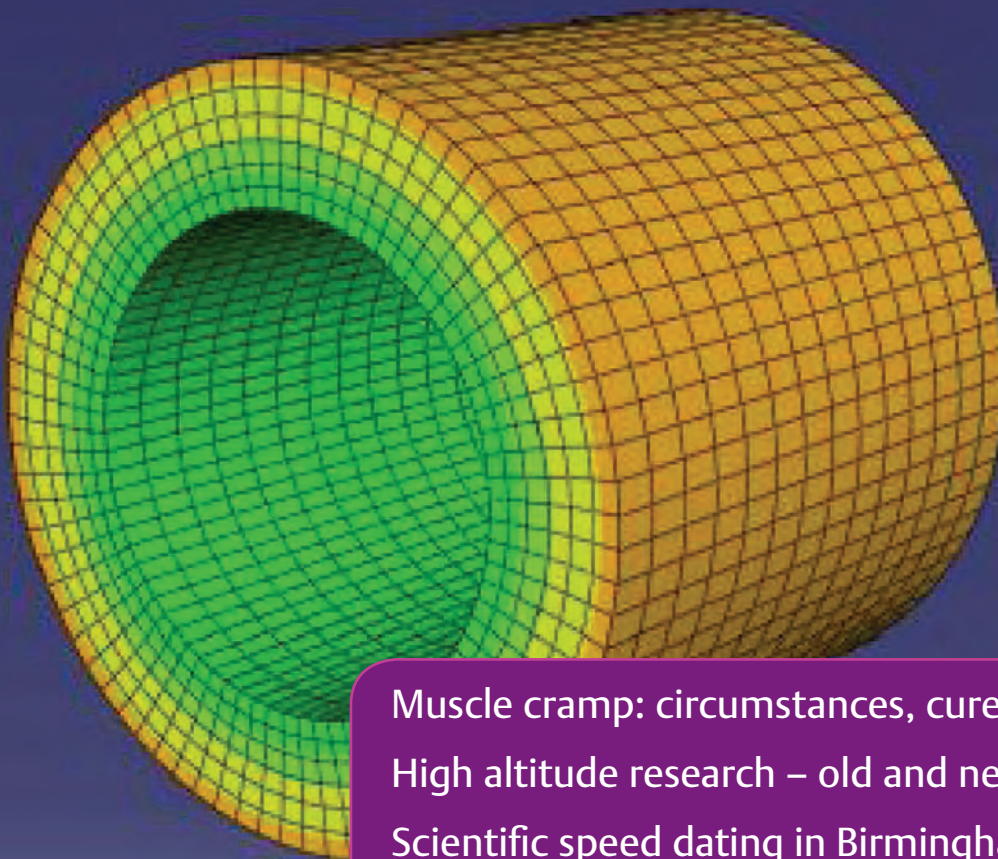
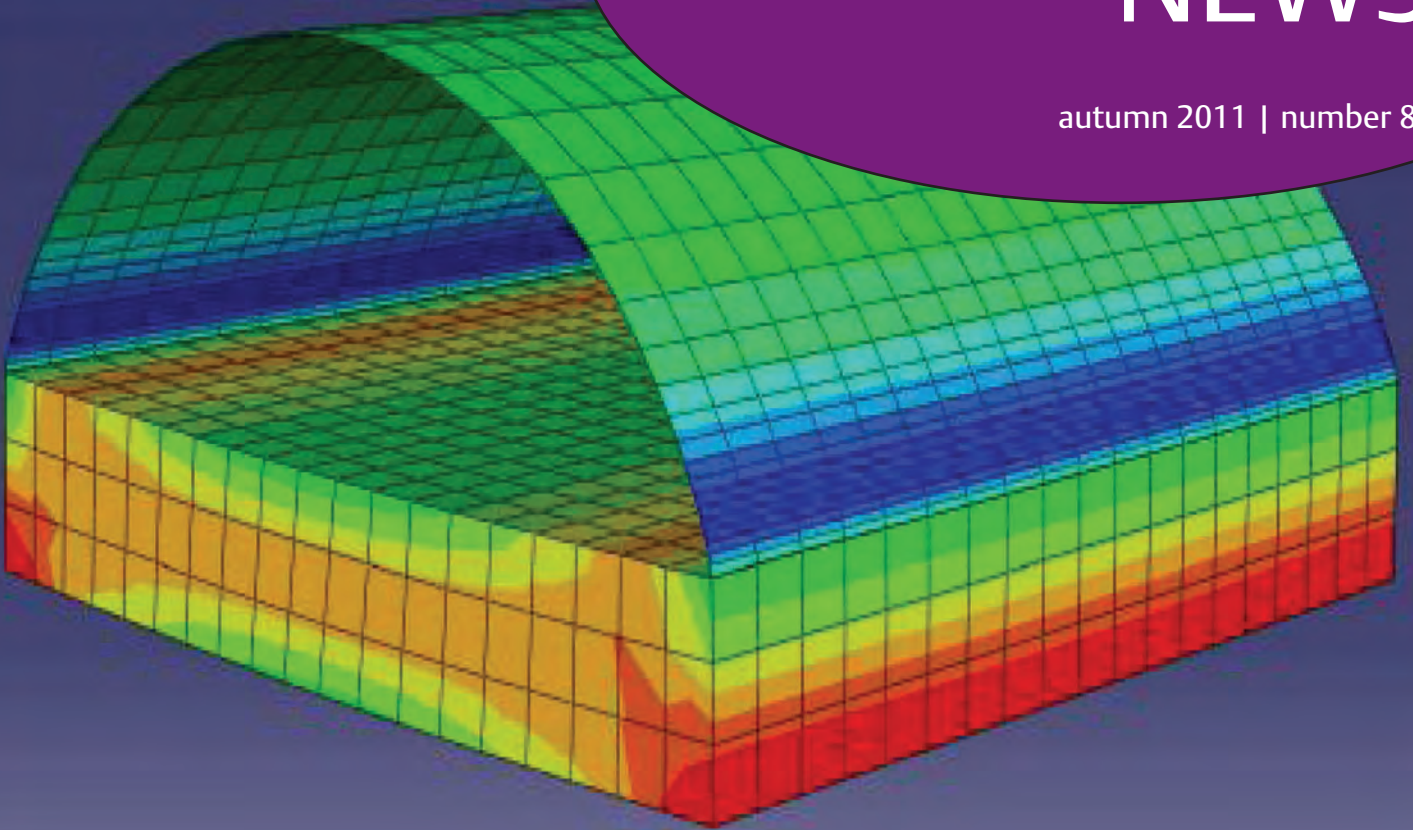



PHYSIOLOGY NEWS

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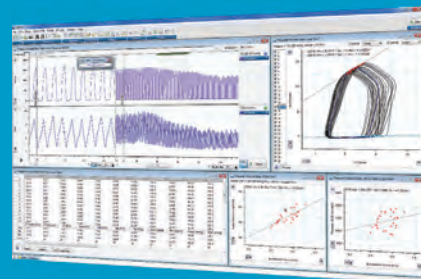
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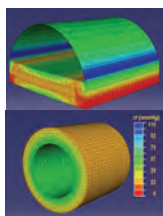
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Cover image: Finite element modelling of the diaphragmatic lymphatic vessel, from p. 30.

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Grants

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

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Physiology News

Deadlines

Letters and articles and all other contributions for inclusion in the Winter 2011 issue, No. 85, should reach the Publications Office (magazine@physoc.org) by **6 October 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 Senior Production Editor or a member of the Editorial Board of *Physiology News* (see contents page for details).

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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>).

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In this issue

Welcome to the Autumn 2011 *Physiology News*.

As anyone who attended the excellent Society debate in Oxford recently will know, the question of the identity, and future, of the discipline continues to preoccupy physiologists. A guest editorial in this issue from the Editors-in-Chief of *J Physiol* and *Am J Physiol* rallies the troops and calls, again, for physiologists to promote the discipline wherever and whenever possible, including with prominent use of the word 'physiology'. Readers with good memories may recall similar exhortations in PN (see e.g. Editorial in PN 75). Perhaps our eminent colleagues have been reading.

This issue we have our usual mix of features: Opinion, Science News & Views, Society news and events, history and the rest. Editors like to think they have a sense of historical perspective, so it is intriguing to me to see that, a half century after the work of pioneers like the late Britton Chance, physiologists are still finding novel ways to use spectroscopy to investigate living cells (pp. 18 and 25).

Another recurring perspective is 'cells to tissues to man' – this issue we feature respiratory chemosensing in action at both the cellular/molecular (p. 32) and whole human (p. 37) level, all the more appropriate on the 100th anniversary of the famous Pikes Peak altitude expedition (p. 48).

Finally, in an age of scarce funding, and fierce competition for airtime and for the attention of the public, funders and potential future scientists, it is pleasing to see so many pages devoted to features on 'outreach' activities (pp. 44–45 and 53–57). Even if you do get publicly rejected (p. 45), or have to invest in a special pair of wellington boots (p. 44)...

Austin Elliott
Editor

Physiology: Found in Translation

Every year, the 12 scientific sections of the American Physiological Society each select a physiologist prominent in their scientific domain to deliver a distinguished named lecture at the Society's annual meeting (Experimental Biology). In 2011, the Environmental and Exercise Physiology section chose Michael Joyner, MD from the Mayo Clinic for this honour, named the Edward Adolph Lecture.

Joyner's lecture appears in the *J Appl Physiol* (Joyner, 2011), and can usefully be read together with a related narrative that appeared a few months ago in *J Physiol* (March 2011, 589, 1005). He used the occasion of his lecture to continue to promote the importance of the discipline of physiology, not just as a discipline that has delivered huge advances in understanding, diagnosing, and treating human and other animal diseases, but as the cornerstone of what is currently the major biomedical research push – translational research. Simply put, physiology and physiological research remain the essential links between genes and clinical care. Translational research just cannot be accomplished without physiology.

Joyner provocatively contrasted the relative failure (to date at least) of the molecular revolution of the past 30 or so years to deliver on its own promises of cures, against the successes of physiology. Whether you buy Joyner's stance or not, there is no question that the genomic revolution has had a major negative effect on the discipline of physiology. In many ways it has been a double-edged sword. The enormous impact of two editorials in 1987 (by then Director of the NHLBI, Dr Claude Lenfant, and Director of the NHLBI Lung Division, Dr Suzanne Hurd) in *Am Rev Resp Dis* (Hurd & Lenfant, 1987a,b) promoting a balance between physiology and molecular biology, but clearly suggesting that investigators wishing for research support through NHLBI had better get molecular, was but one blow to physiology that has since led to the disappearance, reorganization and/or renaming of many former departments of physiology around the world, especially in the USA and UK. We have

come dangerously close to losing the foundations of physiology as the masters who built those foundations have been lost. It was then ironic, to say the least, when Lenfant himself later complained that the molecular revolution had not so far produced adequate results, when he concluded (Lenfant, 2003): 'Enthusiasm for gene-centered medicine is contagious, and I am certainly not immune to it. In my view, however, the fundamental issue remains the same. Enormous amounts of new knowledge are barreling down the information highway, but they are not arriving at the doorsteps of our patients.'

There is, however, light at the end of the physiological tunnel – and not the headlamp of an on-rushing train. The molecular revolutionaries have started to recognize the need for a partnership with physiology. Increasingly, they are coming to those few physiologists left and asking for help in studying the significance of their genetic and genomic discoveries. They have even invented a new discipline – 'Systems Biology' – which of course is physiology. To this point, systems biology mostly addresses the interactions among genes to produce functional effects within cells. This will eventually build into larger and larger units of structure and function, and one day we will proudly know that handshaking between molecular biology and physiology was the key research community transformation that advanced our ability to diagnose and treat disease. Physiology could not have done it alone, and molecular biology could not have done it alone, but they *could* do it together.

While we do see light at the end of our tunnel, remaining passive about our discipline and waiting for reductionists to knock on our doors may not get us there. We think we are in a period of incredible opportunity for physiology, precisely because of the genomic revolution and the resulting push for translational research – but we have to get the word out beyond our own ranks. We just have to become individually and collectively much more active in explaining the importance of our discipline to the rest of the world. And as we all know, that is better done with data than with table banging. This has been recognized for some time, and both the American Physiological

Society and The Physiological Society have been and will continue to be active in meeting with funding agencies and political decision-making bodies to explain the importance of the discipline through examples. Equally importantly, we have, and will continue to, put a lot of societal energy and resources into what we call the 'pipeline' – the physiologists of the future who are currently in primary or secondary school, college or university. They and their teachers need to be exposed to more physiology and thereby become excited by it. In this way, we can play a major role in ensuring the future of the discipline and, as a result, in translating basic discoveries into clinical care.

We cannot let this unique opportunity slip by, and we thank Dr Joyner for his provocative thoughts and timely encouragement. This should be a great springboard to a call for action to all physiologists, wherever you live and work. We need you to promote your discipline, not hide it. We urge you to speak to your local and national politicians and funding agencies about how physiology is essential to successful translation of molecular discoveries. We urge you to visit schools and colleges to promote and maybe even help teach physiology. We urge you to rebadge your Departments of Everything-But-Physiology back to Departments of Physiology. And most of all, we urge you to partner with those molecular biologists (and systems biologists) who are now in need of your scientific expertise, without which translation will not happen.

Peter D. Wagner and David J. Paterson

David Paterson is Editor-in-Chief of *The Journal of Physiology*. Peter Wagner is Editor-in-Chief of the *Journal of Applied Physiology*.

This article has also been published in *J Physiol*; *Exp Physiol*; *Am J Physiol Renal Physiol*; *AJP Endocrinol Metab*; *AJP Heart Circ Physiol*; *J Appl Physiol*; and *Physiology*.

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Hurd SS & Lenfant CJM (1987b). *Am Rev Respir Dis* 136, 542–544.

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Vascular & Smooth Muscle Physiology Themed Meeting in Edinburgh

Society Themed Meetings are a great opportunity to meet and learn from those working in a similar field. December's event is for all those working, or with an interest in, vascular and smooth muscle physiology, and will bring together around 150 scientists and clinicians to share ideas and the latest cutting-edge research. The focused symposium, put together by A. Mark Evans (University of Edinburgh, UK) and Graeme Nixon (University of Aberdeen, UK), adds internationally renowned speakers to explore the topic 'Nanojunctions and calcium signalling in smooth muscle cells: from contraction and migration to gene expression'.



This Meeting will naturally bring together scientists working on smooth muscle in a variety of research fields. It will represent the broadest church in terms of a consideration of regulation, not only of processes that underpin calcium signals, but also of the wide variety of cellular functions that signal control, from contraction, energy supply and migration to gene expression. Therefore, this Themed Meeting will stimulate much discussion across research boundaries and facilitate collaborative ventures between research groups in the UK and abroad.

Surgeons' Hall is based in the heart of Edinburgh – 10 minutes from Edinburgh Castle, Princes Street and importantly, Waverley train station. The King Khalid Building will host the Meeting itself, whereas

Topics covered

- Smooth muscle calcium signalling and contractility
- Calcium, gene expression and smooth muscle proliferation
- Calcium signalling and angiogenesis

the historic Fellows Library in the Playfair Building will be the venue for The Society Dinner. Attending the dinner will give you unique access to museums of surgery and dental surgery that house collections of international importance, and will fascinate medical and lay people alike.



Like all Society Meetings, we welcome Members and non-Members of The Society at any stage in their career, from undergraduates to more senior researchers. Even if this is not your field, but Edinburgh is local to you, why not come along and meet new people and also some members of The Physiological Society team?

www.physoc.org/vs2011

Invited speakers

Casey van Breeman

University of British Columbia, Canada

Mike Zhu

University of Houston, USA

Mark Evans

University of Edinburgh, UK

Iain Parker

University of California, Irvine, USA

David Beech

University of Leeds, UK

Nicola Fameli

University of British Columbia, Canada

John McCarron

University of Strathclyde, UK

Graeme Nixon

University of Aberdeen, UK

Maria Gomez

Lund University, Sweden

Teresa Pérez-García

Universidad de Valladolid, Spain

Alan Knox

University of Nottingham, UK

Teresa Tejerina

Universidad Complutense, Spain



Far left: Fellows Library. Middle: King Khalid Auditorium; the Playfair Building. Above: Playfair Main Hall.

Physiology 2011, held in Oxford

11–14 July



'I wonder anybody does anything at Oxford but dream and remember, the place is so beautiful. One almost expects the people to sing instead of speaking. It is all like an opera.'

WB Yeats

Quotes from the Meeting

Was superb, a great programme with many outstanding researchers attending. Keep up the good work!

It was excellent and I thoroughly enjoyed every aspect of it. I am very much inspired by the talks I attended in the Cardiovascular and Respiratory lectures. I feel that the speakers have motivated me a lot in my work as a PhD student. Also the conference was amazingly organised and it was truly memorable and excellent.

Very good meeting; good value for money and excellent quality symposia

Fantastic meeting!

I liked very much the international mix of speakers at the symposia

Very nice venues, nice to have large poster area. With good weather it was very pleasant to wander between the lecture theatres but because the meeting was spread (compared to Manchester) it meant slightly harder to meet people

We are already planning on bringing our whole research team to the next meeting - this year it was not possible – the atmosphere and discussions were great

Early career social on Monday was great, same again next year!

For me, this was a highly enjoyable meeting, the best general meeting (non topic restricted) meeting I attended in 2011

What I like about the Phys Soc meetings is the bits of information I pick up from fields remote from my own.

This was a very stimulating meeting for me, very good science presented at the symposia

The trade show was good this time, and I think it was good to have it next to the posters

This was a superb meeting. The quality of the organization and scientific programme were excellent. It is rare to attend a meeting that offers diversity and quality. The Plenaries were outstanding

Physiology 2012 – Edinburgh International Conference Centre

2–5 July 2012

1 venue, 21 symposia, 4 plenary lectures



Photos from the Oxford Main Meeting 2011



CGR Advanced Course in Genome-wide Gene Expression Profiling by Array and NextGen Sequencing

10–13 October 2011, University of Liverpool, UK

A new techniques workshop sponsored by The Society and organised by Prof Andrew Cossins and Dr Marta Milo.

The 3-day course is designed to provide a thorough introduction to the use of gene expression profiling: looking at array-based approaches and high-throughput DNA sequencing. The course will consist of lectures, keynote seminars, wet-lab and dry-informatics demonstrations.

Registration for the workshop is free to Members and £50 for non-Members. If you would like to attend, download a registration form from The Society's website: www.physoc.org/education.



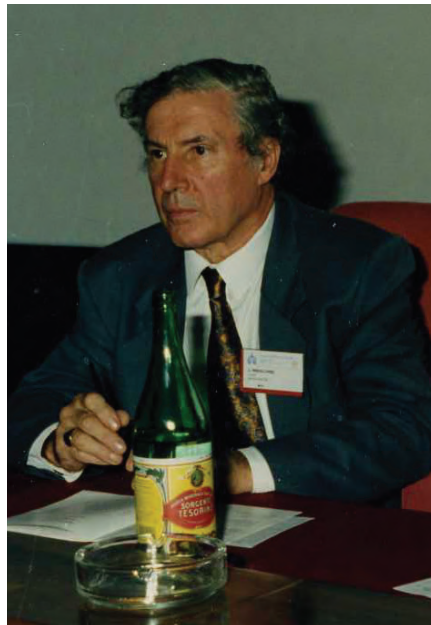
It was the cough that carried him off*

I started research in 1950, and am still active, not in my own laboratory but in meetings, talks and collaborative experiments. What I remember most about these 60 years is not the research, but the people. Of course, you can't have one without the other, although some distinguished physiologists seem to complete their careers with little collaboration. You could say that I enjoyed the research and was devoted to (most of) my colleagues. They came in three overlapping categories.

Mentors

When I was a houseman at Barts in 1949, Ronald Christie was my boss and pointed me towards respiratory medicine. He was a chain smoker but claimed that he never inhaled. Once I said to him 'Professor, you say you don't inhale, but you cough all the time. How so?' He replied 'Maybe, but then no-one knows anything about cough'. Everybody coughs, and 10% of the population are chronic coughers. Yet we knew nothing about it. There was a gap to be filled.

I started research in 1950 at Oxford under Geoffrey Dawes at the Nuffield Institute for Medical Research. He was at the start of his great career in neonatal respiratory and cardiovascular physiology. He was always called 'the Bishop' (but behind his back we called him 'the Pope') because of his pontifical statements. Table 1 lists a few, but they could be multiplied. In those happy days it took only 4 weeks to get a licence to do animal experiments and, to fill the gap, he told me to go to the library and read up the physiology of the oesophagus ('the most neglected tube in the body'), and also to learn German. After four weeks I returned to him and said: 'No-one, including me, is interested in the oesophagus. The tracheobronchial tree is an even more neglected tube and far more interesting. Can I work on cough and respiratory reflexes?' He agreed, and cough and airway physiology and pathophysiology have been my main research interests ever since.



John in a serious mood; only water available.

Until the 1920s cough physiology in animals had been largely neglected probably because opium and morphine were the usual general anaesthetics, and they blocked the cough reflex. There was a handful of papers in the 1920–1940s, but the field was pretty open. I did mainly single-fibre work, building my own apparatus including amplifiers and glassware (Table 1F), doing my own photography, even typing and binding my DPhil thesis. It was a wonderful education, and Geoffrey was an inspired teacher. I learnt a little about cough, and a great deal about research techniques and the wide breadth of respiratory physiology. I think I was the second to study vagal fibres mediating cough, the first being Keller & Loeser (1926); but their neglected paper only had multi-fibre records looking like seismographs, and mine was based on clear single-fibre records. None of the great experts on lung afferent innervation (Adrian, 1933; Knowlton & Larrabee, 1946) had mentioned cough, although unwittingly they recorded from 'cough fibres'. I called the sensors 'cough receptors', but *The Journal of Physiology* referee (John Coleridge) said 'you can't call them that because they don't "receive cough"'. You must call them "irritant receptors" (Table 1C). After my 1954 papers (Widdicombe, 1954a,b,c)

everyone did so for 45 years until the Baltimore group changed the name back to 'cough receptor'. I reviewed some of their papers enthusiastically and accepted the 'new' name; John Coleridge turned in his grave.

In 1953 I was called to do my national service in the RAF, to be trained for a war that had ended 8 years earlier. I had 8 weeks to prepare my thesis (81 hand-typed pages long) and to send three papers to *J Physiol* (60 printed pages long) — all solid stodge. What did they show? That nobody knew much about cough (Christie)! Did a career in cough research await? Not if the RAF could prevent it.

I will draw a curtain over much of my 2 years in the RAF at Porton, at the Microbiological Research Establishment, Salisbury, except to say that I greatly enjoyed them, did almost nothing on cough, but learnt much about lung infection and pathology. My mentor there was Brigadier Frank Buckland — of famous ancestry. I only wore my airforce uniform twice: once to a mess dinner and the Brigadier admonished me — 'It is not done to wear uniform at dinner, Widdicombe!'; then I wore it in the laboratory — 'It is not done to wear uniform in the laboratory, Widdicombe!' It ended up on a scarecrow in my garden. I am sure that also was 'not done'. I especially recall two incidents from the RAF years: when a glamorous young blond technician (called 'Floosy' by sexists) dropped a flask of concentrated plague culture in the corridor, and we all had a week off work; and when the Brigadier was injecting a rabbit with a cream of concentrated anthrax spores and I, as his junior, had to hold the kicking rabbit. I took the injection in my finger. 'I suppose you had better go along to casualty, Widdicombe', was the Brigadier's only comment. The residual scar on the finger has now been nearly hidden by gout.

Next came 5 years back at Barts as Lecturer in Physiology. The work was mainly on lung mechanics and reflexes, and I remember giving a Physiological Society demonstration with an artificially ventilated guinea-pig, No-one (apart from me)

*Footnote for foreign readers: 'It wasn't the cough that carried him off, it was the coffin they carried him off in' (Victorian English saying).

Table 1. Maxims for a novice scientist

Geoffrey Dawes		
	Maxim	My notes
A	Statistics should be a servant and not a master	But not if the statistics agree with your preconceptions
B	Only alchemists claim gold standards	Very applicable in my own field, where there are so many ways of testing cough to assess respiratory disease and the risk of aspiration after brain damage, and many are claimed to be gold standards
C	Language is the first tool of the scientist	This is probably the most important of Geoffrey's adages. If you don't understand your language you literally don't know what you are talking about
D	Never have more than six pieces of information per slide	I once saw a slide with 1250 pieces of information on it. Means and standard errors, and in the few seconds the slide was flashed on the screen I counted 25 rows and 25 columns and multiplied
E	No more than six slides per 10 min	The length of a Phys Soc communication
F	If possible, always build your own apparatus	You will understand it and know its faults. Not easy nowadays
G	Never trust a manufacturer's claims	Difficult to apply nowadays. CG Douglas (Dougie) would each morning take his Haldane blood-gas analysis apparatus on to the roof of the Physiology Laboratory in Oxford and analyse the 'fresh' air. If it didn't come out at CO ₂ 0.03 (0.04 in the contaminated US), N ₂ 79.9 and O ₂ 20.1% he would do no research that day (blaming himself more than the apparatus?)
H	Never give a talk at a meeting you organize	Very good advice, if you think about it, although I have not always stuck to it
I	The audience is more important than the speaker	You can usually tell if the speaker believes this; and see Comroe's advice below
J	German is the language of science	Not true even in 1950. But I am glad Geoffrey taught me this; it led to me translating the Bismarckian German of Breuer and Kratchmer (still on my shelf somewhere) and discovering their genius in spite of the fact that they didn't seem to know that cough existed
Julius Comroe		
K	The Head of the Department should have the second smallest room in the Department	Apart from Julius, I have never seen this done, but I have known HoDs who seemed to spend most of their time in the smallest room
L	At the end of a talk, ask the speaker 'Please summarize your message in one sentence'	This concentrates the mind wonderfully. Privately he told me, 'the longer the sentence the less convincing it will be!'
M	'If you work in the Cardiovascular Research Institute, you may study what you like apart from cornea and cartilage'	Response when I asked him if I might do research on tracheobronchial afferents. And sound advice to Heads of Departments; never discourage the enthusiasm of youngsters

noticed that the guinea-pig had died just before the demonstration started. I don't think then or now you need a licence to work on dead animals. My mentor there was Kenneth Franklin, a wise and benign father figure, who took the Dawes line that young researchers should do what interested them, with his support but without too much interference. I have always tried to adopt that approach.

In 1960 I went for a sabbatical with Julius Comroe, at the Cardiovascular Research Institute (CVRI) in San Francisco. He was a remarkable man (Table 1). He had published a few

important papers on chemoreceptors in Philadelphia before going to San Francisco, where he had no laboratory and never did another day's research. But the CVRI he built up there must have contributed, over 50 years, more to all aspects of physiology, pathophysiology and medicine (except for cornea and cartilage – Table 1) than any other non-governmental establishment. Wives hated him because he insisted on Saturday morning lectures and seminars, but loved him for his personal qualities and for the careers he established for his visiting researchers.

My research, on lung reflexes and mechanics, flourished there, but more important, it established close collaborative and family friendships that continued (to be morbid) until some of them died. To name a few: Jay Nadel, Malcolm McIlroy, John Coleridge, John Severinghaus (all San Francisco) and Abe Guz (London) – all respiratory scientists will know and admire them and their work.

Colleagues

These last names bring me to the most important part of my career – the establishment of my own laboratories and the ability to attract

Table 2. Retirement resolutions (1992)

1	No more committees	Thanks goodness
2	No more lectures to medical students	A sign of my failure at this activity. But I was not as bad as the anatomist John Hunter. His student audience dwindled to one, so he asked the janitor to 'bring in the skeleton' so that he could start his lecture with the conventional 'Gentlemen'
3	No more black tie do's	On retirement I took my DJ to a charity shop (our garden was too small for a scarecrow), and have only had to hire one twice since
4	No more my name on papers when I haven't dirtied my hands in the research	I have given way on this too many times, usually so as not to be rude to friends and visitors, and sometimes to encourage research in countries less affluent than the UK
5	No more invited lectures	I have given way on this a few times (the last in 2010), always with deep regret, especially when I had also to contribute a manuscript (just finished in 2011). Never again, for several reasons

colleagues, often on sabbatical, and to offer them facilities for research, for which I might get a little credit. First, there were 10 years at Oxford as Fellow of New College and University Lecturer in Physiology, and then 22 years as Chairman of Physiology at St George's, London.

My happiest year at Oxford was 1968 when I took a sabbatical year 'in residence'. (Do these still exist?) No lecturing or committees (I was always bad at these, and a vicious circle built up – I knew I was bad so I became even worse). Full time for research, except: I took a Cordon Bleu cookery course, and spent the weekends with a bottle (or two) of wine preparing gourmet meals for an unappreciative family; and I fell ill with virus pneumonia and remember taking deep breaths and feeling the sensation of lungs tearing open like a velcro seal; but no cough except on deep expiratory efforts (even on sabbatical I couldn't escape cough! More later). I contacted Peter Macklem (Montreal) who had just published on the subject, and we remained good friends until his recent sad death.

Why leave Oxford? It was certainly not a lust for power. Heads of Department have too many responsibilities to have much power. Family matters, irrelevant to this paper, were an important factor. I loved the Oxford collegiate life (perhaps too much) but, looking to the future, I did not want to decline into a wheel-chair, even if it had a container for wine attached. It is ironic that I acquired gout about 30 years after I gave up drinking port in Oxford common rooms.

I was the first basic scientist at St George's, which was about to establish a preclinical school. Endless committees! I remember only three with pleasure. (1) My appointment committee to St George's consisted 'only' of the Dean (Alastair Hunter) and the Professor of Medicine (Tony Dornhorst) in the former's office. It lasted about 30 min and then the three of us went for dinner at the Royal Automobile Club. My letter of appointment (presumably pre-written?) arrived the next day. (2) A meeting with the Department of Health to finalize architectural plans for the new Medical School departments. The DoH chairman pulled out an envelope (literally!) and, looking at its back, said to me severely 'Haven't you forgotten your neurophysiology suite?' I blushed and admitted guilt. He then said 'and haven't you forgotten your human physiology suite'. There was no longer need to blush but again I admitted guilt. Finally 'and haven't you forgotten mechanical and electrical workshops?' I was happy in my guilt. Physiology ended up twice as large as any other preclinical department, and I didn't even need to try. (3) The government tried to impose a merger between St Thomas' and St George's and both groups were told to form committees to plan and detail the merger. A dozen of us preclinicals met formally. Within 5 min it was clear we were all opposed to the merger. We had a friendly gossip for an hour or so, listed our objections in our response, and then went to the local pub. The merger never took place. Committees *can* be worthwhile.

In the new preclinical school at St George's I helped to appoint

wonderful colleagues and staff, but there was little time for my own research. Instead I invited physiologists from all around the world to spend sabbaticals with me. I told them, a la Dawes/Comroe; 'Do research on what you like, but remember that cough is the most neglected and important process in the body'. None of them chose to do research on cough, or so they thought! They put my name on many of their papers so, although I also helped them in raising money, planning the research, usually in writing their papers and acting as a glorified technician, I got undeserved credit for their work. I well remember the stony silence that sometimes occurred when I entered the laboratory to help them late in experiments. I have just checked PubMed. In my 22 years at St George's I had my name on 92 multi-authored research papers, of which only two dealt directly with cough, and on only one research paper as sole author. Of chapters and reviews, I was sole author of 108 and joint author of another 28. Only two of the total dealt directly with cough, but many dealt indirectly with cough as one of the respiratory reflexes. Interpret these figures how you like (I know how I do). To complete the picture, at a rough guess I must have attended well over 2000 committees during the same period.

The sting in the tail is that unknowingly the visitors *were* working on cough. Among the subjects they chose were airway mucus secretion (which causes cough); airway epithelial function (which 'tunes' the epithelial nerves subserving

cough); airway vasculature (that influences cough sensitivity); lung mechanics (which profoundly affects and responds to cough sensitivity); control of breathing pattern (which also strongly affects cough sensitivity and pattern); and nasal physiology (which is now established as a strong influence on cough sensitivity). So, adopting Dawes/Comroe, I told my visitors 'do what you want' and they all unwittingly (?) chose topics that were related to cough.

This is not surprising. We now know that cough is influenced by most of the sensory inputs in the body, and in turn modifies most of the motor outputs (Widdicombe *et al.* 2011). In fact, Ranson (1921) suggested this long ago but he has been largely ignored. So nearly all the research done by my colleagues can be related to cough, and in the last 15 years the study of cough, clinical and physiological, has exploded. In 1953 I could discover only six references to cough sensory mechanisms for my DPhil thesis. Now hundreds or thousands are quoted in PubMed (depending on your question). Before 1996 there was only one monograph on cough (Korpas & Tomori, 1979) and there had been no international symposia. Now there are at least 10 books, including monographs, and several international symposia are held each year on the subject. I am happy and proud to have been part of this expansion of interest. We all cough, many of us suffer from it, and we are beginning to understand why.

It is invidious to select names. I omit the British – the list is too long and one can look them up in PubMed – but some of the 'foreigners' who worked with me and remained close friends and colleagues include: Giuseppe and Franca Sant'Ambrogio, and Dan and Lillian Traber (Galveston), Lauri and Anneka Laitinen (Helsinki), Milos Tatar, Jan Hanacek and George Korpas (Martin), and Zoli Tomori (Kosice). I apologise to those I have left out.

I have also travelled widely, especially to Poland, to what was then Czechoslovakia, and to pre-war Iraq, where I think I helped research development. I received many foreign



John happy on the ski slopes of Slovakia.

honorary awards and distinctions, but, more important, I acquired many friends/colleagues who came to St George's to work with me. They nearly always chose subjects seemingly other than cough.

I retired in 1992 and made a number of firm resolutions (Table 2). I have broken them all. And retirement should have been the end of cough for me. But my friends and colleagues (and family?) would not allow this. Life is like a wonderful sabbatical, without even being 'in residence'. And I have made new research friends: Giovanni Fontana (Florence), Robert Addington (Melbourne, USA), Lu-Yuan Lee (Lexington), Brad Undem and Brendan Canning (Baltimore) and Fan Chung (London). I know I am unashamedly name-dropping; but the names are of friends I refuse to drop. Many readers may think 'so what, who cares about cough?' We should – it is part of our lives. My friends who have worked with me on it are an important part of mine.

In my retirement I think back to my 1968 sabbatical-in-residence and my virus pneumonia. It lasted about a week and led to three chance observations. First, that after it I always coughed on forced deflation. This is well known clinically but had never been studied until Giovanni Fontana and colleagues did so in 2010. Their paper (Lavorini *et al.* 2011), on which my name proudly sits, is the first mention of deflation cough in the literature. Second, the sensation of collapsed lung tearing open is quite well known clinically (Macklem), but nobody has identified its afferent pathway. It is not cough, urge-to-cough, volume sensation, pain, irritation or dyspnoea. Do we

have 'velcro receptors' in the lungs (don't turn, John Coleridge)? Third, ever since that pneumonia I have had mild attacks of 'chronic cough' every year or two. I think the virus is sitting in my lungs like herpes can in the skin, waiting to break out. My clinical friends tell me this is nonsense, but how about some evidence? Is there Herpes pulmonis? I will be happy to add my name to a paper describing it.

Family

I have not mentioned family, because in a paper on scientific research it does not seem appropriate. But they are far more important to me than any of the above, and often merge with it. One of the happiest and most memorable events in my life was organized by my wife and family. For the celebration of my 80th birthday they invited family, friends and colleagues from all over the world to dinner and entertainment at a beautiful venue in Ireland. Cough was frequently mentioned (and songs, limericks and anecdotes were devoted to it), but always as a joke. On that occasion we got our priorities right.

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Augustus Waller and the world-wide-web

Physiology's pioneers shared ideas and hardware freely. The success of their 'open-source' philosophy is apparent in the development of physiology through the last century. Some of today's biggest challenges require software rather than hardware development, so the presence of a thriving, fast-moving, web-based, open-source software community is to be encouraged. Can the UK match the pace?

Physiology has evolved as a discipline in parallel with the technologies that are used to study it. From smoked-drum kymograph through to electrometer, cathode-ray tube and computerised data collection, the questions asked by physiologists have changed with their ability to record, visualise and process the data.

Many, if not all, obstacles to data collection have been overcome. In neuroscience, for example, we can now record from single ion-channels or from several hundred neurones simultaneously and electrical recording is often combined with imaging. The volume of data collected has expanded accordingly and this has created new challenges in data analysis and archiving. With data increasingly collected in digital form, it has become easier to share data across the web and new technologies in massively parallel and cloud computing are allowing those data to be processed in ways that were previously not realistic. History is repeating itself, allowing new questions to be asked and physiologists are responding, as they always have done, by addressing those questions. The new systems biology is part of that response. In neurophysiology, information theory is being applied in ways that have previously been too computationally intensive and many web-based resources have grown to support this; one of these, the Neuroscience Information Framework (NIF; www.neuinfo.org/), currently links to 1800 other related sites.

History is repeating itself in other ways too. Much attention is presently focused on how best to visualise the results of the new methods of analysis – extending work on data visualisation that began with the earliest cartographers ~6200 BC as reviewed by Friendly (2009) who begins with a quotation from Harry S

Truman that is salient here: "The only new thing in the world is the history you don't know".

Data visualisation in modern physiology often still mimics the output of the kymograph introduced by Carl Ludwig in 1847. The kymograph allowed a permanent record of the raw data to be made and, as the data were recorded mechanically, the record was not susceptible to human bias. Also, as this record was in a publishable format, others could inspect it. Many improvements and variations to the kymograph were made – several can be viewed in the archives of *The Journal of Physiology*. A frequent feature of these papers is the inclusion of detailed mechanical diagrams allowing the revised devices to be reconstructed in any laboratory. As recording technology evolved through capillary electrometers and string galvanometers, triodes and transistors, this tradition was maintained (see Frank, 1994).

Einthoven's contribution is particularly interesting here. At the 1st International Congress of Physiology in Basel in 1889,



Malcolm Lidieth

Einthoven had observed a demonstration by Waller in which the electrocardiogram, as it later became known, was recorded by means of a capillary electrometer (Fye, 1994). Einthoven saw the limitations of these recordings and resolved to improve on them. He performed an analysis of the effects of the capillary electrometer on the signal and identified the inertia of the mercury as the limiting factor. By characterising the effects of the electrometer, he could remove them from the output and estimate the true input signal by calculation: pre-empting modern signal analysis by effectively deconvolving the output and the system impulse response to recover the input. Einthoven's results are shown in Fig. 1 where the recorded signal,

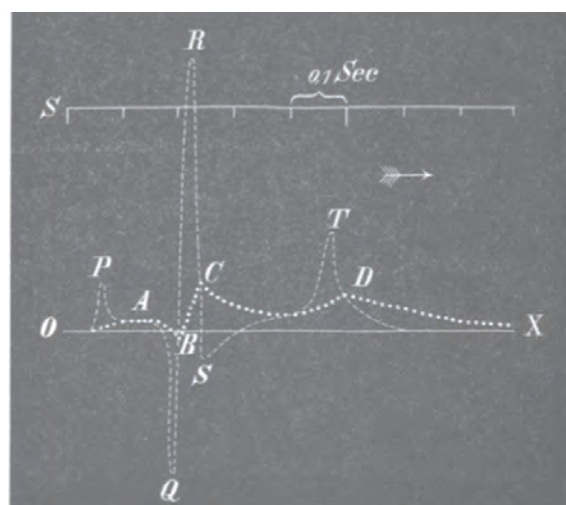
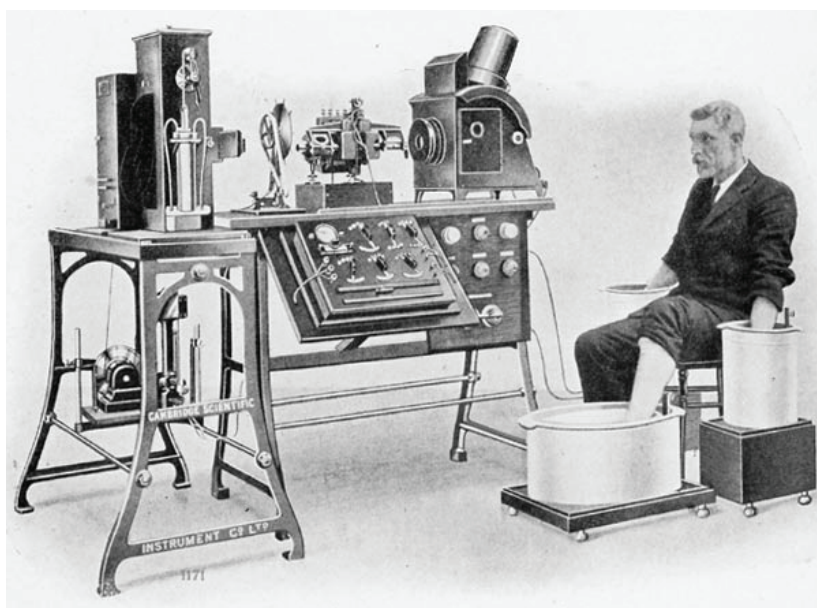


Figure 1. From Einthoven (1895). Construction of an electrocardiogram from the directly registered curve. *ABCD* is the recorded signal. *PQRST* is an estimate of the true signal made by removing the filtering effects of the recording system.



'Photograph of a complete electrocardiograph, showing the manner in which the electrodes are attached to the patient, in this case the hands and one foot being immersed in jars of salt solution.' It was the earliest Cambridge Instrument Company product (1911-1912). It may be the one made for Thomas Lewis at UCH.

ABCD, has been used to derive an estimate of the input labelled *PQRST* which is instantly recognisable as an ECG (the labelling here follows mathematical convention, with *P* as a point on the derived curve; see Hurst, 1998 for discussion). Einthoven published this diagram in 1895 – i.e. before he invented the higher-fidelity string galvanometer that was eventually to make ECG measurement practical in the clinic.

Throughout the first half of the 20th Century, physiologists gave as much attention to instrumentation as to physiology. In his classic paper on the nerve impulse, for example, Adrian (1926) devotes most of the first 10 pages to the amplifier and electrometer. The tradition has not been entirely lost, as evidenced by the number of modern journals dedicated to issues of technique, but fewer physiologists now build all their own recording equipment, relying instead on commercial hardware. This is fine as long as the equipment is adequately, and accurately, specified. When appropriate, the hardware can be characterised, tested and calibrated in the lab.

Can we be as sure of the software used to analyse the recorded data? A quick scan of 29 original papers published in successive

volumes of *The Journal of Physiology* found references in the Methods section to the use of 27 commercial, and 3 free or open-source software packages. Most packages were designed for data collection or statistical analysis. Fifteen of the papers provide few details of how statistical procedures were performed. Presumably, for standard analyses the authors and referees saw no need to provide these details: after all, well-known commercial software is highly unlikely to provide inaccurate results. Or is it? According to McCullough & Heiser (2008), "it is not safe to assume that Microsoft Excel's statistical procedures give the correct answer" (the issues raised are addressed in the latest release of Excel; see Microsoft, 2009). Manufacturers of software regularly post patches and updates correcting bugs, but how many inaccurate results enter the literature untraceably and never to be corrected? If mass-use software can have bugs, what of the niche software used in e.g. electrophysiological analysis?

Plainly, open-source software is no more immune to bugs than commercial software and the pre-conception that it is likely to contain more may contribute to many avoiding its use. However,

as the source-code is available for inspection, it is possible to debug and analyse the performance of open-source code in a way that is not possible with closed-source, black-box commercial software.

In mathematics, the Sage project was begun, in part, for this reason (Joyner & Stein, 2007). It now integrates close to 100 components and aims to be open-source throughout. In mathematics, open-source code may be sufficient: the pudding is in the proof and the starting point is not usually a set of experimental data. The web-page for the Sage project provides its developers' underlying philosophy: "A standard rule in the mathematics community is that everything is laid open for inspection. The Sage project believes that not doing the same for mathematics software is at best a gesture of impoliteness and rudeness, and at worst a violation against standard scientific practices". For laboratory-based sciences, both data and code need to be laid open to inspection. Others can then improve on the original analysis by applying different algorithms and by using different programs to process the same data.

Why has physiology not produced an open-source code project comparable to the mathematicians' Sage project? A clue may be provided in the Report of the Working Group on Biomedical Computing (1999) of the United States NIH which states that: often "...software is cobbled together by graduate students with little programming knowledge, for use by those whose expectations are bound by the immediate problem. The application may be used once, then abandoned when the problem is solved, the graduate student moves on, or the technology changes. The publication goes out, but the tools remain in the laboratory".

For one explanation of why this is so, funding bodies might look close to home. The 'immediate problem' is the cutting-edge problem they fund. Generalizing the code, and documenting it for use by others, is time-consuming but unlikely to attract funding. Once produced, software can no longer be regarded

as novel, so long-term maintenance of that code is even less likely to be funded. If code is maintained, usually because of its value to a broad audience, the novelty and fundability of the code decreases with time. Funding policy therefore militates against the long-term development of a collaborative infrastructure of open-source code.

A second problem is how to generalize code developed in one laboratory for use in others. So far, I have switched without pausing between two problems: data sharing and code sharing because these two problems are not easily separated. An often-cited illustration is the human genome project which, in large part because of its own rapid success, by 1996 "...was foundering in a sea of incompatible data formats, rapidly changing techniques, and monolithic data analysis programs that were already antiquated on the day of their release" (Stein, 2011). If cracking the code of a four-letter alphabet could founder, how will these problems scale to cracking the neural code?

For the genome project the answer was "... to build modular, loosely-coupled systems whose parts could be swapped in and out without retooling the whole system" (Stein, 2011). Many open-source tools now use such a 'pluggable' architecture to incorporate third-party code (ImageJ and EEGLAB prominent amongst them) but the problems, and this solution, are not unique to the genome project or to biology. One of the leading free, general-purpose software development environments is the NetBeans Integrated Development Environment (IDE, <http://netbeans.org/>) coordinated by Oracle. This IDE is entirely open-source. It is also entirely modular, and uses loose-coupling between modules so that any module can be omitted or replaced and custom-modules can be added. With a well-designed NetBeans-developed 'capillary electrometer', Einthoven could have replaced the 'mercurial' module with a higher-fidelity quartz fibre module and not changed anything else. NetBeans takes this to its logical extreme; effectively the IDE itself can be edited to produce

a new application that bears little or no resemblance to the IDE begun with. Some of the many applications developed in this way are showcased on the web (<http://netbeans.org/features/platform/showcase.html>).

In the case of the genome project, the raw sequences at least lent themselves to storage in a common database. For the diverse problems to be addressed in computational neuroscience or systems biology, it is unlikely that any single database model will suffice. US funding bodies seem to have recognised this. The NIH/DHHS-funded NIF (see above) seeks to advance neuroscience research "by enabling discovery and access to public research data and tools worldwide through an open source, networked environment". In systems biology, the Systems Biology Markup Language (SBML) seeks to avoid some of the data compatibility problems that affected the genome project. But, "SBML does not represent an attempt to define a universal language for representing quantitative models. It would be impossible to achieve a one-size-fits-all universal language. A more realistic alternative is to acknowledge the diversity of approaches and methods being explored in systems biology, and seek a common intermediate format – a *lingua franca* – enabling communication of the most essential aspects of the models" (see SBML.org, 2011).

Einthoven's observation of Waller's demonstration in 1889 led to the introduction of the ECG in the clinic. By 1913, Thomas Lewis wrote that "...this new method of examination has become essential to modern diagnosis and treatment of cardiac patients". Franks' (1994) review of the history of electrophysiology shows the part that chance encounters played in the introduction of new technologies. In the era of the web, useful chance encounters, with ideas if not people, are more frequent. The international response has been to encourage and facilitate this through initiatives such as NIF and to improve the fruitfulness of the encounters through common languages such as SBML. Even commercial organizations like Oracle

are encouraging almost anarchic mutilation of their own products like NetBeans. What of the UK? Here, we seem to be moving in the opposite direction. Response-mode funding is declining and centrally dictated strategies rule the day. If the laboratories involved in the genome project could not keep pace with their own output, what prospect is there for large government agencies? Overseas agencies are embracing this dynamic, fast-moving new world and are finding a position for themselves within it. In Britain, we've put the kettle on.

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The orderly recruitment of postganglionic sympathetic neurons

Investigations into discharge patterns of the multi-unit postganglionic sympathetic neural population in humans have been hampered by the poor signal-to-noise aspect of this signal. New signal processing and denoising approaches have now been used to expose such action potential patterns. Such approaches have revealed that variations exist in the conduction velocity of action potentials observed in this neurogram and that a subpopulation of fast-conducting neurons exists for probable recruitment during high stress scenarios.

Order is the shape upon which
beauty depends

Pearl S. Buck (1892–1973)

Blood pressure and blood flow are regulated, to a large extent, by the sympathetic nervous system through its influence on the function of the heart and blood vessels. This influence is exerted through varying levels of sympathetic nerve activity (SNA) and the consequential emission of a variety of chemicals or neurotransmitters. The level of SNA may increase rapidly in response to stressors (the 'fight-or-flight' response), such as changes in posture, emotional arousal, fatiguing exercise and others. Yet, the corresponding cardiovascular responses may vary with the type of reflex. Also, current evidence indicates that the levels of SNA at baseline and during a reflex response are affected by one's sex, age and disease state. Physiologically, there is considerable uncertainty surrounding the manner in which SNA is emitted and how variations in its discharge patterns are regulated, particularly in conscious humans where direct access to neural recordings from the sympathetic nervous system is limited. Nonetheless, variations in SNA discharge are likely to reflect mechanistic determinants of sympathetic recruitment as well as provide an important input to cardiovascular control.

Historically, a major breakthrough in measuring and quantifying SNA discharge patterns in humans came in the late 1960s when adaptations to microneurographic techniques enabled direct recordings from postganglionic sympathetic neurons (Hagbarth & Vallbo, 1968). Using tungsten electrodes penetrating groups of sympathetic neurons in



From top left
clockwise: Kevin
Shoemaker, Aryan
Salmanpour and Craig
Steinback.

peripheral nerves, such as the peroneal (fibular) nerve, it was learned that SNA directed to blood vessels within skeletal muscle (MSNA) exhibits bursty behaviour, with groups of axons discharging more-or-less simultaneously in a manner that is entrained to the cardiac cycle. There is variation in the rate and size of these bursts, suggesting a corresponding modulation of neural recruitment. However, due to the relatively poor signal-to-noise aspects of the SNA signal from human peripheral nerves, analysis of this multi-fibre recording has, to a large extent, been constrained to the integrated neurogram. Through band-pass filtering, rectification and integration, SNA data are typically smoothed to reduce background noise and provide a quantitative measure of sympathetic outflow in terms of the number and size of integrated bursts. However, this approach eliminates all information from individual neurons and the action potentials (APs; often called 'spikes') that make up the total signal. This places a constraint on the ability to study sympathetic neural discharge patterns and recruitment strategies, as well as on the ability to understand the mechanisms linking SNA and end-organ control in humans.

For example, a provocative observation in the integrated neurogram from human MSNA recordings is that the conduction of sympathetic traffic, based on the delay of the burst from a representative R-wave of the electrocardiogram (Wallin *et al.* 1994), is inversely related to burst size. The shorter reflex latency of larger bursts is hypothesized to be due to (a) variations in synaptic delays between the brainstem and peripheral nerve that produce more discharges of the same neurons per burst, and/or (b) more than one population of efferent sympathetic neurons with progressive recruitment thresholds and conduction velocities (Wallin *et al.* 1994). Although articulated in 1994, these issues have remained untested in human or smaller animal models due to methodological limitations for examining SNA at the AP level.

Since 1994, two experimental approaches have been advanced to address SNA discharge patterns and their control in humans. First, the use of higher impedance electrodes enabled the study of single neurons and their behaviour over time. These studies demonstrated that postganglionic sympathetic axons discharge in a probabilistic manner, typically firing only once in a burst of sympathetic activity (Macefield *et al.* 1994); however, multiple within-burst firings of the same neuron may occur with increasing stress (Macefield & Wallin, 1999). From these studies on single-fibre recordings, Macefield proposed that increases in firing probability of active neurons may be the primary mechanism by which integrated burst intensity (size) is augmented. It is also possible that a population of latent neurons is available for recruitment during stress. Although

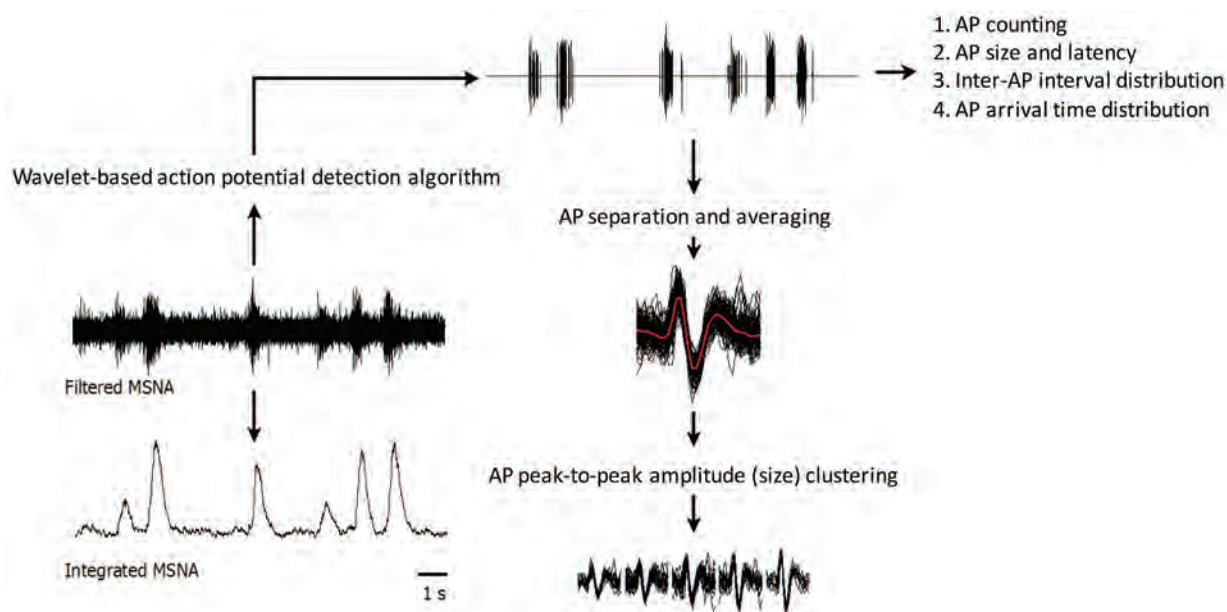


Figure 1. Methodological approach to extracting postganglionic sympathetic action potentials (APs) from the multi-unit microneurographic recordings. The raw filtered signal is passed through a wavelet-based denoising program that indicates the exact location of each AP. Following extraction of these APs from the original raw signal, they are classified on the basis of their peak-to-peak amplitude and binned accordingly.

both of these potential mechanisms have been observed in other neural systems, such as the skeletal-motor system (Henneman *et al.* 1965), such ideas regarding sympathetic neural recruitment required analysis of the multi-unit firing patterns rather than one neuron at a time.

More recently, the advancement of wavelet-based denoising approaches has opened new opportunities to study the patterns of AP discharge within each burst of sympathetic activity. This approach has led to reports of variations in postganglionic sympathetic AP rate, both at rest and during reflex activation (Diedrich *et al.* 2003). However, the ability to study the presence (or absence) of differing populations of postganglionic sympathetic neurons with varying recruitment thresholds or patterns requires additional understanding of the timing and size of APs along with subsequent analysis of properties related to neuronal size, such as its conduction velocity. Recently, our laboratory published new information regarding such discharge patterns using an improved AP detection and classification approach (Salmanpour *et al.* 2010) (see Fig. 1).

With this approach we have studied the question of AP content within bursts of SNA of various sizes and the recruitment of new neurons during severe physiological stress (Steinback *et al.* 2010). Very large increases in muscle SNA are observed during severe chemoreflex stress due to increases in both burst frequency and size. Such a reflex response was considered ideal for the study of AP patterns across a range of reflex stress. Severe chemoreflex stress was produced by a prolonged breath-hold performed by trained free divers. The illustration of the AP discharge pattern as a function of integrated burst size and location are presented in Fig. 2 for a single individual. As a group, these individuals held their breath for 178 ± 37 s resulting in a haemoglobin saturation of $80 \pm 11\%$ and arterial CO_2 partial pressures of 51 ± 3 Torr. The average amplitude of each MSNA burst increased from 0.24 ± 0.04 V at rest to 1.34 ± 0.38 V at maximum breath-hold and the average number of APs per burst of muscle SNA increased from 14 ± 7 at rest to 40 ± 12 at maximum breath-hold. Moreover, we demonstrated that the number of active AP clusters (representing discreet populations of neurons of a particular size) per burst of muscle

SNA increased from 4 ± 2 clusters per burst at rest to 13 ± 3 clusters per burst at maximum stress. Importantly, larger APs became increasingly evident in the muscle SNA neurogram as the chemoreflex stress increased and as integrated burst size increased. In addition, the latency of these APs, relative to their corresponding R-wave, was inversely related to the size of the AP cluster. Thus, the larger APs demonstrated a faster conducting velocity. When considered over the group and breath-hold period, a distinct decaying exponential relationship between AP amplitude and reflex latency became evident (Fig. 3).

The above data were obtained during the progression of chemoreflex stress on the expectation that large APs would be more likely to appear in severe reflex sympathoexcitatory scenarios. However, there is a large variation in integrated MSNA burst size even under conditions of supine rest when physiological stress is considered to be minimal. Also, other reflexes, such as baroreflex activation during postural challenges (in response to a rapid drop in blood pressure), increase the rate of integrated bursts of MSNA but have less impact on

the size of these bursts. Therefore, we have also taken a closer look at AP characteristics both at rest and during high levels of lower body negative pressure to examine the influence of MSNA burst size alone and with baroreflex unloading on such discharge patterns (Salmanpour *et al.* 2011) (Fig. 4). The results indicate that the inverse hyperbolic relationship between AP cluster size and its conduction latency, observed previously during chemoreflex stimuli, is present both at rest and during -60 and -80 mmHg of lower body negative pressure. However, what is significant is the introduction of larger APs at the high levels of

negative pressure, at least in some of the individuals.

Overall, when these new data are coupled with reports from single-neuron recordings, analysis of multi-unit AP patterns and morphology exposes a pattern of regulatory order in postganglionic neuronal recruitment. Specifically, one mechanism of increasing MSNA is to increase the firing rate of already-recruited axons as indicated by increasing numbers of similar-sized APs per burst of SNA. However, there also appears to be a latent population of neurons available for recruitment. Some of these latent

neurons are active at baseline but are likely to be expressed in the larger bursts of MSNA. Therefore, the expression of larger bursts of MSNA at any time occurs because of the increased number of APs and the appearance of new and larger AP clusters. Moreover, the likelihood of these larger APs and additional clusters of larger APs being present in the neurogram appears to increase during severe reflex stress. Importantly, the larger AP clusters are characterized by a shorter reflex latency which supports the premise that they represent larger axons with faster conduction velocities. Thus, this work suggests that larger and

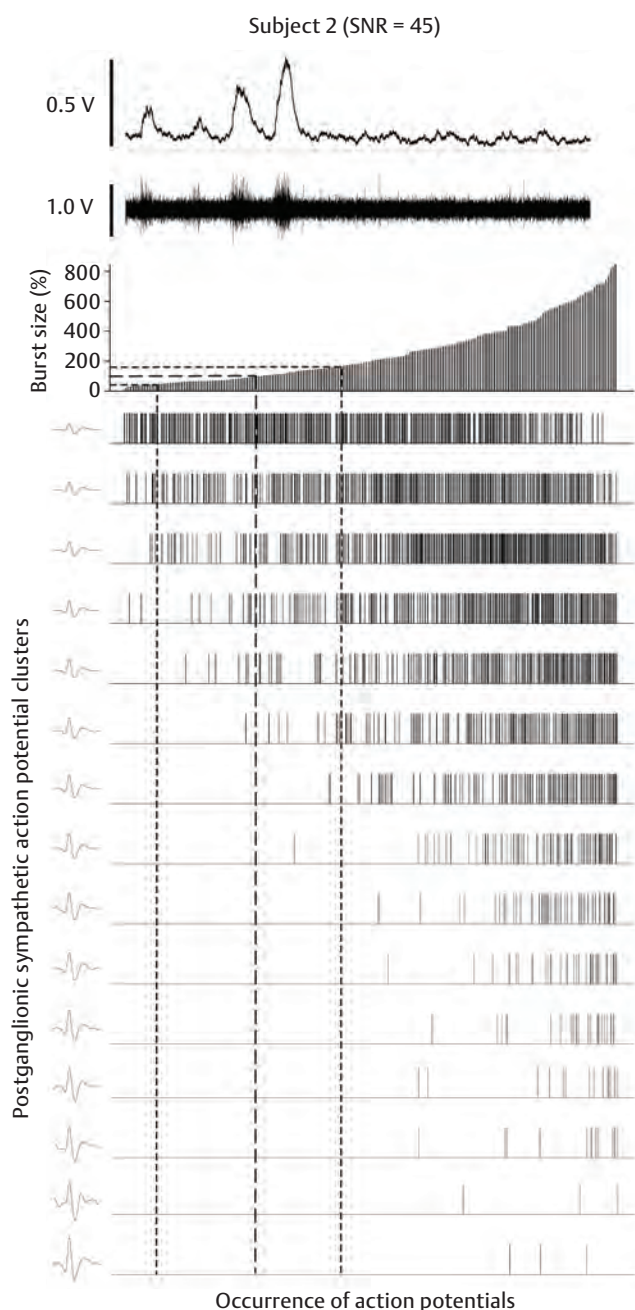


Figure 2. Illustration of action potential occurrence as a function of integrated sympathetic burst size and action potential cluster amplitude with high signal-to-noise ratio, for a single subject on going from rest to chemoreflex activation. Top panel illustrates 10 s of the integrated and raw neurograms obtained at baseline. Dashed lines represent the means and standard deviations of integrated burst sizes at baseline. The action potentials are depicted across the range of integrated sympathetic bursts as they occur in time during the reflex manoeuvre as a percentage of baseline (100%). The occurrences of postganglionic sympathetic action potentials as a function of each integrated burst indicated for each action potential cluster below. Clusters of larger amplitude action potentials are predominately recruited at higher levels of sympathetic activation (as determined by integrated burst size). From Steinback *et al.* (2010).

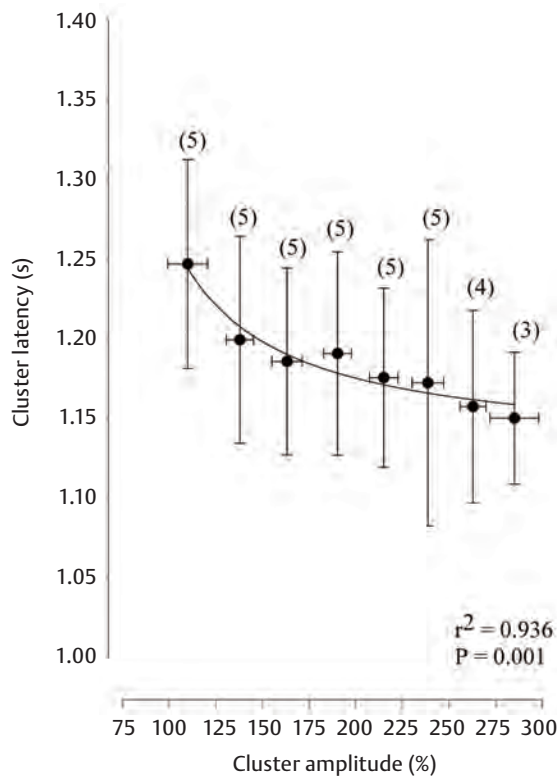


Figure 3. Mean action potential cluster latency, binned across participants, as a function of normalized action potential cluster amplitude during continuous breath-hold. Numbers indicate the number of subjects per bin. The decrease in action potential cluster latency as a function of amplitude was fitted with a modified exponential decay. From Steinback *et al.* (2010).

faster-conducting postganglionic sympathetic neurons (a) are largely latent at baseline, (b) are available for recruitment, (c) have a higher probability of recruitment during

high physiological stress, and (d) are present in larger rather than smaller bursts of MSNA. As this pattern is analogous to previously defined patterns of motor neuron

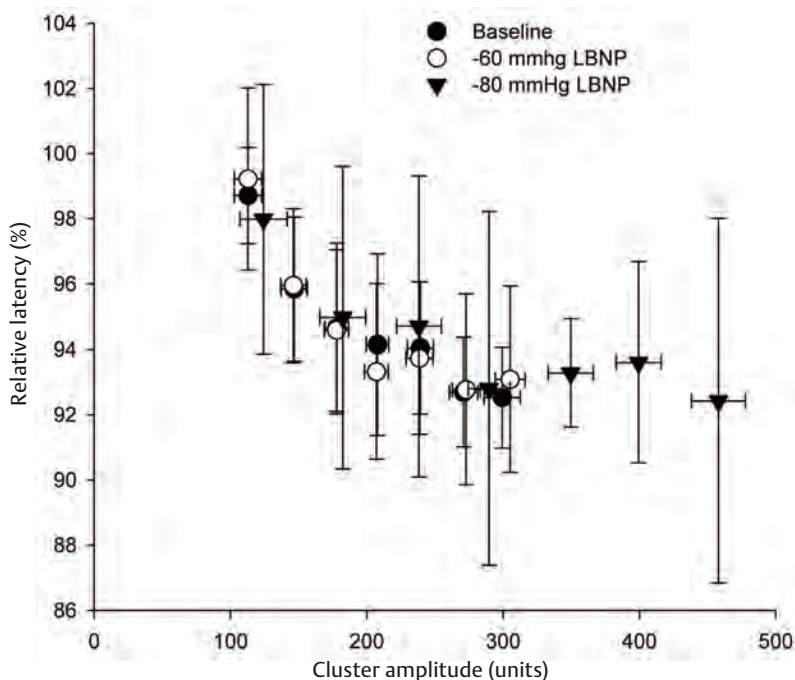


Figure 4. Normalized mean action potential cluster latency as a function of action potential cluster amplitude during supine rest and two levels of lower body negative pressure (LBNP). Adapted from Salmanpour *et al.* (2011).

activation, it appears that this ordered recruitment pattern may be a fundamental feature of excitable neural systems.

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Mitochondrial responses to hypoxia and physiological stimulation of harbouring cells measured optically *in situ*

Understanding the involvement of mitochondria in cellular regulatory processes in living cells and tissues is the hallmark of the current interest in mitochondria. One way to monitor these organelles in live tissue is the newly developed, high-precision, spectral absorption-based method for measuring respiratory pigments' redox state changes. With it we monitored responses of mitochondria, inside the eyes of live insects, to large perturbations like hypoxia, as well as to more physiological ones such as light stimulation.

One of the limitations to physiological research has always been the availability of tools that would allow monitoring of processes hidden inside other structures – processes in organs within the body, in tissues within organs, in cells within tissues and in cellular organelles within cells. A traditional remedy is to dissect the structure, take out the part of interest and study it in an artificial environment, which is as close as possible to the natural one. This approach has many advantages, especially regarding the vast array of experimental methods that can be employed once the obscuring structure has been removed. It is hardly necessary to state that most of our physiological knowledge has been gained this way. The other approach – to observe the whole body and try to extract meaningful information, from often very distorted signals passing through the many structural layers – has been much less popular, due mainly to its inherent imprecision and artefact-proneness. The middle ground has been covered by approaches using various marker probes – from fluorescent molecular to x-ray contrast ones. There are very few methods available that allow reliable insight down to the molecular level within living organisms without the use of additional artificial markers.

All of the above is certainly true for the study of mitochondria. Yet, as we try to understand their involvement in many cellular regulatory processes, it is becoming increasingly apparent that any methods for monitoring mitochondria, without the need for isolation or introduction of potentially function-interfering markers, would be very useful. We have developed such a method, which allows monitoring of mitochondria by simultaneously measuring the



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relative redox state changes of all the respiratory pigments present in the respiratory chain (Zupančič, 2003). It is based on the long-known fact that haems, which are part of respiratory complexes, undergo substantial changes of their absorption spectra with reduction and oxidation (Chance & Williams, 1955). There are, however, a few problems associated with this phenomenon.

First, their absorption spectra overlap heavily. Single wavelength measurements of absorption changes are thus doomed to be inaccurate because all the haems change their redox state/absorption simultaneously when mitochondria are activated or experience any other metabolic challenge. The original solution to this problem was to use wavelength pairs whereby one wavelength served as a semi-isosbestic reference while the other reported on the redox state of a particular haem (Chance & Williams, 1955). Although pioneered in the 1950s, this approach is still being used today. Nevertheless, it remains difficult to simultaneously monitor the whole respiratory chain in this way. In addition, this still does not resolve the problem completely since there are no true isosbestic points in the mitochondrial absorption spectra.

Our solution was to use fast spectral imaging of whole tissue

absorption spectra by a 2048 element linear CCD array and then numerically post-process the acquired time-coded spectral matrix. We used a combination of digital filtering followed by spectral down-sampling, followed by singular value decomposition (or principal component analysis) and fitting of linear combinations of reference haem spectra to the most significant spectral components. The time courses of respiratory pigments' reduction/oxidation were finally obtained by multiplying the matrix of the most significant time course vectors with the matrix of fit parameters (for a detailed explanation see Zupančič, 2003). This approach is only possible because, although cytochrome absorption spectra differ between species, the haem difference absorption spectra are remarkably constant from bacteria to man. Because of this, we could use published haem difference absorption spectra from different species as our references. The side benefit of all this numerical processing was a drastic increase in the signal-to-noise ratio in longer records, where it was improved several hundred times, with respect to the original record (Fig. 1).

Using this method we were able to examine in great detail what is happening to mitochondria inside a living, although immobilised, animal during various stages of hypoxia, as well as during metabolic challenges associated with normal cellular function (Meglič & Zupančič, 2011). We used mutant white-eyed blowflies that have no screening pigment in their otherwise normally functioning compound eyes. Although this feature prevents the animals seeing a sharp picture of their surroundings, it is very useful for optical measurements of tissue absorption changes. Light

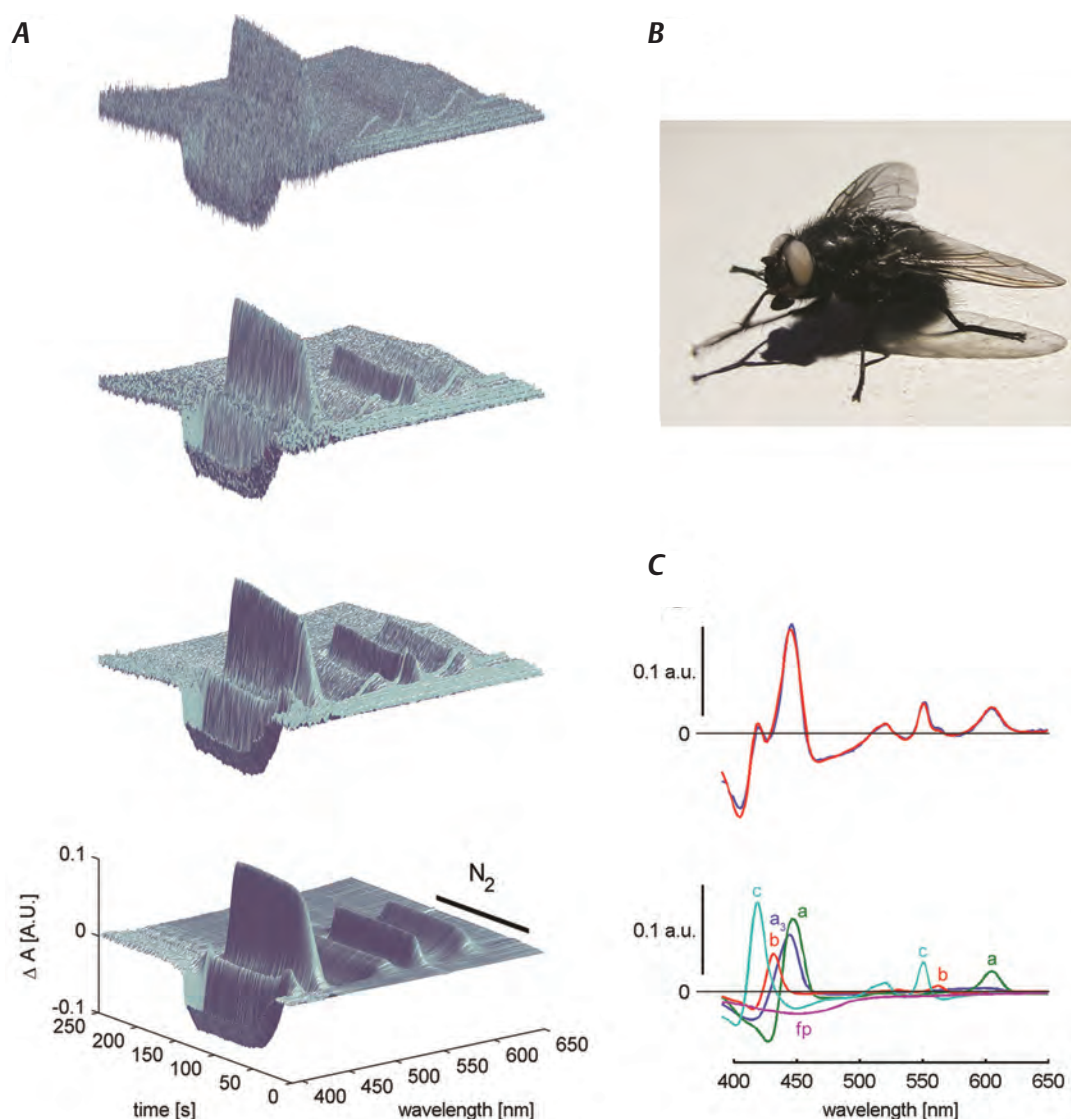


Figure 1. Experimental data are a time series of differential absorbance spectra. These are the differences between the spectral absorbance at any given time and the first recorded absorbance spectrum. They are shown arranged in matrices with a time and a spectral dimension of absorbance change in A. In the particular experiment shown here, the blowfly (*Calliphora vicina* chalky), shown in B, was exposed to pure N_2 for 2 min and the 'white' light reflected from its white eye analysed. The 3-D matrix representations in A show a progressive increase in the signal-to-noise ratio during various stages of numerical processing, with the top corresponding to raw recordings and the bottom one to the final set used for spectral fitting. One spectrum from this set and the corresponding fit are shown in C. Here the blue line in the top panel shows the recorded and post-processed differential spectrum at the end of N_2 exposure and the superimposed red line shows the spectrum reconstructed from a linear combination of respiratory pigments reference difference spectra – actually a sum of the spectra displayed below. Letters a, a_3 , b and c stand for respective haems and fp for flavoproteins.

that enters the eye is scattered many times by the many interfaces between the air in the tracheal system and the tissue, and a substantial portion of it leaves the eye, making it appear white. This light, however, bears also the signature of all light-absorbing molecules present in the eye – rhodopsin/metarhodopsin, cytochromes, flavines, NADH/NAD. The respiratory pigments are especially abundant, since the photoreceptor cells are full of mitochondria, which support the ion pumps in maintaining the ionic

imbalance in the presence of strong light-induced ionic currents.

The insect phototransduction is in contrast to vertebrates coupled to TRP and TRPL channel-mediated depolarization upon illumination, via G_q -protein and $PLC\beta$ (Hardie, 2007). The one in flies is usually exemplified as the fastest known G-protein-coupled cascade due to its high degree of spatial organization. Our experiments were made easier, on one hand, by the fact that the tracheal system brings air virtually to

each cell. Since the O_2 diffusion in the air is approx. 10,000 times faster than in water, this allows fast changes in O_2 content deep within the eye tissue. On the other hand, the very high speed of the transduction cascade produces close to step changes of many cellular physiological parameters – membrane potential, $[Ca^{2+}]_i$, etc. (Gerster *et al.* 1997; Oberwinkler & Stavenga, 2000).

What we discovered was that such a step-like physiological load induces virtually simultaneous changes in

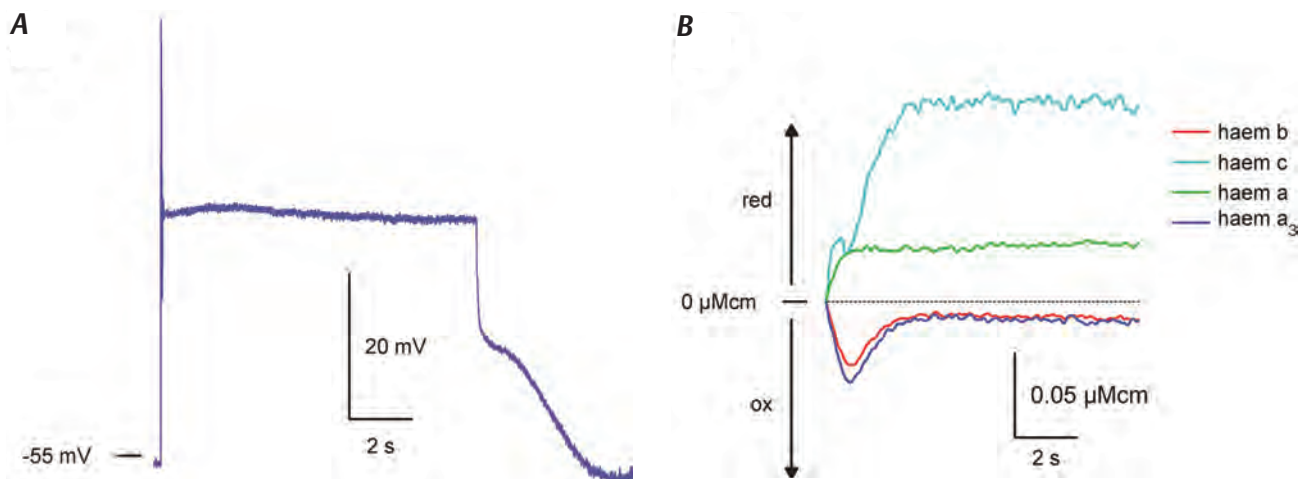


Figure 2. The response of blowfly photoreceptors to 10 s of white light illumination. *A* shows the intracellularly recorded membrane potential of a photoreceptor cell. It shows a typical fast transient depolarization followed by a lower plateau phase, which persists throughout illumination. In *B* the traces represent the average responses of respiratory pigments to 30 instances of 10 s of white light illumination recorded from one preparation. Since the stimulating light was used for absorption measurements as well, the respiratory pigment signals start at the beginning and end at the cessation of illumination. The directions of the change in the redox state are indicated by arrows, with red indicating reduction and ox indicating oxidation.

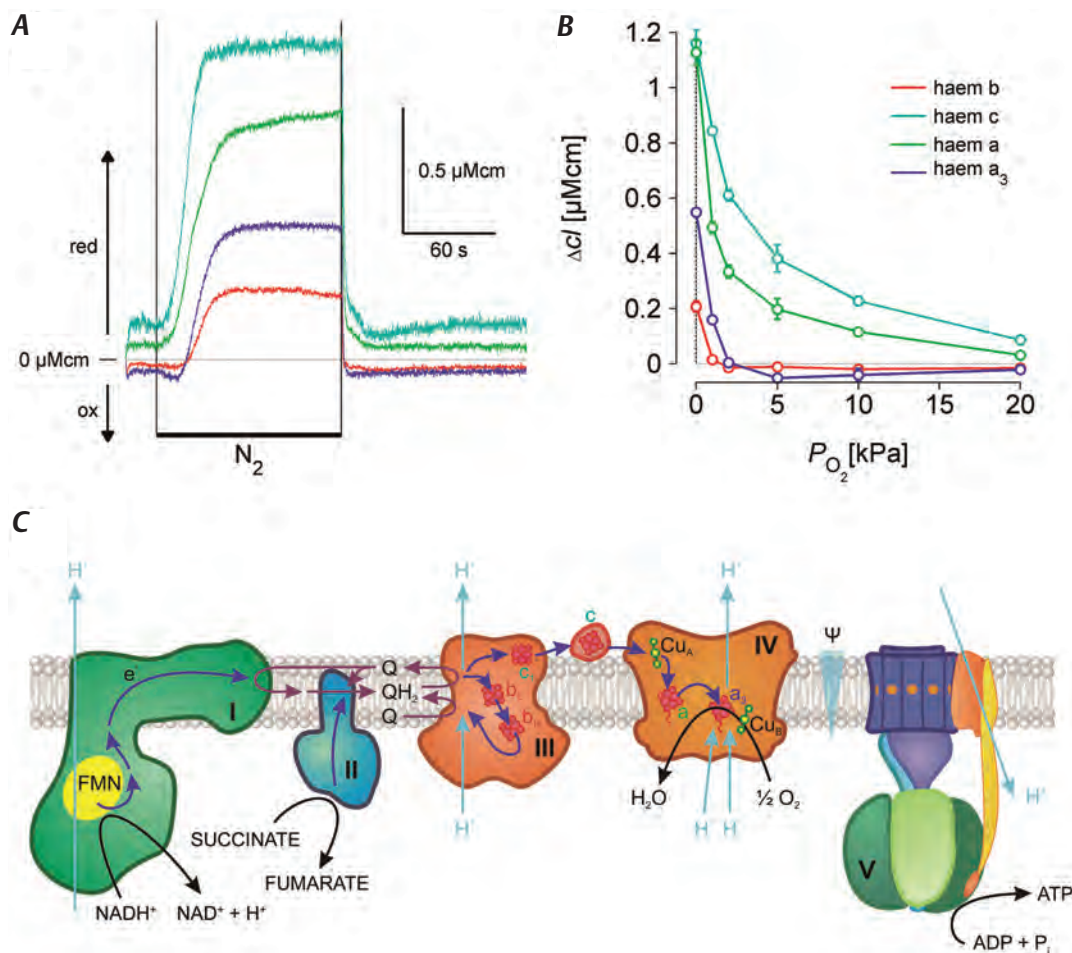


Figure 3. When exposed to complete absence of O_2 in the animal's environment, respiratory pigments eventually get reduced as shown in *A*. The recorded change depends on both the total amount of a particular pigment and its starting redox state. Due to a poorly defined light path, we were unable to determine their true concentration change, hence the units of μMcm . As shown also in Fig. 2, light exposure, necessary for the recording, induces oxidation of haems b and a_3 while haems c and a are simultaneously reduced. Similarly, as shown in *B*, the latter pair is continuously progressively reduced with a decrease in P_{O_2} , while the former two only start getting reduced below 2 kPa P_{O_2} . Such behaviour is at odds with a widespread opinion that upon O_2 absence, the respiratory chain, shown here schematically in *C*, starts filling up with electrons from the rear end – from haem a_3 in complex IV forward. The process is clearly more complex, especially when mitochondria are fully active, as is the case here.

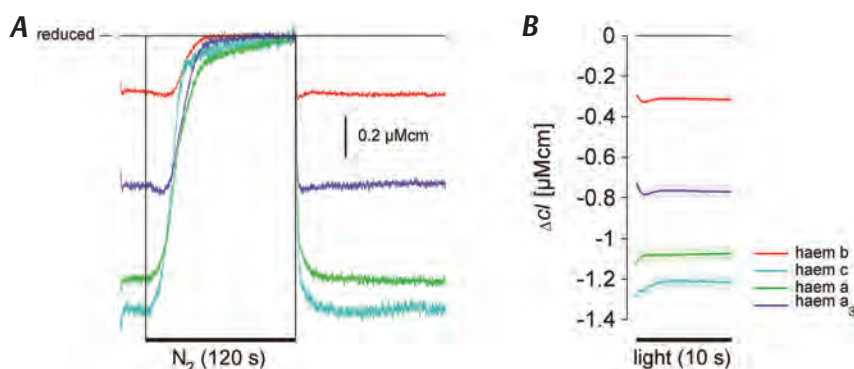


Figure 4. Although there is a degree of uncertainty regarding the true concentration change of a particular form of each haem, we can reasonably assume that, when deprived of O_2 for long enough, all the molecules will eventually be filled with electrons – i.e. reduced. When data are shown this way in A, a striking feature emerges. Haems a and a_3 are equimolar parts of complex IV, yet are reduced upon N_2 exposure by a different amount. This implies that their resting redox states are different. The same holds, although with some caveats regarding stoichiometry, also for haems b and c. From this perspective the changes elicited by light-induced metabolic load shown in B make more sense since the haems reduced more at rest get oxidized and the more oxidized ones get reduced when mitochondria are activated and electrons start to flow down the respiratory chain. The different resting redox states possibly reflect the energy barriers to electron flow imposed by the inner mitochondrial membrane potential, which is relieved during mitochondrial activation.

the redox state of all cytochromes and these are, in part, very similar to changes of flavoproteins and NADH previously measured using their autofluorescence (Stavenga, 1995). What differs is that some pigments (haems b and a_3 , as well as NADH and flavoproteins – from NADH and succinate dehydrogenases) undergo a transient oxidation, while others (haems a and c) undergo reduction without a conspicuous transient (Fig. 2). A minor exception is haem c, which seems to show a combination of both responses – possibly due to the fact that we could not optically differentiate between haems c and c_1 , which is a part of complex III.

The analysis of responses to hypoxia exposed the same two groups in both steady-state and dynamic P_{O_2} change experiments (Fig. 3). In both cases, haems a and c started to get reduced as soon as the environmental P_{O_2} started to decrease below normal atmospheric level. Haems b and a_3 , however, first went into oxidation, which only reversed into reduction, due to accumulation of electrons that could not be passed on to O_2 , below 2 kPa P_{O_2} . Of course, this oxidation is most probably due to the fact that the animals were constantly exposed to measuring light and thereby a physiological load. Nevertheless, it clearly shows that the two groups of haems, situated within the same respiratory complexes, behave very differently. This is further illustrated by combining the two sets of data

– from hypoxia and illumination experiments (Fig. 4). Especially haems a and a_3 – equimolar components of complex IV, the cytochrome c oxidase – have very different steady-state reduction values and they also behave very differently. Haem a_3 , the more reduced one at rest, exhibits a transient oxidation, while haem a, the highly oxidized one, goes straight into reduction when the photoreceptor cells are illuminated. Although less clear, due to the technical problems of separating haems c from c_1 and b_H from b_L , the story is the same with haems b and c. The likeliest explanation is that this separation of redox states is due to an energy barrier to H^+ pumping and electron flow imposed by the inner mitochondrial membrane potential, which is relieved as the mitochondria are activated.

Our results illustrate the fact that, in functioning mitochondria inside a living animal, the conditions are very different to cases where specific protein complexes are studied in great detail in isolation. In the intact mitochondrion within a cell, the particular complexes find themselves in conditions determined simultaneously by many influences like the mitochondrial membrane potential, substrate availability, the respiratory electron flux down the respiratory chain, by the states of electron donor and acceptor molecules, by the interactions with other ions and molecules

etc. Many of these are still poorly explored but we believe they are likely to hide important clues to understanding many mitochondrion-related illnesses. We also believe the approach presented here can help in deciphering at least some of them.

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The physiology of 'ooh' and 'aagh'

(Muscle cramp: circumstances, cures and causes)

Aaaagh! Just back from a marathon on your 25th birthday? Just gone to bed on your 65th? Cramp! Cramp is a condition of two surprising extremes: the old lying very still and the young at full pace. So what is cramp? How can it be prevented? What do we know about its physiology? I'll try to answer all three questions, but first, a definition: cramp is a painful involuntary muscle contraction that occurs in part of a muscle, feels like a hard knot and has an active electromyogram. Cramps can spread from the original focus or start again from a new one.

Circumstances

Most muscle cramps are benign but are also a sign in pathological conditions such as lower motor neurone disorders (e.g. amyotrophic lateral sclerosis: ALS), metabolic disorders (e.g. uraemia) and acute extracellular volume depletion (e.g. in cholera). For further information on these see Miller & Layzer (2005). Writer's cramp and other cramps associated with dystonia are believed to have an origin in the basal ganglia and are not covered here. Cramp occurs in patients undergoing haemodialysis, in response to some drugs, e.g. diuretics and in 30% of pregnant women in the last trimester. It mainly strikes when muscles are fully shortened and it is probably impossible to evoke cramp in a lengthened muscle (Bertolasi *et al.* 1993). Women appear to be more susceptible than men (Jansen *et al.* 1991). Muscles of the calf (84% of cramps) and feet (39%) are the most susceptible followed by the hamstrings and the quadriceps (Jansen *et al.* 1991). Any muscle can be affected as illustrated by Moss (1923) writing about the miners of the Agcroft and Pendleton pits: "If a man is attacked whilst lifting a full tub onto the rails cramp might occur in the arms, legs, or abdomen. If the latter, the man is put out of action immediately, the contortion of the abdominal muscles being so great as to form a lump the size of a cricket ball".

Cramp follows muscle fatigue and endurance athletes often experience cramp towards the end of their competition. At work, cramp often struck at the end of a shift in profusely sweating men doing hard physical jobs in hot environments. Labourers building the Hoover Dam on the Colorado river in the 1930s were particularly prone to attacks. In 1900 the French ship Bugeaud in the Red Sea reported forty-two cases for its stokehold but by 1928 a correspondent to the *Lancet* noted that the change from coal to oil as fuel had dramatically decreased the incidence of engine room cramp. During the early 20th century research into these heat cramps (e.g. Moss, 1923) did not get much further than suggesting that muscle fatigue, low plasma sodium and low plasma volume were the most likely causes.

In contrast to the race track and the stokers' hold, cramps also occur in bed and these rest cramps are more frequent as we get older and can seriously erode quality of life (Jansen *et al.* 1991). In a study of 233 people aged 60 years or more, 40% had cramps more than three



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times a week, and 6% reported daily cramps. The prevalence of nocturnal cramp in old age may be related to reduced limb blood flow as another study showed an association with peripheral vascular disease. Sleeping with the foot plantar flexed will also increase the incidence of calf cramp. The kick in swimming also leads to a very shortened and unloaded calf muscle which may be part of the explanation for swimming cramps. Anecdotally swimming cramps are also more common in cold water.

Cures

Like death and taxes, cramp has always been with us and so have bizarre preventative measures and cures. At the Science Museum in London you can see a medieval cramp ring. These were blessed and distributed by the monarch on Good Friday at the Chapel Royal in the Tower of London and were worn to cure 'the crampe' and 'the falling sickness' (epilepsy) (Fig. 2). Legend tells that a pilgrim returning from Jerusalem presented the original ring to Edward the Confessor and explained its miraculous properties to the king. Much later in 1809 Parr's *London Medical Dictionary* prescribed "tar water every night and morning" or "holding a roll of brimstone". Rather more scientifically, H. Charlton Bastian in Richard Quain's *Dictionary of Medicine* (1894) recommended contracting the antagonist or firm pressure around the thigh or on the sciatic nerve. However, as late as 1972, yes 1972, a letter to the *Lancet* proclaimed the efficacy of a flat horseshoe magnet 3 inches in length placed between the mattress and the lower sheet. Pickle juice has also featured in



Figure 1. A man suffering from cramp in the leg. Coloured aquatint after M Egerton, 1828. Credit: Wellcome Library, London.

recent home remedies. In the past, preventative measures for cramp in the heat were rather more rational and involved the worker taking isotonic drinks or more salt in food, but this in itself may have bad effects not least on blood pressure.

Drugs have, of course, been tried. Wolf first described the positive effects of the anti-malarial drug quinine on myotonia. Early work on isolated preparations found a curare-like inhibitory effect on the postsynaptic neuromuscular junction and a potentiating effect on the twitch action potential of curarised muscle. Quinine also presynaptically reduces the quantal content of the end-plate potential. Quinine is still the most effective drug; however, the US FDA banned over-the-counter sales in 1994 because of drug interactions and side effects such as tinnitus and more rarely low platelet count. In 2010, the UK Medicines and Healthcare products Regulatory Agency (MHRA) issued a drug safety update stating that its use should only be considered when cramps cause regular disruption of sleep and non-pharmacological measures have not worked. Incidentally, Indian tonic water still contains quinine concentrations close to the effective range but patients with compromised neuromuscular transmission should avoid this drink. Other drugs have been tried which aim to partially block transmission at different sites from spinal cord to muscle membrane (Miller & Layzer, 2005). Although success has been claimed (e.g. for the calcium channel blocker verapamil) none are used routinely.

So much for preventative measures; what can be done to relieve an attack? In electrolyte disturbances, saline given orally or, in the case of dialysis, intravenously usually ends the cramp. However, the most immediately effective acute measure is to lengthen the muscle by external force or by contracting the antagonist which will also evoke reciprocal inhibition. It is not yet certain whether regular muscle stretching or stretching before sleeping prevents cramp.

Causes

Cramp is associated with ischaemic-like pain but not paraesthesia. Recent research suggests that ischaemic pain arises when ATP, and lactate, which are released from oxygen-deprived, contracting muscle, increase the ability of acid-sensing channel number 3 (ASIC3) on nociceptors to respond to a subtle decrease in pH. It is very likely that a similar mechanism gives rise to the pain of cramp.

The main feature of cramp is the muscle contraction. Here the major debate is whether it has a central or peripheral origin i.e. spinal or supra spinal vs. nerve endings or muscle fibres. There are several findings which support the central hypothesis. Cramp can be evoked in very susceptible individuals by stimulation of IA afferents using an H reflex technique (Baldissera *et al.* 1994). It is suppressed by contraction of the antagonists (Norris *et al.* 1957) and by cutaneous (Baldissera *et al.* 1994) or tendon stimulation. Norris *et al.* (1957) reported synchronous firing of different motor units and Ross & Thomas (1995), using tungsten electrodes, recorded potentials indistinguishable from those found in voluntary contractions. All these findings suggest coordination from the spinal cord.

The peripheral hypothesis also has experimental findings in its favour. Cramps spread outwards from a focus into neighbouring muscle tissue whereas voluntary contractions activate motor units throughout the muscle (Roeleveld *et al.* 2000). Some of the best evidence comes from work on twitch-like muscle fasciculations. Cramp is often associated with spontaneous benign fasciculations and the mechanisms are probably identical. Denny Brown & Foley (1948) found that single fasciculation potentials changed shape suggesting locally activated fractions of motor units. Another result showing the peripheral origin of some fasciculation potentials was their observation that potentials moving antidromically from the periphery occasionally cancelled voluntary potentials descending from

the spinal cord. Later, authors using experimentally induced collisions, showed that most fasciculation potentials have a peripheral origin. Finally, Bertolasi *et al.* (1993) reported a critical result favouring the peripheral hypothesis. They found they could still evoke cramps by electrical stimulation distal to a complete peripheral nerve conduction block. They also noted that even in muscle with a complete nerve block, lengthening still suppressed the cramp, suggesting a peripheral mechanism for this effect as well.

If the initiation of cramp is peripheral where and when does it occur?

A working hypothesis is that spontaneous activity (ectopic firing) arises in peripheral nerve endings which then spreads to neighbouring excitable tissue by direct contact (ephaptic spread), a process which probably also has a role in epileptic seizures. There is some evidence which allows us to narrow down to a possible site. For example, the ectopic foci for fasciculations are probably at or beyond the motor nerve endings as peripheral nerve block does not abolish them (Minetto *et al.* 2010). Curare and botulinum toxin both block cramp so the foci must be proximal to the muscle membrane and prior to the endplate. The most likely region is the last unmyelinated part of the nerve endings where sodium and potassium channels are located.

Ectopic and ephaptic activity is most likely to occur when: axons are closely apposed; there is high extracellular resistance; electrolyte balance is disturbed; and there is prior repetitive discharge. All of these conditions are also associated with cramp. A shortening and contracting muscle will press axons and muscle fibres closer together. It has been calculated that pressures in a fully contracted human gastrocnemius can rise to 43.79 kPa (328 mmHg), which is more than sufficient to also cause ischaemia. Osmotically induced cell swelling will also crush muscle fibres and nerve endings together and increase the likelihood of ectopic firing. For example,

hyposmolar solutions increase, and hyperosmolar decrease, ectopic type firing in hippocampal slices. Stewart *et al.* (1972) suggested that such cell swelling occurred in dialysis and could be responsible for dialysis cramps. Volume expansion with either saline or hypertonic dextrose relieves these cramps. The equal effectiveness of dextrose highlights the role of low plasma volume as a cause of cramp.

There is considerable evidence that extracellular ionic disturbances are related to ectopic firing in nerve and fasciculations in muscle. The exact contribution of potassium and sodium concentrations and currents has not been definitely determined but these must be prime candidates. There are some clues, however. The fasciculations of ALS appear to be due to both persistent sodium currents, and reduced potassium currents. Potassium accumulates extracellularly around fatiguing muscle and prolonged stimulation of axons also results in extracellular K⁺ accumulation. It is also possible that acetylcholine acting presynaptically may generate ectopic activity in motor nerve endings.

Cramp is more common in shortened muscle. A possible explanation is that the local concentrations of these ions and molecules may be increased by reduction of extracellular space in extreme shortening, thus making ectopic and ephaptic activation more likely. Finally, ephaptic spread is also more likely if cells fire repetitively and in this context it is interesting to note that the higher the frequency the shorter the duration of the tetanic stimulation required to evoke cramp (Bertolasi *et al.* 1993).

From the discussion so far it would appear that the hypothesis for a purely 'peripheral' origin of cramp has the most direct and circumstantial evidence in its favour. However, recent careful work by Minetto *et al.* (2010) has challenged this view. They showed that electrically evoked cramps do occur with a proximal nerve block but only lasted less than 3 s. Only in the unblocked condition did full-blown cramps lasting 30 s or more occur. A sensible interpretation of these results is a synthesis of the peripheral and central hypotheses in which the initiation of cramp is local

but its development needs spinal connectivity.

In summary, it is surprising that we still know so little about cramp, a common phenomenon which will certainly dash the dreams of many a 2012 Olympic hopeful. Any future breakthrough in understanding and treatment will require research on both the mechanisms of ectopic foci, and on the role of spinal circuits in both animal and human models of cramp.

In the meantime keep your muscles stretched, your drinks long and your salts balanced!

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Figure 2. Queen Mary I blessing cramp-rings. After a miniature in Queen Mary's Manual, Westminster Cathedral Library. By HM Hayman. Credit: Wellcome Library London.

Pulsed infrared radiation: a potential tool to reveal cellular secrets

Pulsed infrared radiation (IR) has been shown to stimulate excitable cells through activating key intracellular signalling events. Data suggest the presence of fundamental IR-sensitive mechanisms that generalize across cell types. Our recent results demonstrate the importance of IR-modulated intracellular Ca^{2+} signalling in cardiomyocytes and inner-ear hair cells. Application of pulsed IR to modulate intracellular Ca^{2+} may be particularly useful to study events controlling synaptic transmission and excitation–contraction coupling.

Arsonval (1891) was the first to demonstrate endogenous photosensitivity of cells. It was later recognized that the responses to 'light' are widespread and even occur in cells that are normally shielded from optical radiation. For example, it has been known for five decades that optical radiation can evoke action potentials in neurons (Arvanitaki & Chalazonitis, 1961), and that optical responses can be exploited to identify functional connections in neural networks. Since the invention of the laser, radiations of various wavelengths have been used to irradiate and stimulate neurons. The primary mechanism of action in these early studies was thermal responses of ion channels and the heat deposited by optical radiation. The non-specific thermal origin of responses dampened broad use of optical stimuli. The discovery of direct light-activated ion channels (Nagel *et al.* 2002) and the development of techniques for photocontrol of neurons *in vivo* (Boyden *et al.* 2005) has provided a new set of optogenetic tools for photo-excitation and -inhibition of cells independent of heat. But heat itself can be a powerful stimulus. New laser and optical technologies have enabled the delivery of very short focused pulses of IR, a stimulus that delivers transient pulses of thermal energy (dT/dt) without large increases in accumulated temperature of cells or tissue. Pulsed IR activates endogenous physiological mechanisms within the cell, and the mechanisms may vary based on the heterogeneous absorption spectra of the key molecules expressed. IR stimuli have been successfully applied for neuroprostheses (Wells *et al.* 2005; Rajguru *et al.* 2010), cardiac



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pacemaking (Jenkins *et al.* 2010) and studies of intracellular signalling (Dittami *et al.* 2011).

Wells *et al.* (2005) applied pulsed IR to evoke action potentials in regions of the rat sciatic nerve to elicit contraction of distinct muscle groups. Pulsed IR has been used to stimulate the facial (Teudt *et al.* 2007) and auditory nerves (Izzo *et al.* 2006). Although it is clear that pulsed IR delivers transient kinetic energy to molecules comprising the cell, which specific molecules are responsible for excitability has been a subject of debate and probably

varies between cell type, location of the radiation and the wavelength. Transient receptor potential channels (TRPs), stretch-activated channels and biochemical kinetics (e.g. Arrhenius model) may all be involved. It is clear that IR applied to the cell body or synapse modulates intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$). We have reported evidence in rat neonatal cardiomyocytes that 1862 nm pulsed IR activates mitochondrial Ca^{2+} flux and that blocking the mitochondrial Ca^{2+} uniporter (mCU) or the mitochondrial Na^+ – Ca^{2+} exchanger (mNCX) blocks the major component of IR excitability in these cells (Dittami *et al.* 2011). Assuming that this IR-modulated Ca^{2+} signalling is a general principle, we expected the inner-ear hair cells to also be sensitive to IR due to their high mitochondrial density and the Ca^{2+} -dependent synaptic machinery. In the hair cells, intracellular Ca^{2+} plays an important role in

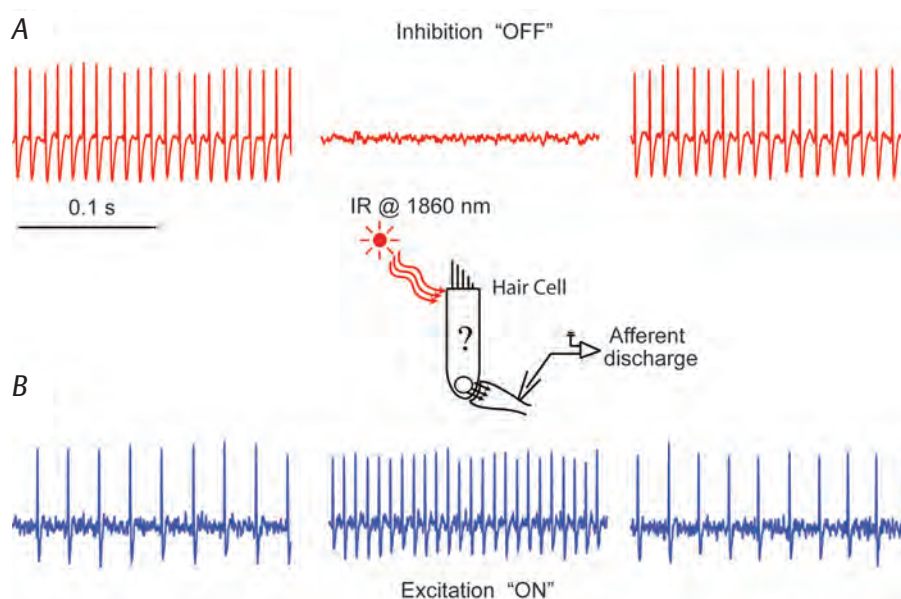


Figure 1. Sample records showing the responses evoked in two different post-synaptic afferents by pulsed infrared irradiation (IR ~1860 nm) of the vestibular horizontal canal hair cells: inhibitory 'off' (A) and excitatory 'on' (B). The response type was independent of the rate of IR stimulation or the amount of radiant exposure. The mechanism by which IR induces the intracellular changes and leads to the observed responses remains in question.

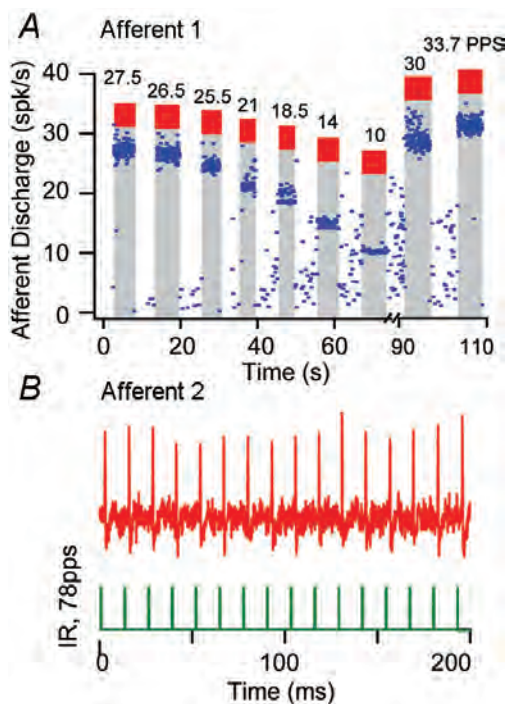


Figure 2. Afferents phase-locked to IR pulse trains. **A**, example phase locking of afferent discharge at various IR pulse train frequencies (10–33.7 pps). **B**, second example showing phase locking at ~78 spikes s⁻¹ in response to 200-μs-long, 610 μJ per pulse, IR pulses at 78 pps (Rajguru *et al.* 2011).

signal transduction. Increases in intracellular Ca²⁺ have been shown to be involved in several important processes in mammalian hair cells, including adaptation of the mechanotransducer current and in triggering neurotransmitter release.

In a recent study published in *The Journal of Physiology*, we applied pulsed IR (1860 nm, pulse durations ranging from 200 to 750 μs and with pulse repetition rates of up to 100 Hz) to stimulate presynaptic hair cells in the vestibular organs of the toadfish, *Opsanus tau*, and recorded evoked responses from the post-synaptic afferent neurons *in vivo* (Rajguru *et al.* 2011). The radiation was delivered to the vestibular epithelium using a 200 or 400 μm optical fibre coupled to a diode laser. This non-contact method allowed stimulation and neural recordings to be carried out for many hours. IR stimulation of hair cells resulted in robust single-unit semicircular canal afferent responses (Fig. 1). Some afferent neurons were excited, some were inhibited, and some showed a mixed inhibitory–excitatory pattern. Afferents that were tested for phase-locking to pulsed IR up to 100 Hz, responded pulse-by-pulse with an action potential (Fig. 2). The responses of the post-synaptic afferents did not diminish after many

hours of stimulation. Responses were not due to overall changes in accumulated temperature of the sensory organ, but probably were evoked by heterogeneous changes in transient molecular thermal kinetic energy imparted by the IR. IR pulse-by-pulse evoked responses suggest that the transient energy relaxed prior to arrival of the subsequent IR pulse. Also, the IR-evoked responses persisted when the whole-body temperature was reduced to the extent expected to close key TRP channels. In rat neonatal cardiomyocytes, IR evoked intracellular [Ca²⁺]_i transients, distinct from spontaneous [Ca²⁺]_i events and persisted after blocking the Ca²⁺-activated Ca²⁺ machinery of the cell. These results raise the possibility of using pulsed IR to study certain events related to excitation–contraction coupling and synaptic transmission that are difficult to examine using conventional approaches.

Recent results using pulsed IR stimuli clearly demonstrate its potential for basic research and suggest promise for the future development of therapeutic interventions. Realizing the full potential of IR stimuli, however, remains limited by inadequate characterization of stimulus-dependent cellular/molecular mechanisms.

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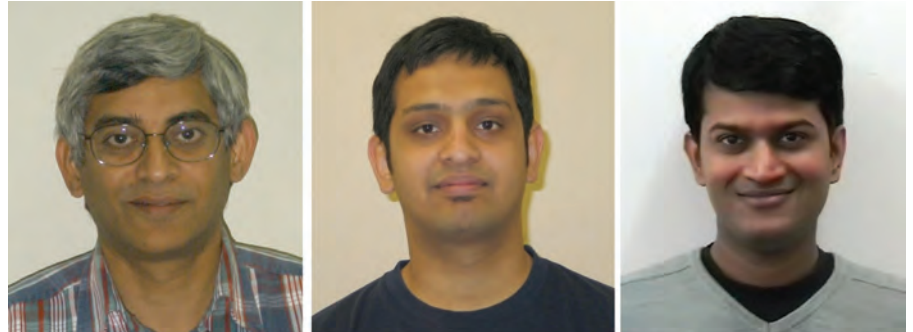
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How does the visual brain code contours?

Neurons in the primary visual cortex of mammals show a remarkable selectivity for the orientation of contours in the visual scene. The neuronal basis of this property has been the subject of intense debate for nearly half a century. Our recent work throws new light on this problem.

Various parts of the cerebral cortex, whether they receive visual, auditory or other sensory inputs or signals from other parts of the cortex, do not fundamentally differ in structure, barring minor variations. Thus, the distinctive function of a cortical region may be determined largely by its inputs and not so much by any intrinsic structure specific to each cortical area. This suggests that there exists a canonical microcircuitry of the cortex that enables the impressive transformations in the coding of the sensory signals arriving at the primary sensory areas. The major breakthrough in our understanding of such a fundamental circuitry came with the studies on the mammalian primary visual cortex by Hubel and Wiesel (1962). Their discovery of the remarkable selectivity for the orientation of an edge or a line shown by primary visual cortical cells and an elegant model they put forward to account for this has inspired a whole host of



From left to right, Trichur Vidyasagar, Jaikishan Jayakumar and Sivaram Viswanathan.

experiments over the last 50 years. It has also remained as one of the most intensely debated schemes in all sensory neuroscience. Many laboratories, including ours, are now bringing us closer to a comprehensive understanding of a basic circuitry of the way sensory signals are coded by primary sensory areas.

Hubel and Wiesel suggested that the orientation selectivity of single cortical cells arises from an excitatory convergence they receive from neurones in the dorsal lateral

geniculate nucleus (LGN) of the thalamus, that themselves have circular receptive fields and lack any orientation selectivity. In this scheme, the receptive fields (RFs) of these LGN cells were assumed to be along a row in visual space, so that a long contour of the right orientation that stimulates this row of LGN RFs would best excite the cortical cell that they all project to (Fig. 1A). There have been two other schools of thought that have consistently questioned this scheme: (1) Intracortical mechanisms, especially excitation from cells tuned to the

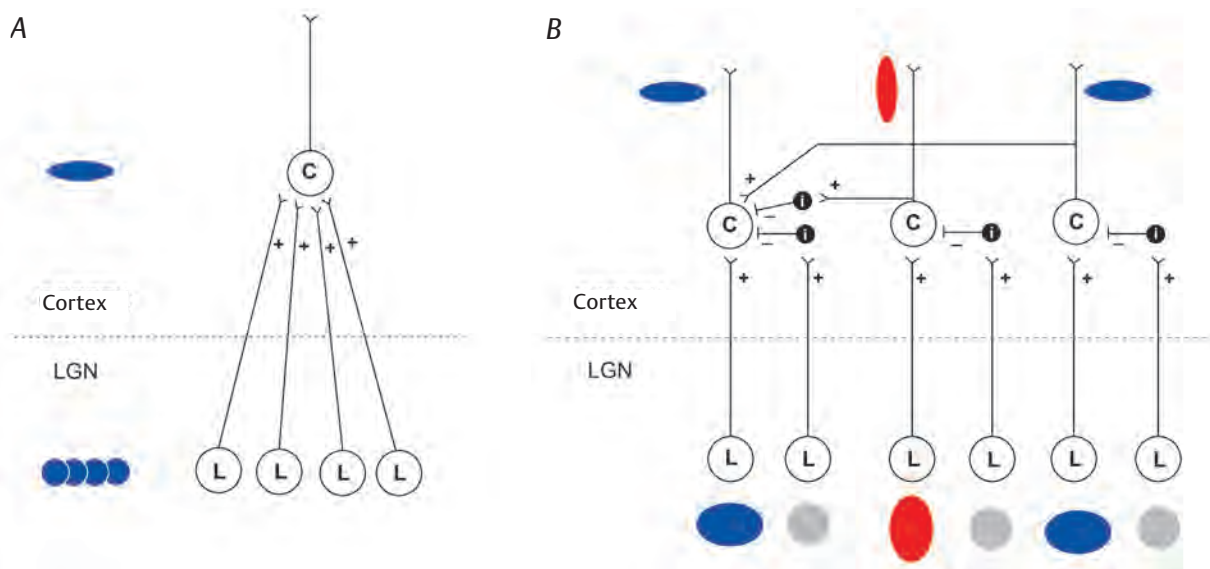


Figure 1. A, Hubel and Wiesel's (1962) scheme where orientation selectivity of a cortical cell (C) is generated by a group of LGN cells (L), whose receptive fields are arranged in a row in visual space. B, alternative model we favour where orientation selectivity is based on the orientation biases of the LGN receptive fields. This orientation bias is sharpened by a disynaptic inhibition from the LGN via intracortical inhibitory interneurons (i) and possibly further sharpened by intracortical mechanisms such as cross-orientation inhibition and iso-orientation excitation. +, excitatory connections; -, inhibitory inputs.

optimal orientation and inhibition from cells tuned to orthogonal orientations, act on an excitatory input from the LGN that is not tuned to stimulus orientation (Creutzfeldt *et al.* 1974; Sillito *et al.* 1980; Douglas *et al.* 1991); (2) The mild degrees of orientation sensitivity that are already present in single thalamic neurones, when acted upon by intracortical inhibition, can lead to sharp orientation selectivity at the cortical level (Vidyasagar &

Heide, 1984; Vidyasagar *et al.* 1996) (Fig. 1B).

Though the debate has been dragging on without a general consensus, recent experiments may be taking us towards a resolution. Kara *et al.* (2002) invented a clever protocol to 'isolate' the raw geniculate input to the cortex without any cortical influences by applying a particular train of electrical stimulation to the LGN. Such stimulation

excites, among other cells, cortical inhibitory interneurons and leads to a profound suppression of all intracortical interactions. Kara *et al.* (2002) found that the orientation selectivity of cells receiving direct geniculate inputs was not significantly reduced by the electrical stimulation in the LGN. They interpreted this as support for Hubel & Wiesel's scheme of excitatory convergence, since the stimulation had effectively 'silenced'

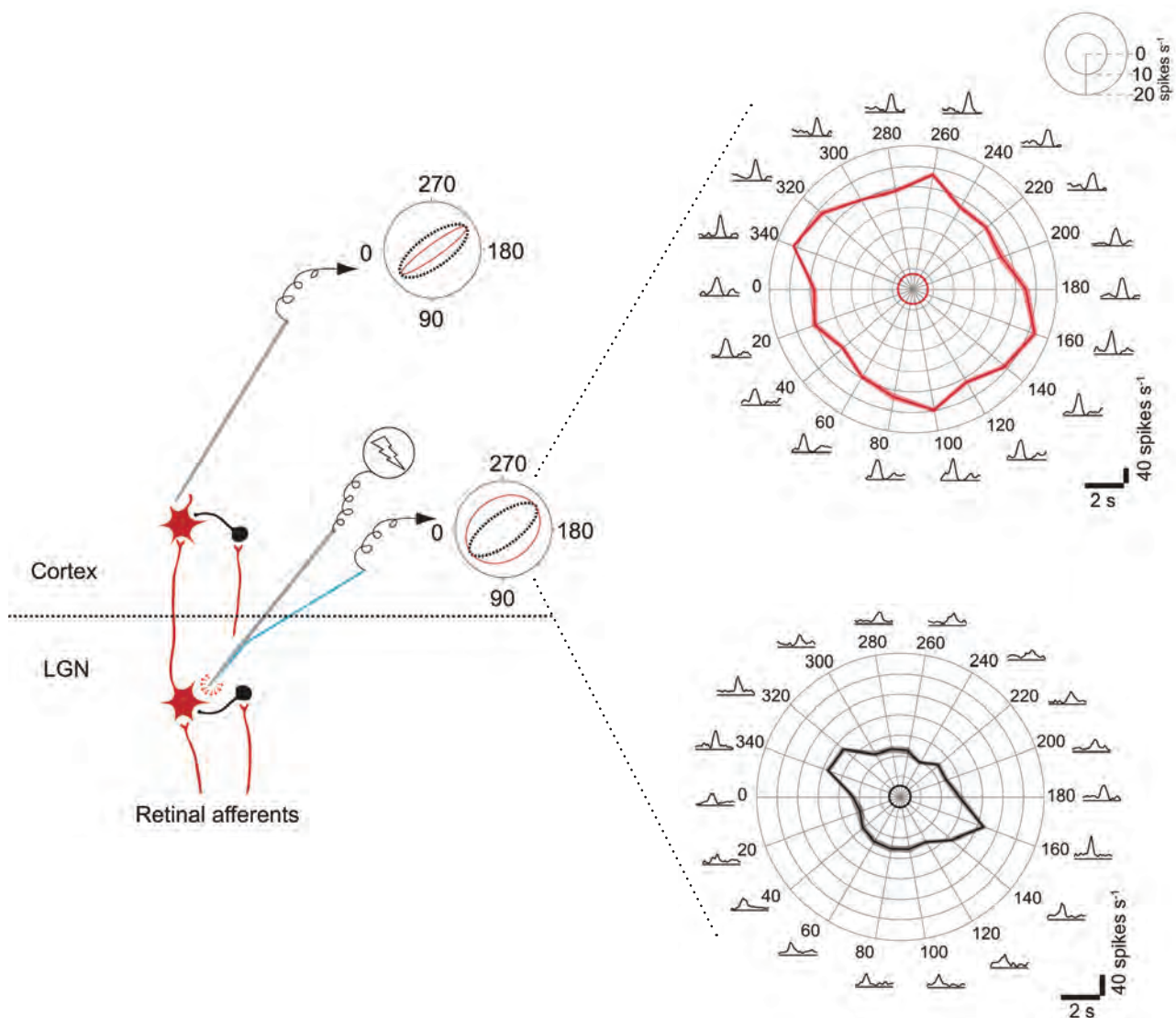


Figure 2. Schematic diagram of our experimental protocol, which is a modified version of a protocol by Kara *et al.* (2002). The original Kara *et al.* protocol (represented by grey electrodes) consisted of electrical microstimulation of the LGN and simultaneous recording from visual cortical neurones. Our modified protocol consisted of a dual-electrode assembly in the LGN (blue electrode) which permitted recording of single LGN neurones while stimulating the LGN at the same place. Hypothetical orientation selectivities of cortical and LGN cells are shown next to the respective electrodes as polar diagrams (control responses in red and responses during electrical stimulation in the LGN in black). As predicted by the scheme in Fig. 1B, LGN selectivity gets sharpened and the cortical selectivity broadens (see Viswanathan *et al.* 2011 for details). The inset on the right represents the responses of an LGN cell whose orientation bias (top panel, red tuning curve) was considerably sharpened during electrical stimulation (bottom panel, black tuning curve). The peristimulus time histograms of the responses to bars of different orientations are also shown. (Figure adapted from Viswanathan *et al.* 2011.)

the cortex. However, an alternative interpretation that we favour is that the electrical stimulation in the LGN also activates intrageniculate inhibition which would sharpen the orientation bias that the geniculate cells inherit from the retina. In that case, during the electrical stimulation, the signal reaching cortical cells in single geniculate afferents could already be sharply tuned for orientation. To test this suggestion, we constructed special electrodes that had a recording electrode glued to the LGN stimulating electrode and we recorded the orientation selectivity of the LGN cells themselves before and during electrical stimulation in the close vicinity (Viswanathan *et al.* 2011). Figure 2 (left panel) shows our variation on Kara *et al.*'s design. We found that LGN cells showed a significant increase in their orientation selectivity during local electrical stimulation (Fig. 2, right panel). Thus, the sharp tuning seen in the raw geniculocortical input (Kara *et al.* 2002) could reflect to a large extent the sharpening that happens in the LGN itself and need not be due to the pattern of geniculocortical convergence originally suggested by Hubel and Wiesel.

Our results imply that any non-specific inhibition, such as that happening within the cortex during normal visual stimulation and acting on an orientationally biased input to a cortical cell, can lead to sharp orientation selectivity in the cortex. Consistent with this suggestion, we also found that iontophoretic application of the inhibitory transmitter, GABA, near an LGN cell could also markedly sharpen the orientation selectivity of the cell. The sharper, band-pass spatial frequency selectivity of cortical cells compared to the low-pass LGN and retinal cells indicates that non-specific inhibition from a larger surround does occur at the cortex, as indeed it has been shown in experiments using an antagonist of GABA-mediated inhibition (Vidyasagar & Mueller, 1994). It is also now well established that the orientation bias seen in

the LGN (Vidyasagar & Heide, 1984; Soodak *et al.* 1987) and retina (Levick & Thibos, 1980) are apparent mainly towards the higher spatial frequency end of the broad spectrum of spatial frequencies that these cells respond to. Thus, non-specific intracortical inhibition simultaneously bestows on the cell selectivities for both spatial frequency and orientation (Vidyasagar & Heide, 1984).

Other recent experiments (Jin *et al.* 2011) have suggested that the receptive field scatter of thalamic afferents in the cortex is less than what was believed based upon the classical scheme of Hubel and Wiesel, and hint at a substantial role for subcortical mechanisms in generating the cortical selectivity for stimulus orientation. If orientation is indeed coded in the retina and sharpened further at thalamic and cortical levels, there are some constraints if the system is to preserve resolution and sensitivity and still have a broad range of orientations represented in the cortex. Orientation can be coded by retinal ganglion cells only in a limited number of broadly tuned channels, as is done for processing of colour by the three cone types. Consistent with this, we find that retinal and LGN cells are not only broadly tuned for orientation, but their preferred orientations seem to be close to the radial orientation (i.e. pointing towards the centre of the retina) or its tangential orientation (Levick & Thibos, 1980; Vidyasagar & Urbas, 1982; Shou & Leventhal, 1989). Thus, the remarkable orientation selectivity of visual cortical cells first described by Hubel and Wiesel in a pioneering article in *The Journal of Physiology* almost 50 years ago may turn out to be the outcome of a number of mechanisms that build upon the receptive field asymmetry present in retinal ganglion cells.

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Tissue biomechanics as a modulator of lymphatic function

The lymphatic network represents a good example of how biological structures maximise their efficiency by exploiting the anatomical and biomechanical properties of their microenvironment. Indeed, the stiffness of the extracellular matrix may directly affect the absorptive and/or propulsive nature of a lymphatic vessel, thus exerting an important modulatory role in lymphatic function.

The diaphragmatic lymphatic network represents a good example not only of the mechanisms of lymph formation and transport along the complex lymphatic microvasculature, but also of how the biological structures maximise their efficiency with minimal expense by exploiting in the best possible way the anatomical and biomechanical properties of their biological microenvironment. In the extracellular compartment, freely moving water is distributed between the intravascular plasma and the interstitial fluid filling the porosity of the three-dimensional extracellular fibrous matrix. Interstitial fluid derives from plasma and is mostly filtered through paracellular pathways between adjacent endothelial cells, down a favourable transendothelial pressure gradient resulting from the imbalance of hydraulic and colloid osmotic pressures. A crucial element of this process is interstitial fluid pressure (P_{int}), a complex parameter which simultaneously depends upon tissue hydration, the mechanical behaviour of the matrix and the osmotic forces exerted by the matrix itself. At normal tissue hydration, baseline P_{int} is usually subatmospheric and may change, either towards more positive or more negative values, in response to the displacement of the surrounding tissue, thus affecting the transendothelial fluid filtration process. For example, in the lung parenchyma, characterised by very negative end-expiratory P_{int} values, fluid filtration occurs across both the arteriolar and venular side of the capillary and further increases when P_{int} drops on inspiration. Convective fluid filtration from the microvasculature to interstitium is accompanied by solutes and protein fluxes. Therefore, progressive departure of



Daniela Negrini (left) and Andrea Moriondo

plasma volume and proteins and an increase of interstitial fluid volume (a condition called oedema), would occur if excess interstitial fluid and solutes were not continuously removed into the initial lymphatics, small dead-end saccular structures dispersed among the matrix fibres. The lymphatic system has developed in the majority of body organs of

vertebrates to remove fluid, solutes and even cells from the tissues and return them to the venous blood stream, thus maintaining fluid and solute homeostasis in both the extravascular and intravascular environments. Lymph flow is sustained by pressure gradients ($\Delta P_{\text{TM-lymph}}$) developing when intraluminal lymphatic pressures drop below P_{int} ; the newly formed lymph is thereafter directed, with the aid of unidirectional intraluminal valves, into larger collecting lymphatics, equipped with contractile smooth muscle cells. Favourable $\Delta P_{\text{TM-lymph}}$ levels develop as a consequence of cyclic contraction of lymphatic smooth muscle cells and/or of tissue displacement (Schmid-Schönbein, 1990; Aukland

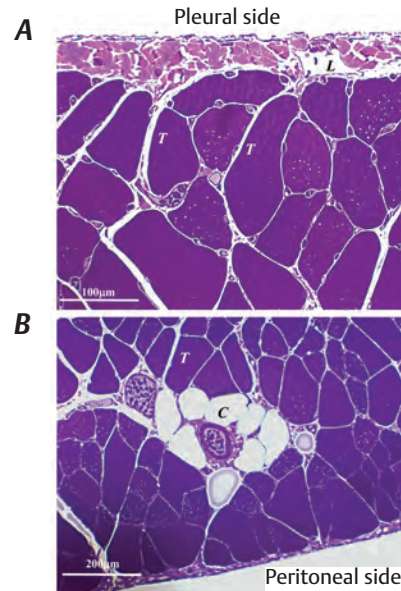


Figure 1. Arrangement of the diaphragmatic lymphatic network. *A*, semi-thin cross-section of rat pleural hemi-diaphragm stained with crystal violet and basic fuchsin. Submesothelial lymphatic lacunae (L) are located within the interstitial space between the pleural mesothelial surface and the skeletal muscular fibres plane. Transverse lymphatic vessels (T) running through the diaphragmatic muscular fibres connect the superficial lacunae to the deeper collecting lymphatic ducts (*B*). *B*, semi-thin cross-section stained with crystal violet and basic fuchsin showing the central part of the central diaphragm and the peritoneal mesothelial surface. Transverse lymphatic vessels (T) leaving from the pleural side (on top, not included in the section) and the peritoneal submesothelial lacunae empty into central lymphatic collectors (C), located in the central diaphragm adjacent to the main blood vessels, recognizable in the section by the presence of red blood cells in their lumen.

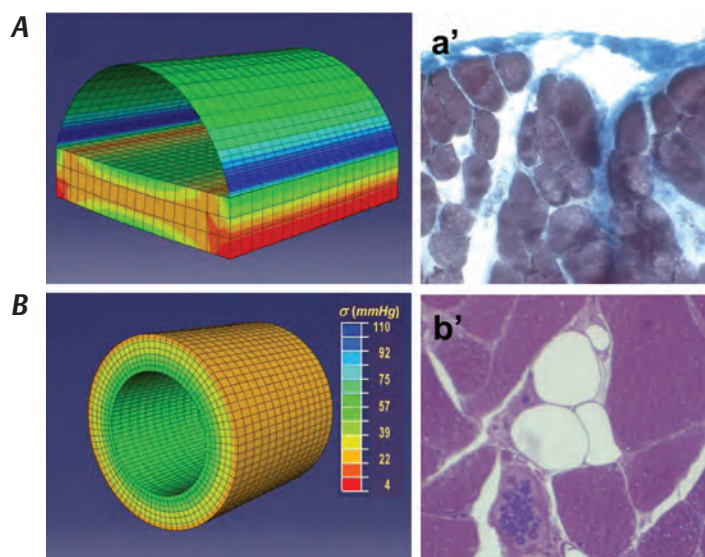


Figure 2. Finite element modelling (FEM) of the diaphragmatic lymphatic vessel wall. A, stress distribution map, obtained through finite element modelling for a diaphragmatic initial lymphatic delimited (as in the subpleural lacuna presented in panel a') on the top side by the compliant mesothelium and on the bottom side by the stiffer muscular and/or tendinous plane, as shown in the section of panel a'. B, stress distribution map of the wall of a deeper collecting lymphatic entirely delimited by a stiff isotropic skeletal muscular tissue in the central diaphragm (as in section b'). The circumferential stress (σ) distribution is identified by colours on a scale from red (low stress) to blue (high stress) as indicated by the colour scale in B. Circumferential stress and wall deformation is higher in submesothelial superficial compliant vessels (A), where opening of the unidirectional valves is favoured by the high tangential stress (blue area) developing at the junction between the mesothelial and muscular planes. In deep vessels with a stiffer wall (B), circumferential stress is lower and homogeneously distributed over the entire surface. Therefore, submesothelial compliant vessels seem mechanically more adequate to absorb fluid from pleural/peritoneal cavities, whereas deeper and stiffer vessels would more efficiently exploit local tissue forces to propel fluid through the lymphatic network.

& Reed, 1993). For example, during spontaneous breathing, the average $\Delta P_{\text{TM-lymph}}$ in intercostal lymphatics over a minute ventilation is positive (~ 4 – 10 mmHg) (Moriondo *et al.* 2005), suggesting that rhythmic expansion of the chest favours lymph formation. However, when the same tidal volume is attained through mechanical ventilation with positive alveolar pressure, $\Delta P_{\text{TM-lymph}}$ is nullified (Moriondo *et al.* 2005), indicating that not simply the degree of tissue displacement, but rather the magnitude and direction of local forces developing across the lymphatic vessel wall is critical in supporting lymphatic function.

For tissue displacements to be able to support the development of $\Delta P_{\text{TM-lymph}}$, thereby promoting lymph formation and further progression along the network, local tissue stresses must be transmitted to the

lymphatic lumen and converted into intraluminal pressure waves. In turn, force transmission is influenced by the mechanical properties of the vessel wall and is expected to be more effective in stiff vessels characterized by a lower compliance compared to distensible, highly compliant structures. Even if belonging to the same drainage network, the diaphragmatic lymphatic vessels display a rather remarkable heterogeneity. Indeed, deepening from the pleural and peritoneal mesothelial surfaces into the diaphragmatic tissue, the lymphatic network is organised in: (a) submesothelial lacunae, located above or partially within the submesothelial interstitial space (Figs 1A and 2A); (b) deep central collectors partially equipped with smooth muscle cells (Figs 1B, and 2B), transverse lymphatic ducts (Fig. 1A and B) running

through muscular and tendinous diaphragmatic fibres and connecting the submesothelial lacunae to the central collectors (Grimaldi *et al.* 2006).

A finite element model of the mechanical properties of the vessels based on direct measurements of wall compliance in lymphatic vessels running over the pleural diaphragmatic surface and of the elastic modulus of the tendinous and muscular relaxed diaphragmatic tissue (Moriondo *et al.* 2011) revealed that these vessels exhibit changed mechanical behaviour in relation to the different structure of their wall and of the surrounding tissue. Vessels delimited by a compliant tissue act as distensible fluid reservoirs, whose walls are subject to high tangential stresses favouring the opening of primary unidirectional valves in the vessel wall (Fig. 2A); these vessels, corresponding in the diaphragm to the submesothelial diaphragmatic lacunae, are therefore favourable sites of drainage of fluid from the pleural and the peritoneal cavities. In contrast, vessels surrounded by stiff tissue, such as those running deep among diaphragmatic muscular or tendinous fibres (Fig. 2B), are characterized by a fast and efficient transmission of tissue forces to the vessel lumen and are therefore better suited to propel the newly formed lymph along the lymphatic collecting network. Therefore, more generally in the lymphatic system, vessels supplying skeletal muscle or stiff actively moving tissues, such as the lung, may not necessarily need the phasic synchronized contraction of lymphatic smooth muscle cells usually encountered in most collecting ducts to sustain local lymphatic function.

Based on these considerations, the arrangement of the matrix fibres and their mechanical properties emerge as an important factor in setting, maintaining and modulating the local lymph flux in initial lymphatics and therefore in the entire lymphatic network. It is worth

recalling that, even in collecting lymphatics, wall stiffness and therefore the mechanical response of the vessels is both physically and tonically increased by contraction of smooth muscle cells in the external vessel layer. Therefore, a large variety of pathophysiological conditions, such as neuromuscular failure, skeletal motor weakness, disturbance of smooth muscle contraction, disorganization of the macromolecular composition and/or arrangement of the fibrous tissue scaffold, like that observed during development of pulmonary oedema (Negrini *et al.* 1996), by modifying the mechanical features of the lymphatic vessel wall and adjacent tissues, may greatly impair normal lymphatic function, thus contributing to the development of oedema and exacerbation of tissue and organ damage.

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The trouble with CO₂

We breathe for two reasons: to take in O₂ and to regulate the amount of dissolved CO₂ in the blood. This latter function is fundamental to the control of the pH of blood and hence is a vital homeostatic function. For many years the quest to understand the regulation of breathing by CO₂ has concentrated on how the brain measures the pH of blood. New evidence now suggests that the brain also directly measures the level of dissolved CO₂.

The trouble with CO₂ is that it readily combines with water to form H₂CO₃, which dissociates rapidly to H⁺ and HCO₃⁻. In any solution therefore, the partial pressure of CO₂ (P_{CO_2}) will be in equilibrium with, and thus inescapably related to, the pH and the concentration of HCO₃⁻ of that solution. In terms of physiology, the P_{CO_2} in blood will be an extremely important determinant of the pH of all bodily fluids including the cerebrospinal fluid in the central nervous system. It should come as no surprise that physiological reflexes control the levels of P_{CO_2} via the frequency and depth of breathing, as excretion of CO₂ via the lungs is the main mechanism to control its levels in the body. If P_{CO_2} in arterial blood increases, so does the respiratory rate and tidal volume. If the converse happens and P_{CO_2} in arterial blood decreases, then there is a corresponding reduction of respiratory rate and tidal volume.

Any physiological reflex requires at its initiating apex a sensory mechanism – in this case a mechanism of ‘chemosensory transduction’. Here the trouble with CO₂ comes to the fore – what does the body measure: CO₂, pH, HCO₃⁻, or some combination? All three are inextricably linked and in that sense any one of them would ‘do’. Without knowing the key molecules that are detected by the chemosensors, identifying the molecular transducers involved in the chemosensory reflex is challenging. To make life even more difficult for observers of this field, the phrase ‘CO₂ chemosensitivity’ is used loosely to denote responses to changes in P_{CO_2} irrespective of whether this is mediated by direct



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detection of CO₂ or indirectly via consequent changes in pH or HCO₃⁻.

The great German physiologist Loeschcke published an influential review in *The Journal of Physiology* in 1982, in which he outlined ‘reaction theory’. According to Loeschcke, the respiratory chemosensitive reflexes depended upon the detection of pH and pH alone. This primacy of pH in chemoreception is reflected in other fields of physiology including, for example, CO₂ cerebrovascular reactivity and the control of vasodilatation. In the following years pH has been largely regarded as the chemosensory signal and thus much research effort has hinged on studying pH-sensitive ion channels as potential molecular transducers of respiratory chemoreception. So far, genetic manipulation of these channels has failed to provide evidence for their involvement in central mechanisms of chemoreception.

In addition to this identification of pH as the key chemosensory signal, Loeschcke, Schlaefke and Mitchell made the major advance of identifying the ventral surface of the medulla oblongata as being a key site of central CO₂ chemosensitivity. The carotid body, of course, provides a peripheral location for CO₂ and O₂ chemosensitivity, but for CO₂ the

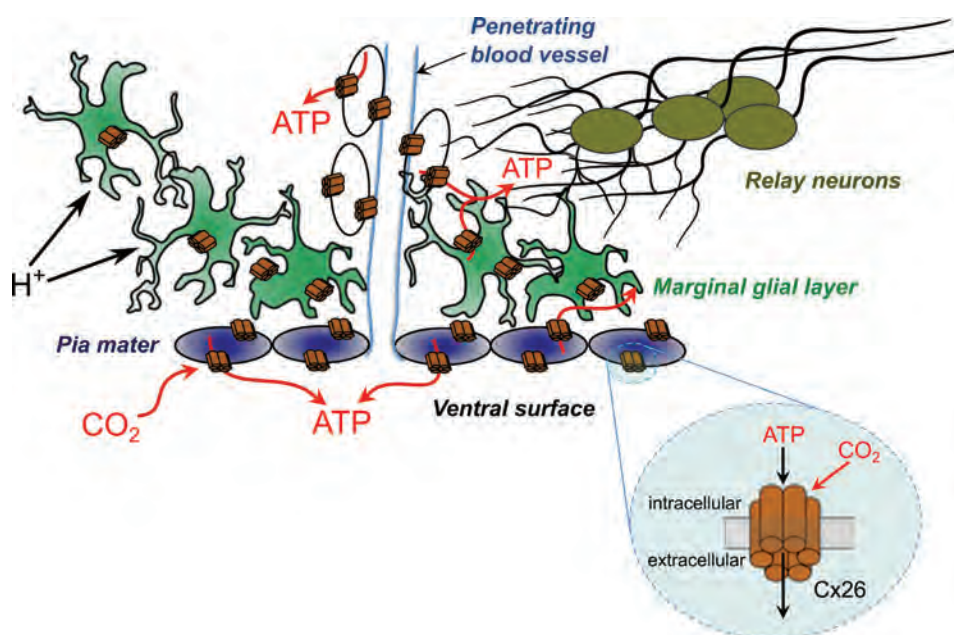


Figure 1. Chemosensory mechanisms at the ventral surface of the medulla oblongata. Connexin 26 (Cx26) is found in the cells of the pia mater (blue cells), the astrocytes of the marginal glia (green irregular-shaped cells) and cells at the external surface of penetrating blood vessels (white cells). CO₂ acts directly on Cx26 (expanded inset) to cause it to open and release ATP which can then act on P2 receptors of relay neurons to cause the adaptive change in breathing. The astrocytes of the marginal glial layer are also sensitive to changes in pH. This gives two parallel pathways of chemoreception at the ventral surface of the medulla.

central sites of chemosensitivity are thought to predominate. Yet even within Loeschcke's 1982 review, and in his own prior work as well as the subsequent work of other groups (e.g. Eldridge *et al.* 1985), there are strong indications that pH is not the only chemosensitive signal. For example, comparison of the effect on respiration of applying acidic saline to the medulla oblongata with inhalation of additional CO₂ to induce the same pH change in the medulla showed that the combination of an increase in P_{CO_2} and a decrease in pH was a more powerful stimulant than a decrease in pH alone. In retrospect, it seems puzzling that Loeschcke did not acknowledge that these observations were discordant with reaction theory but instead spent considerable effort in 'arguing them away'. To quote Eldridge *et al.* (1985): "We conclude that the e.c.f. [extracellular fluid] [H⁺] does not represent the unique stimulus to the central chemoreceptors. We discuss several alternate mechanisms for the action of CO₂ and [H⁺] on central chemoreceptors but none can be considered definitive at the present time."

It is also curious that in the intervening years, reaction theory has rarely been challenged and the contradictory evidence has faded from the collective consciousness. Perhaps one reason for this is that it is easy to think about detection of pH in molecular terms – there are many pH-sensitive channels and receptors (e.g. K⁺ channels of the TASK and inward rectifier gene families and the ASICs (acid-sensing cation channels)). By contrast, CO₂-dependent modulation of proteins has remained unfamiliar to physiologists.

However, these interactions are not unknown. Insects such as mosquitos are sensitive to CO₂ – thought to be mediated through a G-protein-coupled receptor – but the mechanisms of interaction of CO₂ with the receptor remain unknown. The now very old discovery of the effect of CO₂ on the affinity of haemoglobin for O₂ (the Bohr effect) seems to have been overlooked by physiologists in the sense of what it might mean for the direct detection of CO₂ in other contexts. In the Bohr effect, CO₂ reacts to form a

carbamate moiety (via a rather labile covalent bond) on each of the terminal amines of the two α and two β globin chains. In other fields of biology, notably microbial enzymology and in the fixation of CO₂, activation of enzymes by formation of a carbamate moiety on specific lysine side chains is well known. These examples give plausible mechanisms by which CO₂ could interact with proteins and thereby be directly detected in physiological systems.

This forms the background to our discovery that connexin 26 (Cx26) – a protein that can form gap junctions – may be a hitherto overlooked receptor for CO₂. Connexins are one of two multigene families that can form gap junctions. Gap junctions comprise two hemichannels in each cell that dock together to form a large channel that connects the intracellular contents of such coupled cells. For a long time gap junction hemichannels were rather ignored – seen as being en route to forming a fully-fledged gap junction. However, hemichannels can indeed have functions of

their own – for example, there is well-documented release of ATP through both pannexin and connexin hemichannels. At first little credence was given to physiological roles for hemichannel-mediated ATP release; rather that this was a phenomenon associated with pathology. However, hemichannels can indeed release ATP under normal conditions e.g. in the developing retina and inside taste buds.

Our starting point was the discovery made with collaborators at University College London (Mike Spyer and Alex Gourine) that ATP is released in response to hypercapnia from the chemosensitive areas in the ventral medulla oblongata (the classic areas discovered by Loeschcke and Mitchell). Furthermore, pharmacological data suggested that this ATP release was causally linked to the adaptive respiratory response. Our further analysis of the mechanisms of CO₂-evoked ATP release led to the discovery that Cx26 was fundamental to the whole process (Huckstepp *et al.* 2010a). Firstly, Cx26 is localized in the right place – in specialized glial cells at the ventral surface of the medulla and the membrane (the pia mater) that covers the surface of the medulla. Secondly, ATP release was mediated via a process that was independent of extracellular Ca²⁺, and could be blocked by hemichannel antagonists, especially those with activity at Cx26. The ATP release depended on P_{CO_2} rather than extracellular pH, and was unaffected by modifiers of intracellular pH. Thirdly, we demonstrated the CO₂-dependent loading of carboxyfluorescein into both glial cells and the pia mater at the ventral surface of the medulla – independent evidence that CO₂ was able to open channels in these cells large enough to allow ingress of the fluorescent dye, which is otherwise membrane impermeant. Finally, we used the connexin blockers that could antagonize ATP release in response to elevated CO₂ to demonstrate that these same blockers, when applied to the ventral surface of the medulla *in vivo*, blocked both ATP release and

the adaptive respiratory response evoked by hypercapnia. Thus, hypercapnia-evoked ATP release at the ventral surface of the medulla in large part appears to be mediated via Cx26 hemichannels.

In a companion paper (Huckstepp *et al.* 2010b) we analysed the properties of Cx26 themselves. Here again we had a surprise. Although our initial thinking had been that Cx26 was the conduit for ATP release, it turned out that Cx26 had a more fundamental role. Cx26-expressing HeLa cells, unlike their wild type counterparts, are CO₂ sensitive – they exhibit CO₂-dependent changes in whole cell conductance that have a dose dependence *versus* P_{CO_2} almost identical to the dose dependence of ATP release from the ventral surface of the medulla; Cx26-expressing HeLa cells can also be loaded with carboxyfluorescein in a CO₂-dependent manner; and expression of Cx26 in these cells is sufficient to give them the capacity to release ATP in response to elevated levels of CO₂ (the ‘medulla in a cell’). This evidence collectively points to Cx26 being both the CO₂ sensor and the conduit for ATP release. Perhaps the strongest evidence for this comes from our demonstration that Cx26 hemichannels still respond to both decreases and increases in P_{CO_2} in excised patches. While we cannot exclude the possibility that there might be an accessory protein necessary for the CO₂ sensitivity of Cx26, the simplest interpretation of our data is that Cx26 is indeed directly sensitive to CO₂. Interestingly, the closely related connexins Cx30 and Cx32 also exhibit CO₂ sensitivity, whereas much more distant connexins (Cx36 and Cx43) are not opened by increases in P_{CO_2} .

Could our discovery of the CO₂ sensitivity of Cx26 be the alternate mechanism hypothesized by Eldridge *et al.* (1985) and quoted above? In the light of our results it seems likely that there are parallel ‘modalities’ of chemoreception – one based on detection of pH and

another based on direct detection of CO₂ (via Cx26). Although our evidence points to an unexpected locus for chemosensitivity, the pia mater and the astrocytes, a very recent paper from Alex Gourine and collaborators (2010) supports that view and suggests that astrocytes, in addition to being CO₂ sensitive, can also respond to pH. This raises the prospect that these parallel modalities of chemoreception may coexist in one class of cell type (Fig. 1).

This is an exciting time to be in the field of chemosensory transduction. The recent data showing the importance of astrocytes, the leptomeninges and direct detection of CO₂ move the field away from some of its older, more established concepts that assume the primacy of neurons and detection of pH in respiratory chemosensitivity. It will be fascinating to see how prevalent the sensitivity of ion channels to CO₂ turns out to be; and how the parallel modalities of pH sensing and CO₂ sensing are integrated into complete physiological systems.

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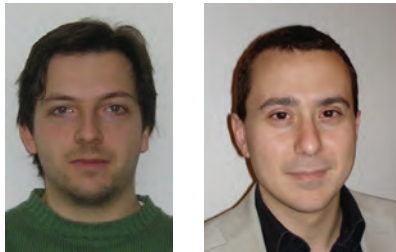
Transmission of cortical oscillations to motoneuron output for force control

The precise control of muscles for movement execution requires reliable transmission of information in the central nervous system. At the spinal motoneurons, information transmission is partly linear because the input is common to a large number of neurons. This generates coherence between the EEG and EMG signals, a measure whose validity is, however, debated.

The motor cortex controls the generation of voluntary movements through the direct and indirect activation of spinal motoneurons. Direct corticospinal projections on alpha-motoneurons have been demonstrated by histological findings (Lemon, 2008) and electrophysiological measures (Conway *et al.* 1995; Halliday *et al.* 1998). Moreover, the observation that the motor cortex activity recorded by the magnetoencephalogram (MEG) and the electroencephalogram (EEG) is coherent with the surface electromyogram (EMG) detected over the active muscle indicates that the cortical command is transmitted to the spinal motoneurons in a partly linear way (Fig. 1). Despite this observation which has been confirmed in several independent studies and various conditions, its explanation is not obvious.

The neurons, which are the main components of the central nervous system, are non-linear systems. Therefore, why is the coherence between EEG and EMG significant in certain frequency bands? Coherence analysis is indeed a linear tool that indicates linear correlation in the frequency domain between two signals.

Because of the intrinsic properties of neurons, the input to a neuron is not directly proportional to the generated output, but it depends on several parameters, such as the discharge statistics. Moreover, if several inputs are integrated together, as for motoneurons, the resulting output is not, in general, the summation of the individual responses. For such reasons, the control of neural systems based on the tuning of individual inputs seems an impractical problem. However,



Francesco Negro (left) and Dario Farina

since population coding has been demonstrated to play a key role in the decoding of external stimuli (and thus in the transmission of information) in several regions of the central nervous system (Averbeck *et al.* 2006), it is likely that it also plays a key role in the control of voluntary movements.

Spinal motoneurons discharge at relatively low frequency during moderate muscle contractions. Therefore, since the motoneuron behaves essentially as an integrate-and-sample process, any input signal with fast variability is under-sampled by each individual motoneuron. However, if the input is common to a population of motoneurons, the actual sampling rate is increased by a factor that is associated with

the number of motoneurons in the population and to their discharge rates. Moreover, the noise which is not common to all motoneurons is attenuated in the cumulative output spike train.

We have recently investigated the corticospinal transmission using EEG and single motor unit recordings in humans during isometric low force contractions (Negro & Farina, 2011). Starting from an analytical derivation previously proposed for an integrate-and-fire neuron (Nakao *et al.* 1997), it was demonstrated that under the assumption of sinusoidal inputs, the output of the motoneuron pool reproduced the input with greater fidelity (greater linearity) when the number of motoneurons that received the common input increased. Moreover, the level of interference created by the spiking processes was, at least partly, overcome by integrating the input with a relatively small number of motoneurons. The experimental results confirmed the analytical findings, showing that the significant level of coherence between the EEG activity recorded

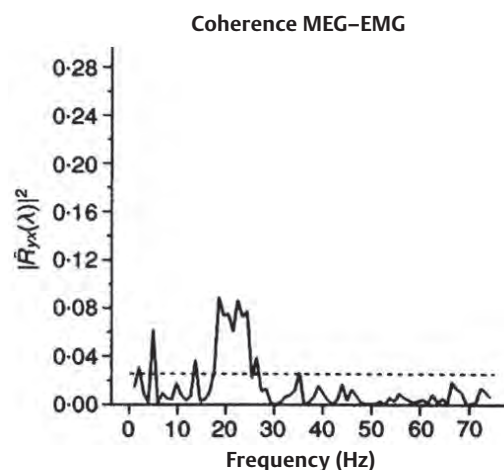


Figure 1. Coherence between the MEG and the rectified EMG signals recorded over the contralateral first dorsal interosseous muscle during an isometric contraction. Significant peaks in the beta band (15-30 Hz) are evident. From Conway *et al.* (1995).

over the motor cortex and the motor unit spike trains in the beta band increases with the number of spike trains used in the calculation but that after summing more than a few output spike trains, it reaches a plateau value (Fig 2). These results completed previous observations on low-frequency oscillations of motoneuron spike trains (De Luca *et al.* 1982; Negro *et al.* 2009) and demonstrated that the input to motoneurons is largely spread across the entire pool.

These results, however, leave some open questions. For example, it is not clear why the cortical oscillations in the mu/alpha band are usually not found to be coherent with motoneuron activity, contrary to oscillations in the beta band. Recently a potential contribution of spinal circuits and out-of-phase afferent feedbacks have been pointed out to account for this observation (Williams & Baker, 2009). Moreover, it has recently been shown in simulation that the presence of other common inputs to motoneurons, independent of the cortical input, could decorrelate the output, which is another way of stating that afferent input common to the motoneurons can decrease

or cancel the coherence of cortical input with motoneuron output (Negro, 2011).

Beside the discussion on which frequency bands are represented in the EEG–EMG coherence, one fundamental question remains unanswered: why are oscillations in the alpha and beta band transmitted to the motoneuron pool if most of their power is reduced anyway by the low-pass filtering effect of the muscle dynamics (Baldiessa *et al.* 1998)? For example, in a recent study, we have shown that the frequency content below 2 Hz in the cumulative spike train of motoneurons accounts for more than 60% of the total variance of the generated joint force whereas higher frequencies in the spike trains had negligible influence on force (Negro *et al.* 2009). The functional significance of oscillations at greater frequencies transmitted to the neural drive to muscles is thus unclear. Several hypotheses could be discussed; however, the simplest answer may be that these oscillations are only a by-product of the spiking nature of the neurons and are filtered out by the muscle contraction to perform a stable and precise movement. This

hypothesis is partly in agreement with the observed beta, but not alpha, band coherence. Since the cut-off frequency of the muscle contraction is indeed at around 3–5 Hz, it is necessary to delete the presence of oscillations in the alpha band with some spinal interaction (Williams & Baker, 2009) because these oscillations would not be completely removed by the muscle dynamics. It is interesting to note in this respect that alpha oscillations correspond to pathological tremor frequencies (Elble & Randall, 1976), so that it can be hypothesized that pathological tremor corresponds to the inability of neural mechanisms (such as afferent pathways) to suppress such oscillations. On the other hand, the beta band is completely attenuated by the muscle dynamics and thus it is not necessary for the system to attenuate it with neural mechanisms, which may explain the reason why it remains in the neural drive to muscle and is observable in the EEG–EMG coherence analysis.

In conclusion, there is evidence that oscillations present at the input of motoneurons can be transmitted partly linearly to the output if the input is common to the motoneurons. This explains the presence of coherence between EEG and EMG as well as between EEG and cumulative motor unit spike trains. Nevertheless, the actual functional significance of the observed linear association between cortical oscillations at relatively high frequencies and the neural drive to muscles is unclear due to the filtering of the neural drive by the muscle dynamics. We propose here the hypothesis that these oscillations may be a by-product of the transmission of information from different parts of the central nervous system. This hypothesis, which is purely speculative, needs to be proven (or disproven) in further experiments.

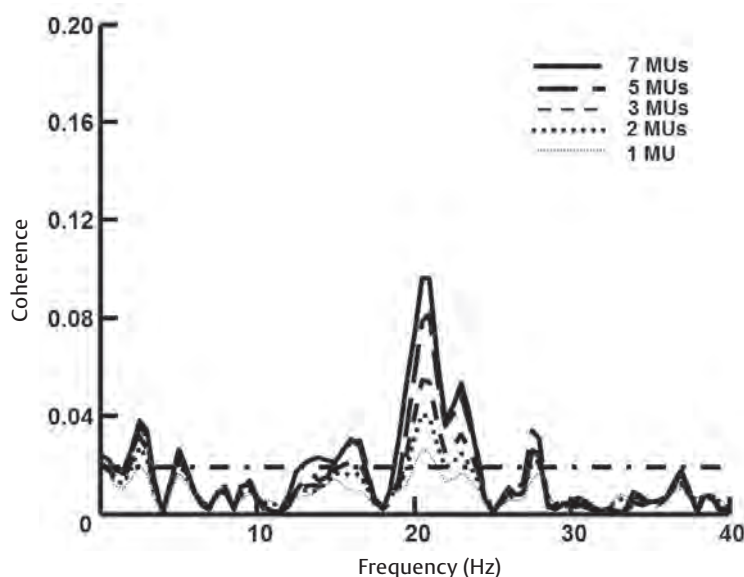


Figure 2. Coherence between EEG and the cumulative spike trains of motor units in the abductor digiti minimi muscle. The coherence is shown when using 1, 2, 3, 5 and 7 motor unit (MU) spike trains. The results are averaged over all possible combinations of motor unit spike trains. The horizontal line in the coherence plots represents the confidence level. From Negro & Farina, 2011.

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Research at high altitude

The factors that regulate brain blood flow at high altitude are unclear. Our recent findings show that the balance of oxygen (O₂) and carbon dioxide (CO₂) pressures in arterial blood explains 40% of the change in brain blood flow upon arrival to high altitude (5050 m). We have also shown that blood vessels in the brain respond to changes in CO₂ differently at high altitude compared to sea level – a factor that can influence breathing responses as well.

Research at high altitude provides an excellent means to examine chronic and acute adaptation to hypobaric hypoxia. Humans native to high altitude provide an ideal cohort in which to study biological adaptation to the chronic environmental stress of living at high altitude. Given the time necessary to study any chronic adaptation as well as the profound limitations on quality of life and related expense, studying the effects of chronic hypoxia at sea level using hypobaric or hypoxic chambers is not feasible. The Pyramid Research Laboratory in Nepal (Fig. 1), located at an altitude of 5050 m above sea level, is an ideal in-the-field laboratory in which to conduct high-altitude experiments. A central focus for our work is on the influence that cerebral blood flow (CBF) has on breathing control, the changes that occur at high altitude and how this relates to the development of periodic breathing.



Sam Lucas and Philip Ainslie

Overview

Since first reported by Severinghaus and colleagues (1966), increased CBF upon initial exposure to high altitude and subsequent normalisation after a period of acclimatisation has been well described; yet, the underlying mechanisms for these changes remain unclear. The myriad of factors involved (e.g. neuronal, biochemical and mechanical), the nature of their interaction, and their progressive changes with acclimatisation underscore the complexity of this



Figure 1. The Ev-K2-CNR Pyramid Laboratory in the Khumbu Valley, Nepal (5050 m). Data presented here from our research expedition to Nepal was carried out within the framework of the Ev-K2-CNR Project in collaboration with the Nepal Academy of Science and Technology as foreseen by the Memorandum of Understanding between Nepal and Italy, and thanks to contributions from the Italian National Research Council and the Italian Ministry of Foreign Affairs.

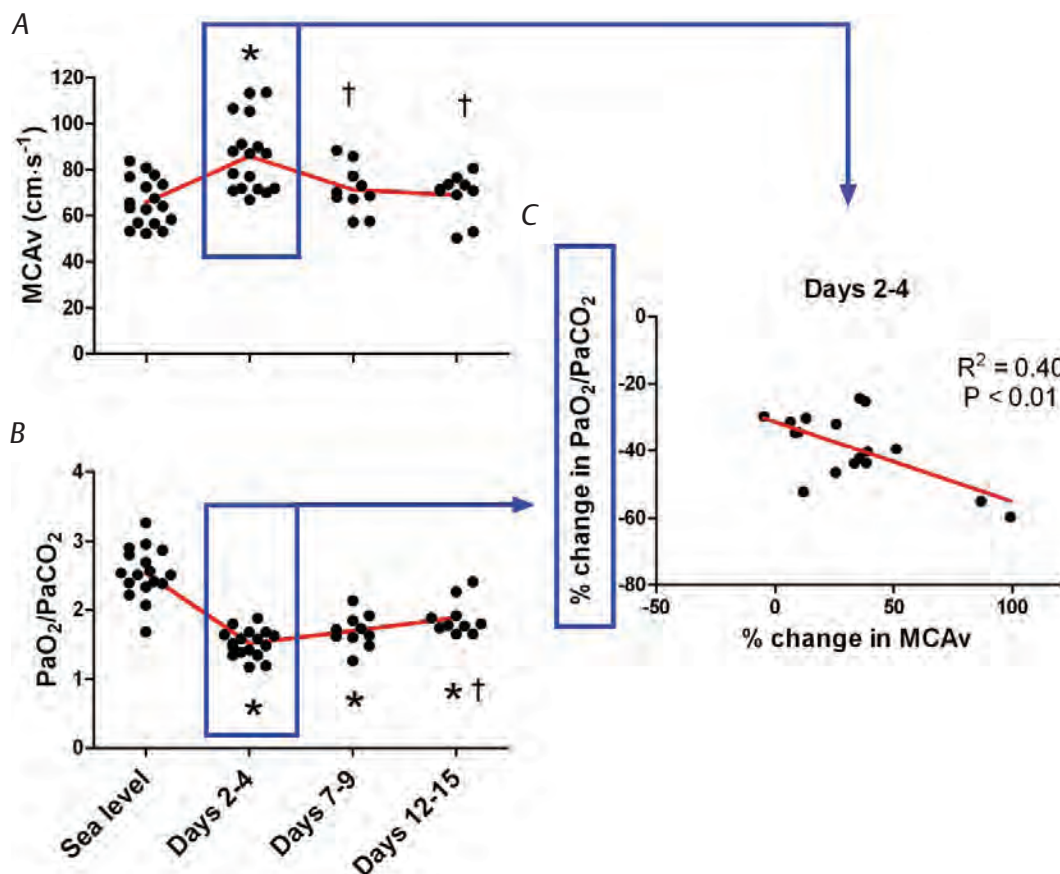


Figure 2. Cerebral blood flow velocity (MCAv, A) and ratio of arterial blood gases (P_{aO_2}/P_{aCO_2} , B) for eupnea (room air) at sea level and upon initial arrival (2–4 days, $n = 17$), and following 7–9 and 12–15 days ($n = 10$) of living at high altitude (5050 m). Upon arrival to 5050 m, changes in the arterial P_{aO_2} to P_{aCO_2} ratio explained ~40% of the variance underlying the initial elevation in MCAv (C). A low P_{aO_2} to P_{aCO_2} ratio indicates more hypoxic vasodilatation for a given hypocapnic vasoconstriction. Adapted from Figs 1 and 2 in Lucas *et al.* (2011). * $P < 0.05$: difference compared with sea level; † $P < 0.05$: difference compared with days 2–4 at high altitude.

process. The regulation of CBF during exposure to high altitude will, at least partly, depend on the degree of ventilatory sensitivity

(i.e. hypoxic and hypercapnic ventilatory responses) and on the cerebrovascular responses to hypoxia and CO₂.

Ventilatory control of CBF

The partial pressures of arterial carbon dioxide (P_{aCO_2}) and oxygen (P_{aO_2}) play a major role in CBF

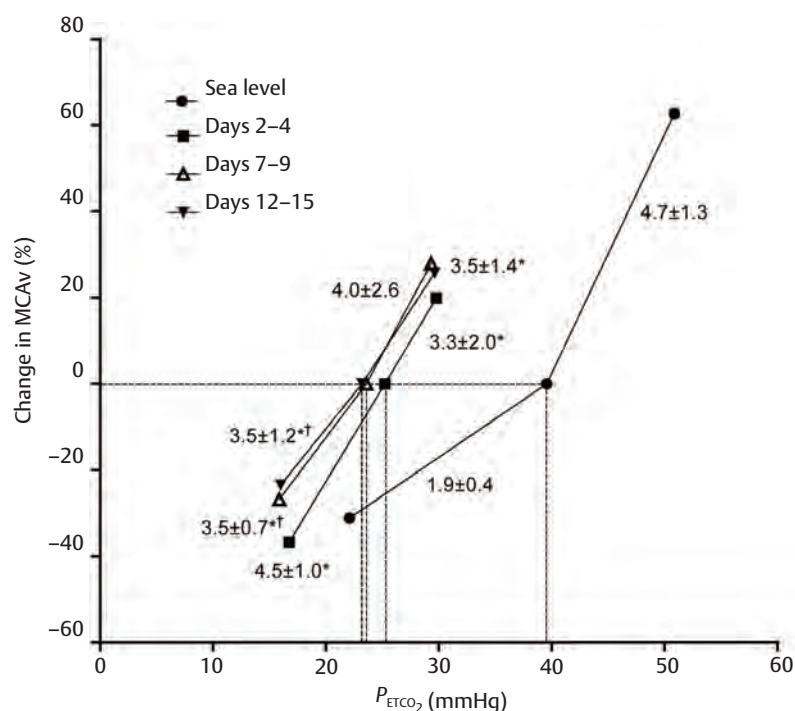


Figure 3. Changes in MCAv from baseline (eupnea) during a 5 min voluntary hyperventilation (hypocapnia) and a 4 min steady-state hypercapnia (7% CO₂) at sea level and after 2–4, 7–9 and 12–15 days of living at high altitude (5050 m). Group cerebrovascular reactivity (% MCAv/mmHg CO₂; mean ± SD) for each slope (hypercapnia and hypocapnia) at sea level and during 2 weeks at high altitude. * $P < 0.05$: difference compared with sea level; † $P < 0.05$: difference compared with days 2–4 at high altitude. These data indicate that cerebrovascular reactivity to hypercapnia and hypocapnia is differentially affected by high altitude exposure and remains distorted during partial acclimatisation. P_{ETCO_2} , partial pressure of end tidal CO₂. Adapted from Fig. 3 in Lucas *et al.* (2011).

regulation. Upon arrival to high altitude, those individuals who exhibit a 'brisk' ventilatory response will have a higher P_{aO_2} and lower P_{aCO_2} than those individuals who have a 'blunted' ventilatory response. Thus, changes in CBF at high altitude may be related to the balance of P_{aO_2}/P_{aCO_2} – a balance determined by an individual's ventilatory sensitivity to the hypoxic environment.

Cerebrovascular sensitivity

The degree of CBF responsiveness to acute changes in P_{aO_2} and P_{aCO_2} (i.e. cerebral reactivity) is another important factor that determines CBF. Experimental studies that have examined the influence of exposure to hypoxia on cerebral reactivity are limited and have variable results, while the changes that occur across acclimatisation have not been investigated to date.

In a recent publication in *The Journal of Physiology* (Lucas *et al.* 2011), we report on the relation between changes in CBF and the balance of arterial blood gases upon ascent to high altitude and during a two week acclimatisation period to 5050 m. We observed that the changes in the balance of arterial blood gases explain approximately 40% of the variance underlying the initial elevation in CBF at high altitude (Fig. 2). Furthermore, our data indicated that, within 7–9 days of living at high altitude, normalisation of CBF occurs due to hypoxia-induced elevations in ventilation. This influence leads to a higher P_{aO_2} -to- P_{aCO_2} ratio and therefore less hypoxia-induced dilatation and more hypocapnia-induced constriction in the cerebral circulation. The additional factors that account for the 'other' 60% are likely to include neuronal and local factors (e.g. endothelium-derived nitric oxide) as well as CBF changes that are driven by alterations in blood pressure, given the reported reductions in cerebral autoregulation at high altitude (Van Osta *et al.* 2005).

At sea level, steady-state cerebrovascular reactivity is more sensitive to increases in P_{CO_2} (i.e. hypercapnic reactivity)

than decreases (i.e. hypocapnic reactivity). Our high-altitude data indicate that this relation is reversed upon initial arrival to high altitude; notably, however, following partial acclimatisation, hyper- and hypocapnia cerebrovascular reactivity become similar to one another and both different from sea-level responses (Fig. 3).

Implications

The implications of alterations in CBF and cerebrovascular reactivity to CO_2 are numerous, including a potential impact on breathing stability. For example, reductions in cerebrovascular CO_2 reactivity develop a heightened ventilatory response to CO_2 and an unstable breathing pattern in patients with congestive heart failure (e.g. Xie *et al.* 2005) and obstructive sleep apnoea (e.g. Burgess *et al.* 2010).

Tight control of the cerebrovascular CO_2 reactivity provides an important protective reflex to minimise changes in brain $[H^+]$ at the level of the central chemoreceptor, and thereby stabilise breathing during fluctuating levels of chemical stimuli (Ainslie & Duffin, 2009). Consequently, a reduction in CBF hypercapnic responsiveness to CO_2 and/or an augmentation in the hypocapnic range, as we observed in our recent study, may account in part for the development of periodic breathing commonly observed in newcomers to high altitude. The causative link between altered cerebrovascular function and the occurrence of breathing instability at high altitude is incompletely understood and thus requires further investigation.

Future directions

To date, research on CBF responses at high altitude has almost entirely focused on the effects during wakefulness. Extrapolation of these findings to the sleeping state, however, should be done with caution given the dynamic changes in CBF during sleep and the potential for differences in CBF responsiveness between wakefulness and sleep. We also know that the wakefulness drive to breathe is vital for maintaining

rhythmical breathing, and in its absence (i.e. during sleep) levels of P_{aCO_2} become critical in regulating the breathing pattern (Skatrud & Dempsey, 1983). Thus, given the link between breathing pattern and CBF, both of which are altered at high altitude when awake, the changes in breathing stability and CBF that occur during acclimatisation may be important in the development of periodic breathing at high altitude. Therefore, obtaining sleeping state measures of CBF responses at high altitude seems like a logical step for improving our understanding of the pathophysiology of periodic breathing, and has the potential to provide insight into why symptoms of altitude sickness are more prevalent after waking.

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An update on Directive 2010/63/EU and its implementation in the UK

The current UK legislation governing the use of animals for research purposes, the Animals (Scientific Procedures) Act 1986 (commonly known as 'ASPA'), was the UK's implementation of EU Directive 86/609, which came into effect the same year. Within a few years it became clear that there were significant shortcomings with the Directive; for instance, there was no real control of accommodation and care of animals, nor of licensing procedures, etc. One result was considerable variation in the implementation between different member states, which in turn resulted both in variations in welfare standards and in practical and/or legal restrictions on scientific mobility between EU states.

One major prong of moving towards new standards was the work put into care and accommodation of lab animals. After something like 7 years of discussions between the varied groups with interests in the issue, a set of standards was signed off in 2005, under the auspices of the Council of Europe, as ETS123 – see <http://conventions.coe.int/Treaty/en/Treaties/html/123.htm>. At the time these were seen as desirable targets but with no legal obligation for implementation.

The background work on a new directive started around the millennium. For historical reasons it was the Environment Directorate in the Commission that was responsible for driving this forward – an issue for the bioscience sector since there was little apparent insight into the workings and requirements of the scientific method. Over several years there was intermittent background activity in response to various 'leaked' documents, as well as an open consultation in 2006. The more formal consultations between Brussels and the sector only really got going when the EU Parliament became involved in discussing the drafting, which resulted, after much discussion between and lobbying by the bioscience sector across Europe,

in the final Directive 2010/63/EU that came into force in late 2010. The text can be found at <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:EN:PDF>. Translations of the Directive can be easily selected at http://eur-lex.europa.eu/Result.do?T1=V1&T2=2010&T3=63&RechType=RECH_naturel&Submit=Search which can provide interesting comparisons where subtleties of wording are likely to affect the final operation of the Directive.

In a parallel strand of activity by the bioscience sector, there were moves under the previous government's 'better regulation' agenda to try to get the operation of ASPA streamlined. To push this agenda forward, a 'UK bioscience sector coalition' was formed to coordinate the views of academia, industry, funders and patient groups, with strong support from the Research Defence Society, now restructured as Understanding Animal Research (UAR). The coalition has operated very successfully to produce joint papers and submissions that have carried far more weight in government than separate submissions from the various parties would have done. When the Directive arrived on the scene, the coalition turned its attention to how the UK could best respond. As a result, there is an ongoing series of meetings with Home Office officials in ASPD (the Animals (Scientific Procedures) Division and ASPI (the administrative team) and Inspectorate. It has been gratifying to see just how much consensus there is over the major issues, not only across the sector, but also between the sector and the Home Office.

Transposition of the Directive into UK legislation

The Directive states that all EU countries must 'transpose' the Directive into national laws that are to take effect from 1 January 2013.

The Coalition in June 2010 submitted to the HO a useful short



Max Headley

summary of what it saw then as the key principles for transposition. That emphasis hasn't changed materially. See http://www.understandinganimalresearch.org.uk/page/download_document/?document_id=64

UAR in November 2010 produced a guide for institutions (see http://www.understandinganimalresearch.org.uk/page/download_document/?document_id=53)

The UK government is committed to holding public consultations prior to the development of all such legislation. The consultation over the Directive has been anticipated since late 2010, but ongoing delays between government departments caused it to be released only on 13 June, with a closing date of 5 September. The document is available at: <http://www.homeoffice.gov.uk/publications/about-us/consultations/transposition-protection-animals/>

The HO has made it very clear that it is the weight of argument, not the weight of postbag, that will influence them. At the time of writing, the coalition is working to coordinate a primary response that we hope will reflect the views of the sector and be signed off by all major parties (including The Physiological Society). No doubt many groups will wish to make supplementary submissions, but it is in everybody's interests to get as strong a single document as we can manage.

What are the implications of the Directive for us in the UK?

Much of the Directive was clearly modelled on what the UK already had, so the changes that will be

implemented will seem much less vigorous to us than to colleagues in many other EU member states. Nonetheless, change is clearly on the way. In general we hope for some reductions in bureaucracy (e.g. with personal licences) and anticipate (and welcome) a greater emphasis on the 3Rs of refinement, reduction and replacement (such as with clearer training standards). The situation regarding Ethical Review Committees and Project Licences is currently rather fluid. One important change will be that the terms of ETS 123 will become mandatory, which will have significant cost implications (for some species much more than others). The best feel for the issues can be gained by reading the UK bioscience sector coalition's draft response to the consultation, which (as flagged in The Society's July newsletter) is accessible to members on The Society's site at <http://www.physoc.org/site/cms/contentChapterView.asp?chapter=139>. By the time this article appears, the final version of the coalition's submission needs to have been prepared and submitted to organisations for sign off; once submitted by 5 September it will be posted on UAR's website at http://www.understandinganimalresearch.org.uk/policy_issues.

An area of contention is bound to arise over those aspects where existing UK legislation is stricter than the Directive. The Directive permits member states to retain those where they serve welfare, so the question arises as to what promotes welfare and what is bureaucratic control that does not. Resolution of these aspects will require some delicate negotiation given the potential adverse publicity of 'diluting' the current regulations.

How will all this move forward?

ASPD is already drafting the legislation and in doing so will bear the responses to the consultation in mind. The UK bioscience sector coalition will continue to meet with the HO on a regular basis to discuss the issues and try to resolve the areas of concern, which can perhaps

be summarised as containing costs, minimising bureaucracy and protecting confidentiality.

It is deemed likely that the proposed UK legislation will be presented to parliament for comment, with the opportunity for each House to accept or reject but not modify. Acceptance seems likely. However, HO officials think it likely that the Minister will take note of issues raised in the Houses and potentially modify the guidance documents to incorporate them. Although lobbying of MPs is not currently required, it is quite likely that the political temperature will be raised as the issues of contention become clearer once the various submissions for the HO consultation are looked at in September. It is therefore possible that the sector may need to start a vigorous campaign of lobbying MPs over the winter and into next spring; the bioscience sector coalition and UAR will be monitoring the situation and will involve societies such as ours if and when the need is felt to arise.

In general terms the Directive is a sensible compromise that has a clear focus on the 3Rs and improving animal welfare while also minimising the restrictions on research. The UK bioscience sector coalition is therefore likely to push to get the UK legislation to follow the Directive as closely as possible.

Any such legislative change is a challenge, especially when there are firmly held views by different parties who each claim a right to determine the outcome. What our sector must hold to is the support of regulation where the evidence shows it promotes welfare, but push to minimise controls that restrict research. The different parties, of course, see that balance rather differently, and so discussions over the coming months are likely to focus on trying to gain consensus in those areas. The hope is still that we can achieve new legislation that will promote both the 3Rs and good science – the two aspects that I would hope we can all sign up to.

Max Headley
University of Bristol

Society Noticeboard

Scientific Meetings – 2011

Epithelia & Membrane Transport Themed Meeting

University College London, UK
1–3 September

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
(Joint Meeting of The Physiological Society, the British Pharmacological Society and Wiley-Blackwell)

Physiology 2012

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

The Journal of Physiology Symposia 2011

Synaptic Mechanisms in the CNS – Symposium to honour Roger A. Nicoll

Silverado Resort, Napa Valley, CA, USA, 18–20 September

Cortical inhibitory neuron 'basket cells': from circuit function to disruption – Satellite Symposium held in conjunction with the Society for Neuroscience Annual Meeting

Walter E. Washington Convention Center, Washington, DC, USA, 11 November

Society-sponsored meetings – 2011

FEPS 2011 - The Society is sponsoring two symposia on 'Cellular dysfunction in heart disease' and 'Mouse models of vascular permeability control'

Istanbul, Turkey, 3–7 September

Motoneurons, their inputs and outputs

Warsaw, Poland, 5–7 September

Travel Grants

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

The 3Rs

Do you think of the replacement, reduction and refinement of animal research (commonly referred to as the 3Rs) as a regulatory hurdle, an ethical necessity, a sop to the anti-vivisection organisations or an opportunity to improve your science?

In my experience, more and more scientists, **through the work of the NC3Rs**, are recognising the 3Rs as a framework for improving the utility and efficiency of animal models, and for exploiting the use of new technologies and approaches which reduce reliance on *in vivo* research.

The NC3Rs is the UK's largest funder of 3Rs research. Since the Centre was launched seven years ago, we have committed almost £20 million in grants across a broad range of disciplines from neurodegenerative disease to cardiovascular models. This year, in addition to doubling the number of PhDs we support and introducing a new pilot project scheme, we are also launching two new routes for funding.

The first is the David Sainsbury Fellowship scheme (www.nc3rs.org.uk/fellowships), named in recognition of the former Science Minister's role in establishing the NC3Rs. The scheme will fund five exceptional early career scientists each year. The scheme will be open to scientists with less than three years post-doctoral experience and is intended to support the transition to independent researcher. Our goal is to embed the 3Rs in the training and development of the research leaders of the future. Awards of up to £200k over three years are available.

The second new scheme is a research competition called CRACK-IT (www.nc3rs.org.uk/crackit) which will exploit the move in industry for greater collaboration and open innovation. It will catalyse and foster new partnerships between academic and industrial scientists in the challenge of using the 3Rs to solve global business problems relating to the use of animals. *In vivo* models



Vicky Robinson

are regularly cited as bottlenecks in pharmaceutical discovery and development. In other industry sectors, the regulatory environment poses significant pressure in terms of animal use. In the consumer product sector, the ban on animal use for cosmetics presents a potential hurdle to innovation. The Registration, Evaluation, Authorisation and restriction of CHemicals (REACH) legislation for the chemicals industry, on the other hand, could drive increased animal use across Europe by many millions.

Under CRACK-IT we have identified six research challenges with companies such as AstraZeneca, Lilly, Roche and Unilever. The first CRACK-IT call will include, for example, developing new tools to study bipolar disorder which avoid the use of the existing poorly translatable animal models; technological innovation to allow wireless recording of cognitive function in rodent psychiatric disease models; and improved detection of kidney toxicity. The research competition will include funding from the NC3Rs (with a total budget of £5 million for 2011) and in-kind contributions from the sponsoring companies, including data, samples, compounds and access to equipment for example.

Further information on the work of the NC3Rs, including the David Sainsbury Fellowship scheme and CRACK-IT can be found at www.nc3rs.org.uk.

Dr Vicky Robinson is Chief Executive of the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs).

A degree of choice

Students choosing courses in chemistry, physics and engineering can opt for degrees that are accredited by the relevant professional body. With the exception of biomedical science courses, students who are choosing biological science degrees don't currently have that option.

With a more market-based approach to university finance, students are likely to become increasingly demanding in return for the investment they make in their education. Among other things, their interest in employability will grow. The Society of Biology is keen to enable students to make informed choices and to be more certain of the employability prospects they can expect from their university education. This has been one of the contributing factors to the development of the Society of Biology's Degree Accreditation Programme for the biosciences.

With a strong emphasis on academic rigor and research experience, the Society's Accreditation Programme is specific in its aims:

- to highlight degrees that provide graduates with the skills and experience required to progress to employment in academic or industrial research and
- to ensure a pipeline of skilled graduates into areas of particular national need and international importance.

To achieve these aims the accreditation criteria put a strong emphasis on academic excellence and, critically, time spent in an active research environment, focusing on four year undergraduate degrees with substantial research placements either in academic or industrial research groups. This rigorous assessment procedure will recognise outstanding courses across the UK that focus not only on core knowledge but also on experimental and analytical skills.

This scheme doesn't only stand to benefit students. A survey carried

out by the Society of Biology last year indicated that employers ranked 'lack of work experience' as the main reason for not employing a graduate with a BSc or MSc qualification. The Accreditation Programme will enable employers in both industry and academia to better identify graduates with the required research experience, skills and interest. The Programme also benefits employers by targeting bioscience disciplines where reports^{1,2} have highlighted national graduate skills gaps. In June 2011, the Society of Biology launched a pilot Accreditation Programme, initially focusing on two key areas of graduate skill shortages – biochemistry and *in vivo* science. This will help ensure that skills demands are better met in both academia and industry, and supports the Government's recommendations for a growth agenda. The project has the backing of the Department for Business, Innovation and Skills (BIS) and the Biotechnology and Biological Sciences Research Council (BBSRC), and was highlighted in HM Treasury's 'Plan for Growth'³ as a key strategy for growth of UK industry.

The Society of Biology has consulted a full range of stakeholders in the biosciences, including academics and universities, industry, learned societies, funding bodies, sector skills councils and students. We have been working closely with relevant learned societies such as The Physiological Society to provide the discipline-specific expertise needed. During the development of the pilot, The Physiological Society was asked to nominate members to help draft the *in vivo* specific assessment criteria and to act as assessors during the pilot.

Since the early stages of development of the Programme, we have received much interest from higher education institutes and through this we have selected a manageable number of degree courses to enter the pilot. We have been keen to include a variety of institutions and a mixture of course types in order to thoroughly test the process. Following a successful outcome from the pilot, we will



Eva Sharpe

open the scheme to all UK higher education institutions in 2012.

The Accreditation process is not prescriptive; we will not be dictating to universities how they should teach a subject or the particular topics they should cover. Instead we focus on the learning outcomes we expect from degree programmes. Through the Accreditation Programme we hope to highlight and share best practice and we see that Accreditation has the potential to drive up the already high standards of teaching and learning in biology higher education, cementing the UK's position as a leader in life science training and research.

In-built flexibility ensures that the Programme also remains inclusive of a range of bioscience disciplines, and we aim to expand the Accreditation Programme into a wider range of strategically important research disciplines with identified skill shortages in the future. Accreditation will not be appropriate for all courses and, as with other subjects, many students will continue to take excellent non-accredited degrees, especially if aiming for non-research careers.

The Society of Biology operates a mailing list for updates on the Accreditation Programme as well as a list of institutes interested in applying for Accreditation in the future. For more information please contact Dr Eva Sharpe, Higher Education Policy Officer at the Society of Biology, at evasharpe@societyofbiology.org or visit www.societyofbiology.org/education/hei/accreditation.

We look forward to celebrating the announcement of the first *in vivo*

science and biochemistry courses to be awarded Accreditation by the Society of Biology in Spring 2012.

Eva Sharpe

HE Policy Officer, Society of Biology

¹ABPI – *Skills needs for biomedical research* (2008). www.abpi.org.uk/our-work/library/industry/Pages/skills-biomedical-research.aspx

²Semta, Cogent, Skills for Health – *Life sciences and pharmaceuticals: a future skills review with recommendations to sustain growth in emerging technologies* (2010). <http://www.cogent-ssc.com/research/Publications/LSPReport.pdf>

³HM Treasury *Plan for Growth* 2011. http://cdn.hm-treasury.gov.uk/2011budget_growth.df

The policy of The Society

Since the concept of accreditation was first established, The Physiological Society has been an active supporter of the idea for *in vivo* intensive degrees. This work has been actively led by The Society's Policy Committee and coordinated with sister societies.

In principle, The Physiological Society supports the introduction of accredited degrees, with the understanding that the primary aim is to produce graduates with a higher level of practical training, whilst also leveraging additional funding for qualifying courses. However, we do have significant reservations on two issues:

- First, the current criteria for accreditation are not as transparent as we would hope – in particular, they lack sufficient core and mandatory aspects to differentiate accredited degrees;
- Second, it is critical that evaluation criteria are open and transparent and should have been widely available from the initiation of the pilot, aligned with the agreed objectives of accreditation.

Our view is that much work remains to ensure the accreditation system is fit for purpose, and we are continuing to work with the Society of Biology toward that end. If you have a view, or want to contribute to this work more actively, please contact our Policy Manager, Michelle Brook, on mbrook@physoc.org

Lab coats, muddy boots and a lot of questions!

Can you sell years of research in just one sentence?

That was the challenge posed on the application form for the Wellcome Trust-funded public engagement project 'I'm a scientist...get me out of here'. After thorough consideration I described my neuroscience PhD at the University of Manchester as trying to *'understand the secret double agent inside your body which is attacking your brain... Your immune system!'*. But would it be good enough to appeal to a tough judging panel of high school students, choosing only the most 'interesting' scientists to be involved this June? Luckily for me it seemed to do the trick.

'I'm a scientist' is an 'X-factor'-style science project which allows direct communication between scientists and students (13–18 year olds) across the UK via online questions and live web chats, giving students the chance to ask real scientists anything and everything they want to know. Then students get to vote for their favourite scientist. Twenty-three zones individually compete, with five scientists and around 400 students allocated to each zone. In the 'copper zone', I was competing against scientists from very diverse scientific backgrounds and career stages. Our collective knowledge spanned broadly from theoretical physics to reproductive hormones.

The one thing I hadn't accounted for when I applied for 'I'm a scientist' was that it would be two of the busiest weeks of my life. I had to juggle day-long experiments, answering questions, live web chats and even managed to squeeze in a trip to Glastonbury festival, but don't let that put you off, as it was also brilliant fun.

As the competition got in full swing the questions came at an alarming pace. Some were predictable; *"Why did you become a scientist?"* Some



needed a bit more consideration; *"Why is it that we tend to lose our memory as we age?"* Others got a little personal; *"Do you find relationships hard with you being so nerdy?"* However, the most exhilarating part of the competition was talking directly to the students in live web chats, being under concerted pressure to think quickly and answer accurately. It was under these conditions that I was able to get a real sense of the students' enthusiasm and some controversial topics were raised, such as the use of animals in research, which sparked excellent online debate.

The whole experience was addictive and I eagerly anticipated finishing my experiments each day so I could log in and answer questions. I even placed a 'do not disturb' sign above my computer for the intense live chats. The second week of the competition proved even more challenging, as I left the lab behind and found myself submitting answers from the muddy trenches at this year's rain-ravaged Glastonbury festival. The organisers were very happy that I was under-cutting some of the social perceptions of the image associated with a scientist by portraying a young scientist's lifestyle: someone who must manage the delicate balance of completing important experiments whilst still maintaining a social life. Even though there was a lot going on around me, I couldn't wait to hear news of the daily evictions and was grateful for the support I received from friends, colleagues and the 'I'm a scientist' twitter community.

The final eviction soon came and I found myself, amidst a sea of strangers waiting to see a band, frantically checking my phone for news of the pending eviction. To my utter surprise – and that of the people around me when I screamed in sheer delight – I was the winner in the copper zone. However, the story doesn't end there, as the crowning glory is that the winner of each zone is awarded £500 to spend on public engagement. Although still early in the planning stage, I have decided to use the prize money to develop a community project highlighting the risk factors for stroke and how they relate to the lifestyle choices we make.

I would wholeheartedly encourage any Phys Soc Member to get involved in 'I'm a scientist' 2012; it is a fun and interactive scheme that aims to break down barriers between scientists and the general public. Personally I think this is the best public engagement scheme I've been involved in – and the benefits aren't just for the students. You may start thinking about your research in a whole new light after having to explain and defend it to the harshest of critics.

Emily Robinson

Neuroscience PhD student, The University of Manchester

Scientists and schools can sign up to take part in future events at the 'I'm a Scientist...' website:
<http://imascientist.org.uk>

Personal reflections on “I’m a scientist, get me out of here!”

“That was one of the best things I have ever done,” I said to myself, having just had a pink banner announcing my eviction from the Sports Zone of “I’m a scientist, get me out of here!” slapped across my avatar’s face. It followed an exhausting two weeks in which working scientists and schools linked up through a website where the school pupils were in total control: they were the ones who questioned the scientists and they were responsible for choosing which scientists to evict. I must confess I had little knowledge of the event until Alice Sheppard (@penguinalgaxy) tweeted me suggesting that I apply to be in the event. A bit of extra arm-twisting courtesy of the Phys Soc’s Louise Crane and *Physiology News* Editor Austin Elliott convinced me to register my interest, and soon after I was told I was one of the chosen five for the zone. Overall, some 600 scientists applied for the 115 places available, so competition was fierce before the event even began.

Within a few days, I received a briefing pack and I had to build my profile (<http://sportsj11.imascientist.org.uk/profile/markburnley>). Soon after that, the questions started to arrive. These varied from the challenging: “What is the biological basis of consciousness?”; “Has anyone disproved your theories?”), to the odd (“If you eat oily food will it make your joints less ‘rusty’?”; “Is it possible to kick something so hard your leg came off?”) – but even these questions provided an angle from which to educate the pupils on the science underpinning what they had asked. Other questions were more general: “hi i would like you to tell me with one work [word] how you would describe science and then say why you have



Mark, before and after being evicted.

chosen this...!?!? thank you ... :)”. It is worth pointing out that moderators quite rightly avoided editing questions for grammatical errors, and left the scientists to interpret what the pupils meant.

Knowing the audience, and answering accordingly, was the most important part of this for me, and also the hardest bit to get right. This was especially the case during the live webchats, which were pre-booked by the schools themselves. It is hard to describe what a challenge that was, but it felt like trying to read a set of TV credits on fast-forward. Somebody would pluck up the courage to ask the first question, followed by seven others asking two questions each. By the time I had answered two of these, 14 more would pile in. At this point I would apologise for being slow (leading to several “lolz”¹ in reply), before trying to trace the next in line. After what seemed like six seconds of chat, the moderator announced it was all over. My watch told me I’d been chatting for 40 minutes.

The first week was eviction free, and within a few days it felt like I was getting to know the other scientists contesting the zone with me. Some of the questions were aimed at

individual scientists, but others were asked of all of us. By the end of the week, there was a distinct camaraderie building amongst us (even though I have never met any of them). If one of us answered a question first and we could do no better, the rest of us would chip in with “Yeah, what Jenni said...”. I say this because at no point did I consider the other scientists rivals, and when the first eviction was announced, I didn’t feel relieved to have got through, but rather sad to have lost a colleague. It also ramped up the pressure to answer questions, which were still coming through thick and fast.

In the end, I reached the final day of evictions (the ‘live final’), and sat at my computer at 3 pm waiting for the results to be announced. Ten minutes passed and then the ‘evicted’ banner was added to my avatar. I didn’t feel disappointed in the slightest, because everything about the previous two weeks had been positive: I managed to do more public engagement in those two weeks than in the rest of my career combined.

So how would I sum it up? Well, I think that “I’m a Scientist...” captures something very special indeed. The late Richard Feynman ran an unofficial class at Caltech called Physics X, in which he would turn up and simply ask “Any questions?” and see where things would go from there. “I’m a Scientist...” does just that with school children, and the results are incredible. The politics underpinning ‘public engagement’ may be off-putting, but with events like “I’m a Scientist...”, blogging, Twitter, Cafe Scientifique, Skeptics in the Pub and the like, there really has never been a better time to talk to people about science.

Mark Burnley

Department of Sport and Exercise Science, Aberystwyth University

¹Text language for ‘laughs out loud’, for older readers.

Young Life Scientists go scientific speed dating in Birmingham...

On the 26th May 2011, more than 75 young life scientists from across the UK converged on the University of Birmingham to attend a YLS symposium on 'Autonomic Control in Health and Disease', organised by postgraduate students from the School of Clinical and Experimental Medicine and the School of Sport and Exercise Sciences. Communications and poster presentations from postgraduate scientists were interspersed with keynote lectures from top researchers in the field: John Coote, James Fisher and Keith Brain.

Throughout the day there were many opportunities to talk about our own work and to share experimental ideas with other young researchers as well as some principal investigators. The scientific 'speed-dating' event, something I'd never done before, was particularly successful, and enjoyable too! In this session, which involved everyone, selected poster presenters were visited by a small group of other delegates and had five minutes to explain their work and answer questions before the group was moved on. Then the whole routine started again, as a new group of delegates came to listen to the presentation of the poster. After several cycles, roles reversed. As a poster presenter who always has too



Poster speed dating at YLS 2011: (left to right) Mike Ambler, Kieran Brack and Jennifer Clancy meet Peter Sidaway.

much to say, the experience of being forced to condense three years of my research into a five-minute-long chat proved quite a challenge. But it is a really useful skill to have acquired, and one that should prove invaluable at a forthcoming international meeting.

Following a fantastic buffet lunch and more opportunity to view the posters and talk to other scientists in a more leisurely manner, there were two parallel workshop sessions. The first one, run by Steve Watson provided an insight into successful grant writing. The other workshop entitled 'Science as a career' by Prem Kumar, explored different scientific careers away from the academic laboratory. These workshops were highly useful, and perfectly pitched for an audience of young researchers considering the next direction on their career path.

After a highly informative and enjoyable day, we relaxed with a glass of champagne and the prizewinners were announced,

before all going out for dinner to sample some authentic Birmingham cuisine at a local Indian restaurant – a really lovely way to end such a fantastic day.

The standard of YLS presentations was outstanding in both scientific content and delivery, creating a difficult task for the judges who were choosing prizewinners. The YLS prizes for best oral communications were awarded to Sarah Robertson (University of Edinburgh) and Prajini Sadananda (University of Bristol) and the YLS poster prize was awarded to William Seligman (Imperial College, London). To mark its centenary celebration, the Biochemical Society also awarded an additional poster prize to Maria Fragiadaki from Imperial College, London. Congratulations to all the prize-winners and to all those who presented posters and gave talks.

From a personal perspective, the opportunity to present my own work in a very relaxed and informal atmosphere has improved my confidence in speaking in public when discussing my data, and also gave me the experience of fielding questions from scientists from different research backgrounds. I also made useful contacts with other scientists working in a similar area of research to myself, and I am looking forward to meeting up with some of them again at an international conference in a few months time.

This event would not have been possible without the hard work and dedication of the YLS organising committee. On behalf of the YLS symposium delegates 2011, I would like to thank, Ella Stone, Andrew Holmes, Doreen Hartwich and William Rook for organising an excellent and enjoyable day. I would also like to thank The Physiological Society, the British Pharmacological Society, the Biochemical Society and the College of Medical and Dental Sciences, Birmingham who provided sponsorship for the event.

Laura-Joy Gooch

The University of Sheffield



The YLS Birmingham home team. Symposium organizers (from left to right) Will Rook, Andy Holmes, Ella Stone and Doreen Hartwich with speakers (from left to right) Prem Kumar, Keith Brain, John Coote, Steve Watson and James Fisher.

Workshop report: Quantification of nucleic acids by real- time PCR

This Easter's 2-day real-time qPCR workshop held at King's College London brought together 18 physiologists (mainly postgraduate students and postdoctoral fellows) from many parts of the UK including London, Cork, Loughborough, and abroad – Lagos and Osijek to name a few. The course developed our skills and essential understanding in all aspect of quantitative assessment of gene expression by PCR and transformed us from physiologists to fledgling molecular physiologists.

Each day comprised a mixture of theory, practice and application of techniques. The course is loosely based on a real research project and covers the background and practical work involved in all of the processes from RNA extraction to detection of gene expression by qPCR.

Funding to run the workshop was generously provided by The Physiological Society. With over 250 physiologists benefiting from this course, which has run each year since 2001, these workshops continue to provide essential training to anyone wanting to measure accurately gene expression using quantitative real-time PCR.

Lunchtime provided the opportunity for participants to discuss various points of interest from the morning's tutorials/practicals and to get to know each other, and of course to gossip about old friends. After lunch, the afternoon was spent in the lab completing reverse transcription of the RNA that we had extracted in the morning. This session covered theory and practical elements as well as the background to real-time PCR including primer design, various detection strategies and the vital issue of avoiding contamination.

The workshop ended about 6 pm and after some free time to get a well-earned drink after a busy day,



Participants of the real-time qPCR workshop in April.

everyone met up for a meal in a local Turkish restaurant. We all had great fun with a delicious selection of food; this also gave us an opportunity to really get to know one another and it was an ideal forum for networking.

The next day began with a series of practicals which involved preparing DNA standards for real-time PCR alongside the theory of making these and the important issue of calculating copy numbers of DNA. After lunch we then set up and ran our real-time PCRs. While these were running and we were waiting to see if we had managed to successfully get a good standard curve, we covered the background on the essential MIQE guidelines (Minimum Information for Publication of Quantitative Real-Time PCR Experiments, for the uninitiated), which are now required by many journals in order to get qPCR data published. The last session of the workshop was the moment of truth, when we analysed our data and between us demonstrated clearly several good – and one or two not so good – standard curves. Finally, a feedback session followed, in which participants were encouraged to share their opinions on the workshop and in particular to

suggest improvements for the next time.

Overall the meeting was an undeniable success, as corroborated by all the positive feedback from all participants, including those, like me, who had some previous experience and those who were new to the techniques. Finally, we must all thank Drs David Sugden and Patricia de Winter for running such an informative, useful and fun workshop which I strongly recommend to others who are thinking of or are currently in the early stages of running real-time quantitative PCRs.

Hiten D. Mistry
King's College London

Physiology News

If you have enjoyed this issue of *Physiology News* please don't throw it away. Put it in your coffee room so that others may see it too.

We are always looking for interesting features, meeting reports, news items and photographs. Contact us (magazine@physoc.org) with your suggestions.

100th anniversary of the Pikes Peak expedition to high altitude

Just 100 years ago on the 12th of July, four physiologists from Oxford and Yale Universities and Colorado College took the cog railway up to the summit of Pikes Peak, altitude 4300 m, for a historic study of acclimatization to high altitude. The participants included the eminent physiologist J. S. Haldane from Oxford who was accompanied by his younger collaborator C. G. Douglas. A few years earlier these two scientists had established the critical importance of P_{CO_2} in determining the normal level of pulmonary ventilation. Two other investigators were Y. Henderson from Yale University who had an interest in high-altitude physiology, and E. C. Schneider who worked in a laboratory at Colorado College near Pikes Peak. Mabel FitzGerald, also from Oxford, ascended to the summit but did not stay there with the men (Fig. 1).

The choice of the venue had an interesting twist. Haldane had met Henderson at the International Congress of Physiology in Vienna in 1910, and they discussed the possibility of a high-altitude expedition. Haldane was adamant that he needed 'a nice comfortable mountain' which sounds rather whimsical. However, he was probably alluding to the fact that the two European high-altitude stations, the Capanna Margherita on Monte Rosa and the Observatoire Vallot on Mont Blanc, had very difficult access and furthermore provided very spartan living conditions. Haldane argued that what was needed was an environment where the only variable compared with sea level was the low barometric pressure, and the hotel on the summit of Pikes Peak fulfilled this criterium well. The mountain had a substantial altitude, access was easy via a cog railway, there was comfortable living and laboratory accommodation, and Colorado College was nearby in case additional equipment was necessary.

The design of the expedition was classical. Measurements were first

made at sea level in Oxford or New Haven, and then after a short period at Colorado Springs, the four men ascended to the summit of Pikes Peak where they remained for five weeks. At the conclusion of this time they descended to New Haven to study the deacclimatization process.

The scientific program was extensive and was reported in a relaxed style in no less than 133 pages (Douglas *et al.* 1913). This is easily accessible by JSTOR today and still makes good reading. The report begins with a section on the symptoms and signs of acute mountain sickness including headache, loss of appetite, nausea, vomiting, periodic breathing and cyanosis. These features were noted in all the investigators and particularly in the numerous visitors to the summit. However, after two or three days most of the symptoms disappeared with the exception of breathlessness on exertion. There were many measurements of the P_{O_2} of alveolar gas and arterial blood on the summit. At the time, Haldane believed that the lung secreted oxygen and the paper stated that the P_{O_2} in arterial blood exceeded that in alveolar gas by about 36 mmHg. Of



John West

course, this was an error related in some way to the indirect method of measuring arterial P_{O_2} from the level of carbon monoxide in the blood after the subject had been exposed to a low concentration of this gas. Nevertheless this error should not detract from the important results of the other studies.

There was an extensive description of the changes in alveolar P_{O_2} and P_{CO_2} first with acclimatization and then deacclimatization after descent. For example, the alveolar P_{CO_2} fell over the period of five weeks to about 25 mmHg and took two to three weeks to recover after return to sea level. The deacclimatization data are particularly interesting because few studies of this are available. The investigators were greatly impressed



Figure 1. Members of the Anglo-American Pikes Peak Expedition of 1911, together with M. P. FitzGerald. From left to right, J. S. Haldane, M. P. FitzGerald, E. C. Schneider, Y. Henderson and C. G. Douglas. The cog railway line can be seen in the background. (See also *Physiology News* 83, p. 42, where this photo first appeared as part of the Paton Prize Bursary Photographic competition.)

by the degree of hyperventilation, but they were not able to explain it convincingly because the peripheral chemoreceptors were not discovered until some 15 years later (Heymans & Heymans, 1927). There were also extensive observations of periodic breathing and the fact that this could be returned to normal by oxygen administration. Attempts were made to determine changes in cardiac output by indirect techniques and the conclusion was that there was very little alteration in the circulation rate. There were also extensive measurements of changes in the blood including haemoglobin concentration, oxygen capacity of the blood and blood volume. It was recognized that the initial increase in the haemoglobin concentration was caused by concentration of the blood as a result of dehydration but that later there was an increase in the total amount of haemoglobin. In the summary of the report, they stated that the three main factors in the acclimatization process were the oxygen secretion by the

lung, the lowering of the exciting threshold of alveolar P_{CO_2} as a result of the diminished alkalinity of the blood, and the increased amount of haemoglobin in the blood.

One of the most colourful aspects of the expedition was the involvement of Mabel FitzGerald. She did not stay with the men on the summit, allegedly because she was not chaperoned, although it is more likely that the reason was that it would have complicated living conditions in the relatively small space available. Instead, she carried out a study of alveolar gas by visiting various Colorado mining camps accompanied only by a mule. It is difficult to imagine a more hazardous environment for a woman on her own than that. However, it is remarkable that her data are still cited today because few measurements of alveolar gas have been made at relatively low altitudes. Mabel FitzGerald was then lost sight of until the Haldane Centenary Symposium

was organized in Oxford in 1961. Her address was found in the Oxford telephone directory and she resurfaced to be given an honorary MA degree from Oxford at the age of 100. She was also elected a Member of The Physiological Society.

In summary, the Pikes Peak Expedition was one of the most influential of the early high-altitude expeditions, and on its 100th anniversary the report still makes rewarding reading.

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Creating Modern Neuroscience: The Revolutionary 1950s

By Gordon M. Shepherd

OUP USA; 1st edition
£40, 304 pages, hardback

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ISBN-13: 978-0195391503

Gordon Shepherd is the pre-eminent writer in the field of neuroscience, his previous books including *Neurobiology*, *The Synaptic Organisation of the Brain* and *Foundations of the Neurone Doctrine*. In this current book, based on a course taught at Yale, Shepherd describes how modern neuroscience emerged from key discoveries made in the 1950s, in the author's view the pivotal decade in modern biology.

The book opens with a chapter on the justification for studying history, and in particular, what was so special about the 1950s. This chapter brings together apparently disparate events and disciplines and moulds them into a cohesive narrative. Subsequent chapters follow a rough outline of increasing complexity such that the chapter on DNA is followed by chapters

on signalling molecules, synapses, the action potential, synaptic and receptor potentials, neural circuits, learning and memory, neurology, neurosurgery, neuropsychiatry and theoretical neuroscience.

Shepherd is a wonderful storyteller, and his chronicle is made all the more interesting by the fact that he worked with some of the greats in his formative years (he grew up a block away from John Atanasoff, the inventor of the digital computer), before becoming himself one of our greatest neuroscientists, his laboratory in Yale a training ground for numerous talented individuals.

Given the chapter structure, the book is not arranged chronologically, but rather each chapter can be considered an independent review, which introduces key characters and their respective contributions. In this way Shepherd reveals who did what, why they did it, when and where they did, and what followed on from their findings. What is particularly enjoyable are descriptions of the personalities of various researchers. Here is what Shepherd has to say about Eccles confrontational attitude

to competitors: 'The reason for his harsh attacks, I believe, goes back to his training in England, where they could take place between colleagues within the clubby atmosphere of the Physiological Society. In the outside world, they were interpreted as, and had the effect of, doing unnecessary harm.'

Given the scope of the subject covered, some topics are not described in as much detail as one would hope, but this is an understandable economy. Readers interested in the topic are directed to the Historical Archives on the Society of Neuroscience website for more detailed autobiographical chapters of individual scientists. In summary, this book is compulsory reading for those in the field of neuroscience. It is particularly important for students/postdocs to have a single volume that clarifies the key experiments and techniques that are the foundation of modern neuroscience. Hopefully Shepherd is at work on a new book. Would 'A History of Twentieth Century Electrophysiology' be too much to hope for?

Angus Brown and Lauren Murray

100 years ago in *J Physiol*

On the relation of the refractory period to the propagated disturbance in nerve. J Crichton Bramwell and Keith Lucas (1911). *J Physiol* 42, 495–511.

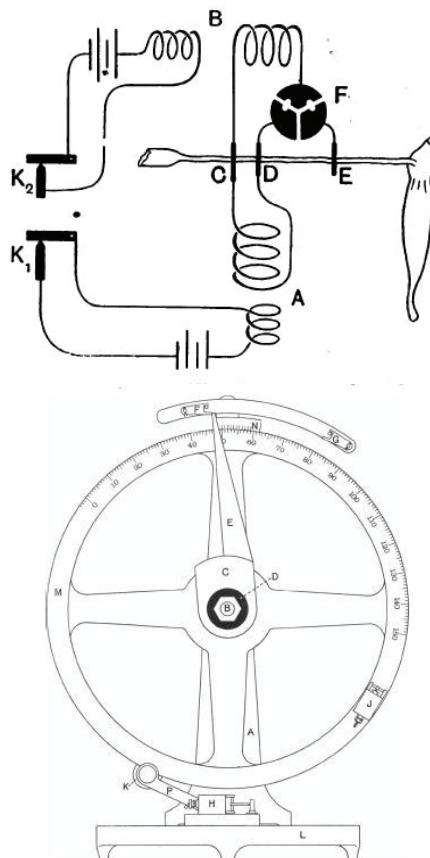
Of the 22 papers that Keith Lucas (1879–1916) published in *The Journal of Physiology* in the decade from 1904 to 1913, only three have a co-author. This highly select band comprised GR Mines, who like Lucas was to die tragically young (see *Physiology News Archives PN 71* and **83**); John Crichton Bramwell, co-author of the present paper; and Lucas' most famous pupil, and scientific 'inheritor', Edgar Adrian.

I will not dwell on Lucas' work and influence at length here – interested readers can consult archives **PN 74**. However, it is impossible to over-stress the significance of his work, or the esteem in which he was held by his fellow physiologists. Here, for instance is Edgar Adrian, accepting the Nobel Prize in 1932:

"I cannot let this occasion pass by without recording how much I owe to [Keith Lucas'] inspiration. In my own work I have tried to follow the lines which [he] would have developed if he had lived, and I am happy to think that in honouring me with the Nobel Prize you have honoured the master as well as the pupil."

Lucas worked exclusively on excitable cells, reflecting his focus on the problems of excitation and conduction, and nerve and nerve-muscle preparations, mostly from the frog, were his experimental system of choice. In this he was assisted by his great skill as an instrument designer and builder.

Nowadays, many of the experiments that workers like Lucas struggled to carry out a century ago are easily reproduced in undergraduate physiology practicals. An example is paired stimulation of a nerve – say with two pulses of millisecond duration applied a few milliseconds apart. However, in Lucas' day, the apparatus to deliver such carefully calibrated millisecond stimuli was decades away. Precisely timed stimulation of nerve or muscle had therefore to be accomplished by the rapid making, or breaking, of circuits that delivered a DC voltage. In the 1911 study, two stimuli were applied, at a defined time interval, and at either the same or at different points along a



Top panel, Fig. 1 of Bramwell & Lucas (1911) showing the experimental set-up. Bottom panel, front view of Keith Lucas' rotating pendulum (Lucas, 1908).

sciatic nerve. The basic set-up can be seen schematically in Fig. 1 of the paper. The circuits A and B were struck open in quick succession by a circular pendulum which Lucas had built and described in an earlier publication (Lucas, 1908; see Fig. 2). The design was obviously robust, since AV Hill was still using a similar pendulum, given to him by Lucas, some half a century later!

The focus of the 1911 paper is the processes underlying the refractory period (see **PN 79**). Though Hodgkin and Huxley did not record the first action potential until 1939, and the details of transmission were not worked out until the early 1950s, Lucas and his contemporaries fully appreciated that some change in excitation must propagate along the nerve. The question was whether the refractory period was simply a consequence of the passing of this 'propagated disturbance' (the action potential) or whether some separate local consequence of the process of electrically stimulating the nerve contributed as well. The idea of giving the nerve paired stimulation at two different points was to distinguish these possibilities. Bramwell and Lucas also measured nerve conduction velocity

at different temperatures, and visualised nerve compound action potentials – the 'propagated disturbance' – using a capillary electrometer. Their overall conclusion was that:

'It appears... that the diminished excitability which follows an effective stimulus is entirely due to the passage of the propagated disturbance over the tissue, and is not caused to any measurable degree by a local action of the exciting current.'

Lucas' co-author on the paper, John 'Jack' Crichton Bramwell (1889–1976), was the equivalent of a modern MSc student, having graduated from Cambridge in Natural Sciences in 1910. Bramwell came from a prominent Edinburgh medical family and in 1912 he was a graduate entrant to medical school in Manchester – where he was to remain for the rest of his career apart from WW1 military service. After WW1, Bramwell was appointed to the staff of the Manchester Royal Infirmary to run their first Electrocardiography Department, ultimately becoming a staff physician and cardiologist. He was elected to The Physiological Society in 1922.

Though he published no more papers in *J Physiol*, in his long career Bramwell worked on many physiological cardiovascular problems. Over the years 1920–23 he collaborated with AV Hill, then Professor of Physiology in Manchester, on the measurement of arterial pulse wave velocity in man. It seems virtually certain that Bramwell would have known Hill from the Cambridge Physiological Laboratory a decade earlier. Their joint work, presented at several Phys Soc meetings of the period, led Bramwell and Hill to derive the important Bramwell–Hill Equation, still in use, which relates arterial pulse wave velocity to the arterial stiffness. Later in the 1920s Bramwell studied the hearts of athletes, comparing them to non-trained individuals, and he also investigated the use of quinidine to treat atrial fibrillation. Bramwell was the first elected member of the British Cardiac Club, later the British Cardiac Society, and chaired both societies. Following WW2 Bramwell became Manchester's, and the UK's, first Professor of Cardiology, retiring in 1954 (Campbell, 1956).

Austin Elliott

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A cynical scientist applies the two cow paradigm to a range of scientific problems

To call a creative person derivative is an insult. As a scientist, with an unspecified personality disorder, my restrained answer to the same accusation would be “poncy bastard, if it pays the bills, so what?” When, as a narcissistic youth, I fancied myself as a political theorist, I came across the two cow paradigm, a parable that attempted to explain a range of political ideologies. In its political context most of us have come into contact with various examples of the two cow paradigm. Two I remember are:

Communism: There are two cows. One is bigger and produces more milk than the other. In an effort to correct this outrage the government sends the more productive cow to a re-education camp. The other cow then becomes very frightened and fails to produce milk.

Toryism or Republicanism: You have two cows, your neighbour has none. So what?

While in its original incarnation, the cows were a metaphor for the inadequacies of a range of political ideologies, variations of this paradigm have been used to explain a range of problems. Below I have deployed the two cow paradigm to feebly satirise a range of problems of a scientific nature. Here, rather than being a metaphor for a range of politically inspired ideological deficiencies, the cows below are a conduit for my disturbed views on an entirely similar range of scientific inadequacies.

Deep sequencing: The lab has two cows. One cow deep sequences the other. This generates 15 terabytes of information. The cow then has to employ 10 highly paid bioinformaticians to process the data. The data look very pretty but nobody can work out their biological significance, the lab goes bankrupt and both cows starve.

Translational research: A lab has two cows. Both are doing identical research. One cow has narcissistic personality disorder and is therefore politically devious and attention seeking. It lies and claims that its research is ‘translational’ and can therefore be used to treat a number of human diseases. The British government gives the politically devious cow all its money, the other cow starves.

Pharmaceutical development: A lab has two cows. Both are engaged in pharmaceutical research on a drug designed to cure depression in cows. One cow’s funding comes from a charity, one comes from a pharmaceutical company. The cow whose funding comes from a pharmaceutical company obtains results that suggest the drug is very effective at low concentrations and has minimal side effects. The cow whose funding is obtained from a charity finds exactly the opposite – this cow is ignored. Drug company then buys the head of department and the first cow extremely expensive swivel chairs. Millions of cows develop bi-polar disorder.

Computer modelling: A lab has two cows. They have Asperger’s syndrome and are mathematical geniuses. The two cows spend enormous amounts of money devising an elegant mathematical model which purports to explain how cows behave when in a herd. Being mathematicians, the two cows forget that herds of cattle can behave irrationally when startled and when the model is tested in the real world, both cows are trampled to death in a stampede. Mathematicians then blame the ‘real world’, close ranks, the herd is killed and the two mathematically gifted cows are posthumously awarded a Nobel prize. The computer model then becomes the ‘standard model’ for explaining cattle behaviour and in a 5 year period a further 317 cows, 47 pigs and 27 small children are trampled to death. The British government completely fails to notice this development and adopts the same mathematical model to predict the levels of risk involved in lending money to underperforming cows in



the Eurozone. Millions of cows are bankrupted.

A lab for legal reasons I never ever worked in 1: A lab has five cows. One cow has blonde hair and a very attractive-looking udder. An ageing professor becomes obsessed with the blonde cow, neglects his work and forgets to apply for government grants. The blonde cow gives birth to a calf, professor is divorced by a vengeful wife and the laboratory is closed. All the remaining cows starve.

A lab for legal reasons I never ever worked in 2: A lab has two cows. One has been sired by a famous scientist; the other cow’s father worked in a factory. One cow is encouraged to apply for prestigious fellowships; the other is told that their application will not be considered. Guess which cow gets the fellowship and which one starves.

A lab for legal reasons I never ever worked in 3: A lab has two cows. One commits scientific fraud in full view of the other, who attempts to report him to the authorities. The totally innocent cow is told by the management and trade union representative of his institution, “don’t cause trouble it might affect funding”. The cow continues to commit fraud, is caught and then every other cow on the surface of the planet is blamed.

And, finally, this never happened at any scientific institution I ever worked in ever: An institution has two cows. It is 3pm on a summer’s afternoon. The head of the institution is drunk and says to the cows “I bloody hate this place”. He falls over. The two cows laugh.

For legal reasons, none of this article is true.

Dr Keith Cormorant

Ask a physiologist!

“Why can’t you tickle yourself?” (Chloe (age 17), Flora (14) and Phoebe (12))

Dr Austin Elliott, University of Manchester, replies:

The short answer is that your brain already knows how it will feel (because it is you doing it – your brain knows this, since it ‘told’ your body to do it!). So the sensations the tickling produces (the ‘sensory feedback’, as the touch and pressure receptors in your skin are stimulated by the tickling motion) are completely as expected, and therefore it isn’t ticklish.

This is an example of something that goes by the (rather obscure) technical name of ‘efference copy’. ‘Efferent’ is a word that describes ‘signals sent out from the brain’, typically what scientists call ‘motor commands’ (instructions from your brain to your muscles to move). When you make the movement to tickle someone, including yourself, your brain ‘keeps a detailed copy’ of all the commands it sent out to move your arm and fingers. It also seems to make a detailed ‘map’ of what it *predicts* this will feel like (i.e. what sensory feedback in your body the movement will produce). When the real sensory information starts coming back following the movement (including from the touch and pressure receptors, and even from pain receptors, in the tickled area), this is compared to this predicted ‘copy and map’. For you tickling yourself, these sensations closely match the prediction (the sensory map) so your brain knows it was you doing the tickling. There are no ‘unexpected’ sensations, hence no feeling of being tickled.

One of the ways this can be shown is by using a robot arm, remotely controlled by your own arm, to tickle your own foot. Your other foot can be tickled by another arm, *not* controlled by you, as a ‘control’. The tickling by the arm you control feels much less ticklish. However, the experimenter can programme a delay into the tickling arm you are controlling. If this is done then, as the delay gets longer, the feeling gets *more* ticklish, better matching the ‘control’ foot. This is thought to be because the brain’s ‘copy and map’ of your action, and how it should feel, is very short-lived and is being continually updated and replaced. The delay means the predicted map has been changed, so the real feedback (as the arm tickles the foot) is now not a match – hence it gets properly ticklish, and more so the longer the delay becomes.

How does muscular dystrophy occur and how can we help prevent or slow down the process and is there any possible cure that could occur in the future? (Thomas, age 17)

Professor Dominic Wells, Royal Veterinary College, replies:

Muscular dystrophy (MD) is an inherited condition in which the muscle fibres are particularly vulnerable to damage associated with activity. Over time this leads to muscle wasting, formation of scar tissue and loss of function. Mutations in a number of genes have been shown to cause MD and the most common childhood form is Duchenne muscular dystrophy (DMD). The DMD gene is located on the X chromosome and most mutations lead to DMD, a condition that affects young boys who are confined to a wheelchair by 12 years old and who only live to their mid 20s to early 30s.

As MDs are, by definition, inherited, it is possible to prevent many cases by appropriate genetic counselling. However, conditions such as DMD have a high spontaneous mutation rate that limits the effectiveness of genetic counselling. Corticosteroids can be effective in a number of MDs as they slow the rate of muscle loss, although there are often side effects. Current drugs can be used to delay the cardiac failure that is common in many MDs. Intermittent positive-pressure ventilation can support respiratory function and does much to improve the quality of life in older patients.

Research has focused on developing therapies that can halt the progression of the condition, with a particular emphasis on DMD. Such experimental therapies include gene therapy with transfer of a modified version of the DMD gene, cell therapy with implantation of cells from a donor or the patient’s genetically modified cells, drugs to ‘read-through’ mutations that prevent translation of the mRNA into dystrophin and the use of antisense to modify the gene product to produce a functional version of dystrophin (exon-skipping). In addition, a number of groups are working to develop drugs that increase the expression of other genes, such as utrophin, or modify other elements of the disease process. A number of these novel therapies have entered clinical trial and although it is too early to predict when a cure might be found, there is considerable hope that we will find effective treatments for these conditions.

Why does a rabbit twitch its nose? (Hannah, age 11)

Dr Anne McBride of the University of Southampton replies:

It is true, the twitch, twitch of a rabbit’s nose is a very obvious characteristic, and very important to its survival. Not only does it draw air in to fill its lungs and breathe, in the same way as we do, but it also helps the rabbit detect danger, and identify friends and potential mates.

When we sniff/smell something our nostrils expand, lifting upwards and outwards. The same thing happens in rabbits, but is more obvious because they are constantly sniffing the air, rather than just breathing it in. Rabbits have over fifty million receptor cells in their nose, compared to our meager six million. These enable rabbits to detect predators well before they may even see them. This is why, if you want to watch rabbits (or other animals) without being noticed, approach downwind from them. That way the wind will go past the rabbit before it picks up your scent.

Rabbits, like many other animals, have two types of scent detection cells in their nose. Olfactory sensory cells detect ordinary airborne odours, while a specialized group, the Jacobson Organ, pick up heavy moisture-borne molecules and pheromones.

Moist air carries more scent. You may have noticed that flowers smell stronger when the dew has settled in the early morning and evening, and woods smell of vegetation in the damp of autumn. When rabbits breathe in, their split top lip parts and moistens the air as it passes. This enhances any scent and helps the rabbit discover more about the ‘smelly’ world around it – who is nearby, friend, foe or female ready to be mated, or any scrumptious food. As rabbits communicate mainly through scent, a good sniff of each other no doubt is a bit like a long human chat!

Rabbits are prey animals and their acute senses help them stay alive. The eyes, located on the side of their head and slightly above the mid-line, enable them to see behind and above them; their huge, mobile ears can pick up the slightest of sounds, though that constantly whiffing nose is perhaps the most important of all.

Report on a visit to The University of Trieste, under the Erasmus Staff exchange Scheme

27 May to 15 June 2011

Following a formal invitation from Giampiero Leanza and Enrico Tongiorgi, Coordinators of the new International Masters degree programme in Neuroscience at the University of Trieste, Italy (http://www.biologia.units.it/~sc_biologiche/neuroscienze/index.php), I was involved for the third year, in delivering a total of 18 hours of lectures to the Masters students, over a period of two weeks, covering the following topics: 'Local anaesthetics, General anaesthetics, Illegal drugs of abuse, Heroin and related opiates, Volatile substance abuse, Pleasure/reward/reinforcement pathways and drug addiction mechanisms' and 'Control of appetite and obesity'. All lectures were presented in English using PowerPoint computer projection. I also conducted a 3 hour session on the 10th June, where I was involved in formally assessing oral PowerPoint presentations made by the student groups as part of their coursework requirement, based on five recently published research papers, pre-selected by me, relating to the areas covered in my lectures. Each group of two students took turns to present the introduction, methods and discussion/main conclusions of each paper, and to handle the questions from the student audience and from myself and Dr Tongiorgi, who was also present. The individual groups were then given immediate feedback about their performance, particularly on the clarity of their presentations (in English), the quality of their scientific interpretation of their assigned paper, and their general understanding of the relevant field as revealed during discussion. Each student group received a credit for their contribution, and the best-performing group were further rewarded by an extra 'honours' credit. For some students, this was the first occasion that they had ever presented in this manner in English,



This year's MSc Neuroscience Course in Trieste with Andrew Constanti (centre left) and the whole class of 2011.

and they all agreed that it was a highly useful experience, and of great benefit to their scientific training.

In general, the overall standard of the presentations was very good, and English comprehension was also good, although there were often careless spelling errors in the slides. Students also handled my specific critical questions and other discussion points quite well, although it was notable that questions from the student audience were not immediately forthcoming. Clearly, Italian students are not used to being critical of their peers or their lecturers. I also met the students informally to discuss course progress, and they were generally happy with the content, although some felt it was too intensive. For example, there were no scheduled periods for tutorials and feedback, which was not really conducive to the learning process.

In all, I think the sessions went very well, and the students were grateful for the information that I was able to provide them with. Dr Tongiorgi was also happy with the content of my lectures and had received good feedback from the students. I understand that the format for the current course will be repeated for the next cohort of Masters students in 2012. This is the third year that I have taught on this exciting new course, that is now attracting much international attention, particularly as it is being taught in English, and is stressing the use of English for making presentations, dealing with discussions and for writing reports and examinations. This year's cohort

were an intelligent and motivated group of students, who I am sure, will do well in their future careers in neuroscience.

Andrew Constanti

Reader in Pharmacology, The School of Pharmacy, University of London, London, UK

My trip was kindly supported by The School of Pharmacy Erasmus Staff Exchange Scheme and also by a travel grant from The Physiological Society.

For further information about Travel Grants, go to: www.physoc.org/grants

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

Focus on physiology: The Society's new outreach publications

We are pleased to announce that the new edition of The Society's booklet '*Understanding Life*' finally rolled off the presses earlier this year. '*Understanding Life*' provides an introduction to the subject of physiology, how it is studied and how knowledge is used. The booklet is aimed primarily at young people aged between 15 and 18, but it is also widely distributed to older students and the general public at events such as Science Festivals and Careers Fairs. You may even find it a useful way of explaining to your friends and family what it is that we physiologists do! The booklet gives an insight into the central role of physiology in the progress and understanding of biological and clinical sciences. It also explains how physiologists are involved in the detection, prevention and treatment of disease and disability. This new edition has been rewritten and updated to reflect developments in physiology since the first edition was published in 2006. It contains new material, with contributions from at least 20 Members of The Society that include images from their labs, descriptions of their work and brief case histories. Everyone we asked to contribute to '*Understanding Life*' did so willingly and promptly, which made this aspect of the process far easier than we had expected.

Once the content began to take shape, we worked with a design company to produce a booklet that would be inviting and exciting to read. The struggle between the designers' vision of 'lots of clean white space' on the pages and our determination to ensure a detailed and rigorous content was resolved amicably through at least seven draft versions of the booklet – not including corrections of typos, repositioning of our logo according to 'corporate' guidelines and the last-minute inclusion of the latest Nobel Prize-winning physiology. We hope you will agree that the final product is a triumph of both style AND substance!

We have also produced a separate careers booklet this year entitled '*The Science of Life*'. The booklet provides an introduction to studying physiology at university; it outlines the skills that can be gained and the range of career options that a degree in physiology can open up. It also includes 'career stories' from 10 people in a broad range of jobs who use their training in physiology in their everyday work. The booklet is designed to help secondary school pupils make decisions about which subjects to study at school and university, and help them realise that Medicine is not the only option for students who enjoy science A-levels.

You can read both '*Understanding Life*' and '*The Science of Life*' on The Society website, or hard copies are available by emailing: education@physoc.org

Sarah Hall

Education and Outreach Committee

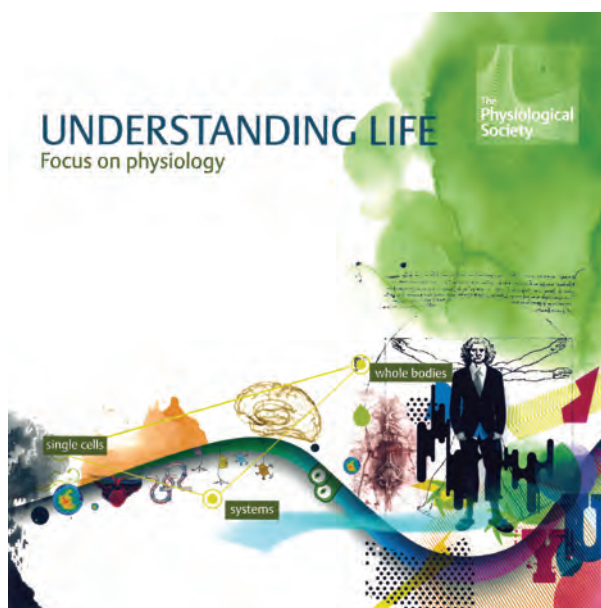
Prize-winning cover designs



Mars, the cover designer.

The cover of the new '*Understanding Life*' was designed by Tianshu Song (also known as Mars), who is an undergraduate at Central St Martins College of Art and Design. With the help of staff at the college, The Society ran a competition for their BA Graphic Design students to produce a cover conveying the message that physiology is a fun, contemporary and relevant science. The Education and Outreach Committee considered a shortlist of designs and chose Mars' concept as the winner because it includes modern and classical images of physiology in an arresting and intriguing format.

As competition winner, Mars received a prize of £300 and an acknowledgement on the back cover of the booklet. In addition, he was then invited to design the cover for our careers booklet, '*The Science of Life*' (see p. 58). The challenge here was to produce a cover that complemented his cover for '*Understanding Life*', but with its own distinct identity. Mars worked with us cheerfully and willingly to refine and modify his original concepts for both booklets, and he may even have learned a little physiology during the process!



Baby boomers and ageing – Phys Soc at the Cheltenham Science Festival

What influences how we age? And what does an ageing generation mean for society? These questions, among many others, were addressed at a Society-sponsored event held last month at *The Times* Cheltenham Science Festival.

The event, co-organised with the Academy of Medical Sciences, filled Cheltenham's largest auditorium – attracting an audience of over 220. Professor Linda Partridge from UCL opened with the statistic that a baby born at midnight today will live an extra six hours compared to a baby born at midnight yesterday. She introduced the scale of the UK's ageing 'epidemic', where there are now more people in the UK aged over 60 than there are under 18.

Linda's research is investigating genetic influences on ageing and age-related diseases in fruit flies and worms. Her research has identified certain mutations which increase lifespan and delay the onset of age-related diseases – these findings have the potential to be applied to humans to prevent the diseases associated with ageing – the question is would we want it?

The event was hosted by BBC *Radio 4*'s Geoff Watts, who polled the audience on this issue: the majority said they would like to

Geoff Watts, Linda Partridge and David Barker on the stage.

live longer if they could, with most voting for 85 as the perfect age to live to.

Genetic factors, it seems, can influence why someone lives a long, healthy life and others die young. However, according to David Barker from the University of Southampton, genetics are not the sole cause. His research explores the effects of nutrition in the womb on ageing. His presentation outlined that before birth almost every organ and system of the body goes through a critical period of growth. The first 1000 days of a baby's life are crucial for future development and susceptibility to diseases in later life. It's the nutrition of the mother throughout her whole life, not just her pregnancy, that affects a baby's ability to live to an old age.

David also outlined some unusual trends gleaned from longitudinal studies, such as how the height of a boy by the age of seven is a strong

predictor for how long he will live – taller boys living longer.

The lively discussion that followed both presentations covered issues such as would we want medical interventions in order to live a longer life, if we are not functioning at our peak? And whether trends such as height to indicate a longer life are useful given that they could be used against us; for example, to discriminate against a person's life- or medical-insurance eligibility. For the first time too, questions were also submitted via Twitter. The Society posted updates on the discussion as it unfolded, enabling online followers to engage with the debate. This proved a successful experiment in broadening the reach of our event; it is something The Society will be doing increasingly – to keep up to date on Society activities, you can find us on Twitter as @ThePhySoc

Clare Kingston
Head of Media & Communications



Otto Hutter Physiology Teaching Prize

The Otto Hutter Physiology Teaching Prize provides a vehicle for The Society to recognise an individual's contribution to teaching undergraduate physiology.

Since the launch of the Prize in 2009, two prizes have been awarded: to Professor Mary Cotter at the University of Aberdeen (2009) and to Dr Neil Morris at the University of Leeds (2010) (see right).

We are now welcoming nominations for the 2011 prize. Please visit The Society's website for more information about how to nominate yourself or a colleague for this prize:

www.physoc.org/education



Neil Morris receiving the 2010 Teaching Prize from Otto Hutter himself.

All a Twitter about body clocks

To complement Professor Russell Foster's sell-out Annual Public Lecture at Physiology 2011, The Society ran a very successful trial to extend the reach of Russell's fascinating research through the social media site, Twitter.

A live Q&A session followed the lecture and helped promote both The Society and Russell's work on the body clock to a wider audience.

Users on Twitter communicate with a series of 140-character 'tweets' to share links, news, information and opinions. The Society invited the world of Twitter to send in their questions to be answered by Russell in a live Q&A session. This provided a great opportunity for those who couldn't make the lecture – and even those who did, but wanted to learn more – to get in touch.

The response was brilliant, thanks in part to the *Guardian's* coverage of the lecture and promotion of the Q&A session. Over 35 questions were submitted and answered in just 45 minutes. Russell's inexperience with Twitter quickly evolved into expertise as he summarised his answers into bitesize tweets. Questions ranged from the fundamental: "Is 24 hours



Russell Foster (middle), who gave the Annual Public Lecture in Oxford, with Louise Robson and Mike Shipston.

the natural period of the body clock?" to complex, with "Are circadian rhythms a by-product of photoreception in early Cambrian (or earlier proto-) eyes? Which came first?" – sent in from Russell's former PhD examinee, BBC science presenter Adam Rutherford. The reaction to the session was excellent, with a plug from *Nature's* online science editor, Ananyo Bhattacharya – which all helps to increase The Society's profile among members of Twitter, young and old.

One measure of success on Twitter is the number of followers a person or organisation has – that is, followers who read your tweets. The Society's Twitter account increased by 60 followers around the time of the session and is continuing to steadily grow. The Society is hoping to build its Twitter following over the coming months with similar activities to convey the variety of The Society's work and reach new audiences – look out for us @ThePhySoc to find out more.



Sports Lab visit

Sports Lab is a family-friendly exhibition that opened in January this year, partly sponsored by The Physiological Society. It was created by Museums Sheffield and Sheffield Hallam University to provide a fun, fascinating look at the way sport plays an important part in our cultural history – and it involves more than a little bit of science.

The exhibition makes great use of artefacts held by Museums Sheffield, like the series of pedal bikes that form the central exhibit. It's amazing to see how much bicycles have developed since the penny farthing, thanks to the application of aerodynamic engineering. An old hunting spear is mounted next to a Viking javelin, the type that will be used in the Olympics next year. It shows how sport has developed from something ancient and primitive.

The Society's contribution to this exhibit has enabled the museum to develop several interactive exhibits that focus more on the physiology of sport. There is a virtual reality bike ride around Weston Park that provides an opportunity for you to compete against fellow exhibition visitors, and you can also test your reaction speed in a Batak test.

There is also a printed exhibition trail for younger visitors, which engages them with some of the scientific concepts behind the exhibition. One activity gets them to consider what roles nature and nurture play in the development of an elite athlete. There is an interactive game within the exhibition where visitors can



Photo courtesy of Gavin Joynt

Sports Lab exhibition at the Weston Park Museum, Sheffield.

design their own athlete. They can combine, for example, long legs with short torsos and big feet, and see what kind of ability this athlete would have. The exhibition trail asks visitors to consider whether athletes are successful because they are born good, whether it's due to training, or even technology. This is explored more in a 'find out further' sheet, which gives the exhibition increased educational value.

There are some exhibits that are real gems: for example, a zoetrope of an Eadweard Muybridge series makes the visitor realise just how useful slowed-down movement analysis is for elite athletes. Was Muybridge the original biomechanist? The exhibition explores biomechanics further with a look at prosthetics for disabled athletes, and asks whether running blades for leg amputees could become even more effective than natural legs in future.

Sports Lab is a really engaging, informative combination of the history, culture and science of sport and brings physiology to an audience generally interested in learning more about the world around them. The historical and cultural exhibits, like the football that was used in the 2010 World Cup and a collection of beautifully wrought ice skates, are intriguing and the science holds its own.

The exhibition is open at Weston Park Museum, Sheffield until November 20th and will transfer to the V&A Museum of Childhood, London between 31 March and 2 September 2012.

Louise Crane
Outreach Manager

Young Life Scientists' Symposium 2012

The Physiological Society, the Biochemical Society and the British Pharmacological Society are looking for a group of four or five PhD students or postdocs to be the organizers of YLS2012.

Organizing YLS provides an opportunity for emerging scientists to work with the three societies to host a conference within their own research area. Applicants must submit a complete meeting proposal, as well as a CV for each applicant and a letter of support from supervisors, by **24 October 2011**.

To give you an idea of what you could achieve, visit the YLS2011 website: www.yls2011.com.

For more information contact education@biochemistry.org



Photo courtesy of Gavin Joynt

On Your Bike! interactive exhibit at *Sports Lab*.

Launch of a new competition for schools

The competition, entitled '*The Science of Sport: How to Win Gold*', is open to 16- to 17-year-olds studying for A-levels or equivalent qualifications. They will be required to carry out a practical research project into an area of sports physiology, which can be related to a topic within the curriculum or a subject outside the curriculum that they are interested in.

At the meeting, the projects will be judged and gold, silver and bronze prizes awarded for the top three entries. Prizes include a 'Train Like a Champion Day' at an English Institute of Sport High Performance Centre.

A Facebook page, focusing on education in schools, has also been established recently (www.facebook.com/understandinglife). This will provide another means of communication with students and teachers – initially focusing on the competition and facilitating discussion between applicants, teachers and The Society.

Deadline for registration for this competition is 31 October 2011.

For more information about this competition, please visit www.understanding-life.org or contact scienceofsport@physoc.org.

As part of our support for school students entering the competition, 'The Science of Sport: How to Win Gold', we would like to provide them with an opportunity to ask a scientist a question about physiology, or even to meet them in person and visit their lab. All Members (including Affiliates) and non-Members would be very welcome to join this group.

If you or any of your colleagues are interested in taking part, please contact scienceofsport@physoc.org



The Science of Life is a careers publication aimed at students aged from 11 upwards. It provides an introduction to studying physiology, the skills you can gain through a physiology degree and the wide range of career options that a degree in physiology can open up to you.

Download it from The
Society website:
www.physoc.org/education

Sessions include*Cardiac, respiratory and vascular aspects of performance*

Daniel J Green, Liverpool, UK
 Benjamin Levine, Dallas, USA
 Markus Amann, Salt Lake City, USA
 Douglas Seals, Boulder, USA
 Daniel Theisen, Luxembourg

Drugs in sport

Carsten Lundby, Zurich, Switzerland
 David Cowan, London, UK
 Fawzi Kadi, Örebro, Sweden
 Martial Saugy, Lausanne, Switzerland

Thermoregulation

Jose Gonzalez-Alonso, London, UK
 Mike Sawka, Boston, USA
 Lars Nybo, Copenhagen, Denmark

Genomics and exercise

Claude Bouchard, Baton Rouge, USA
 Jamie A Timmons, London, UK

Neuromuscular function, muscle phenotype and mass regulation

Simon Gandevia, Sydney, Australia
 Peter Zammit, London, UK
 Stefano Schiaffino, Padova, Italy
 Jan Lexell, Lund, Sweden
 Marco Narici, Manchester, UK

Exercise metabolism

Michael J Rennie, Nottingham, UK
 Bente Kiens, Copenhagen, Denmark
 Erik Richter, Copenhagen, Denmark
 Marty Gibala, Hamilton, Canada

Sports and exercise medicine

Steven Blair, Columbia, USA
 Magnus Karlsson, Lund, Sweden
 Michael Kjær, Copenhagen, Denmark
 Peter Magnusson, Copenhagen, Denmark

The Biomedical Basis of Elite Performance

London 19–21 March 2012

The Queen Elizabeth II Conference Centre, London, UK

Abstract submission opens 1 November 2011

Plenary speakers

Frank Booth, Columbia, USA

Scott Drawer/Christian Cooke, UK Sport, UK

Bengt Saltin, Copenhagen, Denmark

www.bbep2012.org



Joint Meeting of
 The Physiological Society, the
 British Pharmacological Society
 and Wiley-Blackwell. Supported
 by Gatorade Sports Science
 Institute

Published by *The Journal of Physiology*, *Experimental Physiology*, *British Journal of Pharmacology* and *The Scandinavian Journal of Medicine and Science in Sports*

New Society staff members

Louise Crane



I joined the Society in May 2011 as Outreach Manager. I am responsible for our outreach and public engagement activities, which means communicating physiology to new audiences. I'll be managing the events we put on at science festivals and encouraging Members to do their own outreach activities through our Outreach Grants scheme.

I previously worked at the Wellcome Trust, where I was a biomedical

picture researcher and was seconded to work on the Trust's 75th anniversary project for six months. In my spare time last year I produced the Geek Calendar, a celebration of British nerdishness that raised over £16,000 for the Libel Reform campaign.

I love knitting and baking, am trained in ballet, tap and jazz and can often be found (in a personal capacity) on Twitter as @lulucrumble

Michelle Brook



I joined The Society in July 2011 in the role of Policy Manager – this is a new position at The Society, reflecting plans to raise the profile of physiology amongst stakeholders and the Government. I will be engaging with issues such as the transposition of the EU Directive on Animal Experimentation, Diversity in Science and changes to Higher Education; and will be looking for input from the membership to ensure the views of the physiology community are appropriately represented.

After graduating with a degree in Biochemistry, I began working within science policy – an area that fascinates me – most recently working as a Policy Officer for the Biochemical Society.

I spend my weekends exploring London, and am mildly addicted to hummus and chocolate.

Experimental Physiology

Translation and Integration

2010 Impact factors

The 2010 impact factor (IF) lists released on 29 June brought good news for both journals. *The Journal of Physiology*'s IF rose by 8% from 4.764 to 5.139 while that of *Experimental Physiology* rose 7% from 3.17 to 3.33. Within the Physiology list, the average change from 2009 IFs was 0.04, indicating a 4% inflation in IFs within the list. Against this background, the improvements in our journals measured by the IF calculations are significant.

For *The Journal of Physiology*, this year's IF rise is the culmination of a programme of editorial changes aimed at improving quality in general and the IF in particular. Former Editor-in-Chief William Large, who instigated the changes, commented "This year's rise shows it can be done and there is no reason why the IF cannot improve further with better editorial decision making". David Paterson, who has taken over as EiC, acknowledged his debt to William's policies: "It is pleasing to see this recent uplift in Impact Factor as JP continues to build on the hard work of William Large's earlier strategic initiatives. JP is a world-class journal and this increase reflects the quality of research published within it. I hope to sustain and build on *The Journal's* reputation over the course of my tenure."

David can take the credit for the rise in *Experimental Physiology's* impact factor, as this reflects changes he initiated while EiC. During his term of office, the IF for *Experimental Physiology* rose from 2.3 in 2006 to its present value, which puts the journal into direct competition with the American Physiological Society journals. David focused on a more rigorous initial assessment of submitted papers and high-quality editing to raise the quality of research content, along with a carefully structured approach to commissioning invited content.

The Journal of Physiology

Improving visibility

While authors focus on a journal's impact factor in deciding where to publish, as readers they may be totally oblivious to the publication hosting the article they are interested in. In today's online article-oriented world, visibility is increasingly important for journals. This is particularly challenging for general journals which may not be readily associated with specific subject areas.

Virtual issues

One method of signalling a journal's interest in a specific topic is to publish a virtual issue that collates articles on the topic published at different times. Our journals recently published a joint virtual issue on the *Biomedical Basis of Elite Performance* to flag the forthcoming joint societies meeting on the topic and highlight existing articles on this topic. The issue has been promoted to a selection of societies and institutes with an interest in human performance physiology.

Dedicated neuroscience issues

Another initiative is being trialled to raise the profile of *The Journal of Physiology* as a neuroscience publication. Although over 40% of our content is in our neuroscience section, we have a relatively low visibility amongst the neuroscience community. To remedy this situation, the 1 September issue of *The Journal* will be dedicated to neuroscience. The issue will hold a selection of reviews, commentaries and original research papers, according to our usual format. We will also add a 'Neuroscience' strapline to the cover to alert readers to the focus of the content. We anticipate publishing about eight such issues a year. The initiative is a natural extension of our Symposium and Special Issues which contain review series on specific topics, often together with several related research papers. If the experiment proves successful, it will be repeated for other subject areas.

The Journal of Physiology New Editors 2011

John Floras



I am Professor in the Faculty of Medicine at the University of Toronto and Cardiology Research Director for the University Health Network and Mount Sinai Hospital. On graduation in Medicine from Toronto in 1977, I accepted a Rhodes Scholarship to read for a DPhil at the University of Oxford. My thesis, supervised by Peter Sleight, incorporated experimental and human integrative cardiovascular physiology and pharmacology. After clinical training in Toronto I was awarded, in 1983, a Fellowship in Cardiology by the Royal College of Physicians. A Canadian Heart Foundation Fellowship supported post-doctoral training at the University of Iowa Cardiovascular Research Center in the laboratories of Francois Abboud and Allyn Mark. On returning to the University of Toronto in 1985 I established the first laboratory in Canada capable of recording human muscle sympathetic nerve activity. From 1995 to 1998, while Mount Sinai Hospital Cardiology Division Head, I assembled a unique research complex, adding cardiac catheterization and sleep laboratories also dedicated exclusively to human cardiovascular investigation. My primary research interest is the neural regulation of the heart and circulation in human health and disease, with particular emphasis

on hypertension, heart failure, renal failure, heart–lung interactions and sleep-related breathing disorders and their treatment in men and women. In 2004, I was awarded a Tier 1 Canada Research Chair in Integrative Cardiovascular Biology. A Past-President of the Canadian Hypertension Society, I am presently Chairman of the Board of Trustees of the Banting Research Foundation.

Andrew King



I am a Wellcome Trust Principal Research Fellowship and Professor of Neurophysiology in the Department of Physiology, Anatomy and Genetics at the University of Oxford. I'm also a Fellow of Merton College and Director of the University of Oxford's doctoral training programme in neuroscience. After studying physiology as an undergraduate at King's College London, I obtained a PhD in the Division of Neurophysiology and Neuropharmacology at the National Institute for Medical Research in Mill Hill. In 1984, I moved to what was then the University Laboratory of Physiology in Oxford, where I have held a series of research fellowships from the Science and Engineering Research Council, the Lister Institute of Preventive Medicine and the Wellcome Trust. I have also worked in the USA as a visiting scientist at the Eye Research Institute in Boston. I received the Layton Science Research Award from King's College London and the Wellcome Prize in Physiology, and, earlier this year, was elected a Fellow of the Academy of Medical Sciences.

My research employs an interdisciplinary approach, combining behavioural, physiological and anatomical methods, to investigate the neural basis of auditory perception. This includes examining

how auditory and visual signals are combined and integrated in the brain. I am particularly interested in the plasticity of auditory processing and in the capacity of the brain to adapt at different ages and over different timescales to changes in its sensory inputs. While focusing on the fundamental question of how information about the auditory world is represented in the brain in a dynamic and adaptive fashion, my research is also aimed at understanding how the brain responds to loss of hearing and its restoration.

In addition to being a member of the Editorial Board of *The Journal of Physiology*, I am an Associate Editor of the *Journal of Neuroscience* and a Review Editor for *Frontiers in Systems Neuroscience*. I recently became the Chief Research Adviser to the charity Deafness Research UK and am a member of both the Wellcome Trust's Basic Science Interview Committee and the Medical Research Advisory Panel at Action on Hearing Loss (formerly the Royal National Institute for Deaf People). I was the Convenor of The Physiological Society's Special Interest Group on Sensory Functions from 1993 to 1997, a member of the National Committee of the British Neuroscience Association from 2004 to 2007, and have served on the organizing committees of a number of international conferences, including 3 years as Chair of the programming committee for the annual auditory satellite that precedes the Society for Neuroscience meeting in the USA.

Jamie Vandenberg



I am a cardiac electrophysiologist with a particular interest in the molecular basis of cardiac arrhythmias. My laboratory works

predominantly on hERG K⁺ channels with projects focused on genotype–phenotype relationships in long QT syndrome type 2 (caused by mutations in the hERG K⁺ channel), the molecular basis of drug binding to the hERG K⁺ channel and development of computer models of drug binding to hERG.

I studied Medicine at the University of Sydney and completed an internship and residency in Sydney before moving to the UK to do a PhD at the University of Cambridge. In Cambridge, I worked with Peter Morris and Jim Metcalfe, looking at cardiac metabolism using NMR spectroscopy. I moved to the University Laboratory of Physiology, Oxford in 1993 where I developed an interest in cardiac electrophysiology whilst working with Trevor Powell. I subsequently moved back to Cambridge, where with Andrew Grace, I helped establish the Cardiac Arrhythmia Research Group. In 2002, I returned to Sydney, and established the Mark Cowley Lidwill Cardiac Electrophysiology Research Program at the Victor Chang Cardiac Research Institute.

I am currently a National Health and Medical Research Council Senior Research Fellow, Faculty member and co-Deputy Director of the Victor Chang Cardiac Research Institute, conjoint Professor in the Faculty of Medicine at the University of New South Wales and President of the Australian Society for Biophysics.

2011 Council Elections

The following have been elected by Members to serve on Council for four years with effect from the AGM on Wednesday 13 July:

Rebecca Burton
William Colledge
Michael Evans
Stewart Sage

The following have been elected by Affiliates to serve as Affiliate Representatives on Council:

Keith Siew
Jamie McPhee

The Journal of Physiology



- **New Editor-in-Chief (David J Paterson)**
- **New Look**
- **New Editors**
- **New Opportunities**

<http://jp.physoc.org>



Lab Profiles: Welcome to the Exercise Physiology Laboratory, University of Chichester

Following on from Samantha Passey's laboratory profile that appeared in the last issue of *Physiology News*, I have been invited to highlight the research being conducted within the Department of Sport and Exercise Sciences at the University of Chichester based on the southern coast of England in West Sussex.

Since October of last year I have been working for my PhD in Exercise Physiology supervised by Stephen Myers. In recent years, the University has been building up its research repertoire and is hoping to increase its number of research students in the coming few years. Currently the Department conducts research within four main domains pertaining to sport and exercise – physiology, psychology, biomechanics and sociology. However, as part of the on-going theme of *Physiology News* you will be pleased to know I was invited to only talk about the physiology research domain.

In the Department of Sport and Exercise Sciences at Chichester, physiology researchers have access to a variety of specialist equipment, including an environmental chamber that allows for controlled changes in temperature from extreme cold (-20°C) to extreme heat (50°C), humidity (25% to 95%) and oxygen concentration (8% to 21% O_2) during exercise, and a swimming flume in which the speed of water flow can be adapted for research interests. Other equipment available ranges from basic to sophisticated devices which allow for the measurement of exhaled gases, blood lactate, body composition, hydration, heat-induced stress, cardiovascular function and skeletal muscle function.

Research within the Department is varied and includes aspects of both clinical, exercise and performance physiology. Research projects



Carla Gallagher

have tackled exercise in the aged, loaded carriage exercise, endurance performance, eccentric exercise and performance enhancement through β -alanine supplementation. Current research interests include the effects of hypoxia on weight loss and the effects of eccentric exercise-induced muscle damage in men and women.

Mandy Gault and Sam Blacker, both previous postgraduate researchers at the University of Chichester, have recently completed their research programmes. Mandy was interested in examining the acute physiological effects of eccentric exercise in older adults using a self-selected walking speed protocol. She examined the physiological and functional adaptations to a 12-week controlled exercise intervention that consisted of level (i.e. concentric contractions) and downhill (i.e. eccentric contractions) treadmill walking. Sam's research interests, however, lay in performance physiology, specifically examining the physiological effects of prolonged load carriage by carrying of a backpack, with a particular focus on the recovery of neuromuscular function, assessed using surface electrical stimulation techniques. His research impacts on our understanding for occupational settings that involve extended periods of load carriage such as the military.



One of our research trips to Aguille du Midi ridge.

Current research includes that of my own and that of another first year postgraduate research student James Gavin. Over the past eight months James' research has involved examining the repeatability of running protocols to standardise exercise intensity. Next, he will investigate the effect of glycogen depletion on neuromuscular responses during controlled eccentric contractions. My research ultimately aims to determine the efficacy of using hypoxia to enhance weight loss in obese populations; this work is carried out using a normobaric hypoxia chamber simulating a high-altitude environment. A combined effect of hypoxia and exercise will be examined. I now find myself, following completion of a study in Chichester looking at a range of techniques to control exercise intensity in hypoxia (e.g. cardiovascular and respiratory variables), in the laboratory at the University of Bedfordshire, collaborating with the Muscle Cellular and Molecular Physiology Group. In the group's laboratories I am conducting experiments examining the effects of hypoxia on *in vitro* skeletal muscle. This combination of both *in vitro* and *in vivo* work should provide useful insights into the effect of hypoxia on weight loss and help to address the increasing problem of obesity. This research work also aims to shed light on the mechanisms underpinning the weight loss experienced by visitors at high altitudes.

The research carried out at the University of Chichester has largely an applied focus and the laboratories are part of the Chichester Centre of Applied Sport and Exercise Sciences. Collaborations with other universities and institutions with complementary specialities are proving insightful and exciting, and along with the continuing development of the exercise physiology laboratories, provide exciting opportunities for current and future research students.

Carla Gallagher

Postgraduate Researcher at the University of Chichester, West Sussex

Ian Campbell Roddie

1928–2011

Ian Roddie was Chairman of the Committee of The Physiological Society from 1986 to 1988, a Sherrington Lecturer and an Honorary Member from 1998.

His introduction to physiology came in the second year of his medical studies and was strengthened when he took an intercalated BSc in Physiology (First class honours, 1950) in the early years of David Greenfield's outstanding contribution as head of Physiology in Queen's University, Belfast. After qualification he returned to Physiology, working under David Greenfield in the actively advancing field of human circulatory physiology to obtain an MD with gold medal (1957), and just five years later DSc. When Professor Greenfield vacated the chair in 1964, it was a natural progression for Ian to become Dunville Professor of Physiology at Queen's at the age of 35.

Ian led research in three major areas related to the circulation – effects of mental stress, thermoregulation and circulation of lymph. The papers he published (largely in *The Journal of Physiology*) were classics which are still widely quoted some 50 years later.

The stimuli for mental stress were originally ingenious and tailored to be alarming to the individual subject. When the Head of Department (democratically) volunteered to be a subject, there were background mutterings at the appropriate moment of a fire that had just broken out in an adjoining room. However, in due course it was found that a series of questions in mental arithmetic produced similar physiological responses and this sanitized version was subsequently used over the years. It was so reliable in provoking forearm vasodilatation that it could be demonstrated to a random volunteer in a class tutorial about venous occlusion plethysmography.

The studies on thermoregulation included assessment of sweating by having subjects lie on a couch whose weight (together with that of the subject) was constantly and extremely accurately recorded. In thermoneutral conditions the record showed a steady small loss of weight due largely to



Ian Roddie, taken in 2001. With kind permission of Mary Roddie.

insensible loss of water from lungs and skin. With active sweating induced by heating, or by mental stress, the rate of loss of weight increased markedly and the effects could be studied in relation to their physiological control mechanisms.

The circulation of lymph was studied in both isolated lymphatics and *in vivo*. It was shown clearly that lymphatics with their multiple valves behave like multi-chambered hearts, sucking in tissue fluid and pumping it proximally towards the neck veins.

All this work was shared not only with junior colleagues working for a doctoral degree, but also with many medical, dental and science undergraduates whom he provided with original research projects in their final honours BSc year. The talented, meticulous, fundamental, but 'fun' approach to research spilled over into day-to-day lectures to undergraduates, who greatly appreciated his lucid, no-nonsense communication of principles which would stand them in good stead in subsequent professional careers. Ian's involvement in basic physiological education was also reflected in his contribution to textbooks and a multiple choice publication which ran to six editions from 1971 to 2004. In collaborating with him in this (from the days when original hand-written texts would be laboriously typed and retyped by secretaries to later international email exchanges) it was very satisfying to argue the merits of sentence A *versus* sentence B and end up with a much better and briefer sentence C.

Ian's scientific precision was also much in demand for administrative posts, including Dean of the Faculty of Medicine and Pro Vice Chancellor at Queen's University, Belfast, and member of the General Medical Council, the General Dental Council and the Medical Research Council. He took a keen interest in separating the precious metal from the dross in the changing fashions of educational methods and assessments, and was external examiner in many universities in the British Isles and abroad. He examined for many years in the Fellowship examinations of the Royal Colleges of Surgeons.

After retiring early from Queen's, he spent further years abroad, initially as Visiting Professor at the Chinese University of Hong Kong and then as Medical Director and Head of Medical Education in Jeddah. Latterly his influence extended worldwide, particularly in developing countries, when he advised the World Bank, the Asian Development Bank and various governments and institutions in some 30 countries, from Guatemala to Vietnam, and from Poland to South Africa.

His many contributions were recognized nationally when he became Commander of the Order of the British Empire.

He took great delight in his family, who were particularly supportive of him in his final months battling prostatic cancer. Typically, at this stage he sent a final 'circular email letter' to friends, informing us that his condition had now reached the palliative stage. In true physiological fashion he described objectively the effects, and positive aspects, of the various features of his illness and how he coped. He referred particularly to the support of family and it was good that they were able to be present as he passed peacefully away.

I would like to thank his daughter Mary for copies of his CV and publications, including some 70 original research articles, and for the photo; also Professor Mike Joyner of the Mayo Clinic for a transatlantic perspective.

William F. M. Wallace
Professor Emeritus of Applied
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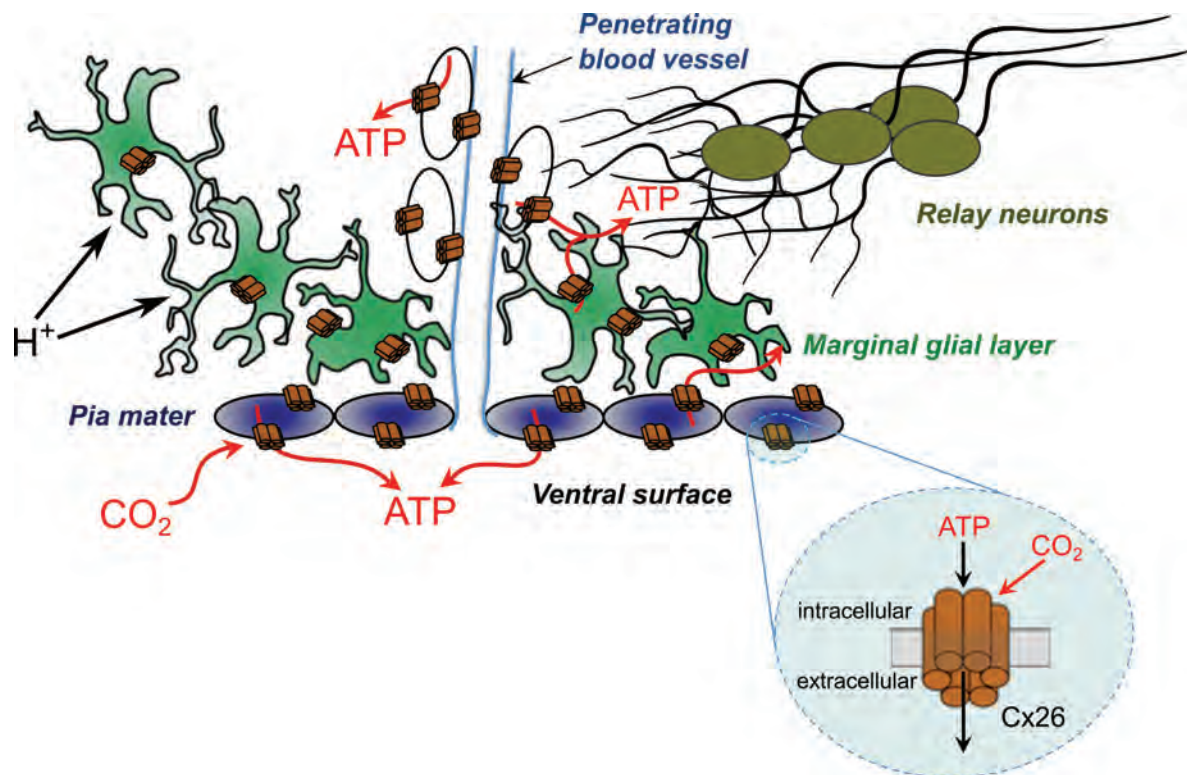


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Chemosensory mechanisms at the ventral surface of the medulla oblongata (p. 32).