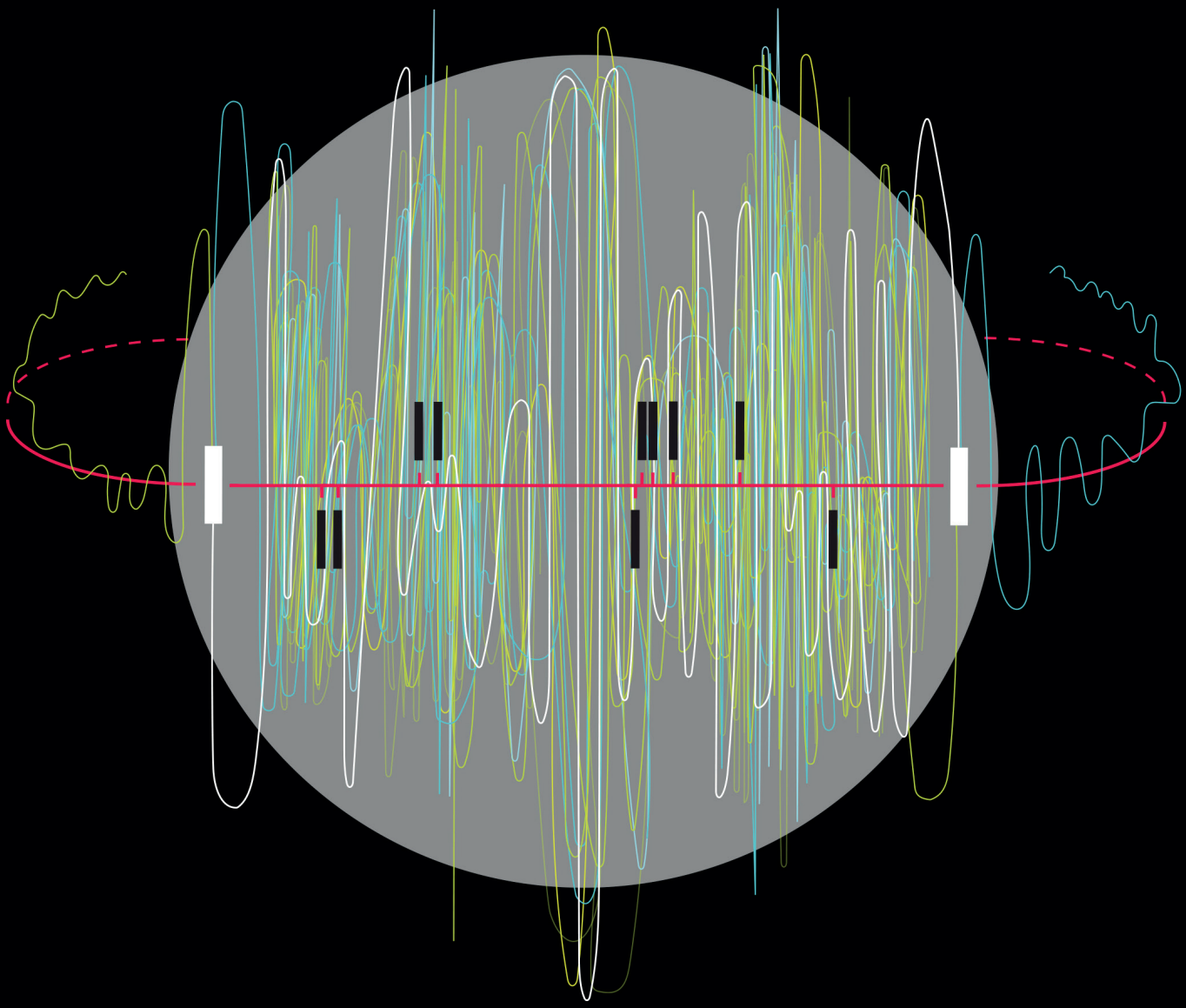
PHENOTYPE

Issue 34 | Michaelmas 2019 & Trinity 2020 | Double Issue



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LETTER FROM THE EDITORS

Issue 34 | Michaelmas 2019 & Trinity 2020

Dear Readers,

After 12 years and 33 issues, *Phenotype* is going global! For those of you picking up an issue of *Phenotype* for the first time – welcome! We are Oxford scientists and Oxford alumni writing about the life sciences for other scientists and the general public.

This issue features a special interview with Professor Alastair Buchan, Director of Oxford-in-Berlin, who has previously served as the Pro-Vice-Chancellor of Brexit Strategy at the University of Oxford. Find out about future collaborations between Oxford and Berlin on page 27.

This issue also features an exclusive interview with Professor Maria Leptin, Director of EMBO, who shares her story about becoming the first woman in the history of EMBO to lead the organization. Maria offers candid advice to the next generation of scientists (page 6).

In the Research Highlights section (page 10), Laura Garmendia Sanchez writes about two recently-published articles in Neuron by Oxford scientists.

On page 12, Abigail Wilson takes us inside the heart to tell us everything we need to know about cardiomyocytes, while on page 22, Komal Yasmin sheds light on CpG islands.

A fascinating piece about AI, Machine Learning, and the Digital Healthcare Revolution is a must-read for anyone interested in the future of medicine, written by Andrew Creagh (page 14).

5-10% of us will eventually come down with Carpal tunnel syndrome. Find out what it is in an informative piece by Dr Akira Wiberg. This article is a special contribution by the Oral Presentation Winner of the 2019 Oxford Medical Science Division DPhil Day (page 17).

Have you ever wondered why Himalayan people have redder cheeks? Find out from Dr Martine I. Abboud, Junior Research Fellow in Chemical Biology (page 19). This is accompanied by an interesting Research Infographic that illustrates the history of oxygen sensing in cells, a discovery awarded the 2019 Nobel Prize for Physiology and Medicine.

Turn to page 24 which features a write-up by the Gold-medal winning Oxford iGEM team writing about their war against antibiotic resistance.

On page 32, Dr Verena Heise, Intermediate Fellow at the Nuffield Department of Population Health, discusses reproducibility in science/research.

Science meets Art. Dr Siv Vingill talks about a career diversification from being a scientist to an artist, and describes the new chic neuro-art scene in Berlin (page 36).

We also interviewed Patricia Alison Hartley, General Manager at HRA Pharma and former General Manager of Consumer Healthcare (DACH) at Sanofi, who has been voted as one of the top 50 female managers in the pharma branch. She discussed with us what it takes to be a good leader (page 39).

Dr Jessica Ocampos discusses her journey from being a PhD student at Cambridge to establishing a successful startup on sustainability (page 42).

What lies in the future of an academic journey? A brief report by Dr Maddalena Comini from a joint-departmental postdoctoral retreat can be found on page 39.

We end with a crossword by Dr Siv Vingill. Email us your attempt to win some exciting prizes!

Last but not least, you can access all our previous issues and subscribe to *Phenotype* on our website www.phenotypejournal.org.

Sonia Muliyil and Marina Kolesnichenko
Co-Editors-in-Chief of Phenotype

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Words from our cover artists...

What does it mean to be part of a community, and do we speak the same language? Can a language of science and art be evolved to be mutually beneficial to the development of both?

Phenotype appears to us as a fleshy geography, representing different voices across the world. We are learning the urgency of science finding a way to communicate its findings as it continues to oscillate between microscopic and cosmological.

Ela Man and Olivia Williamson are finalists at the Ruskin School of Art at Oxford, and collaborate on multidisciplinary projects at the threshold of art, design and architecture. They recently completed "Details of Light", a site-specific installation commissioned by the Oxford Dunn School of Pathology Art Award.

A DIRECTOR'S JOURNEY

Meet the Director of EMBO

excellence in life sciences

Professor Maria Leptin

Professor Maria Leptin is a biologist with her research spanning areas of developmental biology and immunology. She was appointed the Director of European Molecular Biology Organisation (EMBO) in 2010, the first woman to be appointed to the position.

Interviewed by Sonia Muliyil, Co-Editor-in-Chief.

***Phenotype*:** How would you define your career path? Some feel you had a very unconventional one.

Professor Leptin: Many people I know did not have a straight career path leading them to where they are now. There is no such thing as "by the book". I wanted to do something completely different – wanted to be an interior decorator, a dancer and so on. Then, I went into biology and maths because that is what I was good at, and what I enjoyed. I had no intention of doing research at that point but thought I'd go into teaching. But when the time came to be a teacher, I freaked out. I just saw in front of me days and months and years of doing the same thing. I suddenly realised that this is not what I wanted. I had also done a really good practical course during my penultimate year at university. The guys who taught this course offered to do an advanced course the following year and a bunch of us signed up. However, by the time they came around to conduct that course, almost everyone had dropped out, with only me left. So, they made me work in a lab instead. At that time, this was quite unusual. I just loved it. It was fun! One of them had suggested I should consider a research career, so eventually, I asked him whether I could join his lab. That is how I started my PhD.

***Phenotype*:** Was there someone who inspired you to become a scientist? Maybe some incident or life-changing experience that propelled you to take up an academic career?

Professor Leptin: Not at all. In fact towards the end of my PhD, I got very depressed. It was incredibly hard. The Basel Institute for Immunology was an unusual institute – 50 scientists and 50 technicians and only 2 other students. People highly selected from all over the world. To me they were all superstars! As far as I could see, everybody published papers left, right and centre. The only person whose experiments were not working was me. It got very depressing. But giving up was not an option I would have considered. It was clear to me that I had to finish my PhD. So, I finished. But towards the end of my PhD, I already started to look for options for going back to dancing. But everybody said that Post-Doc is the best thing you could ever do and it's so much fun. I thought there's no harm in applying and meeting interesting people. It's not that I did not think that science was exciting. I used to go to the Biozentrum in Basel and listen to talks. I had begun to hear talks about *Drosophila* and all the amazing things that were possible. Genome walking had just begun, so had cloning and P-element transformation. The other big thing at that time was transcription and transcriptional regulation. So, I interviewed with several labs engaged in this line of research and decided to give it a try.

***Phenotype*:** At any stage of your life, did you feel that mentorship played an integral part or do you think it's simply overrated?

Professor Leptin: I had good advisors. I did not miss out on anything. I had friends. But I don't think I'm good at listening to advice. Usually, when people tell me not to do something, I want to do it. Recently in a talk relating to an invention, the speaker had a sample in a falcon tube, and passed it around to everyone in the audience stating that we should simply look at it, and not try to smell it. I just had to open it.

***Phenotype*:** So, you basically like to go against the tide. That's interesting.

Professor Leptin: I think so. If I am asked not to do something, I like to find out why not.

***Phenotype*:** I guess that defines why you are a scientist. Because at the end of the day, research is mostly curiosity-driven. Right?

Professor Leptin: Exactly! I think that's important. I know there are people who want to cure a particular disease, or do research to help mankind in other ways. I just came back from the Falling Walls Conference in Berlin. Lots of people presenting stuff that they invented, or other breakthroughs they had made. It's great. Many of these people are doing things that are immediately useful. But one physicist reported on his success in generating exceptionally low temperature. The audience asked him what it was good for. He said that there were three reasons – one of which was to satisfy his own curiosity and before he could outline the other two, his time ran out, so we never got to know what were the other two objectives. But I thought it was good that he stated "curiosity" first!

***Phenotype*:** But in the current scenario, where funding agencies and grants dictate what research you actually engage in, do you feel there is at all a space for curiosity-driven research?

Professor Leptin: Well, absolutely! We do have funding agencies for that. Many national funding agencies fund basic research; the ERC as well. It's all curiosity-driven. Any country that does not support basic research would be making a huge mistake, in my view. If they want to fund applied research, what will they apply if they don't have any ongoing basic research?

***Phenotype*:** On that note, moving to a more controversial topic, i.e. Brexit. Keeping in mind all the stuff that's happening, a lot of people are re-considering the future of Science in the UK. EMBO fellows, myself included, and those funded by European agencies have been contemplating their future in the UK. What are your thoughts on this?

Professor Leptin: There are other EMBO member states that are not in the EU, so Brexit won't directly affect EMBO fellowships. Of course, it's a disaster if the UK leaves the EU. It's a stupid decision! But that does not mean that science will come to a standstill. Even if the economy dips a bit, there will still be a lot of money in the UK. EMBO fellowships will be available post Brexit, as well as lots of other fellowships and grants. But yes, access to EU funding will be lost.

***Phenotype*:** Do you envision it will impact collaborations in any way? ▶

Professor Leptin: I don't think so. For instance, the ERC Synergy grants can include one team outside the EU. Yes, life will be worse for scientists, going back and forth between places will be more difficult. But, it will sort itself out. It has to!

***Phenotype*:** Going back to your career choices, I realised from your CV that you did have some training in Mathematics before you transitioned to Biology. Did that influence the way you approached certain problems in science?

Professor Leptin: I think it does. You tend to think differently. I was quite lousy at Maths at university. But even so, the way of thinking stays with you. This just means that it's easier for me to talk to people who take mathematical approaches to solve a problem. That influences my thinking, but not in a big way. I know people who are not very good at Maths, but can think more creatively and logically than myself. There are so many different ways of doing science that I think it does not really matter in the end. Chemistry, I think, is probably more valuable to biologists than Maths.

***Phenotype*:** How was your experience transitioning from a solely academic position onto assuming additional responsibilities as the first female EMBO Director?

Professor Leptin: It was not so difficult. In principle, you learn to run things anyway. I had been acting as head of the Institute at Cologne. You learn how to do things like that. It proved easy not least because the staff at the EMBO are fantastic.

***Phenotype*:** Do you think enough support exists for female scientists or is there scope for improvement?

Professor Leptin: There're still stereotypes and biases; sometimes it's shocking to hear what people think. There is still scope for improvement in the heads of people. But the situation has improved a lot already. For example, as part of its 50th anniversary celebrations, the EPFL at Lausanne hosted a wonderful open science day with a programme of illustrious speakers, and 50% of them were women. So it can be done, even though I often hear "there aren't enough women in this field to invite". These things don't happen without making an effort. And again, just looking at meetings I recently attended, also at the Falling Walls Conference in Berlin last week, about half the presenters in the "Labs" competition were women from all over the world, Africa, Asia, South America. So powerful! I think there isn't much of an excuse for women not to take up science as a career. What's not going to get easier is the fact that children do need a lot of time and attention. If you talk about hard, the hardest time in my life was when our children were small and my husband and I both moved to Cologne from different places. We both had to set up our labs in a university setting that we were not familiar with, while wanting to be there for our children as well. That was tough. There were really days when I thought that if somebody comes up with another problem, I would simply scream. So, that was the only period I would call really difficult.

Professor Leptin currently leads two research groups, one at EMBL Heidelberg (pictured) and another at the University of Cologne.

***Phenotype*:** What would be your advice for early-career scientists, who are juggling parenthood with science?

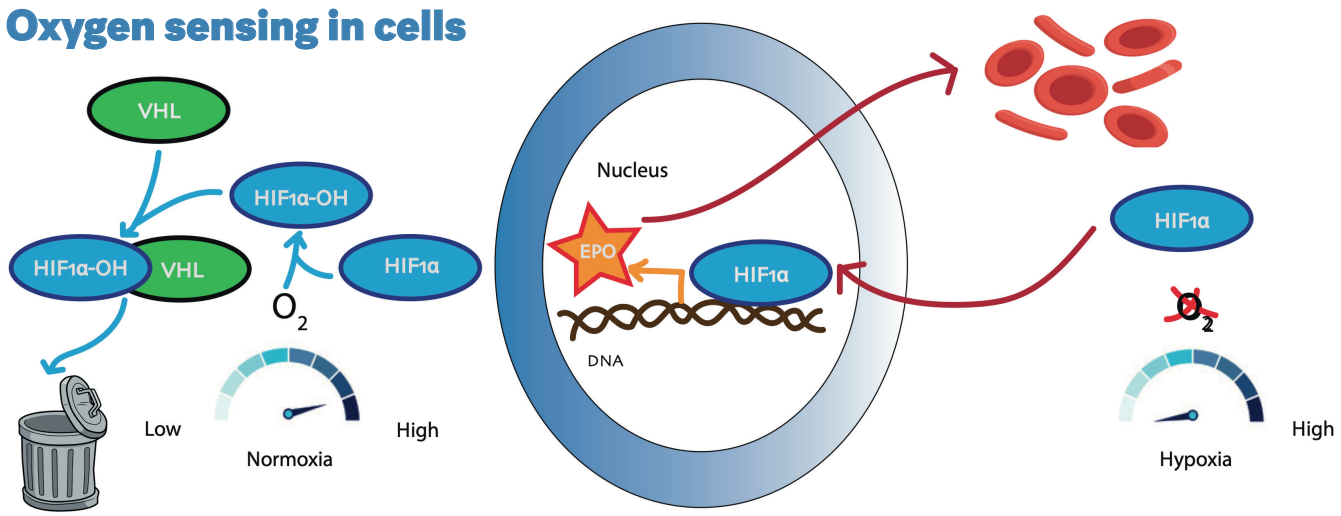
Professor Leptin: I don't understand why people don't pay to get help. We had a full-time nanny. People around us thought it was crazy to "waste" my entire salary on that. Yes, sure, a good part of our income went into that. But so what? If I had stayed home, we wouldn't have had that income either, and this way I was able to continue to work, and the children were happy. I did not have the time anyway to buy new clothes or go to the hairdresser or dine in expensive restaurants. So, my advice would be – pay pay pay! Buy help; don't complain; just get on with it. My husband did help a lot. We really shared the workload at home. And that's true for many couples now.

***Phenotype*:** Thanks a lot for being so open and generous with your time. ■

RESEARCH INFOGRAPHIC

By Linda van Bijsterveldt and Sam Durley. Linda and Sam are a DPhil student and a postdoctoral researcher at Tim Humphrey's group at the Department of Oncology, University of Oxford.

William J. Kaelin, Peter J. Ratcliffe and Gregg L. Semenza were awarded the Nobel Prize in Physiology or Medicine 2019, in recognition of their leading contribution to the field of oxygen sensing. Below is a summary of how the field evolved and the contributions by the laureates. More on oxygen sensing on p.19.



In 1858, **Louis Pasteur** showed that animal cells require oxygen to survive. In 1882, French physiologist **Paul Bert** discovered that being in oxygen-poor conditions at high altitude increases the red blood cell count, crucial for providing cells with enough oxygen to survive. In 1986-1987, **Maurice Bondurant**, **Mark Koury** and **Jaime Caro** showed that low O_2 causes an increase in a hormone, erythropoietin (EPO), which leads to an increase in red blood cells. It took over 100 years to identify the mechanism for oxygen sensing in cells.

In 1992, **Gregg Semenza** discovered Hypoxia-Inducible Factor 1α (HIF1α), which bound the *EPO* gene, and in 1993 both he and Peter Ratcliffe showed this leads to increased transcription of the gene. The expression of HIF1α was not affected by oxygen levels, however, so the question as to how red blood cell production was regulated under changing conditions of oxygen, remained.

In 1999, **Peter Ratcliffe** and colleagues observed that, in normal oxygen levels, HIF1α is degraded through an interaction with VHL, a protein identified by **William Kaelin**'s laboratory in 1995. In 2001, both Kaelin and Ratcliffe demonstrated that the interaction between HIF1α and VHL is dependent on the presence of oxygen, and it results in HIF1α and VHL being degraded by the proteasome, thereby providing a mechanism for oxygen sensing.

RESEARCH HIGHLIGHTS

By Laura Garmendia Sanchez. Laura is a DPhil student at Gero Miesenböck's lab at the Centre for Neural Circuits and Behaviour (CNCB), University of Oxford.

Defining the functional role of $Na_V1.7$ in human nociception

McDermott & Weir *et al*. (2019). *Neuron* **101**, 905–19.

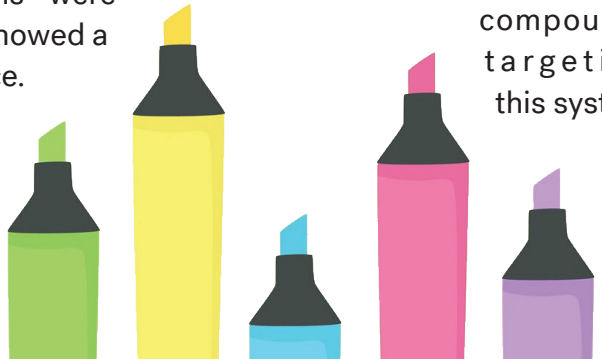
Loss of function mutations in *SCN9A*, the gene encoding the voltage-gated sodium channel NaV1.7, cause congenital insensitivity to pain (CIP). Strikingly, while people with inactivating mutations in $Na_V1.7$ don't feel pain in response to noxious stimuli of any kind, the only other clinical feature they experience is the loss of the sense of smell, either partially or totally (anosmia). This has spurred increasing interest in developing analgesics that selectively target this channel. However, to facilitate drug development, it is key to first understand how $Na_V1.7$ mutations lead to CIP.

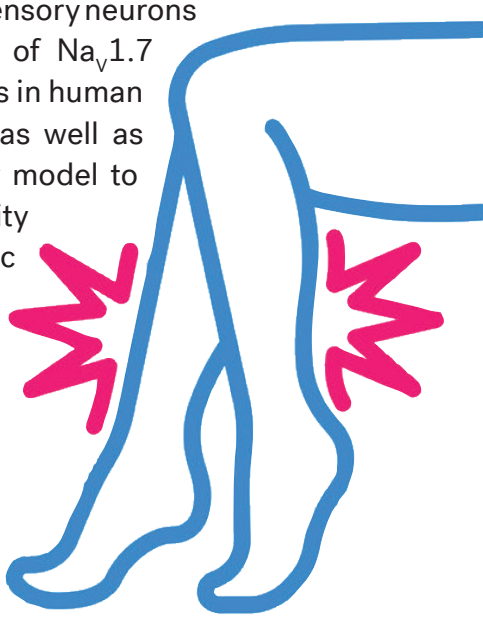
To this end, McDermott & Weir *et al*. (2019) used a multi-modal approach to detail the functional role of $Na_V1.7$ in the human nociceptive system. Three CIP participants with confirmed *SCN9A* mutations, predicted to affect $Na_V1.7$, were recruited to assess their sensory nerve function. This revealed an impaired function of small fibres and preserved large fibre function. Consistently, C-fibre recordings from the CIP patients did not detect C-nociceptor characteristic traits. Additionally, brain imaging was performed to assess the brain activity changes in response to noxious stimuli (capsaicin and thermal stimuli) and, while healthy individuals showed changes in brain activity consistent with a painful experience, this was not observed in the CIP subject.

To study CIP at the cellular level, whole-cell patch-clamp recordings of the various mutant $Na_V1.7$ channels were performed, all of which showed a decrease in conductance. Furthermore, nociceptor neurons derived from induced pluripotent stem cells (iPSC) from CIP and healthy individuals were generated. These revealed that $Na_V1.7$ is localised in specific neuronal compartments within nociceptors and that the channel plays a crucial role in regulating excitability to generate action potentials. Finally, they showed that human iPSC-derived nociceptors are a useful cellular model to probe the selectivity of potential $Na_V1.7$-selective inhibitors aimed as analgesics.

Co-first authors of the paper Lucy McDermott (right) and Greg Weir (left), are members of David Bennett's group (middle) at the Nuffield Department of Clinical Neurosciences, University of Oxford.

In summary, McDermott & Weir *et al*. use cellular models and somatosensory neurons to show the impact of $Na_V1.7$ CIP-related mutations in human nociception *in vivo*, as well as developing a cellular model to assess the selectivity of novel analgesic compounds targeting this system.





The hippocampus and neocortical inhibitory engrams protect against memory interference

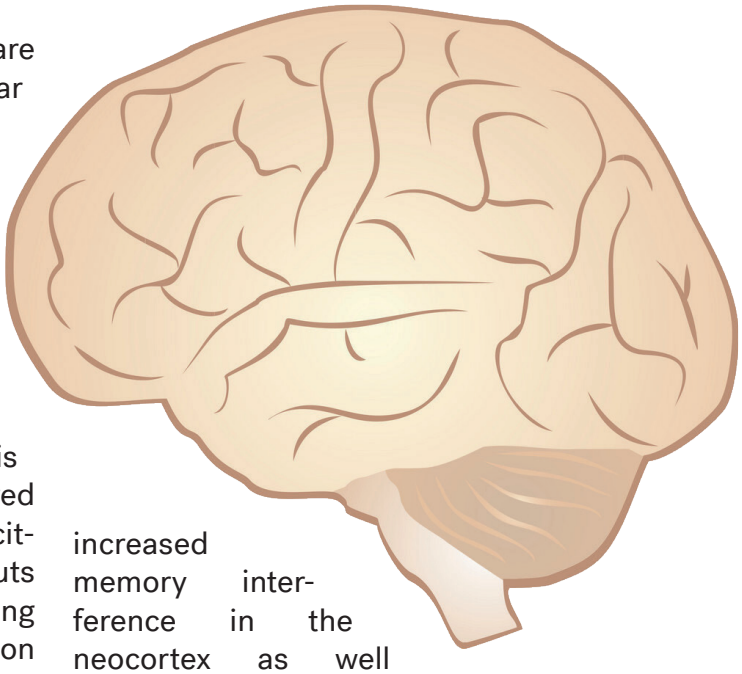
Koolschijn *et al*. (2019). *Neuron* **101**, 528–41.

Our past experiences often overlap. Yet we are capable of distinguishing them from similar memories. The neuronal mechanisms that help prevent interference between overlapping memories and allow precise memory recall remain unclear.

Previous research has suggested contextual representations as a means used by the brain to reduce memory interference, but how information is organised within these representations is not clear. Additionally, it is thought that when new information is stored in the brain through the strengthening of excitatory connections, opposing inhibitory inputs are used to maintain balance when new learning occurs, suggesting a potential role for inhibition in protecting from memory interference. Building on this, Koolschijn *et al*. (2019) investigated the role of the hippocampus and of neocortical inhibition in preventing interference between two context-dependent overlapping memories.

For this, they first trained human patients to encode two context-dependent overlapping memories across two consecutive days and performed ultra-high-field 7T MRI imaging on the third day. Using the BOLD functional MRI technique, this revealed an increase in hippocampal activation during periods of potential memory interference, which, in turn, correlated with a higher percentage of errors in the memory interference task.

Additionally, the maximum separation was found in hippocampal representations of stimuli that had different relational positions access the two memories, suggesting that contextual representations are structured in the hippocampus using a relational code. Finally, through brain-stimulation, they reduced the concentration of GABA in the neocortex and found that this correlated with increased memory interference in the neocortex as well as in the behavioural responses.



In conclusion, Koolschijn *et al*. find two mechanisms used by the brain to reduce memory interference, one involving the separation of memories in the hippocampus and another one involving neocortical inhibition. ■

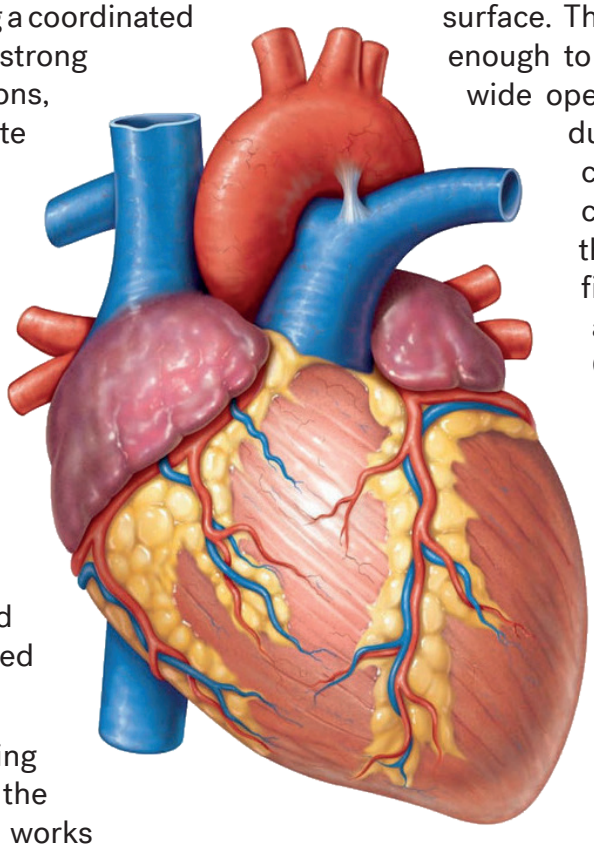
First author of the paper, Renée Koolschijn (left), and Helen Barron (right), lead contact of this work.

HEARTY COLLABORATION

Taking a look inside the cardiomyocyte

By Abigail Wilson. Abigail is a DPhil student in Rebecca Sitsapesan's research group at the Department of Pharmacology, University of Oxford.

The heart is a fantastic piece of coordinated machinery, which consists of an estimated 2–3 billion individual muscle cells called cardiomyocytes. Cardiomyocytes are fixed together like bricks in a wall, forming a coordinated structure that induces strong and rhythmic contractions, which ultimately translate to pumping and relaxing during every heartbeat.



Each cardiomyocyte is a huge cell, inside of which is a vast compartment called the sarcoplasmic reticulum (SR). Embedded into the SR are cardiac ryanodine receptors (RyR2), the largest ion channel in your body, that creates a gated pore, which can be opened and closed [1].

Calcium is a critical signalling ion for the contraction of the heart. A cardiomyocyte works very hard to maintain its cytosolic calcium concentration inside the cell at around 100 nanomolar, an estimated 100,000 times lower than outside the cell. By contrast, the SR compartment inside the cell has a high calcium concentration of around 1 millimolar, therefore providing a large chemical gradient of about 10,000-fold across the SR [1, 2]. This large gradient allows cardiomyocytes to change the cytosolic calcium concentration very rapidly.

RyR2 channels form clusters on the surface of these SR compartments, holding their pores tightly shut, waiting for their signal. An electrical message causes a small amount of calcium to enter the cardiomyocytes from their outer surface. This minute increase in calcium is enough to trigger the huge RyR2 to snap wide open, a process called calcium-induced calcium release [2]. This causes an enormous cascade of calcium to flood out from the SR, through the pore of the RyR2, filling the inside of the cell with a higher calcium concentration. Calcium binds to the muscular components of cardiomyocytes to squeeze each cell together. The additive effect of every cardiomyocyte across the heart contracting together is a powerful pumping action, forcing blood out of the heart and round to the circulatory system.

There are a plethora of ways to explore the function of cardiomyocytes. You can add a chemical dye that permeates inside the cell and fluoresces when calcium binds to it. Fluorescence intensity can then be used as a measure of the calcium flux within the cell. You can also "patch" cardiomyocytes. This is where you seal onto or pierce through the outer membrane of the cardiomyocyte, and then measure changes in membrane voltage or current. However, it is impossible to use these techniques to record the activity of RyR2 alone as they reside deep inside the cardiomyocyte, rather than on the surface membrane.

An example single-channel bilayer recording of an RyR2 channel gating. The upward deflections show RyR2 channel opening via calcium flux through the channel.

The only way to accurately measure the gating kinetics of RyR2 is through the single channel bilayer technique. To do this, heart tissue is homogenised and SR membrane vesicles containing intracellular ion channels are isolated. Next, these SR vesicles are incorporated back into an artificial lipid bilayer [3]. This allows us to mimic the environment of RyR2 inside cardiomyocytes, whilst still having absolute control of the conditions on either side of RyR2. Our laboratory uses this single-channel bilayer technique to study RyR2, making it possible to measure the minuscule currents of calcium that flow through these ion channels. We can gain so much information by zooming in to this channel and observing its behaviour at the molecular level. It is here, that we can compare the activity of healthy and diseased RyR2. For example, if we observe that a dysfunctional ryanodine receptor is much more sensitive to calcium activation, then that wave of calcium entering a cardiomyocyte can be triggered at the wrong moment. This will result in devastating effects such as irregular heartbeats and even sudden cardiac death.

Each technique in science comes with its own limitations. As for single-channel bilayer experiments, they are technically difficult. Removing the RyR2 channels from their cellular environment is an obvious caveat. However, interdisciplinary science holds the answer to understanding the full picture. Our laboratory is often contacted by other groups for means of collaborations to establish proof of concept. For example, a group working on a model of ryanodine receptor disease might have made a convincing phenotypical model of the particular disease, but only the single-channel bilayer technique allows the molecular study of the differences in single-channel behaviour. As another example, a research group might have a potential drug they think works on a whole cell or tissue level. The single-channel bilayer technique provides the only way to directly measure a drug interaction with an intracellular ion channel, excluding the drug's effect on other parts of the cardiomyocyte [4].

This puts our own technique at the crosstalk of interdisciplinary science. Recent cryogenic electron microscopy studies have revealed the molecular structure of RyR2, and advances in computational modelling and molecular dynamics can predict the behaviour of RyR2. But how does this relate to its function? Are computational studies recapitulated by its actual activity in the bilayer? The only way to know if a computational study is truly accurate is to see the evidence repeated in single-channel bilayer experiments.

The best way to understand RyR2 disease is to put together data from a range of interdisciplinary techniques to take a look inside the cardiomyocyte. It is critical to use the strengths of each research technique to further our understanding of health and disease in the field of biophysics. This rationale can be applied to science on the whole; although each technique has its own strengths and weaknesses, integrated knowledge is certainly the most powerful. ■

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AI and the digital healthcare revolution uniting disciplines

By Andrew Creagh. Andrew is a DPhil student with the Computational Intelligence in Biomedical Monitoring (CIBIM) Laboratory, led by Maarten De Vos at the Institute of Biomedical Engineering, University of Oxford.

We are at the precipice of a digital healthcare revolution. The promise that machine learning and artificial intelligence hold to transform medical science is extraordinary. We are in the age of big data, and the bigger the better; the larger the data sets, the greater the opportunity we have to find cures to chronic conditions, create innovative solutions to tackle scientific problems and create truly personalised care, tailored to the individual.

In recent years the blending of computer science, data science and medicine has ignited a revolution in healthcare and forged a cross-disciplinary field. Data scientists and engineers are starting to work closely with clinicians and medical scientists to understand this new wave of big data. Big data in a medical sense can mean any number of things, but the fundamental concept is that there needs to be a lot. As technology has developed, more and more terabytes can be stored, alongside algorithms with new abilities to compute this information more efficiently and meaningfully. This flow of new scientific data is coming from a vast range of sources, including MRI images, electronic healthcare records, basal DNA and genetic data, and patient-generated data such as from smartphone health apps, wearable devices and sleep trackers.

Machine learning (ML), often categorised under the broader tag: "Artificial Intelligence (AI)", is the application of algorithms to learn patterns within these data and make inferences based on those learnings. Deep Learning, which is a subfield of ML, is arguably the most powerful AI tool at our disposal [1]. Like Lego, layers and layers of non-linear blocks are stacked upon each other. Each "neural network" layer learns and transforms the data from the previous layer into a more abstract representation of what it once was – searching for patterns within patterns beyond anything a human can do – allowing highly complex functions to be learned from the original raw data. However, AI is not here to replace clinicians, it is here to give them a superpower.

Recently, deep learning has achieved and exceeded physician-level performance in identifying patients needed for referral using retinal optical coherence tomography scans [2]. Other research has shown the successful ability to distinguish between benign and malignant breast lesions in ultrasound images, as well as pulmonary nodules in CT images [3]. These studies demonstrate the power of AI architectures; reaching and beating the performance of human assessors essentially equips clinicians with an extra pair of eyes. Now a huge pipeline of clinical images can be evaluated rapidly by machines to potentially alert of suspicious tissues, offer a second opinion and to help ease the burden on doctors so they can focus on treating patients. This is where the real value of AI lies.

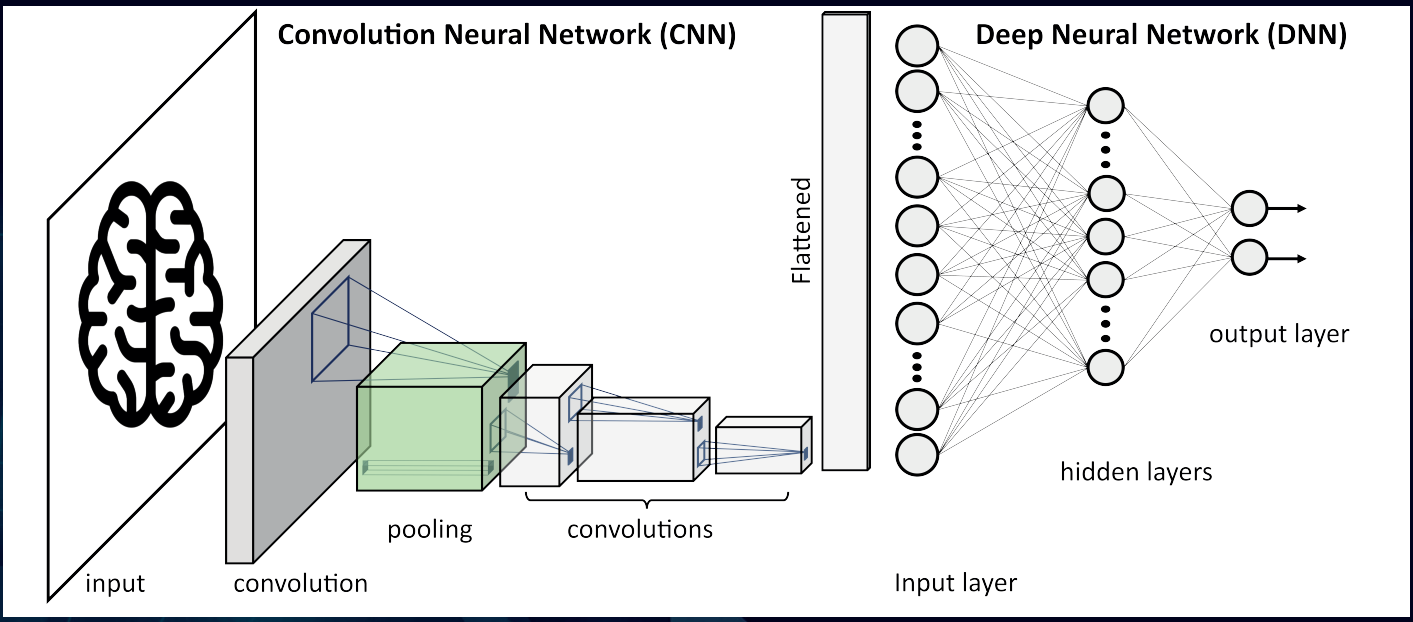

Figure illustration of a CNN-DNN model, a commonly used architecture in deep-learning that are inspired by the connectivity patterns in the animal visual cortex. Convolution Neural Networks (CNN) are a class of algorithm which can learn patterns (termed "features") within the data. In this example, a brain input image can be sequentially transformed (left-to-right) using a series of convolution and pooling operations into flattened vectors. A simple Deep Neural Network (DNN) can be stacked together consisting of hidden layers (hence a "deep" artificial neural network, denoted by the circles), and takes in data at the input layer. As this data flows through each node of the network it will again be non-linearly transformed. The elements of the final output layer (in this case a classification "softmax" later) represent the probabilities of the presence of disease versus healthy. During the training process, the internal parameters of the network layers are adjusted to improve the model accuracy. Different output layers can perform separate tasks such as classification (e.g. disease vs no disease) or regression (e.g. how diseased is this brain). CNN and DNN models can by typically combined or used separately in deep-learning approaches.

Communication of data and techniques used will play a vital role in developing the understanding of AI and the bridging of disciplines. We now have a fundamental need for clinicians and engineers to work in creative and collaborative ways. Engineers will need to assemble stories that can be understood from fragmented, sometimes sparse and heterogeneous data. We will need to work together to harness the real value from these data, to build expert knowledge and trust by opening the so-called "black-box" algorithms.

The biggest challenge of acceptance deep-learning faces is the inherent difficulty in interpreting the decisions that lead to a prediction. Models can be highly complex, and patterns learned are so abstract that they become difficult to understand. It is our job to improve the communication of these models and the reasoning behind their use. Breakthroughs in visualising deep networks have paved the way for these explanations, where heat maps of 'relevant' parts of data can be built by decomposing the internal neural network nodes. Recently, this has been applied to interpreting relevant parts of the brain responsible for the predictions of Alzheimer's disease using MRI images [4]. Interpretation of network decisions could greatly develop clinicians' fundamental understanding of diseases like Alzheimer's.

Collecting a lot of data from varied sources is vitally important for the effectiveness of AI. It allows algorithms to train more effectively, to identify characteristic signatures and to generalise to data it has never seen before. Perhaps the most extraordinary example of building trust in AI has come by allowing people to collect their own data. Smartphones are ubiquitous and this technology has allowed researchers the opportunity to collect a new type of medical data on a large scale that is needed. Crowdfunding user-generated data from technologies such as smartphones, wearable devices and sleep trackers has opened up possibilities for people to monitor their ▶

own health, help train algorithms and engage with AI. For example, patients with multiple sclerosis or Parkinson's disease have been given smartphones and smartwatches which can help monitor their symptoms; in the latter case, patients need only download an app and perform a series of walking, voice or cognition tests every day [5, 6]. Over 10,000 people, mostly healthy, downloaded the app and contributed meaningful data. These types of remotely captured tests can help clinicians to augment routine healthcare assessments and even help monitor and identify signs of illness or degeneration before they occur – with the help of machine learning.

As a result of the influx of more data, the endless possibilities of personalised healthcare are becoming more achievable. Mining of this information may allow the clustering of people with similar characteristics, such as how they respond to various treatments, or discovery of hidden patterns within genetics which can be exploited to create innovative ways to further drug discovery and treatment regimens.

Much work has already begun in uniting the fields of genomics and deep-learning [7]. More data and these state-of-the-art methods offer the potential to revolutionise traditional biomarker techniques that can characterise the onset and progression of diseases. Models have already been successful in predicting the sequence specificity of DNA- and RNA-binding proteins and of enhancer and regulatory regions [8, 9], along with predicting intermediate molecular phenotypes – for example, gene expression and splicing control [10]. I believe that these advances in fundamental science will be augmented and united with this new flow of digital data – data capable of being captured on a large and longitudinal scale, in a clinic or remotely from a diverse population.

The close collaboration of data scientists and engineers with clinicians, medical scientists and the general public will be crucial for the success of this new digital age of personalised healthcare. Trust in the data and AI must be generated from all sides. Interpretations from experts will be depended upon to build reliable models. We, as engineers and data scientists, must learn how to communicate with each other and engage those experts with the data. Most of all, we need to allow people the ability to generate, engage with, and take control of their own medical information. With this, we can break down the barriers and open up the black-box of AI. The digital healthcare revolution has arrived. ■

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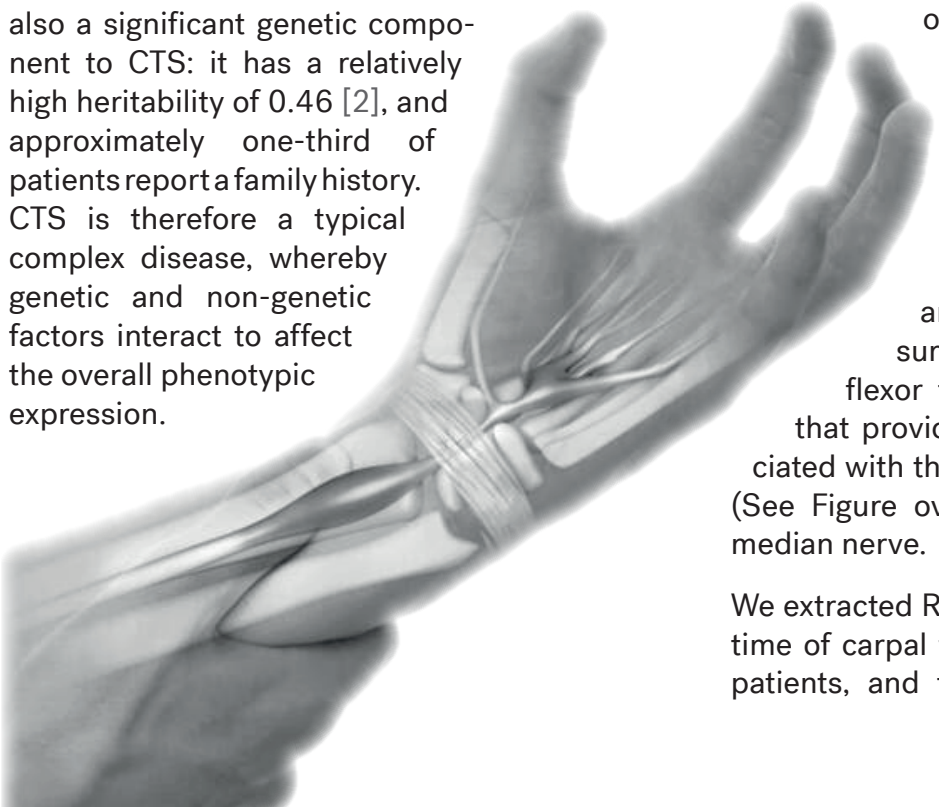
The genetic landscape of carpal tunnel syndrome

By Dr Akira Wiberg. Akira is an NIHR Clinical Lecturer in Plastic Surgery, and was a DPhil student jointly supervised by Dominic Furniss in the Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences (NDORMS) and David Bennett in the Nuffield Department of Clinical Neurosciences (NDCN), at the University of Oxford.

Carpal tunnel syndrome (CTS) is caused by compression of the median nerve as it travels through the carpal tunnel, a narrow anatomical tunnel in the wrist. Patients experience pain, tingling and numbness in the fingers, and weakness in the thumb, which eventually leads to functional impairment of the hand. CTS is very common, with an estimated prevalence of 5–10%; as it affects many patients' ability to work, there is a considerable socioeconomic cost to this disease.

The pathophysiology of CTS is poorly understood. There are certain diseases such as diabetes and rheumatoid arthritis that are associated with an increased risk of CTS, and certain occupational factors are also believed to predispose to the disease [1]. However, there is also a significant genetic component to CTS: it has a relatively high heritability of 0.46 [2], and approximately one-third of patients report a family history. CTS is therefore a typical complex disease, whereby genetic and non-genetic factors interact to affect the overall phenotypic expression.

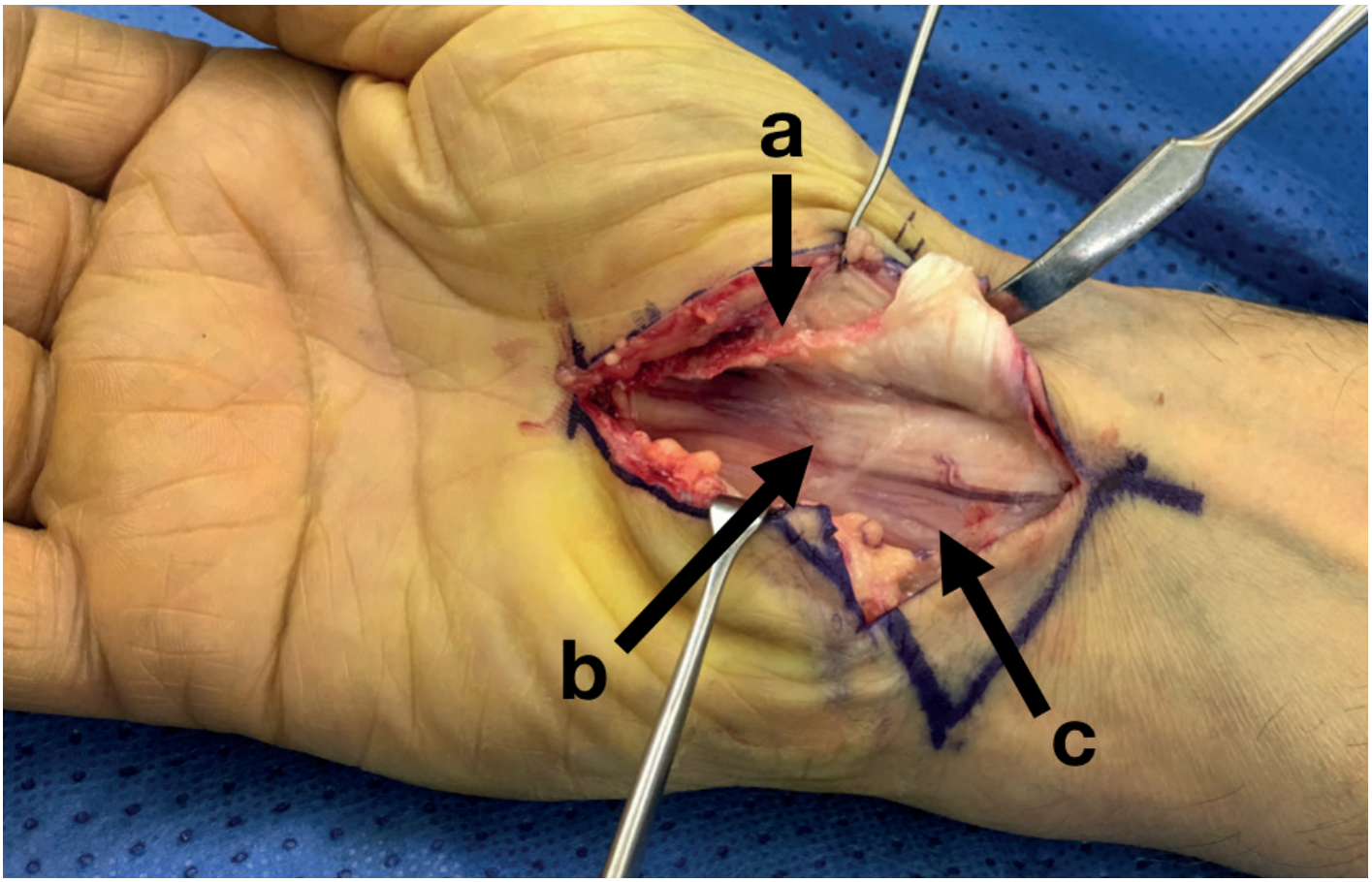


With the aim of interrogating these genetic factors, we performed the first-ever genome-wide association study (GWAS) in CTS. We used ~12,000 CTS patients in UK Biobank, a prospective cohort study of ~500,000 individuals for whom we have access to genotype data and medical diagnoses.

Sixteen genomic loci were significantly associated with CTS, and using a suite of bioinformatics tools, we mapped 25 candidate "risk genes" to these loci. These included two genes in the *ADAMTS* family (important in extracellular matrix remodelling), *EFEMP1* (which encodes a matrix glycoprotein), and three genes in the TGF-β/SMAD signalling pathway. When the candidate genes were analysed in a gene-set analysis, there was a disproportionate enrichment for gene ontologies related to the extracellular matrix.

This finding mirrors the literature on the pathophysiology of CTS, which has focused on aberrations in the sub-synovial connective tissues (SSCT). These are extracellular matrix-rich tissues surrounding the median nerve and flexor tendons within the carpal tunnel that provide a gliding surface. CTS is associated with thickening and fibrosis of this tissue (See Figure overleaf), causing tethering of the median nerve.

We extracted RNA from the SSCT resected at the time of carpal tunnel surgery in a cohort of CTS patients, and found that the candidate risk ▶



Carpal tunnel decompression surgery. The transverse carpal ligament forms the roof of the carpal tunnel, and this structure is divided to relieve pressure on the median nerve. (a) The divided edge of the transverse carpal ligament. (b) The median nerve, showing a characteristic bruised and constricted appearance as it enters the carpal tunnel. (c) A flexor tendon adjacent to the median nerve: the sub-synovial connective tissues overlying the tendon are thickened and hyperaemic, as is frequently seen in CTS. *Photograph provided by Prof. Dominic Furniss*.

genes are highly expressed in this tissue. We genotyped these patients and found that seven of the genes demonstrated allele-specific expression, whereby carrying a greater number of CTS risk alleles leads to over- or under-expression of the gene. These genes are therefore potential therapeutic targets for preventing the changes that occur in the SSCT in the progression of CTS.

In addition to this, we performed bioinformatic analyses to shed more light on the aetiology of CTS. Genetic correlation studies found that there is a positive correlation between the genetic architecture of CTS and obesity, whereas there is a negative correlation with height. The latter finding was consistent with our observation that CTS patients are on average 2 cm shorter than controls within the UK Biobank.

Through a Mendelian randomisation analysis, we demonstrated that shorter height is not merely associated with CTS, but it is causally implicated: a 1 s.d. increase in height is associated with an odds ratio of 0.79 for CTS. Shorter height is correlated with having shorter hands, and CTS patients have been observed to have a rounder wrist shape. We therefore suggest that genetic variants that result in shorter height also result in altered growth of the wrist and forearm skeleton, creating an anatomical configuration that leads to greater pressure being exerted on the median nerve.

This work, the first to use bioinformatics in the study of CTS biology, has demonstrated that there is a robust polygenic architecture underlying this disease. There is a causal role played by altered skeletal growth in the genetic predisposition to CTS, and also a role played by aberrant expression of extracellular matrix genes within the sub-synovial connective tissues that surround the median nerve. ■

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HUFFING AND HIFING

Why is the hypoxic response NO_2bel-worthy?

By Dr Martine I. Abboud. Martine is a Junior Research Fellow of Kellogg College, working at the Department of Chemistry, University of Oxford.

O_2 and life...

We need oxygen (O_2) to survive. In the lungs, oxygen is passed into the bloodstream, where it is carried around the body in red blood cells. Without oxygen, cells cannot produce the energy they need to survive.

For instance, at the top of high mountains, the atmospheric pressure is lower, so you breathe in less oxygen with each breath than at sea level. How do we survive at higher altitudes? Can we sense differences in oxygen levels? Have you ever wondered why do Himalayan people have redder cheeks?

When the air is thin, we initially compensate for the lower oxygen levels (hypoxia) by increasing our heart and breathing rates. The carotid body, adjacent to large blood vessels on both sides of the neck, senses the blood's oxygen levels. In 1938, the Nobel Prize in Physiology or Medicine was awarded to Corneille Heymans for discovering how blood oxygen sensing via the carotid body controls our respiratory rate.

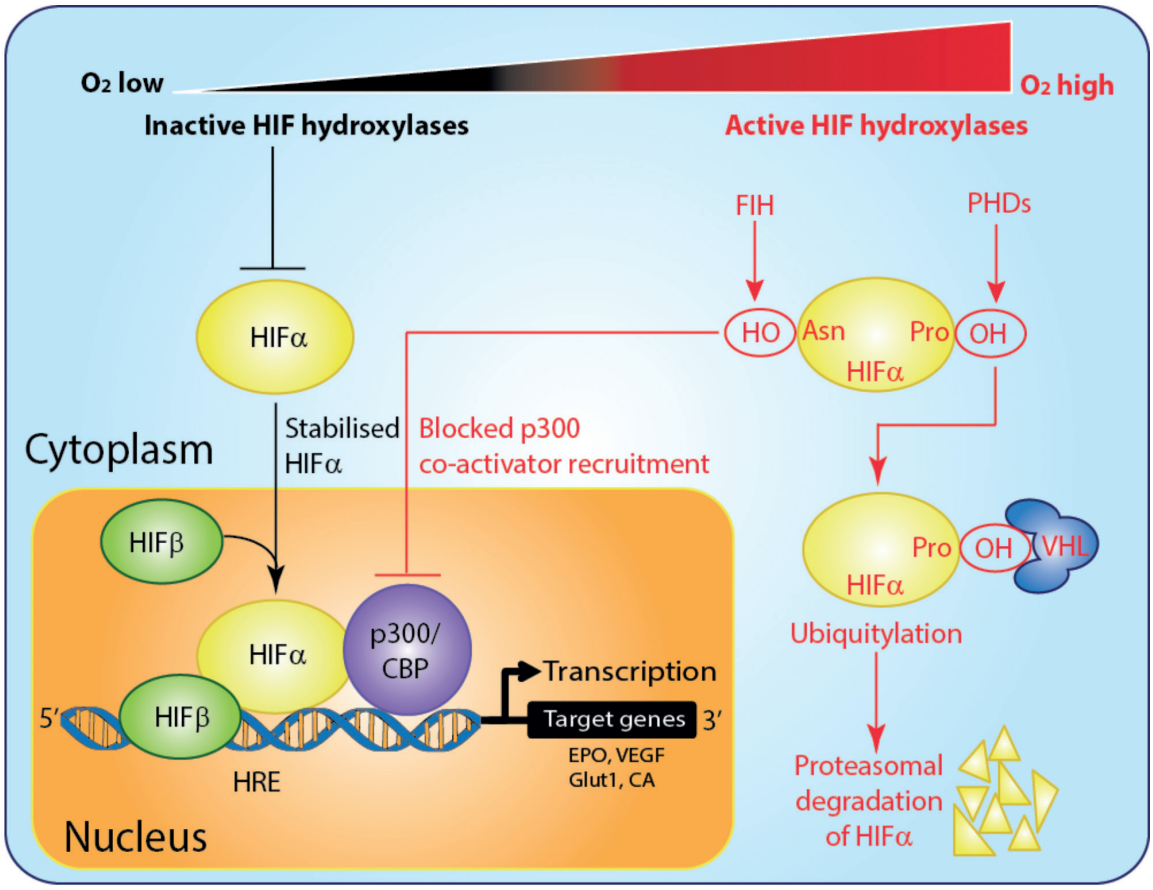
In addition to the carotid body-mediated acute adaptation to hypoxia, there are important chronic adaptations to hypoxia. These adaptations were the subject of the 2019 Nobel Prize in Physiology or Medicine awarded jointly to our own Sir Peter J. Ratcliffe, William G. Kaelin Jr (Harvard) and Gregg L. Semenza (Johns Hopkins) and their research groups.

What is the hypoxic response?

A chain of events enables cells to respond to hypoxia by boosting their blood supply and improving the efficiency of their metabolism. A master regulator of the chronic hypoxic response is HIF, the hypoxia inducible factor, which is a heterodimeric α,β-transcription factor.

Under hypoxic conditions, the levels of HIFα subunits increase, they translocate to the nucleus and dimerise with HIFβ. The α,β-HIF complex binds to hypoxia response elements (HREs) DNA and promotes the expression of HIF target genes. These encode for biomedicinally important proteins that will compensate for the lower oxygen levels, such as the vascular endothelial growth factor (VEGF) and erythropoietin (EPO); these enable the production of blood vessels and red blood cells, respectively (hence, leading to red cheeks in people living at high altitudes!).

Under normal oxygen conditions, HIFα subunits are modified (hydroxylated) by the PHDs (the prolyl hydroxylases). The hydroxylated HIFα ▶



Outline of the machinery involved in the cellular response to hypoxia in animals. A simplified scheme depicting the cellular response to hypoxia and normoxia in animals. PHD: prolyl hydroxylase domain, HIF: hypoxia inducible factor, FIH: factor inhibiting HIF, VHL: von Hippel-Lindau protein, CBP: CREB binding protein, HRE: hypoxia response element. EPO: erythropoietin, VEGF: vascular endothelial growth factor, Glut1: glucose transporter, and CA: carbonic anhydrase are examples of the many HIF target genes.

subunits bind to the von Hippel-Lindau protein (VHL) which causes their degradation by the proteasome, and hence, no HRE genes are upregulated.

What is the Nobel Laureates' contribution to our current understanding of the hypoxic response?

Gregg Semenza studied the *EPO* gene and how it is regulated by varying oxygen levels [1]. Sir Peter Ratcliffe also studied O_2-dependent regulation of the *EPO* gene, and both research groups found that the oxygen sensing mechanism was present in virtually all tissues, not only in the kidney cells where EPO is normally produced. Semenza discovered HIF and that it binds to a DNA sequence (HREs) in an oxygen-dependent manner; he gave HIF its name [2]!

William Kaelin Jr. showed cells lacking a functional VHL gene express abnormally high levels of hypoxia-regulated genes. This proved that VHL was involved in controlling responses to hypoxia. The Ratcliffe research group then made a key discovery: demonstrating that VHL interacts with HIF-1α and is required for its degradation at normal oxygen levels in cells [3].

The Ratcliffe and Kaelin [4] groups separately showed that under normal oxygen levels, modified HIF-1α can interact with VHL, allowing for its degradation. But what protein senses oxygen levels and is responsible for HIF hydroxylation?

How did Oxford contribute to further our understanding of the hypoxic response?

Further research by the Ratcliffe and Christopher J. Schofield groups (Oxford, Chemistry) identified the proteins responsible for mediating HIF hydroxylation, the PHDs [5]. The first structure of a PHD (PHD2) was solved by the Schofield group in Oxford [6]. The PHDs belong to the same family of enzymes that make the penicillins and related β-lactam antibiotics, which of course were the subject of an earlier Nobel prize to which Oxford contributed! Studies with proteins, cells, and animals showed the PHDs to be molecular "hypoxia sensors" for the HIF system; in the sense

that their activity is limited by O_2 availability – they manifest an unusually slow reaction with O_2. Prolyl hydroxylation of HIF-α substantially increases the strength of its binding to VHL (by ~1,000 fold).

The PHDs are Fe(II)- and 2-oxoglutarate (2OG)-dependent oxygenases, with the same core protein fold and general mechanism as the β-lactam biosynthesis enzymes, including those involved in the biosynthesis of the cephalosporins, which were discovered in Oxford by Edward Abraham. Like the antibiotic biosynthesis enzymes and the PHDs, members of the Fe(II)- and 2OG-dependent oxygenases family are involved in many important biological processes and mutations in them are linked to several diseases, including cancer and Ehlers-Danlos syndrome.

What is the medical relevance of the hypoxic response?

Patients with kidney diseases suffer from anaemia because their kidneys do not make EPO. As a result, their cells do not receive enough oxygen. Diseases of the circulatory system can limit the supply of oxygen to the tissues, resulting in cell death. A stroke or brain haemorrhage, for example, causes degeneration of the brain tissue. Manipulating the HIF system might be able to slow this process.

On the other hand, oxygen falls to low levels in some aggressively-growing tumours. This limited oxygen supply in tumour cells stimulates the growth of new blood vessels into the tumour to supply it with oxygen. Drugs designed to turn off HIF might be able to stop the formation of new vasculature in tumours.

Diseases might be treated by manipulating the oxygen sensing mechanism: by boosting the delivery of oxygen in patients with anaemia or cardiovascular disease, or by turning off oxygen sensing in cancer. Pioneering work in Oxford demonstrated that analogues of their 2-oxoglutarate co-substrate can inhibit PHDs, an approach which has been developed into a new clinically approved treatment for anaemia. ■

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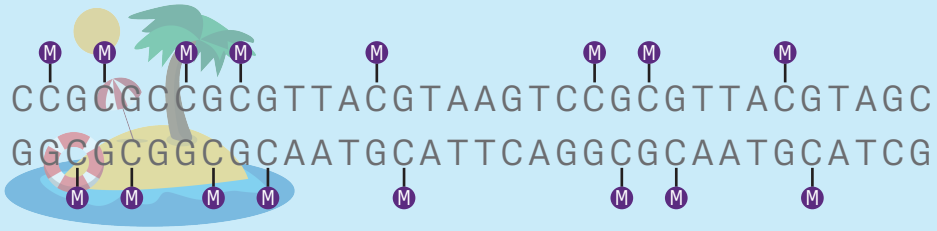
2019 Nobel Laureates in Medicine. (From left to right)
William G. Kaelimn Jr, Sir Peter K. Ratcliffe, Gregg L. Semenza.



The mystery of CpG islands

By Komal Yasmin. Komal is a DPhil student in Neil Brockdorff's lab at the Department of Biochemistry, University of Oxford.

As the cells specialise into different lineages or fates they selectively keep the genes required for lineage-specific functions on, while the remaining genes are turned off. The exact course of events that occur during development and cell fate determination is poorly understood, however it appears to be a fairly robust process where the cells stably maintain the transcriptional state of the genes over multiple rounds of mitotic divisions. Behind these transcriptional changes, a cascade of epigenetic mechanisms is at play, which locks the genes into this on or off state. One of these epigenetic modifications is DNA methylation, which keeps genes into a transcriptionally silenced state. In mammals, DNA methylation occurs at regions rich in CpG dinucleotide sequences. While the majority of these sequences are found methylated across the genome, some domains of CpG regions found in the vicinity of gene promoters are found hypomethylated; hence termed as CpG Islands. CpG Islands are associated with 70% of the genes and their methylation corresponds to their transcriptional silencing [1]. What determines the hypomethylated status of these CpG islands while the CpG rich region elsewhere in the genome are methylated?

The mechanisms behind the maintenance of hypomethylated CpG islands remain unclear. One hypothesis is that CpG islands are refractory to DNA methyltransferases (DNMTs), the enzymes responsible for DNA methylation across the genome. This would indicate that these regions intrinsically hold a quality that repulses DNMTs. It is highly unlikely that the DNA sequence of CpG islands determines their hypomethylated status as CpG islands represent the most suitable substrate for DNMTs. Direct evidence comes from the observation that CpG islands exhibit cell type specific methylation status during development. CpG methylation is acquired during X inactivation where one X chromosome in female mammals is randomly selected for silencing, resulting in a mosaic body pattern in terms of inactive X chromosome. Interestingly, sequences from the bacterium *E. coli* having GC content similar to CpG island behaved like CpG islands when integrated in mouse embryonic stem cells (mESCs) [2]. Another possible explanation is that CpG islands are inaccessible to DNMTs due to the presence of transcription factors at these regions. Support comes from experiments promoting the perturbation of a ubiquitous transcription factor, Sp1 binding site, which lead to ectopic methylation of CpG islands for mouse ARPT locus [3].

There is evidence indicating that chromatin modifications may also play a role in repulsing or excluding DNA methylation form CpG islands. DNMTs possess an ADD domain (ATRX, Dnmt3, Dnmt3L domain) which has been shown to be unable to bind chromatin with lysine 4 methylation on histone 3 (H3K4me3), a histone modification widely found associated with transcriptionally active genes [4]. The role of H3K4me3 in transcription is itself complicated as studies have revealed that it can act both as a cause and consequence of transcription. Unmodified H3K4, however, acts as a substrate for binding of methylation machinery. Contrary to the role of H3K4me3, H3K36me3, another histone modification known to be associated with transcription has been shown to recruit DNMTs via its PWWP domain. H3K36me3 accompanies transcription and is found enriched on gene body, providing an explanation for DNA methylation found across the genome. CpG islands have also been shown as upstream the establishment of some histone modifications. Studies show that CpG islands recruit Zf-CxxC domain possessing proteins, like Kdm2, which have H3K36 demethyl-

ation activity. Deletion of Zf-CxxC domain in Kdm2 proteins (and hence inhibition of their recruitment at CpG Islands) did indeed result in elevated levels of H3K36 methylation on CpG islands and rise in gene expression suggesting that Kdm2 proteins maintain low levels of H3K36me3 on CpG islands which in turn may keep Dnmts from being recruited at these regions. This removal/exclusion of Kdm2 proteins from CpG islands however does not seem to be sufficient for the hypomethylation of CpG islands, indicating redundant mechanisms at play [5]. Contrary to this, study on the intergenic CpG islands in the mouse alpha globin locus revealed transcription coupled gain in H3K36 methylation as a major determinant in methylation of these CpG islands and suggested this pathway as a mechanism to keep spurious/cryptic transcription in check [6].

A completing hypothesis against CpG islands actively excluding Dnmts, is that Dnmts methylate all CpG regions, however, CpG islands act as recruiting site of factors possessing DNA demethylation activity. The most obvious candidate for DNA demethylation activity are the Ten Eleven Translocation (Tet) proteins which oxidize the 5 methyl cytosine modification on DNA into 5 hydroxy methyl-cytosine and then later to 5 formyl-cytosine and 5 carboxyl-cytosine; eventually relying on the base excision repair pathway to replace it with an unmodified cytosine. One study conducted on HEK293 cells showed that the overexpression of Tet proteins did indeed lead to loss of methylation across the genome and CpG Islands [7]. Studies aimed at mapping the binding sites for Tet proteins discovered that Tet1 was bound to sites correlated with high CpG regions at the gene transcription start sites and had very weak binding at the gene bodies, supporting the hypothesis that Tet proteins are recruited to gene promoters and are involved in maintaining the hypomethylated status of CpG islands. This argument is further supported by genome wide studies on the distribution of 5 hydroxymethyl cytosine, which revealed enrichment at gene promoters, thus hinting towards the active conversion of CpG methylation on gene promoters by Tet proteins. Embryonic cells knocked out for all Dnmts had low levels of hydroxy methyl cytosine modification as well, indicating that indeed, 5 hydoxymethyl cytosine in the genome are generated as by products from the oxidation of 5 methyl cytosine. In order to test if Tet proteins do regulate the transcription on the promoters by maintaining them in a hypomethylated state, Tet1 mutant embryonic stem cells were generated; however, the gene expression profiles did not show significant change upon Tet1 perturbation. Similarly, the loss of Dnmts is not related to de-repression of silenced gene [8]. These observations indicated that methylation is not directly regulating transcription. Paradoxically deletion of Tet proteins led to silencing of genes; further investigation on this observation revealed the role of Tet proteins in facilitating the recruitment of sof polycomb proteins, known as gene silencing histone modifying complexes.

Therefore, CpG islands are at the heart of pathways regulating transcription and chromatin structure. The fact that DNA methylation is tightly regulated during development and cell fate determination and the observation that Dnmts and Tet proteins are frequently found mutated in cancers, emphasize on the significance of maintenance of accurate methylation of CpG islands. Although recent studies have revealed many putative players in the regulation of CpG island methylation, the understanding of maintenance of hypomethylated state of these regions and differential methylation of CpG islands is far from complete. ■

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TARGETED THERAPEUTIC

Synthetic biology in the war against antibiotic resistance

By Oxford iGEM Team. The Oxford iGEM team was awarded a gold medal at the iGEM final conference, and also got nominated for the best therapeutics project.

The Oxford iGEM Team at the "Jamboree" on 4 November 2019. Team members include:

Jonathan Chan (1st Yr Biochem.)
Katharina Novikov (1st Yr Biology)
Quentin Guéroult (2nd Yr Chemistry)
David Schramm (2nd Yr Biochem.)
Dániel Felföldi (1st Yr Biochem.)
Abigail Goodship (1st Yr Biomed.)
Calin Dragoi (2nd Yr Biochem.)
Minakshi Ashok (2nd Yr Engr.)
Natasha Cooke (2nd Yr Biochem.)

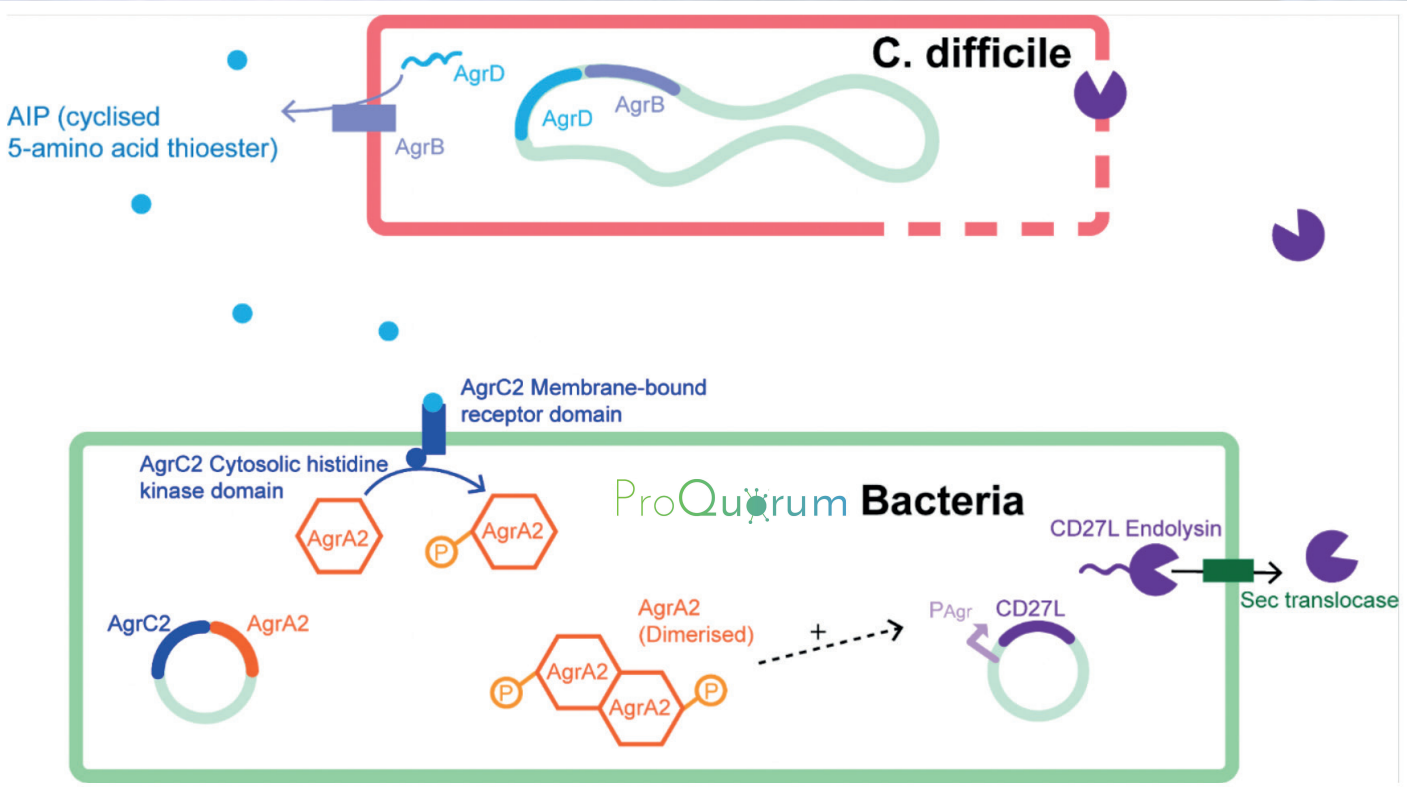
(From left to right)

Standard medicinal weapons, namely antibiotics, are rapidly losing the battle against bacterial resistance. Preventing attacks by spying on and deciphering the secret messages between pathogenic bacteria is a promising alternative approach. As part of the iGEM (International Genetically Engineered Machines) competition, nine Oxford undergraduates from biology, biochemistry, biomedical sciences, chemistry and engineering are declaring war on *Clostridioides difficile* (*C. difficile*) bacteria.

iGEM is a synthetic biology competition which sees thousands of students from across the world compete to engineer organisms that perform novel tasks. This year, more than 300 teams from over 40 different countries will combine experimental work, mathematical modelling and human practices to research their project and engage with its impact on society. The iGEM competition allows interdisciplinary teams of students to push the boundaries of synthetic biology in the hope of tackling 21st century challenges.

C. difficile is a Gram-positive, spore-forming, pathogenic bacterium which causes severe gastrointestinal symptoms such as colitis and bowel perforation [1]. It represents a huge burden on the US healthcare system, with half a million infections per year and an annual cost of $4.8 billion [2]. In 2015, *C. difficile* was responsible for ~30,000 deaths within 30 days of diagnosis in the US alone [3]. It has also been linked to mental health decline with some patients reporting symptoms of post-traumatic stress disorder and anxiety [4].

C. difficile infection (CDI) is currently the leading cause of hospital- and nursing home-acquired infections in the developed world and antibiotic use is the number one risk factor. Antibiotic overuse is rife in the Western world and increasingly so elsewhere, with prescriptions being handed out for everything from the common cold



The ProQuorum autoregulatory system. The autoinducer peptide (AIP) is recognised by the AgrC2 receptor and causes a conformational change. This induces phosphorylation and dimerisation of AgrA2, which activates the Agr promoter (P_{Agr}) leading to endolysin production and secretion. Lysis of *C. difficile* reduces the production of AIP and completes the negative feedback loop.

to serious surgery. Whilst antibiotic use is necessary for certain circumstances, antibiotic exposure drastically alters the gut flora and patients become more susceptible to opportunistic bacteria like *C. difficile*, which can proliferate and dominate. If a patient shows symptoms and tests positive for *C. difficile*, they are taken off their current course of antibiotics and prescribed others such as fluoroquinolones. However, as early as 2004, strains of *C. difficile* were identified that were resistant to fluoroquinolone antibiotics [5] and these concerns have not abated [6]. The Centres for Disease Control cite *C. difficile* as one of their three most urgent threats regarding antibiotic resistance [7].

Using Synthetic Biology, we are engineering a commensal bacterium *Lactobacillus reuteri* to become a super probiotic, named "ProQuorum"; combining probiotics with quorum sensing, the ability to monitor cell density. Lactobacilli are found in yoghurts and are naturally present in the human intestines. In addition to being naturally beneficial to the microbiome, the bacteria will be genetically enhanced to detect *C. difficile*, and then produce and secrete a specific enzyme called endolysin that will kill *C. difficile* bacteria. As the number of *C. difficile* bacteria decreases, less endolysin will be produced to minimise side-effects. This negative feedback loop allows our system to have an automated, targeted response.

C. difficile detection will be achieved by engineering the ProQuorum bacterium with the *C. difficile* AgrAC gene, which encodes a two-component signalling system. The AgrC2 receptor can bind the Quorum sensing molecule AIP (autoinducer peptide) secreted by *C. difficile*. A certain concentration of this AIP molecule marks the point at which *C. difficile* starts to secrete toxin molecules. A conformational change in the receptor's cytosolic histidine kinase domain causes phosphorylation and dimerisation of AgrA2 proteins. The dimerised AgrA2 then activates the *CD27L* promoter and CD27L endolysin is transcribed with a Sec secretion tag to trigger secretion out of the cell. The endolysin is derived from phage CD27L and specifically recognises and cleaves the peptiedoglycan in *C. difficile* cell walls, leading to targeted cell lysis and death.

This is just one example of the power that genetic engineering techniques have brought to biology, allowing us to combine the DNA of different organisms, conferring new abilities and properties.

The iGEM competition encourages students to utilise the latest tools of genetic engineering to try to solve big problems facing our generation, ▶

For more information, contact us at: oxfordigem@bioch.ox.ac.uk, or visit our wiki: https://2019.igem.org/Team:Oxford

from generating biohydrogen and breaking down micro-plastics, to tackling antibiotic-resistant bacteria and producing affordable lab equipment.

However, these new, seemingly endless possibilities do come with certain risks, which synthetic biologists need to be aware of and take into consideration. Despite the huge assets technology can bring to society, we also need to consider the sociological, political and human factors. In a technologically advanced and globalised world, this leads to enormous complexity. It is important to us that whilst we hope to influence the world with our research, the world has a chance to influence our research in turn. This is why we are integrating advice and research from clinical experts, industry leaders, patients and the public. We have interviewed patients about their experiences with CDI. We also discussed with physicians who treat CDI to see if our proposed therapeutic could make a measurable impact in hospitals. Finally, we have raised awareness and discussed the ethics of synthetic biology by hosting talks and workshops at public events and museums across Oxford. These insights from the public have been integrated into our project design, to help it be safe, effective and viable.

In November 2019, we presented our work at the annual iGEM Conference or "Jamboree". For five consecutive years, the Oxford iGEM teams have returned with a gold medal, and we hope to make it six! We can't wait to report back with more news from our project, but in the meantime, if you have any questions please feel free to reach out via our social media channels or visit our wiki.

Finally, we'd like to extend a huge thank you to all our supporters in this ambitious goal: BBSRC, Wellcome Trust, Department of Biochemistry, Department of Engineering Science, Department of Plant Sciences, Department of Zoology, Sir William Dunn School of Pathology, Integrated DNA Technologies (IDT), The Royal Society, AB Vista, New England Biolabs (NEB), Qiagen, and SnapGene. ■

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#OXBER

Building bridges: The future of Oxford in Europe

An interview with Professor Alastair Buchan, Director of Oxford-in-Berlin, former Pro-Vice-Chancellor and Head of Brexit Strategy.

Interviewed by Marina Kolesnichenko, Co-Editor-in-Chief.

Professor Alastair Buchan, FMedSci, is the Director of Oxford-in-Berlin, having previously served as Pro-Vice-Chancellor and Head of Brexit Strategy at the University of Oxford since December 2016. Prior to his role as Pro-Vice-Chancellor, he was the Head of Oxford's Medical Sciences Division, which has been adjudged (by The Times Higher) to be the world's best medical school for the last six years.

Alastair Buchan was born in Rinteln, Germany and studied medicine at the University of Cambridge, University of Oxford, and Harvard University. He completed his post-doctoral training with Henry J. M. Barnett in London, Ontario and with Fred Plum in New York. During his academic career, Alastair Buchan has published over 300 scientific articles and also co-authored ground-breaking papers on the reproducibility of research and on women in science.

Professor Buchan long list of accomplishments includes the establishment of a fully comprehensive regional Stroke Programme at the University of Calgary. For his services Alastair Buchan was awarded honorary Degrees of Laws (LLD) by the University of Calgary and an honorary degree of Doctor of Science (DSc) by Western University (Canada).

In 2007, Professor Buchan was appointed Dean and Head of the Medical Sciences Division. Under his guidance, several new departments were created at the University, including the Nuffield Department of Clinical Neurosciences, the Department of Oncology, the Nuffield Department of Population Health, the Nuffield Department of Primary Health and the Radcliffe Department of Medicine, as well as the Oxford University Hospitals NHS Trust in November 2011. ▶

***Phenotype*:** Professor Buchan, thank you for giving us this opportunity to find out more about the Oxford-Berlin collaboration. Could you please tell us about this initiative?

Professor Buchan: Just before I get onto Oxford and Berlin, I think it's worth saying that in the world in which we live, with the issues to do with growing levels of populism, the Trump election, the Brexit vote, that the universities are core, and always have been, to examining and separating the truth from myth, and separating faith from certainty. Universities, through accurate data and proper teaching, should be the home of certainty in our world. I believe that politicians should be making policy and decisions based on fact and evidence, and not using the decision-making purely to influence the vote.

I think it is really important that we get back to a society that values experts. The thing that upset me most about the Brexit arguments, going into the referendum, were people like Michael Gove suggesting that we had had enough of experts. And the minute that you think you've had enough of experts – then that country is no longer a safe space. Whether it was in Germany in the 1930s, Russia under Stalin, or whether it was in a time of terrible Cultural Revolution in China, when walls go up, then universities are often the first target.

It's therefore really important that universities do everything they can to be multinational, to be porous institutions. What actually is really important for universities and always has been since the founding of the University of Bologna in 1088 – is the freedom of movement: students, scholars, ideas, access to collections, and portability of funding to do research. And critically, that there is no border when it comes to the dissemination of truth.

In the wake of the referendum vote, I thought it was absolutely imperative for the University to start putting the infrastructure in place to connect the University to Europe, even if the country was going to separate from the EU.

I grew up in medical faculties in the UK, in Canada, in the US, and the ones that work best have always had really strong working relationships between the University and the Hospital. When I came back to Oxford having been away from the UK for 22 years, I found a real gulf between the Hospital structure and the University, and so I worked for 10-15 years to build the interactions to support things like the Biomedical Research Centre. So the analogy I would draw is this: that in the same way that the Hospital and the University had to put together some joint working agreements, some legal understandings, some shared resources, some space, some people, some funding and critically, some strategy that was shared – so did Oxford need to do the same in Europe – and it needed to have partners. It needed to be inside Europe, it needed to have some physical space, it needed to have joint working, it needed to have a legal entity. I started as the University's Head of Brexit Strategy within a few months of the 2016 referendum, aiming to build what I regarded as Oxford's base inside Europe. The concept of being inside Europe is analogous to the Hospital being inside the University and the University of being inside a Hospital.

The partnership that we are establishing here means that we have to build not just the interactions, and the communications, and the workshops, we actually have to build the infrastructure to support it. One, therefore, has to focus where one is going to do this and once we make this work in Berlin, it may be possible for other places.

***Phenotype*:** Why was Berlin chosen for this initiative?

Professor Buchan: Partly because the Berlin universities were coming together to form the alliance, and partly because if you go back a little bit in time, there was a huge migration of scholars from Berlin at the time that the politics turned nasty in Germany.

50 years before the catastrophe of 1933, in the 1880s and 1890s, Berlin was the centre, particularly for philosophy, for mathematics, for physics, for chemistry. And in my world, the Charité [Universitätsmedizin Berlin] was probably the leading research hospital in the world, and so people came to Europe from Harvard, from [John] Hopkins [University] in the summer.

There was a very strong interchange between Oxford and Berlin that was lost. I think it was a mistake that the British didn't build on the interactions and help rebuild not just the country, but help rebuild the academic interchange. When the Wall went up, the response in Berlin was to end up with separate hospitals: the Charité, Virchow Campus, Benjamin Franklin, Buch. [Berlin] ended up with 4 if not 5 very strong public universities: Humboldt, Free University, Technical University, UDK (die Universität der Kunst). The Berlin University Alliance (BUA) has brought three of these four universities together with the Charité and that then becomes one of the major centres of research, of teaching, of the curation of artefacts. The future for Berlin is that Berlin should once again become very much one of the world's leading intellectual centres, as it was previously. And for Oxford to be part of that renaissance makes a lot of sense. Berlin is also, of course, in the centre of Germany, which in turn is in the centre of Europe.

This is, of course, the agenda of the mayor, Michael Müller, and the secretary of state for universities, Steffen Krach: Berlin is going to become a city of brains. This week more announcements were made about huge investments in digitisation, digital future, in climate change and global warming. I was very pleased to hear on 19th July that the BUA, which is the structure Oxford is collaborating with, has become as an alliance "a Centre of Excellence". And that justifies the coming together of the universities, and justifies why Oxford has chosen to work in Berlin. And not least in Berlin, the city of museums, of culture, of music – the things which actually for Oxford is a huge bonus because Oxford is not a capital city, and so while access to the performing arts and to cultural excellence is possible in London, we're not in London, but we are in Berlin!

***Phenotype*:** Could you tell us about your role and what makes you excited about it?

Professor Buchan: My role is building things from the bottom up. It is encouraging people who want to come together to come together, and finding the resources and the means to achieve that. What people want to do, so long as it is good, needs to be supported. Yes, it's good to target certain big areas such as global warming or digitisation, but it's best to make an environment where scholars can do their best and to help them get the support they need. I think the term "research strategy" is an oxymoron because you cannot know what it is you need to do – you've got to be driven by what you find. (That's the epitome of curiosity-driven research).

My role initially in Oxford was to work to bring Stroke research into the hospital. We brought the science of stroke into the clinical treatment of patients by building a new structure "the Vascular Imaging Centre". And because of that, I led – bottom-up – the Biomedical Research Centre, which required the hospital and the University to come together to do something new. This [initiative] is about bringing Oxford as a university together with Berlin to do something new. It was hard enough, in a very focused area like Stroke medicine, it was doubly difficult to bring the Hospital and University together across all the different branches of science to build the Biomedical Research Centre. I am happy to report that it has gone from strength to strength and it's had its funding extended twice. This [Oxford-Berlin Initiative] is another level of complexity because it involves all four of Oxford's academic divisions: Medicine, Mathematical, Physical and Life Sciences (MPLS), Humanities, the Social Sciences, as well as the Gardens, Libraries, and Museums of Oxford to do something for the whole of Oxford (including Oxford's 38 colleges) with the whole of Berlin. That's the ambition. The only way that works is if people want to interact. We've put funding of over 1M euros in place between Oxford and Berlin, and over the last two years, we've had somewhere between 36 and 48 workshops. People from Oxford and Berlin have come together, and that has captured the interest of others from all around the world – from China, from India, from Australia.

***Phenotype*:** What kind of programs or funding are available for PhD students?

Professor Buchan: At the moment we are funding travel for people to get together, we are funding workshops, and we are funding research projects as seed funding to enable people to write applications for more substantial research ▶

Photos by Tobias Rosenberg

programmes. As long as their department heads are willing to sign the funding applications, it doesn't matter whether people are postdoctoral researchers, or junior, or senior academics. We just require them to hold an appointment in the University of Oxford, or at one of the colleges, and in Berlin we must have people from one of the four institutions within the BUA. Others in Berlin can be involved too, such as from non-university institutions like the Max Delbrück or Max Planck. But the main partners need to be employed by one of the universities.

We are now trying to set up an Oxford-Berlin Erasmus-like scheme to get money for undergraduates and graduate students to be able to come to Berlin and students from Berlin to be able to come to Oxford. And I'm trying to set up a system to support doctoral students and postdocs, particularly in medicine, where there is some funding from the Charité, to support research speciality training. We are going to build this to support not just the workshops and the projects, but find a way to replace or augment European mechanisms like Erasmus or Marie Curie to support people. In Berlin I'd like three things: I'd like some research space that people could use as the landing strip. Secondly, I want to create some cultural space, where we can have meetings, lectures and seminars. And ultimately, if we can raise the money, I'd very much like to create something like the Wissenschaftskolleg zu Berlin, an environment where people could stay, have dinners and be able to host people, which could be 50% Berlin and 50% Oxford. I really want to create that college-like environment that's been so important for the last 900 years in Oxford. I'd like to create at least an example of it in Berlin so people can see how Oxford works. At the moment, we are not suggesting that we are building any kind of campus. We are not trying to matriculate Berlin students in Oxford and give them Oxford degrees, likewise, we're not trying to help Oxford students get formal positions in Berlin Universities. But we are trying to support exchanges, sabbaticals, electives, and internships.

***Phenotype*:** What is the outlook for Oxford and Oxford collaborations with Berlin post-Brexit?

Professor Buchan: I don't think anybody can predict exactly what will happen. Just for the sake of the record, we are talking on 25th September 2019. In falling asleep last night I felt that the 24th September 2019 was a very important day for our world. They started the impeachment of Donald Trump, and the judgement on the proroguing of the UK Parliament from the Supreme Court was announced yesterday. The Supreme Court upheld

The **Love Oxford-Berlin Photo Competition** was recently held in parallel in Oxford and Berlin, to allow students to win a visit to the other side.

the Rule of Law, and as of 11:30 today has insisted that the Prime Minister and the government are held to account by Parliament. Yesterday was a good day. What tomorrow brings I'm not certain.

I think the people who voted for Brexit will not go along with the result of Brexit, particularly if that means losing the kind of social services that were put in place after the Second World War. Secondly, I think that people are beginning to be frightened that Brexit and the activities of the current administration have meant that we haven't stuck with the UK's Constitution. And that puts the Crown at risk; and it puts the Union of the UK – England, Scotland, Wales and Northern Ireland – at risk. Thirdly, I think that with the prospect of car plants such as the BMW factory just outside Oxford being closed, people are waking up to the fact that what matters to them beyond the politics, and the criticisms of foreign involvement with our country, the loss of sovereignty, is actually the realisation that the economy and where the wealth comes from depends on the mobility of people, of goods, of services, of money. And of course, the universities are totally dependent on the freedoms on which the European Union was founded. I think it's become very clear to everybody that actually the country for 900 years has always required these freedoms of movement. European law and the Maastricht Treaty started to put those laws into legal language and passed legislation. The difference in Britain is that a lot of the constitution has always been unwritten. People have lived by trust and goodwill, and all of a sudden felt very threatened by external, arguably not properly elected government being given written legal jurisdiction over the UK law. So you have the misalignment between what is the way Britain works and the way that Europe works. But it is very clear, for Britain to work properly, that there is continuing freedom of movement. So we've got to find some way to protect our own identity, perhaps a special status like Quebec in Canada, but we have to be part of a greater whole. And the reality is, and the beauty of what we are doing between Oxford and Berlin is that it does not just creates communication and the movement between Oxford and Berlin, but it also attracts activity from Australia, from Canada, and from the US. It helps to make Britain being what it needs to be, which is to be integrated with Europe and therefore part of the global community as a result. And so Oxford in Berlin is about keeping open the borders with Europe. **It's a way of giving Oxford a foothold in Europe, which helps Oxford to remain what it has always been, a global university.** ■

OPEN RESEARCH

Towards reproducible research, one step at a time

Interviewed by Marina Kolesnichenko, Co-Editor-in-Chief.

Let's talk about robust research. What is it, why should you care about it and what can you do to make your own research more robust? We spoke to Verena Heise, Intermediate Fellow at the Nuffield Department of Population Health, who is involved in several initiatives in Oxford and within the UK to lobby for better research practices.

***Phenotype*:** First things first, please tell us a bit about your background!

Verena Heise: Sure, although that's a bit of a long story. I'm a molecular biologist by training, spent quite a bit of time on wet lab research and worked with rodents during my undergrad. I then went on to focus on the human brain when I came to Oxford to do the MSc in Neuroscience and a DPhil in Psychiatry combining genetics and neuroimaging research to investigate how genetic risk factors for Alzheimer's disease affect human brain structure and function. After a brief stint as a postdoc in Germany, I returned to Oxford and I'm now based at the Nuffield Department of Population Health, where I hold a departmental Intermediate Fellowship. That means I'm not quite independent yet but have the opportunity to develop and focus on my own research projects. I'm also very grateful that I had the chance to do another MSc (in Global Health and Epidemiology) during the first year of my fellowship. I'm telling you all this because I think my background is quite important to the work that I'm doing now. I have seen quite a number of areas of biomedical research and it's fascinating to see how individual areas have similar problems when it comes to transparency and reproducibility and how they would profit from more dialogue and sharing of ideas and solutions.

***Phenotype*:** How did you get interested in *reproducibility* and *open science*?

Verena Heise: I guess, like many of us, I have had some experiences where I wasn't able to *replicate* the result of other groups or even my own results. While we are always quite good at explaining how subtle differences in study design, equipment used and so on can lead to those discrepancies, I started to become extremely sceptical about the biomedical literature in general. There is quite a bit of meta-research (i.e. research on research) literature that tells us that "most published research findings are false" (according to the title of a paper by John Ioannidis) and the frustration with this drove me to ask myself whether academic research was really where I wanted to be. I'm a very optimistic person and I think that we as researchers and the wider scientific ecosystem can change, so I decided that I would try to do something about this. In 2017 I attended a course on "Advanced Methods for Reproducible Science", run by

A mini-glossary of open research

Reproducible: a study result that can be recreated by others using the same data and analysis pipelines (according to https://doi.org/10.17226/25303).

Replicable: same scientific conclusion can be reached using independent data and (possibly) independent analysis pipelines (according to https://doi.org/10.17226/25303).

Replication crisis: An ongoing methodological crisis in different areas of empirical research where large-scale replication projects have shown that many study results are difficult or impossible to replicate. Wikipedia has a nice overview article on this: https://en.wikipedia.org/wiki/Replication_crisis.

Open Science: This is a very broad term and means different things to different people. It started off as being simply about making research outputs openly available (such as publishing with open access, making data and materials openly available) and using open-source software (e.g. for data collection and analysis). It has now evolved to include aspects of inclusivity (who is research open to and open for), so that diversity and equality and Citizen Science, which aims to include non-experts in all aspects of research, can also be considered under this umbrella term. See https://www.fosteropenscience.eu/content/what-open-science-introduction for more information.

HARKING (hypothesizing after the results are known): Practice of presenting a hypothesis based on the results of a study as a priori hypothesis that researchers set out to test in the first place. For a good overview, see https://doi.org/10.1207/s15327957pspr0203_4.

P-hacking: The practice of running many different statistical analyses on data and only reporting those analyses that resulted in a "significant" p-value. Also called data dredging or data fishing or selective reporting. See here for a useful paper: https://doi.org/10.1371/journal.pbio.1002106.

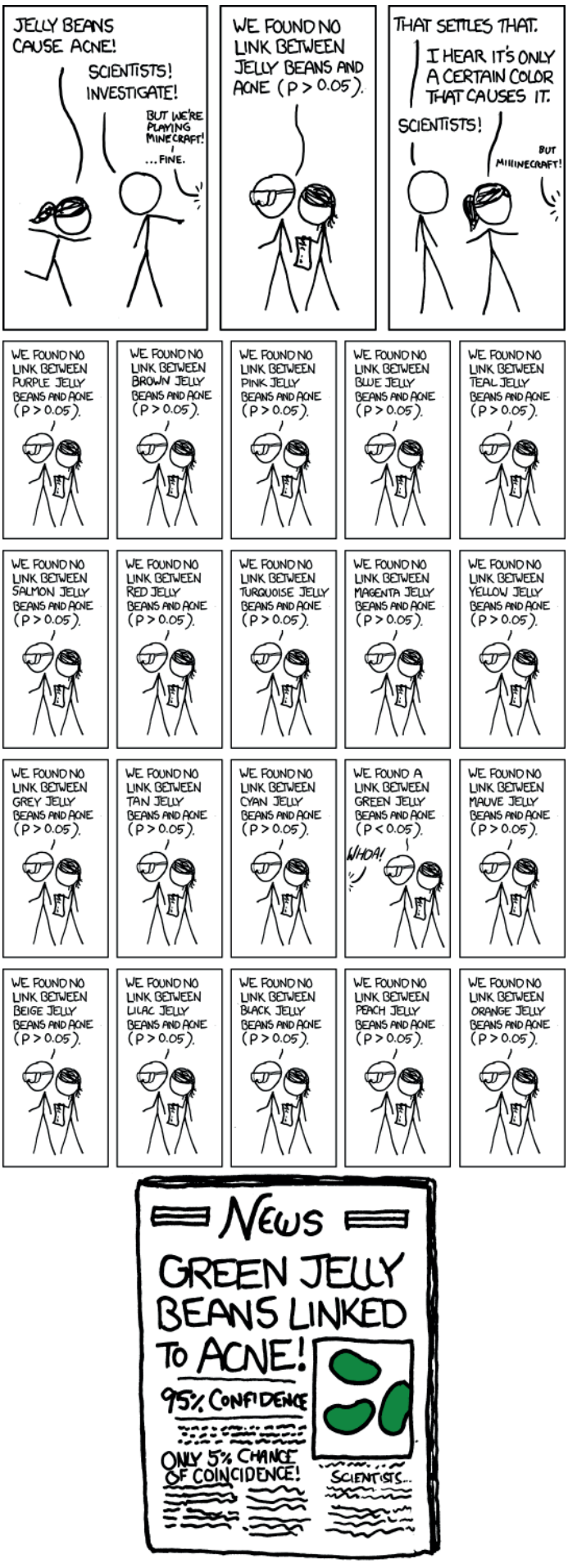
San Francisco Declaration on Research Assessment (DORA, https://sfdora.org): Initiative to improve the ways in which research outputs are evaluated. Specifically recommends that journal-based metrics such as Journal Impact Factors should be eliminated in funding, appointment and promotion considerations.

Marcus Munafò, Chris Chambers, Dorothy Bishop and others who have been lobbying for change for a long time and since then I have tried to not only use those methods in my own research but also develop a network of interested early career researchers (ECRs) at Oxford. Together with other initiatives, this has now evolved into the Oxford node of the UK Reproducibility Network (UKRN).

***Phenotype*:** Great, so before we get to the question of what your initiatives do, why don't you tell us what "Robust Research" is and why we should care?

Verena Heise: I'll start with the second part of that question. I guess I've already alluded to the fact that I think there is enough evidence to show that we are facing a "*Replication Crisis*" in many areas of empirical research. This means that we are wasting a lot of time, energy, money and people's careers on producing unreliable research results. I guess most of us start our career in research because we are curious people and would like to improve our understanding of the world around us. One of the drivers of my own research is that I would like my research results to be of use in the clinic to help patients. But if we do not produce reliable evidence it means that translation into clinical use is going to be difficult or impossible. All those statements in our grant applications and papers about the potential impact of our research will be meaningless if the evidence that we produce is just not reliable. So, this is why "Robust Research" is a topic that should be of interest to all of us.

And I see the solution really in two parts. First of all, it's about more transparency throughout the research cycle. We need to be open and collaborate at the stage of designing research projects, we need to think about transparency from the start of our study: are we going to make data available for others to scrutinize (and I'm the first person to say that there are limits to openness if we're working with human data)? Do we want to make the materials, e.g. code we write to analyse our data, openly available? And if yes, what do we need to do to implement this openness from the start? How do we need to manage our data and materials to make them easily accessible after we finished our project? ▶



xkcd comic on p-hacking
https://xkcd.com/882/

The second part of the solution is really going back to the drawing board to think about what good research practice looks like. Are we doing systematic literature reviews and don't just pick our 2 or 3 favourite papers that confirm the hypothesis that we are working on? Do we have sufficient statistical power and how do we deal with bias and confounding in our study? Do we pre-register our hypothesis and methods to avoid ***HARKING*** and ***p-hacking***? How valid and reliable are our data acquisition and analysis methods? These are just some of the question that we should be able to answer before we even start our research project. And to be able to do all that we need better training.

***Phenotype*:** This is one area where you have been quite active. You recently co-organised the Oxford-Berlin summer school on Open Research. Can you tell us a bit about your experience with this?

Verena Heise: Absolutely. We developed the summer school programme to train ECRs in aspects of robust, i.e. open and reproducible, research that are not covered as part of undergraduate or graduate training. It was a 5-day programme of lectures on topics of interest to everyone in the morning and afternoon workshops that people could pick and choose depending on their interest. This year was the second edition of the summer school, which we are running together with colleagues from the QUEST Center at the Berlin Institute of Health. This has been an incredibly productive collaboration and we are very grateful to the Oxford-Berlin Research Partnership for financial support of the summer school, which meant that we could keep it free to attend for all participants. It has also been great to be able to connect ECRs from Berlin and Oxford as well as other institutions and we hope that some research partnerships will develop from those contacts. The atmosphere this year was absolutely amazing, there was a real buzz and I think most people went home with the motivation not only to change their own practices but also to get involved in culture change at their own institutions.

***Phenotype*:** What's so important about culture change?

Verena Heise: Well, we need to understand where the replication crisis is coming from. I (and others) would argue that it's not just the fact that

Oxford-Berlin summer school on Open Research 2019
Green Templeton College, University of Oxford

we don't train people well enough to be able to produce the most reliable evidence. We also work in a scientific ecosystem that does not value those practices. We are mostly assessed based on publications, on how many we have and which journal they were published in (although institutions like Oxford have now signed up to the *San Francisco DORA*). Deep down inside we all know that science is messy and we rarely get the results that we expected. But we are supposed to write good stories around "significant" results and the more surprising those results the better. Unfortunately, that means we are tempted to p-hack and HARK our way to good stories, and in most cases, the most surprising or "sexy" stories turn out to be based on false-positive results.

***Phenotype*:** So, what's the alternative?

Verena Heise: We should move towards valuing transparency and quality of research design, irrespective of whether someone got a "significant" result, and we need to move towards valuing research outputs other than publications (such as openly available datasets or making study materials available). All that requires changes in how we incentivise researchers (i.e. our hiring and promotion criteria) and we need the right infrastructure in place (e.g. to make our data available). And I'm not even going to start talking about other improvements in working conditions, such as longer-term contracts for ECRs, that are needed to reduce the temptation to cut corners to get ahead of the "competition". But this is why we need culture change at institutions (and not just institutions, other players such as funders and publishers play a very important part, too) to make research results more reliable and one way in which we are trying to achieve this is our Reproducible Research Oxford initiative (RROx).

***Phenotype*:** What is the RROx about?

Verena Heise: The RROx is the local Oxford node of the UK Reproducibility Network, a peer-led consortium that aims to investigate factors that contribute to robust research, to provide training and disseminate best practice. In addition to developing and delivering training in robust research for all researchers (from undergrads to PIs), developing the right infrastructure and incentivising robust research practices, another aim of RROx is to bring researchers together who are working on meta-research questions across the different divisions of the university.

***Phenotype*:** Wow, that's a long to-do list!

Verena Heise: Yes, it is, and nobody is expecting this to be quick or easy. But we're on the right way and we have a wonderful group of ECRs, PIs and librarians here who are keen to work together to make this happen. We have already seen how much of a difference (mostly) ECR-led activities, such as the ReproducibiliTea journal club (https://reproducibilitea.org/) that was developed by colleagues in Experimental Psychology or the Berlin – Oxford summer school can make, and I always hope that we can inspire other ECRs here in Oxford to get involved, too.

***Phenotype*:** So, what is it that ECRs can do who would like to get involved?

Verena Heise: For those who are based at Oxford: have a look at the RROx website (http://ox.ukrn.org/), join our mailing list, follow us on twitter, come to one of our ReproducibiliTea sessions (or start a journal club in your own department) or just email me (verena.heise@ndph.ox.ac.uk) if you're keen to get involved but not sure how. You might already be doing something that we should be aware of and could support, so do let me know about that, too. I'm always super happy to come to lab meetings or departmental seminars to talk about robust research or to just meet for a coffee with anyone who's interested. So, do get in touch, we're here to help.

And for those who are based elsewhere: I've come up with a very brief guide for ECRs (still in development, https://osf.io/pkc6r/) that might help you to think about what you could do. And again, do get in touch if there's anything I can help with. I'm keen to spread the word and very happy to come and visit you at your institution to share ideas and experiences.

***Phenotype*:** Thanks so much, that's a lot of info and good luck with all your activities. ■

By Dr Siv Vingill. Siv is a postdoctoral researcher in Richard Wade-Martin's research group at the Department of Physiology, Anatomy and Genetics (DPAG) at the University of Oxford.

Have you ever looked down your microscope and thought: "That looks like a magical forest..." Or gone slightly over the top on your figure colour scheme and then thought: "Nah, I guess we're back to RGB...?"

A good friend, Mateusz Ambrozkiewicz, did just that for years as a doctoral student before finally embracing his inner artist. During his PhD he studied neuronal development, focusing on genes essential for specifying an axon. On many occasions, he would enthusiastically come over to share a particularly pretty image of a neuron with me. At the Max Planck Institute of Experimental Medicine, he tried his hand at pretty much any staining technique known to humankind to visualise the mysterious paths of axons in the most accurate manner. He has gone from old-fashioned and moody Golgi stains to genetically express fluorescent proteins electroporated into mouse brains; and in the process learned how E3 ubiquitin ligases influence axonal polarity [1, 2] and synapse specification [3].

Mateusz Ambrozkiewicz in front of his work at the "Art & Neuroscience Multimedia Exhibition 2019"

His career then took him to the Tarabykin lab at the Charité University Hospital in Berlin. Being a fan of techno, style and alternative scenery, he immediately thrived in Germany's cosmopolitan capital. Here, exposed to the multifarious artistic points of view, he realised that his images were indeed realist contemporary art. If you are an avid reader of *Phenotype*, you know that many scient-artists feel the same way and submit their microscopic art to our termly cover-image contest. However, few go beyond changing the colours of their images. Mateusz decided there is no reason to leave it there. His art uses scientifically obtained photos, but explores their form to evoke artful interpretations through shapes, titles and descriptions.

Last July an opportunity arose to show his neuro-art to a wider audience. EDGE Neuroscience Art hosts a yearly exhibition where scientists

and artists alike are invited to showcase their work in neuroscience over a four-day long exposition. They are an international bunch studying and researching in Berlin, who see themselves as "a community with varied perspectives on the intersections between art and neuroscience". In keeping with Berlin chic, the exhibition was held partly in a decommissioned power station turned art gallery, and both artists and audience were seldom seen without a glass of bubbly in hand.

I gave Mateusz a call after the show to ask for his perception of science, art and how the two collaborate both conceptually and in real life.

So Mateusz, tell me a bit about the work that you showed at this exhibition.

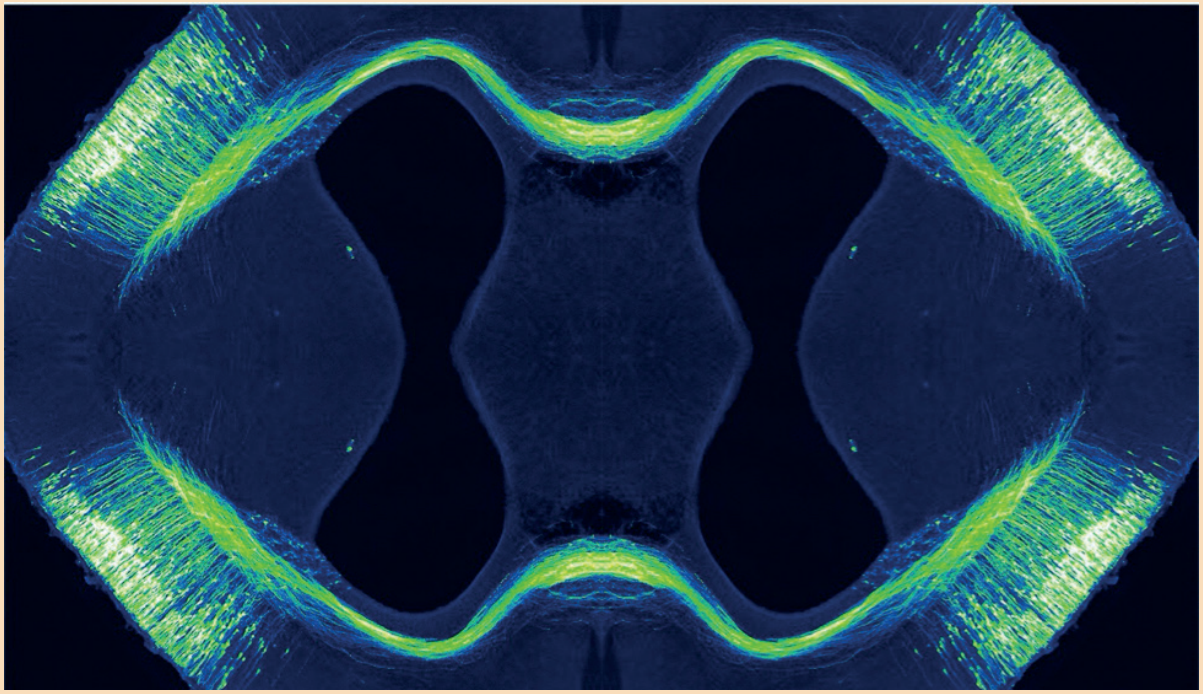
"The images I showed are a part of an art project called FRAIL. They consist of reconstructions or raw images of cortical neurons derived either from an artificial *in vitro* culture, or an intact mouse brain. I find neuronal morphology mesmerising and somewhat intimidating, as it allows for proper brain function; a system so complicated and intricate, that we as scientists fail to fully understand. Each illustration contains a mutant element, so that unaffected "normal" neurons are intertwined with diseased nerve cells derived from mouse models of intellectual disability or autism spectrum disorder. For a lay viewer, all these might seem appealing, decorative and appropriate for interior design purposes, yet the morbid aspect of these poses an element of surprise, hesitation and reflection. I think this project offers deep insight into the work of a neuroscientist, explains the concept of developmental diseases and attracts people to promote discussions and thought."

Imaging is one of the most powerful methods we can use to understand the brain. With the rapid development of new techniques in this area, what do you think we will be able to see in the near future?

"As a scientist particularly drawn to imaging techniques, I am very excited about what's coming. I have been exploring the ever-growing niche of brain clearing and light sheet microscopy, and I truly believe that replacing the conventional good old cryostat and microtome is just around the corner. In my opinion, methods allowing for 3D visualisation are the future of imaging and microscopy. We will also soon find a way to dynamically visualise biological processes in living organisms combining 3D and time-course microscopy. I also believe the future will bring new ways to minimise the invasiveness of current methods."

How has this experience changed you now that you are both a successful scientist and artist?

"I don't think I have changed, because I have always felt like both of those things. A huge ▶



"Symmetry"

The image depicts the corpus callosum – the biggest axonal tract in the brain of placental mammals. In this example, the axonal bundle at the midline is not formed correctly. Neurons extending affected axons bear mutations of genes associated with autism. The original image was modified for artistic purposes.

part of science is about data visualisation and presentation, and to get others interested in your findings, they need to be appealing. In a way, it is like a good film. If a trailer is good, more people will likely watch it. The scientific curiosity is of course the underlying driving force in my work, but the way you execute it, to me is also about aesthetics. Science will never incorporate into the mass culture and society unless it is approachable in a way that a lay audience pays attention to it."

I guess art always finds inspiration in the real world, but is there anything that science can learn from art?

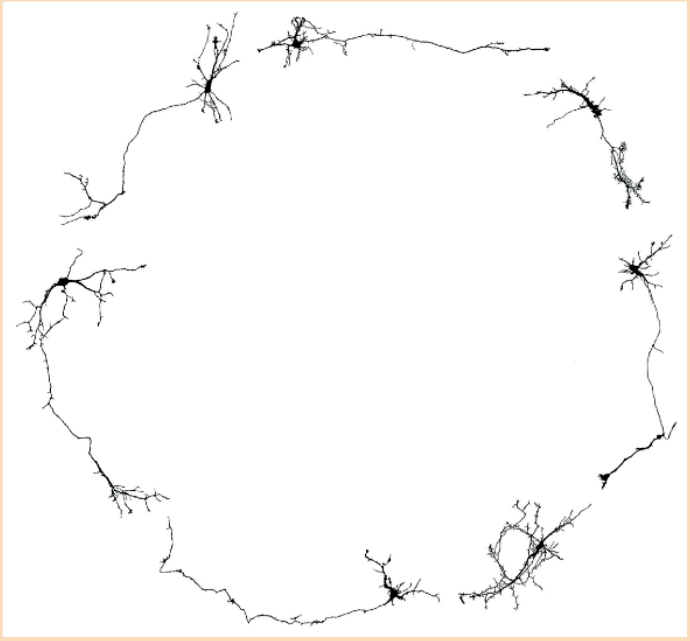
"Currently, I find that there is a gap in communication between the scientific and the wider community, and I think a lot of that stems from the fact that scientists have problems presenting their work in an approachable way. We, as scientists, must promote dialogue and be able to explain the importance of our work. As someone who works with living organisms, I think we must explain why we use these models in our work and what we can achieve through animal research. With exhibitions like these, I am thrilled that I can present my work in a different medium, reach a varied audience and explain why basic research is of invaluable benefit to humankind. Sound research that is beautifully explained is what truly makes the art of science for me."

This exhibition sounds very similar to presenting your poster at a conference, so I'll ask the standard PI question: Did you get good feedback or any new ideas?

"It was quite a challenge to explain these complex models easily, but I really felt people could connect to what I presented, and I could connect with them finding an easy way to talk about neuronal network formation. Many of the other presenters were more artists than scientists, and it was very interesting to see their interpretation of neuroscience in their paintings and sculptures. And in the end, I even had a couple of people interested in buying some of my pieces."

That is probably the best feedback you can get! I guess this means that you will continue to look for new conspicuous brain details also in your future scientific work?

"Definitely. Working with images in this way allows me to connect even stronger to my work, and I hope it can keep on inspiring me to be a fiercer scientist."



"Going in circles"

These reconstructions of neurons are arranged in a circle to illustrate how developmental defects to neuronal polarity, namely difficulty in specifying an axon, affect neuronal circuits in the brain.

So if you are in Berlin, or thinking about going there, check out the links below for information on workshops and upcoming exhibitions. The 2020 participants will be selected from a public call-out already between January and March. The EDGE community also hold monthly meet-ups and workshops in a citizen science bio-lab project space called TOP project space, where creative souls are more than welcome. In the words of the EDGE community:

"Whether incidental or decisive, art in neuroscience is a tool for educating and inspiring, communicating and sharing, for artists and neuroscientists alike."

Visit: https://edge-neuro.art

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You are only a leader if you have people behind you

An interview with Patricia Alison Hartley, General Manager at HRA Pharma and former General Manager of Consumer Healthcare (DACH) at Sanofi.

Interviewed by Marina Kolesnichenko, Co-Editor-in-Chief.

Robert Taylor Photography
taylor-photo.co.uk

I read Classics at Lincoln College 1980–84. During my time in Oxford, I gained my first substantial leadership experience as Editor and General Manager of The Cherwell university newspaper and associated publications. This led to me being offered the first position of my career in marketing by Procter & Gamble. I was with them for 11 years, working internationally in Germany, France, and Spain, before moving into the healthcare sector with Boots Healthcare International, the over-the-counter consumer healthcare division of Boots, first in Spain and Portugal, and later in France. I subsequently took on leadership roles of increasing scope, moving in 2011 to be Global Head of Marketing for the Consumer Health Division of Boehringer Ingelheim in Germany, then the fifth-biggest Consumer Healthcare company worldwide. From Boehringer, I moved to Sanofi, ranked third in Consumer Healthcare globally, as General Manager for Consumer Health in Germany, Austria and Switzerland. I led a team of 200 people in the three countries, with a turnover of €250M, and was Vice President of the Consumer Healthcare Industry Association, representing over 400 healthcare companies in Germany. My current role is with HRA Pharma, a smaller, entrepreneurial healthcare company which is the global leader in emergency contraception. There I am based in Paris as General Manager for France, Benelux, Russia and Eastern Europe.

Phenotype: Alison, thank you for joining us. Since many DPhil students and postdocs aspire to lead teams themselves one day, I am sure they would like to know what makes an effective leader.

Alison: You learn to lead teams by being a team member. It would be extremely difficult to just go from being an isolated individual, who had never worked in a team, to leading. You have to learn by doing. So, I started by being a team member.

I was always working with R&D communities, so as a marketeer my role was always to look for applications – consumer insights – what will be useful to people, what would people value, what people would pay for, or what would improve people's lives. It's always about delivering a product which meets a need for the person who is ultimately going to pay for it.

From that point of view, whether you are managing a washing powder, or a brand of coffee, or science, ultimately you are looking to find a solution to a problem that you identified. What led me into healthcare, which is where I find more scientists working for me directly rather than with me, was

that I always wanted to do something that has a real benefit. And healthcare is something that is of importance to everybody. We might not always think of that, because obviously, when one is healthy, one does not think about it, so you only think about it once you've got a problem. So, moving from one product area to another – for me it's about finding what is important to people. Bringing better solutions to people is very important.

Phenotype: Do you find it different to manage scientists when they directly working for you than other people? Is there a difference?

Alison: Scientists are clearly motivated by the science and by the outcomes for patients. And they are clearly NOT motivated by profit. And in fact, it's an important part of their roles within the pharmaceutical companies, that they are not incentivised by profit. Their rewards never come from selling more because that would be unethical.

Their performance is measured in patient outcomes. But in a company, you clearly have to look for outcomes for patients, but also, at the end of the day, make money, which is a very complex piece of the business.

When I was at Sanofi in Germany, as part of our introduction, I went to make a presentation on what in fact is an over-the-counter (self-medication). We were told that in order to interest scientists we should talk about clinical benefit and patient outcome. If we had gone and given a marketing presentation, we would have shown a lot of pictures, some advertising spots, TV spots and it would have been far more glitzy pictures, appealing to emotions, whereas with the scientists it is very important to be rational and to document that we have good sources that we have good clinical studies, experts, and that we have papers published.

It is understanding how to motivate scientists, which is by being rational, talking about sources, having scientific credentials, and also how to manage them. The key is to give them the space for their creativity and the time to do things their own way. What I learned from managing scientist is to give them the space to invent.

Phenotype: What makes a good leader?

Alison: **The question I always ask myself is why would anybody follow me? Because you are only a leader if have people behind you.** Why do people tend to follow others? In my view, it is because the leaders know where they are going and the destination is inspiring. You have to have a vision and be able to articulate it in a way that makes others want to follow, and you have to make other people believe that you know how to get them there. And not you on your own – because if you try do it all on your own, you are definitely not a leader – you will just collapse under the strain of trying to do it by yourself. The trick of a great leader is spotting (once you are clear on where you want to go), first of all to have a vision which is creative, crafted and refined with the input from others (so you get other people to enrich your vision). Secondly, then, to spot the talents (what do you need, who do you need on your expedition). What skills do other people have that they are passionate to contribute and uniquely able to contribute? **People follow you because they are motivated by the destination, they believe that you can get there, they believe that they can help getting there, and that it will be fun!** There is always got to be something in it for them. And that's where I am getting back again to the scientists – what motivates the scientists I had worked with: on one hand, creating better patient outcomes, and on the other hand, the personal recognition of having papers published or being able to present at conferences, having products that they have invented coming to the market and helping people, and creating an environment where people feel trusted, and have the space to contribute what they want.

People follow if you are humble and if they believe you are open to criticism and to feedback, and you recognise the contribution of others. Leadership is also about letting other people take the credit.

Phenotype: What made you become a great leader? Was there one event or somebody who made a big impact on your life.

Alison: I would never say that when I was growing up, I was a great leader. I just had lots of energy and I could see ways to do things and I had lot of drive. People usually have energy if they are leaders. I think a lot of young people including myself start off being very driven by the task. You have a task that you want to achieve. And people don't really think about how to motivate others. If I tell somebody: if it is important to me, it is bound to be

important to them. Either you end up doing a lot of work yourself because you have not convinced anybody else, or you come flat on your face and think: why don't they do what I asked them when I asked them to do it? I have two things that helped me become a better leader: one was getting feedback from my team members. They could really follow me intellectually and I was winning their heads, but I was not winning their hearts. **And to be a leader you have to win the hearts and the minds.** As a scientist, you can be absolutely brilliant and people would follow your ideas, but if you don't motivate people, and if people don't like you, you won't be a great leader. A tip that I got from one of my bosses was to waste more time. I was very task-driven, and what I was not doing, because I thought it was a waste of time I was not going to somebody's desk and having a chat or having a coffee or just building relationships or asking very open questions because for me it was waste of time. So when he said "waste more time" – that's what he meant. Find our more about people. Find out what's motivating them.

When I talk to people, I always tell them about my hobbies. "I grow many different tomatoes." I tell people that. Now what do tomatoes have got to do with anything? When people for example have to talk to me and they are intimidated because I'm the boss – when they have to sit next to me at a dinner or when they are stuck with me in a lift, and they think: "Oh my goodness what I am going to talk to the boss about?" But if they know I am growing tomatoes, they can talk about tomatoes. It is important that people know you as a human being and not as as the boss. So whatever it is, you give people something to make the conversation easier.

The other thing that made me a much better leader was stop worrying about me. When I started focusing much more on how I made other people more successful, and stopped worrying about my own ego or my own career development, I became a much better leader. Once I realised that for me to succeed, I have to succeed through other people, and I enjoy my greatest legacy will have been developing other people and helping other people to be happier and be a bit more successful. I cannot succeed in my goals if other people are not succeeding in theirs. **Once I stopped worrying about me, I was a much better leader.**

Phenotype: Do you think what there are difficult aspects to being a woman leader?

Alison: I think on the whole I've been fairly lucky. But I definitely have encountered problems and the key problem is that you are being judged by men. And the people I have worked for, have largely been men, and what men look for: characteristics and behaviours, are male characteristics. And that means definitely fewer women get promoted.

In my career, where I am now, I lead primarily a female team. In my business, one of the two key areas is women's health. I do invest time in developing women in women's organisations in sponsoring women, and I think we have to be a bit less naive, and a bit more robust and astute about playing the game. There is no point sitting and saying – I wish people understood me. Now that we see where women can get to, I think it is very important that we keep supporting women, sponsoring women, mentoring women because again, people who are evaluating tend to be men. But that's part of the challenge and that's doable.

Phenotype: What advice would you give to scientists who want to transition to a management role have more leadership?

Alison: I think the question to ask is why – why do people want to manage, what do they want to achieve? For me, management means leading a team – it goes back to what I said earlier, realising that you require other peoples' input to be able to realise a vision which for you is important. And then think about what skills you would need around you, and personalities. You have people who are extraverts, and who are introverts, those who are into detail or great visions. What do you need? And what are YOU bringing? And if you are not sure you have the skills, go get them! ■

Science to startup: Taking care of what really matters

Interviewed by Sonia Muliyil, Co-Editor-in-Chief.

Dr Jessica Ocampos is the founder and President of Camnexus – a company that develops low power digital infrastructure, which allows affordable scalability of connected devices and real-time data using low energy consumption sensors. For more details check out their website camnexus.io.

Phenotype: Jessica, what motivated you to start the company?

Jessica: After working for many years in Latin America, in Europe and the USA, one of the main challenges I experienced was the adoption capability of these technologies. This motived me and a couple of PhD students from the University of Cambridge to create a bridge, a nexus to support local innovation capability building: a bilateral platform. This platform was initially based on a student-led, international academic collaboration, expanded to become Camnexus. To achieve its mission, since 2015, Camnexus works in collaboration with the Cambridge Enterprise's International Outreach Programme and with several international innovation agencies. But such technologies would only make sense if transformed and adapted with local expertise to tackle the real challenges – the global challenges.

We have proven the successful implementation of the first underground wireless sensors network for real-time flooding alert in sewages in cities. This solution is finalising the pilot stage with our client, a water utility company, Aguas Andinas (part of the Suez group), which is interested in larger roll-out for cities. In Brazil, we have a sensors network to alert water quality and improve fertiliser management in hydroponic farming. For this project, we are working with two local universities of Santa Catarina region in Brazil (UNISUL and UFSC) and a local school of Tubarão city, and are training engineering interns, who are learning about sensors development and the benefits of low-power communication systems.

We have recently been awarded another funding from Makerere University in Uganda. Together, we will start a pilot project to manage water and fertilisers in coffee production in Uganda. The project involves the training of farmers and the engagement with the agriculture ministry and local policymakers.

Phenotype: Your company was already recognised for its outstanding accomplishments. Could you tell us about some of them?

Jessica: Camnexus has received several awards, including Cambridge Independent Science and Technology Awards, Highly Commended Cleantech Company of the Year 2019. We were also chosen as part of the Future 20 Programme for Tech for Good companies, and finalist of the Cambridge Wireless Discovering Start-Ups 2018.

Phenotype: How did you transition from an academic career to that of an innovator?

Jessica: I came to Cambridge because I wanted to pursue my PhD. I never imagined that I would become an entrepreneur. In Cambridge, I discovered plenty of extracurricular activities, innovation competitions and talks. Thinking back, I recognise three Cambridge initiatives were key to my personal realisation as an entrepreneur or innovator. The first was Enterprise Tuesdays, a series of talks organised then by the Centre for Entrepreneurial Learning (CfEL) of the Judge Business School. Every Tuesday, an inspiring renown entrepreneur came to share with us their stories and learning. I learnt about the CUE (Cambridge University Entrepreneurs) business plan competition, the second key initiative. I decided to submit my innovative ideas to CUE. After winning in the

Brazilian and UK Camnexus Team installing our first IoT (Internet of Things) network for hydroponic farming in a pilot greenhouse.

first stage, I realised the potential of my ideas, and by being part of the programme, I could be mentored and trained and met more PhD students with similar interest. This was the beginning of my involvement in entrepreneurial programmes. And finally was Enterprise WISE, a programme which was delivered at the Judge Business School. This programme was prepared and delivered by women to female PhDs and postdocs in STEM of the University of Cambridge. The programme made a huge impact on my personal realisation as an entrepreneur. The programme gave me inspiration, tools and training. Moreover, I built a network of amazing women.

Whether we remained in academia or not, we realised the importance of creating more awareness of women participation and diversity in the science, technology and entrepreneurial landscape. We realised our entrepreneurial potential and that it was not a question of changing women but of changing the system. Since 2014, I keep working on this initiative, which now is called Rising WISE, spanning Cambridge and Oxford, to include female PhDs, postdocs and engineers from Cambridge and Oxford University.

I have been always an advocate of inclusion, diversity and gender equality. Participating in the programme made me even more aware of my role and of the impact I could create in others. Since then, I am actively involved in mentoring and training of other women. ■

Jessica gave a TEDx Talk in September 2019
Watch this at: https://www.ted.com/talks/inclusive_innovation_starts_from_within

MANY WAYS OUT

The future of academia is not just academia!

By Dr Maddalena Comini. Maddalena is a postdoctoral researcher in Dave Bennett's group at the Nuffield Department of Clinical Neurosciences, University of Oxford.

"What is the future of Academia?"

"Can academic and commercial research go hand in hand?"

"Are creative thinking, academic rigour and cutting-edge technologies enough to ensure postdocs successful careers as Principal Investigators (PIs)?"

None of these questions has an easy and clear answer. Based on such premises, the postdoctoral societies of the Oxford Departments of Physiology, Anatomy and Genetics (DPAG) and Biochemistry decided to make a joint effort towards organising a retreat that could offer a clearer, or perhaps brighter vision about future job prospects for young researchers.

Indeed, as the academic panorama is rapidly changing, and so is the world of work, postdoctoral researchers feel more and more uncertain about their realistic chances to pursue a career at the bench, and doubt that they can still look appetising for the world of industry and pharmaceutical companies.

Start of the Joint Postdoctoral Retreat

On 18th October 2019, the **Joint Postdoctoral Retreat** took place at the beautiful venue of St Anne's College in Oxford, with about hundred participants including postdocs and speakers. Professor Francis Barr, Head of the Department of Biochemistry, welcomed the audience, highlighting the advantages of the inspiring environment offered by the university and pointing out that nowadays there are loads more opportunities than in the past, hence the call to seize the day and grab the opportunities as soon as they present themselves.

"Be resilient, find a mentor and make a plan" was the welcome speech of Professor David Paterson, the Head of DPAG. The university is an incredible source of role models and great mentors, so **"do not be afraid to approach your supervisor and ask for their advice"** continued David.

The show then continued with the exciting story of Dr Joanna Bagniewksa, a Teaching Fellow at the School of Biological Sciences (University of Reading), who highlighted the importance of staying open-minded, as well as pursuing personal interests and hobbies, as they might one day turn into a career, as she herself experienced.

The discussion carried on with Dr Graham Wynne, a Medicinal Chemistry Advisor at the Department of Chemistry, and Dr Nadia Halidi, the Imaging Facility Manager at Micron Advanced Imaging Consortium. Both focused on communication and networking, key factors in the process of job searching, along with the necessity of having a career plan, yet remaining flexible and not excluding any possibility, *a priori*.

Associate Professor Samira Lakhal-Littleton of DPAG then took the floor and discussed her

academic career, along with an inspirational excursus over her personal story of being a woman and parent. As Samira explained, success in academia and a work-life balance are not mutually exclusive, as long as there is a strong will to make it happen. She also stressed upon the vital importance of asking more senior fellows for advice, as well as having a great mentor. She concluded with the comment **"Listen to your scientific data and results, and do not forget joy and passion in what you are doing."**

The session finally closed with Dr Benjamin Schuster-Böckler, PI of the Computational Genomics group at the Ludwig Institute of Cancer Research. Benjamin engaged the audience by telling his exciting experience as a start-up developer while a PhD student at the University of Cambridge, and again, perhaps not surprisingly, passions and motivations seem to have been the driving force to overcome uncertainties and obstacles.

At the end of this first exciting session, the take-home message was understanding who you are and what strengths you have, along with a good dose of self-awareness and self-confidence.

Leah Thompson (Enterprising Oxford) then raised the interesting subject of women in STEM and how the system should be globally changed – not only at the academic level – in order to offer equal opportunities and to allow women to embrace and develop their entrepreneurial spirit. Another crucial aspect raised was research dissemination and public engagement, in particular through open access and a transparent decision-making and peer-review process, along with scientific rigour and data reproducibility, as emphasised by Sally Rumsey (OpenAccess Oxford) and Professor Tim Behrens, Senior Editor of eLife. This session terminated with Dr Sherie Wright (MRC-UKRI) who outlined an exhaustive overview of the MRC Fellowships, and stressed the importance of young researchers asking for advice and feedback during the application process.

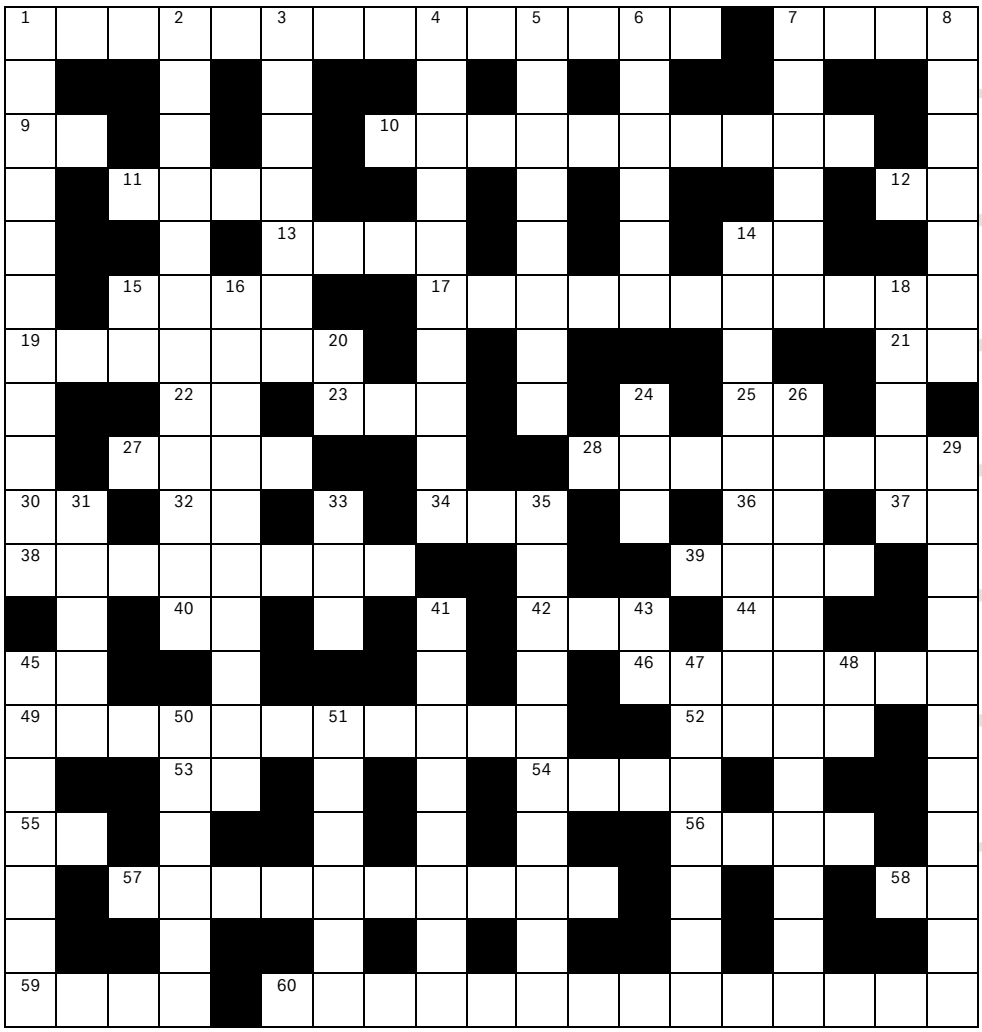
After lunch, morale was still quite high as the networking workshop engaged people in dynamic activities such as learning (and practising) the best strategies for a more productive and less forced networking, as well as an interesting lecture on how to make the best use of online platforms such as LinkedIn.

The third and final session brought examples of young scientists who have decided to leave academia for the so-called "real" world. They were happy to share their personal experiences of the transition. The first speaker was Dr Jonathan Brooks-Bartlett, a data scientist for Deliveroo, who told his tale about starting in academia as a mathematician and ending up finding his dream job in a company. Dr Richard Payne, a former PhD student at the Department of Biochemistry and now a patent attorney (Carpmaels & Ransford), emphasised that doing a PhD allowed him to develop critical thinking skills and a solid foundation of scientific knowledge, both serving as great advantages in now dealing with IPs and patent laws. Dr Pradeep Kumar, a former postdoc in DPAG and now a senior scientist at Oxford Nanoimaging, enthusiastically emphasised that his actual position has finally given him the opportunity to develop people skills along with other important soft skills, to be able to better understand people's needs when providing them with technical support. Finally, Claire O'Brien (Research and Development Management Consultant for Oxstem) reminded the audience that the ability to work in a team, along with problem-solving skills, are extremely valuable competencies that postdocs already have in their basket, and can apply potentially to any area of expertise. Again, motivation and passion just appeared to be the magic words that lead to a successful career, no matter the field of choice.

At the end of a long day, the participants seemed particularly satisfied with the intense yet enriching retreat. The most appreciated aspect was, perhaps not surprisingly, the fact that the speakers had been giving sincere and honest advice, while talking openly about their own very diverse but all inspirational experiences. In particular, the word "failure" appears to be not so frightening anymore, but rather an inevitable and necessary step of what we call "the learning process".

Hence, embrace your future and stay curious! ■

CROSSWORD



By Dr Siv Vingill

Enjoying the crossword?

The first few persons to send over a completed crossword with the correct solution will receive a small cash prize from *Phenotype*! Our email is oxphenotype@gmail.com.

Across

1 Scrutinizing code (9+5)
7 Two thirds of a bowman (4)
9 At (2)
10 Words that remove frost (10)
11 Coalition of countries (4)
12 Collected territories (2)
13 Algae (4)
14 Something knights say (2)
15 Weaken (4)
17 Hearing where things are in the waves (11)
19 Generous German hospital (7)
21 Nobel gas (2)
22 Mic controller (2)
23 Half a mint (3)
25 Redheaded pop phenomenon (2)
27 Kitchen equipment (4)
28 In favour of rapid multiplication (8)
30 From (2)
32 Abbreviated part of kingdom (2)
34 Downtrodden (3)
36 Afterthought (2)
37 Backwards diffusion of water (abbr.) (2)
38 Out of charge (8)
39 A Willy (4)
40 Toxic molecule favoured by Agatha Christie (2)
42 Zilch (3)
44 American State (2)
45 Table salt (2)
46 Strange (7)
49 Ceremonial furniture, say, and indigenous inhabitant give you a substitution (11)
52 Millennial distraction (4)
53 Old English Island (2)
54 Don't use Wi-Fi in Italian capital (4)
55 Empire State (2)
56 Declaration (4)
57 Gilded beast of motion pictures (6+4)
58 Pertaining to two (2)
59 Unclothed (4)
60 German parade below the trees (5+3+6)

Down

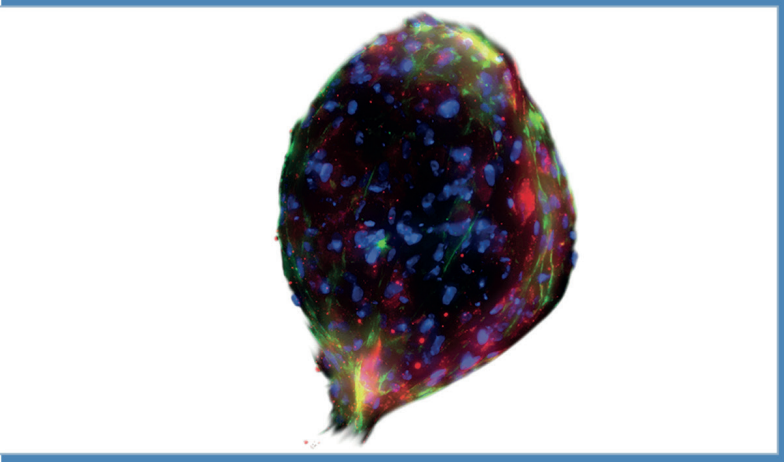
1 Meeting (11)
2 Gary and his brother live together in perfect circumstances (12)
3 British pastime (7)
4 In both directions (10)
5 Bovine crossing brings elite students together (8)
6 Multi-directional (6)
7 Bloodless (6)
8 Alternative body part? (7)
14 Award to right hand gases (5+5)
15 Musical note (2)
16 With which a crossword puzzle can be made (10)
18 Animal found in Latin America and Southeast Asia (5)
20 Latin and (2)
24 Can be wines and humour (3)
26 Renunciation (12)
29 Grocery chain works together (11)
31 In a specific location (5)
33 Mostly N (3)
35 Down the hole to the amazing country (10)
41 Friendly (8)
43 Buffer and broth (2)
45 Person from behind the wardrobe (7)
47 Despicable (7)
48 Do verb again (2)
50 Sad Hundred Acre Woods resident (6)
51 Marine behind (6)

your power for health

greiner BIO-ONE

Magnetic 3D Cell Culture

3D in a 2D workflow



- Fast 3D Tissue Assembly
- Reproducible
- Scalable
- Flat Well Bottom for Optical Imaging
- HTS Compatible
- Automation Ready
- Easy to Handle

Watch our movie about 3D bioprinting!

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Give your cells a **competitive edge**

Sometimes it's the simplest of things that make the difference for cell health.

Anyone who has ever cultured cells in a lab knows that it requires a tremendous investment in time, energy and expense. You just can't leave anything to chance. That's why so many researchers choose Biological Industries (BI) cell culture products. From classic culture media to supplements and reagents, our products are designed to give your cultures superior growth and maintenance performance so you can achieve more consistent and reliable cell health.

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