball – to coordinate automatically to make the action happen faster.

Finally, perhaps you were actually asking how our brain 'makes' our consciousness. That is an even bigger question, and one that lots of scientists would like to solve!

How come our brains are so powerful? (Anna and Connor, age 11)

Dr Stuart Allan, University of Manchester, replies:

The power of the human brain stems from its incredible complexity. Indeed, the human brain is probably the most complex thing on planet Earth. Within the 1.4 kg adult human brain there are 100 billion neurones. These neurones interconnect with their neighbouring cells to form local networks, and these networks can in turn send messages to other networks of neurones in a different brain region, and so on, until whole systems of communication within the brain are established. These complicated interconnecting networks of neurones are what allow the brain to perform diverse functions, such as the control of breathing, recognition of faces, sleep regulation and the formation of new memories, to name but a few.

Each specialised function occurs in specific regions of the brain, and many of these functional areas are shared between humans and other species. However, it is the outermost layer of the human brain, the cerebral cortex, that is most advanced in humans, standing us apart from other animals and ultimately making our brains so powerful.

Neurones pass messages to each other using electrical and chemical signals in a complex process known as synaptic transmission. At any one time millions of neurones in your brain will be firing off messages to each other to allow you to function in everday life. Amazingly, the billions of connections that exist in the human brain are not fixed and neurones have the ability to change how they respond over time. This 'plasticity' of the brain is a key contributor to its amazing power.

However, neurones are not alone in making the brain function. They also rely on glial cells such as astrocytes, microglia and oligodendrocytes to allow them to work properly, and we still have much to learn about how these glial cells contribute to brain function.

Finally, no cell in the brain would be able to work at all without a highly efficient fuel supply, provided by the oxygen and glucose delivered in the blood by an intricate network of blood vessels. Ultimately it is this blood which 'powers' the brain to function in such an awesome fashion.

Why do we have bogeys? (Philip, age 11)

Sam Wilson, Shoreham Academy, replies:

In the air that we breathe there are many small dust particles and millions of microbes, which are not visible to us; however, they could cause us problems if we just inhaled them deep into our lungs. So our nostrils are designed to filter out all of those small particles and microbes that are present in the air. Lining our nostrils are small bones which are crinkled and uneven, a bit like the inside walls of a cave; they are covered in a slimy liquid called 'mucus', otherwise known as 'snot'. So imagine a cave covered in slime, with jagged and uneven walls which are so close together that any air being sucked into the cave crashes into the walls, and any small particles in the air get stuck to the slime.

Now small hairs called 'cilia' brush that slime carrying the dust and pathogens (microbes) down towards the pharynx (throat), where we swallow it; fortunately we are not very aware that we are doing this! The mucus carrying the dust and pathogens is then destroyed by our very strong stomach acid. However, the mucus near to the entrance to the nose dries out quite quickly and can't be moved by the cilia; it is this dried mucus carrying the dust and microbes which forms a bogie.

Now more about the slime. The mucus in the nostrils can be different to the mucus in the trachea (windpipe) and different again to the mucus lower down in the 'respiratory' tract. If you have an infection, such as a cold your respiratory tract produces more mucus, in an attempt to trap more microbes and particles; the mucus often becomes thicker and more sticky

A note of caution:

While we are continually subconsciously swallowing our snot, scientists are not convinced that eating bogeys is a good thing!

The Journal of Physiology

David Paterson, the new Editor-in-Chief for *The Journal*

The Physiological Society is delighted to announce that David Paterson has been appointed the new Editor-in-Chief of *The Journal of Physiology*. David, who is the current Editor-in-Chief of *Experimental Physiology*, will take up his new appointment in April. David will take over the reins from William Large, who has served as Editor-in-Chief for five years.

Mike Spyer, President of The Society, commented on the appointment: 'Personally I am delighted that David has been appointed as the Editor-in-Chief of *The Journal of Physiology*. He will bring energy, commitment and innovation to the role. The Society views *The Journal* as a beacon for physiology and is committed to its development to maintain its international reputation for excellence.'

David is a Professor of Physiology at Oxford University and a Fellow of Merton College, positions he has held since 2002 and 1994, respectively. He completed his doctoral studies in physiological sciences at Oxford as a scholarship winner from the University of Western Australia. He was made a Fellow of the Institute of Biology in 2003 (now the Society of Biology) and the Royal Society of Medicine in 2005.

David's research is in the area of cardiac neurobiology. His team's work looking into the neural causes of cardiac arrhythmia is recognized internationally and is funded by the British Heart Foundation, Wellcome Trust and BBSRC. David has a long association with The Physiological Society. In addition to his five years as Editor-in-Chief with *Experimental Physiology*, he delivered The Society's GL Brown Prize Lecture in 2000.

David says: 'It is a great honour to be given the opportunity by The Physiological Society to lead *The Journal of Physiology* into its next phase. I am of the opinion it is our job as editors to publish the best science presented to us so we can maintain and grow the international standing of *The Journal*, and the discipline of physiology.'

He continued, 'This is a very exciting time to be in physiology. *The Journal* is well positioned to become the opinion leader in the physiological sciences that bridge many of the exciting discoveries made in the post-genomic area. There will be challenges, but also great opportunity.'

An interview with William Large, the outgoing Editor-in-Chief

William Large has been the Editor-in-Chief of *The Journal of Physiology* since 2005, during a time of uncertainty and change in the world of scientific publishing. To mark his retirement in April we asked William about the challenges he has faced over the past 5 years.

1. What were the 'big issues' for *The Journal of Physiology* when you took over as Editor-in-Chief?

When I took up the role of Chair of the Editorial Board in 2005 I had been a member of the Board for 6 years and my major concern was that whereas we published some very strong papers, we were also publishing many papers that were

of limited influence. We embarked on citation analysis and found, for example, that in the 2 years contributing to the impact factor we were publishing about 100 papers (about 10%) that had not been cited. I felt that the overall quality of *The Journal* would be increased by raising the bar for acceptance to eliminate the least influential papers. In addition, I wished to increase the variety of articles to broaden the interest of *The Journal* and to encourage discussion of published articles by more junior members of the research community (leading to the introduction of Journal Club articles). Also, I wished to take this theme further to make *The Journal* (and physiology) of relevance outside our core readership of research physiologists. A first

William, with his wife Kathy, before they set off for California.

step was to introduce Clinical Perspectives to attract clinicians who might not otherwise read *The Journal* regularly. More recently we have started publishing non-technical summaries, which we hope will be of interest not only to high school students and undergraduates but also to members of the general public who are interested in science and health issues.

2. How did you tackle these issues and what problems did you encounter?

These were major challenges and I thought that there needed to be fundamental changes in the organisational structure of the Board to allow editorial policies to be formulated and then enacted. I felt that these policies should be developed by an Editor-in-Chief (not Chair of the Board) together with an Executive Committee consisting of senior members of the Board plus representatives of our publishers and The Society. Moreover, we appointed a team of Senior Editors, experts in their respective fields and who were loosely connected to the Table of Contents subdivisions. The Senior Editors have the final say on acceptance and therefore control the quality of published papers. All of these changes required substantial changes in the governance of *The Journal* which coincided with the establishment of the Publications Committee by The Physiological Society who decided to appoint the Editor-in-Chief instead of an election by the Board.

Inevitably these changes were resisted by some members of the Board because some power was removed from the Board and also scientists are most conservative and often do not like change. A major difficulty was persuading reviewing editors to raise the bar for acceptance and reject more papers. It seemed that some editors have a fixed view of the type of paper that is published in *The Journal* and were reluctant to alter that standard and become much stricter. I believe the appointment of Senior Editors helped to overcome that difficulty.

3. What have you given to *The Journal* during your time/what impact have you had on *The Journal*?

I am not sure that I am the right person to answer this question. I think we made many changes from being much stricter on acceptance, introducing new types of articles – especially those that reach out to individuals that would have not read *The Journal* in the past – to more pragmatic issues such as altering the governance, hence allowing enactment of policies that was very difficult in the past.

4. How have the issues changed during your time as EiC and how has this affected *The Journal*?

Many of the issues are the same, i.e. trying to improve the overall quality of *The Journal* and make it more accessible to a wider readership. When I started in 2005 the issue of 'open access' was a major concern but this seems to have faded over the last few years. I think areas of increasing importance concern the different types of access to *The Journal* via the web, mobile phones, Facebook, Twitter etc.

5. What have been the highlights and low points of your time as EiC?

I gained most satisfaction from working with members of the Board and the staff in the Publications Office to bring about so many changes, which I believe will have a lasting positive effect on *The Journal*. It was a great pleasure to work with so many wise and conscientious individuals who were committed to improving *The Journal*. The greatest headaches were caused by ethical problems and I was amazed that so many scientists seem to be unaware of legal aspects of publication ethics. During my time a much closer relationship with The Society developed which brought some obvious benefits, but I also think the erosion of the independence of the Board has had some downsides that were detrimental to *The Journal*.

6. What makes *The Journal of Physiology* unique from other similar journals?

The Journal has a remarkable history in publishing many of the most influential papers in physiology since 1878 – I think it is fair to say that this is unequalled in the field of physiology. Although it has suffered from the rise of specialist journals (e.g. in neuroscience), which have attracted many authors, *The Journal* still publishes outstanding papers across the full spectrum of physiology.

7. What trends in the physiological community are you most excited about? Any predictions?

I am not sure how wise a comment I can make or whether I can say anything useful but a simple observation is that several years ago the rapid advances in molecular techniques and knowledge pulled many physiologists into these areas and physiology seemed old-fashioned to many scientists. However, it is now evident we still know very little about the function of many gene products and how they interact in terms of integrative physiology (is this what some people call systems biology?) and disease. This is obviously where physiologists will play an enormous role in answering these fundamental problems – in the end it will come to the whole body. Associated with these aims, I believe some physiologists are becoming overly ambitious in their conclusions in papers, often published in high-impact journals. Often data obtained from series of experiments using several diverse techniques may be consistent with a physiological or pathophysiological function. However, in many instances, at least in my field of ion channels in smooth muscle, several of these conclusions have been shown to be at least so incomplete that they are of little value and quite often to be actually incorrect. My guess is this happens more now and in higher impact journals than in the past.

8. What advice would you give David Paterson as he takes over?

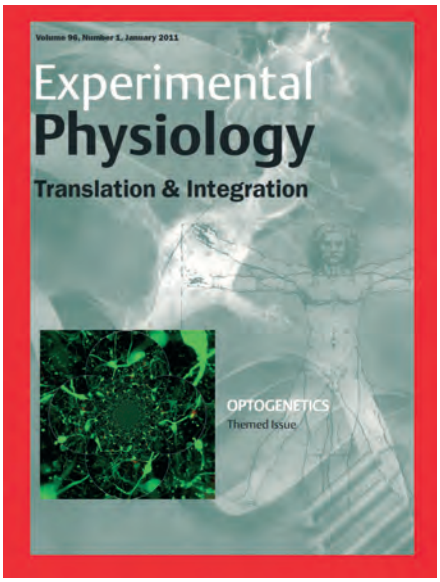
It would be presumptuous of me to advise David who has acted in this role for *Experimental Physiology* but I can make a few suggestions that I found to be valuable. Firstly, use the great wisdom and experience of all members of the Editorial Board and the Publications Office when developing journal policies. Secondly, listen carefully to those arguments that go counter to ones' own idea (not something that happens currently in British universities!) – none of us is always correct. Thirdly, develop achievable aims that can, preferably, be quantified. Finally, enjoy the role – it is a great honour to act as Editor-in-Chief of *The Journal of Physiology*.

9. What will you be doing in retirement?

At present I am adjusting to life in the US after relocationg with my American wife, Kathy, to southern California. In the next few months I will be kept busy renovating a condominium which we have bought in West Hollywood, LA. After that I will have to find something to keep me out of trouble since drinking margaritas in the sunshine is far too attractive. However, I have no firm ideas and I am happy to see what develops.

Experimental **Physiology**
Translation and Integration

Optogenetics Themed Issue



http://ep.physoc.org/content/96/1.toc

The January issue of *Experimental Physiology* contains a set of papers that summarize experiences of a number of internationally recognized laboratories working with the technology currently known as 'optogenetics'.

Contrary to popular belief, optogenetics is not all about using light-sensitive ion channels, such as channelrhodopsin, although this particular method has led to a flood of high-profile publications. Strictly speaking, optogenetics means experimentation which involves a combination of genetic manipulation and optics. Therefore, perhaps, its history should date back to the first days when scientists started experimenting with green fluorescent protein or even earlier. Genetic engineering enables targeted expression, in mammalian cells, of various constructs, which can be subdivided into reporters and unsurprisingly, this approach has been swiftly adopted particularly by neuroscientists. The brain represents a particularly difficult experimental target; there, the numerous cell types are intermingled and interconnected, they are hard to visualize and almost impossible to control selectively other than by using patch clamp on a cell-by-cell basis. Therefore, understanding the complex behaviours of neuronal networks is a formidable task. The recent appreciation of the active role of astroglia has added yet another dimension to it. At the same time, optogenetics deserves a wider application than only neuroscience, and we hope to excite our diverse readership with the possibilities it offers.

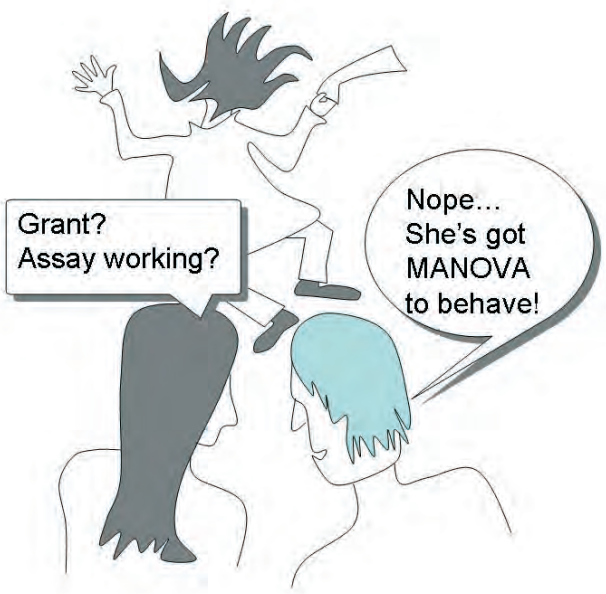
The issue was organised by Editor Sergey Kasparov (pictured above) at Bristol University.

Statistics: Little and often, softly softly, don't scare the horses, oaks and acorns

I came to The Physiological Society from a medical background and editorship in a clinical journal, and I had always been in awe of The Society's ways and its prestige. When I became an Editor, and later joined the board of *The Journal of Physiology*, I remained in awe of the standards and rigour of the science, but ... I had a nagging feeling that in clinical medical publications we had at least been doing one thing better. That was the statistics!

Essentially concurrent with my research career, the use of statistical analysis in medical research has improved greatly. As a statistical novice, I watched the effects of a concerted campaign by well-known statisticians such as Mosteller, Swinscow, Altman, Bland and others, with the help of prominent platforms provided by journals such as the *British Medical Journal* and the *New England Journal of Medicine*. I read their articles with the interest of a budding researcher and watched as there was a substantial improvement in the understanding and use of statistics in medical journals. Of course, these changes were also driven by a variety of other factors, not the least being the increased regulation of data collection in association with drug licensing. It was a long haul. Although we've come a long way, one can still occasionally find fault. However, most journals are now far more rigorous and exacting, giving comprehensive guides to contributors, and are never slow to pick up failings in papers submitted for consideration. Indeed, things may have swung too far: it's almost impossible to avoid the criticism of 'no hypothesis' if one sends in a paper that states 'we studied...' rather than overtly parroting 'we hypothesised that...'.

In physiology, it was soon clear to me that things were different. No-one recognised my quotation "Absence of evidence is not evidence of absence" which to me was the most striking *faux pas* in many papers. Everything was dynamite plunger plots, you could search high and low to find how many individuals were in a study, and as for suspecting that data could be skewed... I started to feel very uneasy. The old aphorism of the lamp post for a drunkard, more support than illumination, came to mind. Thus, when Peter Cahusac launched his broadside against the



t test and the null hypothesis, in *Physiology News* (Cahusac, 2009) it was like the little boy and the Emperor's new clothes! These views would have been considered mainstream and even quite gentle in my personal training arena, the Anaesthetic Research Society, which was at the time not for the faint-hearted presenter. In fact, the point had been made already (Watters & Goodman, 1999).

I was galvanised into action and submitted a paper to the Board of *The Journal*, outlining some suggestions: I collaborated with a statistician who is my co-author and we conducted our own, rather depressing, survey of recent articles, confirming yet again that things 'could do with improvement'. We suggested that we would write a series to see if we could stimulate change. It's been done before, of course: for example, the American Physiological Society did so in 2004 (Curran-Everett & Benos, 2004) and triggered some debate, but less change (Curran-Everett & Benos, 2007). Perhaps it's presumptuous of us to think we can do better, but the approach we have taken is more gentle: a light touch, little and often, nothing too threatening, just enough for a coffee break and no need for an icepack to get to the end. We shall try to avoid equations, and use diagrams to illustrate key messages. *The Journal* Board have been very supportive, as have many others in other journals we have written to, not the least Doug Everett who wrote the APS guidelines, and many others, too numerous to list. Please read the articles, and let me know what you think of them: feedback will be gratefully received and incorporated. I hope to encourage contributions in specialist areas, so volunteers will be valuable.

Gordon Drummond
University of Edinburgh, Edinburgh, UK

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Life as a Physiological Society Affiliate Representative

Well, two affiliate representatives to be more accurate!

Affiliate reps are the voice of the Affiliate Members on The Society Council and committees and we are your current representatives – Samantha Passey from the University of Bedfordshire, and Federico Formenti, currently at the University of Oxford but soon to be jetting off to sunny New Zealand to take up a position as Lecturer at the University of Auckland.

In July 2011 it will be time to vote in a new representative for the affiliate membership. As the existing reps we would like to highlight the activities that we have been involved in to encourage other Affiliate Members to step forward in the coming months to be voted in as a new rep.

I put myself forward for the role of Affiliate rep in Spring 2009 as I was keen to get involved in the activities of The Society, especially in their outreach programme, and also to write for *Physiology News*. I have thoroughly enjoyed myself, met some fantastic and inspirational people and have learned a great deal about the huge range of activities The Society is involved in.

As I understood it the major role of an Affiliate rep was to be the voice of the Affiliate membership at Society Council meetings. These typically begin with a dinner and social event the night before the meeting, which provides an excellent opportunity to meet and chat with the Council members and Society staff in an informal setting before the more official Council meeting the following day. As the Affiliate reps are not full Council members or Society Trustees we do not have voting rights at the meetings, but we can participate in discussions about the running of The Society and it provides an opportunity to raise Affiliate issues and the needs of the Affiliate membership.

Attending the Council meetings has offered a unique and fascinating viewpoint on the running of The Society but for me, sitting on the Education committee and Editorial Board of *Physiology News* has been much more involved. Time consuming in some instances, yes, but hugely rewarding, very enjoyable and totally worth it.

The Education committee meets about 3 times a year, and its remit is to support the learning and teaching of physiology both in schools and at university level. This includes supporting outreach events at schools, universities and public science festivals, running the vacation scholarship scheme for university students, contributing to physiology teaching curricula at universities, and also organising various teaching workshops for Society Members to offer instruction in specific techniques such as PCR, microscopy and *in vivo* techniques. The Education committee is hugely enthusiastic and I was excited to see the range of activities that The Society supports and encourages. It was good to have the opportunity to get involved in this exciting area of The Society's endeavours.

As a member of the Editorial Board for *Physiology News*, I contribute articles and try to encourage other Affiliate Members to contribute too! It is exciting to have the opportunity to play a role in the content and look of the magazine. Along these lines, if anyone has a burning desire to write an article, we are always interested in hearing from Affiliate Members.

So for me, being Affiliate rep has been a hugely rewarding experience and has really opened my eyes to how much The Society does and the different activities that it enthusiastically supports, and also how hard the committee members, Council trustees and Society staff work to ensure The Society runs well and provides useful benefits to its Members. I would whole-heartedly encourage anyone who is tempted to put themselves forward for nomination in the coming months as a new Affiliate rep. In addition to being enjoyable it is great for the CV.

Samantha Passey

University of Bedfordshire and Affiliate representative of The Physiological Society

Dearest Affiliate

I have been honoured to be your representative for The Physiological Society for a fantastic year and a half. It has given me the unique opportunity to see how our beloved Society is run from within, how much effort and care is put into keeping it lively and, we hope, helpful to its Members. Most activities of The Society require a great deal of long-term planning, critical debates and consultation with Trustees, and close constructive collaboration between the scientific experts across the UK and the sparkling Society staff. The Society as it appears to Members and to the public represents only a very small part of their work.

During my time as a rep, I sat on the Meetings committee, where the planning and organization of Society meetings takes place. In addition to participating in discussions on the format and locations of the meetings, I also had the opportunity to put forward ideas on new elements that could be included. Although I was the most junior member of Council and of the

Meetings committee, I was always given a chance to present my ideas, which would then be discussed with other Members. I still think that this is a privilege, as it would not necessarily happen in my country.

I was able to contribute to decisions regarding future activities of The Society. For example, I was involved in the reviewing process of applications for the Young Life Scientists Symposia, which will be run jointly with the Biochemical Society and the Pharmacological Society. They encourage young life scientists (students and postdocs) to organize and run a symposium on a topic within the life sciences. This gives them invaluable experience in what it takes to organize a scientific meeting.

Being a rep gave me the opportunity to meet many authors of papers I had enjoyed reading, to talk about our research, and have a chat about our lives. It was not only meetings and professional discussions. Trustees of The Society give a high priority to human aspects in our work, so there would always be a social component in the plan for the day. Overall, I found being your representative a very valuable experience, which will certainly help me in my future.

Life now asks me to move on to a new job, which starts soon in Auckland, New Zealand. I was told I have good networking skills, and clearly this time I have branched out greatly. Although I will be sad to leave, it opens up a very exciting opportunity for a new Affiliate Member representative, and he or she might be you. This is an experience that most of you would thoroughly enjoy and I most strongly recommend putting your name forward to work for The Society, as I am sure that you would find it a very rewarding and helpful experience.

Obviously I will keep on following the activities and developments of The Physiological Society, now from a new, different perspective (from down under). I enjoyed being in the 'Phys Soc' so much that I am now contemplating the idea of joining our Kiwi friends in the Physiological Society of New Zealand (yes, there is one indeed). I think they also have a dog as a symbol, but it looks white, almost tailless, very fluffy and it finds it very difficult to scratch its ears with its hind feet.

New Zealand has a reputation for being a fantastic country, inhabited by very lovely people. In the seven years I spent in England, my dad visited twice, driving his camper van through Europe, because of his aeroplane phobia. He has now left our hometown Parona di Valpolicella, aiming to be in Auckland for Easter (2012). I might feel the need of some European contact between now and then, so please get in touch if you plan to visit.

Thank you once again for giving me the opportunity to serve The Society, and I wish you all the very best for your future,

Federico Formenti

University of Oxford and Affiliate Representative for The Physiological Society

Understanding Life

A new version of *Understanding Life* is now available. *Understanding Life* is The Society's main educational publication. It provides an introduction to the subject of physiology – why we study it and how we can apply our knowledge. The booklet gives an insight into the central role of physiology in the progress and understanding of biological and clinical sciences, and explains how physiologists are involved in the detection and prevention of disease.

For hard copies, please email education@physoc.org

Alternatively, you can download a copy at: http://www.physoc.org/education

Physiology in the school curriculum

The Society believes it is essential that physiology is accurately represented and recognised as a central component of biology education at all stages. We believe that supporting physiology education, in schools in particular, will promote awareness of the discipline and encourage a high quality entry of school leavers into physiology-related careers.

In recent months, we have been working with awarding organisations, in collaboration with the Society of Biology and other member organisations, to develop their GCSE Science specifications (for first teaching in 2011). Our main focus was to make sure that all physiology content was accurate and to provide recommendations when appropriate.

As yet, none of the GCSE specifications has been accredited: Ofqual concluded they did not represent a clear and significant improvement over current specifications. Awarding organisations are now working closely with Ofqual to ensure that the new specifications are accredited and ready for first teaching in September 2011.

It is worth noting that the coalition government will soon launch a full review of the National Curriculum and The Society expects to make an active contribution to any changes that follow.

The Society has exciting plans to increase support for the teaching of physiology in schools; together with contributing to curriculum changes, we will also be developing resources (including a new website) to support physiology teaching.

If you would like to contribute to The Society's activities in these areas, please email education@physoc.org

IUPS 2013

21–26 July 2013 Birmingham, UK

In 2013, The International Convention Centre, Birmingham will host the largest physiology meeting in the world

Plenary & Keynote Lecture Nominations

You can now nominate yourself or another individual for one of the keynote lectures, the public lectures, or the following named lectures:

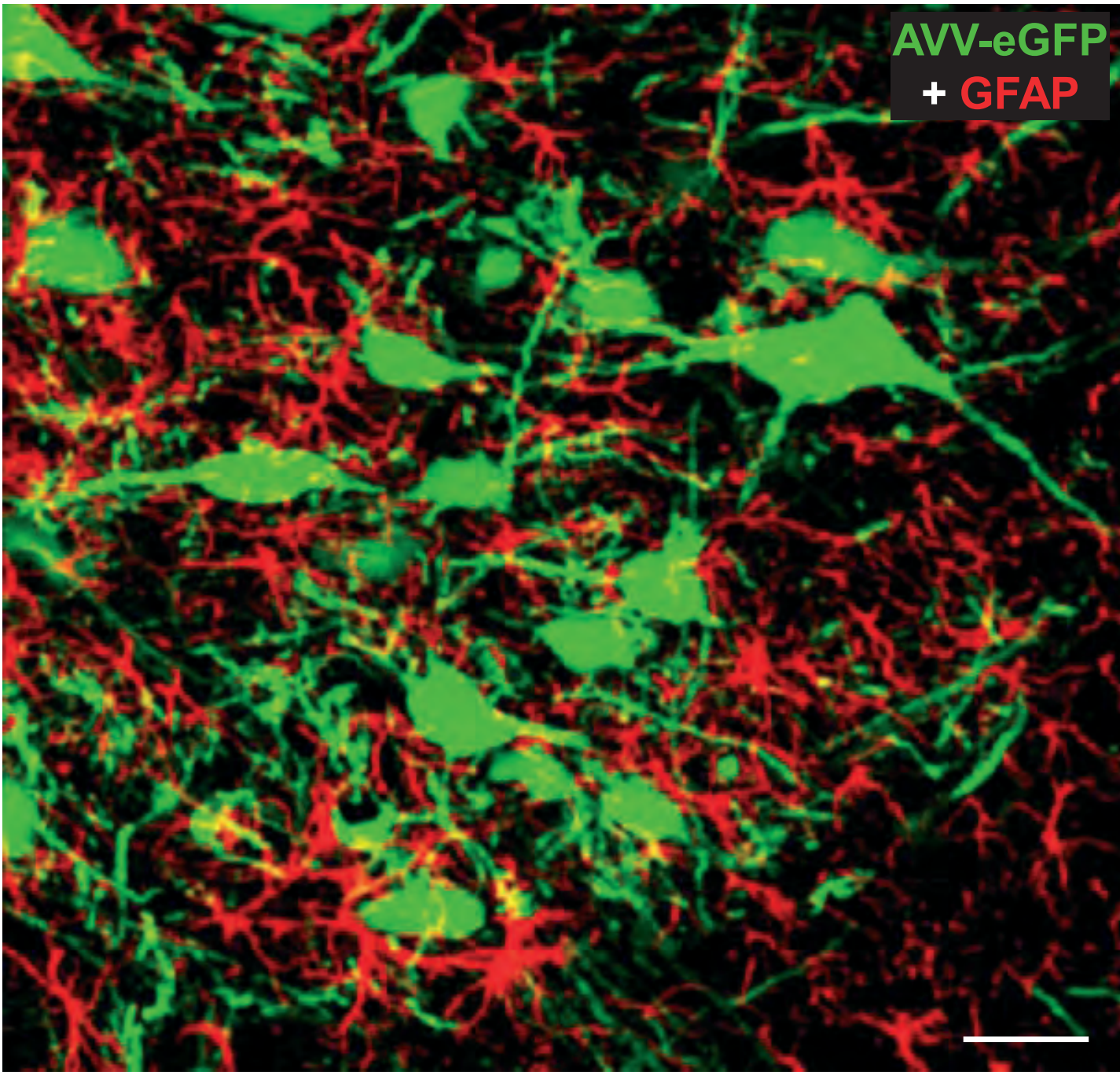
Wallace Fenn Lecture
Ernst Knobil Lecture
TP Feng Lecture
Schmidt-Neilsen Lecture
Robert Pitts Lecture
August Krogh Lecture
FEPS Lecture
Annual Review Prize Lecture

Bayliss-Starling Prize Lecture
GSK Prize Lecture
Hodgkin-Huxley-Katz Prize Lecture
Joan Mott Prize Lecture
Michael de Burgh Daly Prize Lecture
Paton Prize Lecture
Sharpey-Schafer Prize Lecture

Deadline 1 April 2011

For more information or to make a nomination, please visit:

www.iups2013.org/lectures



Confocal photomicrograph after adenoviral administration in the hypoglossal nucleus (p. 39).

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