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Issue 35 | Michaelmas Term 2020

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LETTER FROM THE EDITOR

Issue 35 | Michaelmas Term 2020

Dear Readers,

We are living through challenging times. Now more than ever, it is vital for us as scientists to reach out to the public and provide a source of accessible, factual, and clear information.

The *Phenotype* team came together virtually, joining forces from all over the world, to make this issue. We made a conscious decision to balance COVID-related- with equally important, non-COVID-related content.

This issue opens with an inspirational address from Vice Chancellor Louise Richardson, who informs us about the groundbreaking efforts by Oxford scientists to combat COVID-19. On p. 7 we feature an interview with Jessica Kelley, who tells us about her experience on the frontline, running COVID-19 testing at the Milton Keynes "Lighthouse Lab".

On p. 10 of this issue, Dr Stefania Giussani explores the link between bacterial infection and neuroimmunity. Continuing on the theme of gut-brain axis, Francesca Liva and Raffaele Sarnataro discuss the role of hormones, proteins, and vitamins in modulating sleep. Would you like to know what food may be keeping you up at night? Find out on p. 12. Lastly, our Berlin contributor, Laura Golusda, describes the implementation of nanoparticles in detection of inflammation, including in Inflammatory Bowel Diseases (p. 16).

Our Aspire to Inspire section features an interview with Professor Fiona Powrie, FRS FMedSci, Director of the Kennedy Institute of Rheumatology. Professor Powrie provides a captivating account of her inspirational career.

Once you have read the articles above, we are sure you will feel motivated and inspired to give writing your own grant, or a fellowship application a go. Find out how to begin and what mistakes to avoid on p. 20. Renowned coach and Director of Scriptorium, Dr Julia Staykova offers tips on writing your first grant or fellowship application.

In this issue we are happy to feature a new Op-ed section that is reserved for opinions and letters to the editor. Dr Emma Mee Heyes contributed the Op-ed article, titled "Aducanumab, a fast track to disaster?" (p. 22).

Some stories are vital to share, but due to their personal nature the authors prefer to remain anonymous. We welcome such stories. In this issue we feature a moving reflection by an anonymous contributor on their experience of completing a DPhil with Obsessive Compulsive Disorder (p. 24).

Last but not least, the final contribution is an interview with our former Editor-in-Chief and the current Editor in Chief at a Cell Press journal, Dr Sonia Muliyil. We congratulate Sonia on her exciting new position and thank her for her invaluable contribution to *Phenotype*. Find out about Sonia's journey and what it is like to be a professional editor on p. 26.

Marina Kolesnichenko
Editor-in-Chief of Phenotype

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Words from our cover contributor

Human intestinal epithelium stained against ACE2 (red), CD163 (yellow), TMPRSS2 (green), CD31 (orange), CD8 (cyan), and DAPI (blue) in order to analyse the impact of COVID-19 on patients suffering from Inflammatory Bowel Diseases.

By **Dr. med. Malte Lehmann**, Medical Department, Division of Gastroenterology, Infectiology and Rheumatology, Charité – University Medicine Berlin.

FIGHTING COVID

Photo by John Cairns

Address from Professor Louise Richardson, Vice-Chancellor of the University of Oxford.

Greetings from Oxford. These past six months will go down in the annals of the long history of this university. I hope that they will record the extraordinary way we have adapted to the constraints imposed by the COVID-19 pandemic and how we marshalled our research prowess to lead the global effort to defeat the virus. Our RECOVERY Trail has demonstrated the efficacy of dexamethasone, our statisticians and behavioural scientists are advising the government, our engineers and medics are developing ventilators and novel testing regimens.

Never has the enduring importance of what we do been so evident to the world at large. Never has our research profile been raised so high. For this, we are grateful to the many creative, dedicated and talented researchers at all stages of their careers who form the research powerhouse that is Oxford.

Louise Richardson

For our exclusive interview with Louise Richardson, see Issue 33 of *Phenotype*.

DPhil student reports from a COVID-19 testing facility

Interviewed by Komal Yasmin. Komal is a third-year Wellcome trust-funded DPhil student studying Chromosome and Developmental Biology at the University of Oxford.

Jessica Kelley is a third-year DPhil student in the Klose lab at the Oxford University department of Biochemistry. She was amongst the first wave of volunteers to help run COVID-19 testing at the Milton Keynes "Lighthouse Lab", where she worked for 3 months. During this time, she served as a section lead supervising manual sample processing and the preliminary sorting of samples for automated systems. Phenotype is excited to share Jessica's experience of working at the lighthouse lab.

Twitter: @Jess_R_Kelley

How did you get involved in the testing work? How long were you there for?

As the spread of COVID-19 in the UK gained pace during March, I was very frustrated by the slow ramping up of testing when keeping track of cases was so vital. I knew I had the lab skills to help and was on the lookout for opportunities wherever I could find them! I saw the advert for the Lighthouse labs on April 2nd and I applied immediately. I got a call back within 10 minutes of submitting the application and I drove over to Milton Keynes for my induction day the following Monday, April 6th. I was part of the lab team for nearly three months, leaving at the end of June.

What kind of hours were you working?

I was working 12-hour shifts on 7 days out of 14, in a pattern with a few days on then a few days off. The first couple of weeks were just day shifts, but towards the end of April we transitioned to 24 hour operation and I "volunteered" to take the first stint on night shifts. We were on nights for four weeks, then days for four weeks. It was pretty intense! We did get food during shifts and had rooms provided in a hotel 10 minutes away from the lab. I could go home to Oxford when we had days off, but some people were living in the hotel permanently because they had come from as far away as Newcastle and Glasgow. Milton Keynes is not the most exciting place even when everything is open, so I really admire them for sticking it out!

How many samples were you processing? Do you believe you were working at full capacity?

My first shift was on Easter Monday and we had capacity for around 4,000 samples, but only received 1200 or so. Our capacity grew steadily over the next couple of weeks, but the sample numbers barely increased. It was only once testing was opened up to all key workers and their families that we started actually using the full capacity. By the end of April, we were operating round the clock with about 20,000 samples, and at the end of May we did over 30,000 samples in 24 hours. In mid-June, we hit the total of 1 million samples processed.

There were definitely problems utilising the capacity we had. The lab capacity at the start ramped up far more quickly than the capacity to actually swab people, so it felt like a very slow start right when the pandemic was at its peak. In June, there was a big reshuffle of sample distribution between the Lighthouse Lab sites and our numbers plummeted to less than 10,000 on some days. It was incredibly frustrating sitting around at 2 am waiting for deliveries when we were used to getting through 15,000 samples in a shift. The government decision to set a target for lab capacity rather than tests conducted felt like such a wasted opportunity: there's no point having that capacity if we don't use it! ▶

How does the test actually work?

Each swab is transported in a tube containing a few milliliters of transport medium. We take a small sample of that medium and add it to lysis buffer containing guanidine thiocyanate, which inactivates the virus and releases the RNA. RNA is extracted using a magnetic bead system. The viral RNA is detected by a Taqman RT-PCR assay using probes for 3 different viral genes, with the MS2 phage as an internal control.

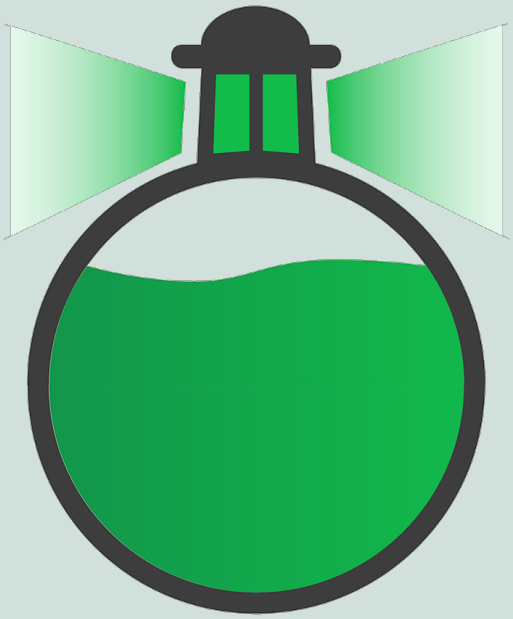
Obviously, the practicalities of actually running the test on tens of thousands of samples a day are a bit more complicated! Each part of the process is handled by a small team of people as a kind of production line. The samples have to be unpacked from their shipping boxes and sorted by tube type, then the tubes are unbagged within a biosafety cabinet and the swabs are removed. Operators load the tubes into a liquid handling robot, which scans the barcodes and assigns each one to a specific well position on a barcoded 96-well plate before pipetting a sample into the lysis buffer. Inevitably some samples aren't suitable for processing by the robots, and have to be pipetted manually. The MS2 phage control and the magnetic beads used for RNA extraction have to be added to each plate manually with a multichannel pipette, and the plates have to be loaded onto the King-Fisher extraction robots. qPCR setup is also done using automated liquid handling systems, but again these need people to operate them! The level of automation makes it possible to process tens of thousands of samples, but there's a lot of manpower involved too.

Which parts of the process were you involved in?

I did a lot of manual lysis plate setup, especially in the first few weeks before the automated systems were fully validated. I also spent quite a bit of time on the RNA extraction station. In my last few weeks I became a section lead, supervising manual processing and sorting samples for the robots. My section was therefore responsible for the initial handling of every sample that came through the lab.

Is there anything people can do to make it easier for the lab to process their test?

Yes! The main thing is to make sure you stick the barcode on the tube vertically, not wrapped around the tube. If it wouldn't scan on a supermarket self-checkout, it won't scan on our machines either! It's also worth checking that the tube you have has enough liquid in – if we can't get 200 µl out of that tube there's no guarantee we can process your test. It's better to request another sample tube than wait a couple of days for results only to be told to take the test again! Also, it may seem obvious, but make sure you screw the lid on the tube properly. Leaky tubes can be processed as long as they still have liquid in, but obviously they are a contamination risk. Whatever you do, please don't send the tube without a barcode, or without the lid on, or without the swab in. That being said, thank-you notes for the lab teams are highly encouraged!



The UK Lighthouse Lab Network. Twitter: @LhouseLabsUK

How accurate and specific is the test?

There has been a lot of talk about high incidences of false negatives from the PCR tests, but it's hard to know what the true figures are. The assay specificity is very high, and if there's RNA in the sample, it should be detected (the lower detection limit is around 15 copies). However, if the swabbing hasn't picked up any virus, there may be false negatives. There's a lot of tests ongoing to see whether saliva samples could provide similar sensitivity with less variability in the quality of sample collection.

Do you know what percentage of tests were actually positive?

When I first started, testing was only open to healthcare workers who had symptoms and our positive rate was about 30%. As testing eligibility was widened to all key workers and then to anyone with symptoms, that number dropped dramatically. It has stabilised over the last few weeks at about 0.5% positive.

Did you enjoy your experience? What were the best and worst things?

I did genuinely enjoy it! The work itself was not difficult and mostly just incredibly tedious, but knowing you're doing something to help thousands of people nervously awaiting results is really rewarding. The team spirit in the lab was amazing. Each shift team was 40+ people from a range of scientific backgrounds and career stages, from final year undergraduates to PIs, but none of those differences matter when you're all working towards a shared goal. The long shifts meant we got to know each other very quickly and we became genuine friends. We have a lot of weird in-jokes!

The worst thing was probably the really gross samples: those swabs have been up people's noses and sometimes you can really tell.

How did this differ from your usual lab work?

The techniques are all broadly familiar, but the scale of the whole operation is like nothing I'm used to. We went through reagents in astonishing quantities! PCR master mix was measured in ml not µl and the stacks of empty tip boxes reached epic proportions. The team dynamics were also very different to what I'm used to. As a DPhil student I have my own project and I'm responsible for every aspect of it, whereas the testing lab is a single shared aim and each person is only ever doing one small part of the process.

Did you have any problems with availability of reagents or PPE?

No, not really. The lab had supply deals with reagent manufacturers so we never had problems there. PPE was also fine - we ran out of masks once for a few days during the 3 months I was there, but that was the only thing. We were much more likely to be short of things like pens or paper towels than anything 100% critical to the process! The main problem for us was that supplies of swabs and sample tubes were under a lot of pressure, so the test sites were just using whatever they could get hold of. There was about a week where we had to process thousands of samples manually because they were in tubes that wouldn't fit on the robots.

There has been a lot of criticism of the centralised lighthouse lab system. What is your view on this?

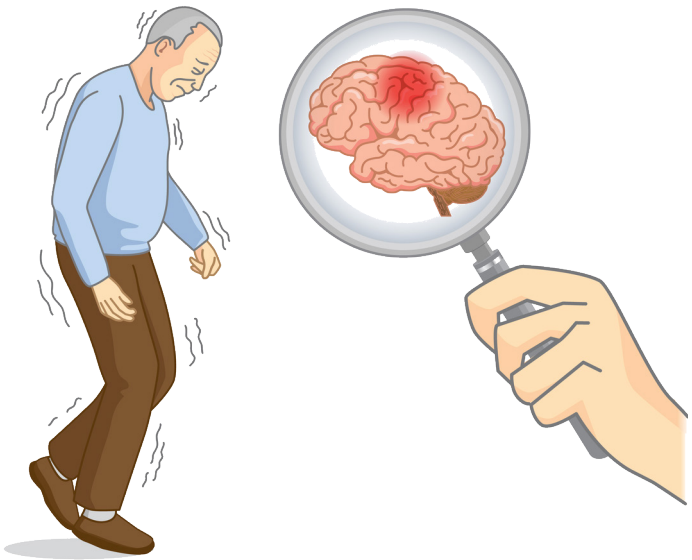
The facility that was set up in Milton Keynes is honestly an incredible achievement and is a testament to the hard work and dedication of a huge team of very talented people. A core group of volunteers built and validated the lab processing from scratch in just a few weeks before I joined. The wealth of expertise in the volunteer lab teams was a huge asset and we worked together to improve processes and built the capacity. Most of my fellow volunteers have now also left Milton Keynes and headed back to their own labs, but I'm confident the new recruits on longer term contracts will keep up the good work!

It is disheartening to think that our hard work is ultimately part of a very poor overall testing strategy. The Independent published several articles at the end of June which summarise these problems far better than I can, even if they somewhat fail to recognise the huge achievements of the lab staff. From my point of view starting in the lab, it felt like too little, too late. I was amongst the first wave of volunteers to arrive in Milton Keynes, on the day the UK death toll topped 5,000. By the time sample numbers were high enough for me to get the call for my first shift a week later, that figure had doubled. The decision in March to halt mass testing undoubtedly cost lives, and we spent all of April playing catch-up when that capacity should have been in place months earlier. It has also emerged that data from the Lighthouse Labs has not been properly shared with regional public health teams, limiting the effectiveness of local responses. I feel incredibly proud to have made my contribution in what way I could, but it's difficult to know that failings in the wider system didn't take full advantage of all our hard work. ■

Neuro...immunity? A focus on Parkinson's Disease

By Dr Stefania Giussani. Stefania is a postdoctoral researcher in the Wade-Martins research group at the Department of Physiology, Anatomy and Genetics (DPAG) at the University of Oxford.

Sometimes our immune cells are more polite than they should be and present antigens that should have remained unknown. The presentation of the wrong antigen in the absence of a functioning regulation can cause our immune system to overreact against a harmless self-antigen. This is the case of autoimmune disorders, such as systemic lupus erythromatosus (SLE), amyotrophic lateral sclerosis (ALS) and many others, in which our immune system decides to attack our own complement components, surface receptors or even mitochondria-derived fragments. The latter is of great interest. There are an average of 1,000 mitochondria inside a single human cell and all of them undergo a life cycle that terminates in a process called mitophagy, which is similar to autophagy, but with a cooler name. One of the best characterised pathways for mitochondrial clearance is controlled by PINK1 (PTEN-induced kinase 1) and Parkin, two proteins that are notably related to genetic forms of Parkinson's Disease (PD). Loss of function mutations in Parkin and PINK1 are some of the most studied monogenic forms of PD. PINK1 is a kinase, while Parkin is a ubiquitin ligase. Under normal conditions, cytosolic PINK1 is targeted to and imported into the mitochondria via the translocases of the outer and inner membrane (TOM and TIM) complexes, which in turn rely on the maintenance of the membrane potential in order to work correctly. Upon mitochondrial damage the inner membrane becomes depolarised, allowing PINK1, which normally exploits the difference in potential gradient to overcome the inner membrane, to remain stuck with TOM. PINK1 then recruits Parkin which starts to target mitochondrial proteins to degradation, thus initiating proper mitophagy that will result in the inclusion of the damaged mitochondrion in an autophagolysosome [1]. If this mechanism is disrupted due to failure in its regulation, mitochondria could either contribute to the accumulation of toxic species (ROS) in the cytoplasm, or form vesicles that will be targeted to late endosomes, leading the digestion products to be loaded on the Major Histocompatibility Complex-1 (MHCI), and thus resulting in presentation of self-antigens [2]. This process is known as Mitochondrial Antigen Presentation (MitAP) and has recently been correlated to specific monogenic forms of PD, strengthening the idea that Parkinson's is a disease harbouring autoimmune features. MHCI is the "go" signal for $CD8^+$ T cells, the hitmen of our immune system, that will lyse and kill all cells that are exposing a class I antigen. This therefore makes the hypothesis of such T cells attacking dopaminergic neurones in PD very appealing.



What happens – immunologically speaking – if either PINK1 or Parkin doesn't work? It has been recently demonstrated that LPS-stimulated Bone Marrow-derived Dendritic Cells (BMDCs) from *Pink1* KO mice show an increase in MitAP, rendering the immune response to a self-antigen much more likely **(Figure)** [3]. This possibility has been investigated by the same group using an interesting strategy to increase inflammation: they exploited a combo of *Pink1* KO mice infected in their gastrointestinal tract with Enteropathogenic *E. coli* (EPEC), which is a common human pathogen that can be correlated to the presentation of the mitochondrial 2-oxoglutarate dehydrogenase (OGDH) antigen and was used as a MitAP indicator [4]. What's really interesting about this work is that BMDCs from the KO mice would display OGDH-derived peptides on MHCI only if combined to another insult (*E. coli*). Moreover, OGDH-specific $CD8^+$ T cells were detected not only in the spleen, where immune cells can normally be found, but also in the brain, regardless

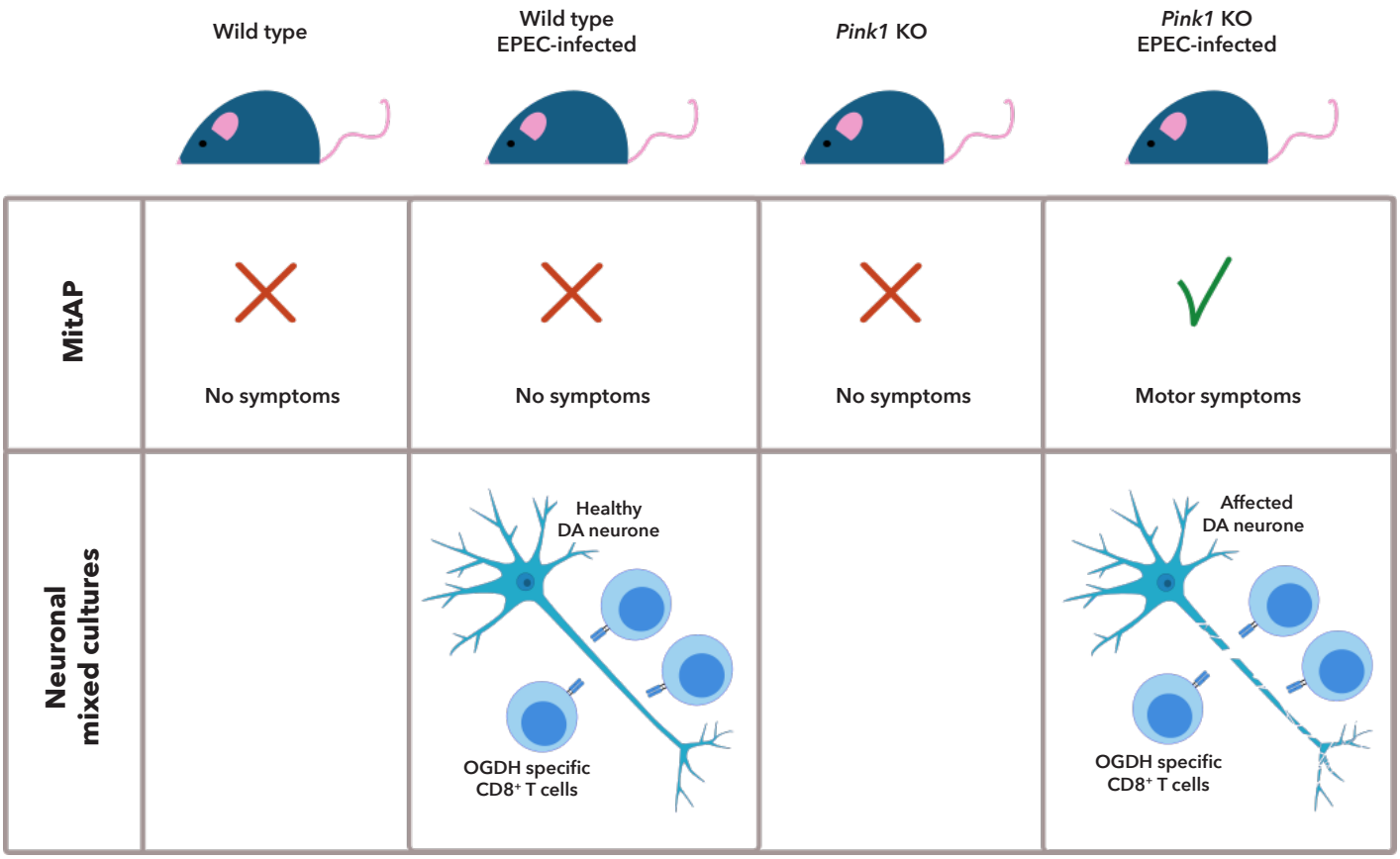


Figure. Schematic representation of the main experimental bullet points. *Upper panel*: Mice were tested for the extent of the mitochondrial antigen presentation after infection with EPEC. *Pink1* KO mice responded to the stimulus and developed motor symptoms 4 months after the infection. *Lower panel*: Neuronal mixed cultures of neurones derived from treated mice were incubated with OGDH-specific CD8 T cell clones. Only DA neurones from the *Pink1* KO mice were targeted by the cytotoxic T cell clones and showed signs of degradation.

of their genotype (*wt* or *Pink1*–/–). Nevertheless, only in *E. coli*-infected *Pink1* KO mice the dopaminergic (DA) neurones were selectively attacked by self CD8$^+$ T cells, leading to the development of PD-like motor symptoms that were promptly reversed by L-DOPA administration **(Figure)**. Additionally, with a decrease in the number of autoreactive CD8$^+$ T cells, DA neurones started to recover, indicating a possible role for autoimmunity in PD progression. As the majority of very good and interesting studies do, this work triggers even more questions than it answers. Why do T cells end up in the brain after the mice are exposed to the insult (*E. coli*)? In this work, they do say that T cells from the spleen start to display the receptor for CX3CL1, which is a chemoattractant normally expressed in neurones during inflammation. But, why and how do T cells migrate to the brain and attack only the dopaminergic neurones in the *Pink1* KO mice? What's the correlation between PD-like motor symptoms and a bacterial infection? You will say: "gut-brain axis!" and I agree, as the link between the gastrointestinal tract flora and the central nervous system reportedly influences the brain at a molecular and behavioural level [5]. But what we need to answer is: "how?" An oversimplification of the results would be: *Pink1* KO mice are fine until they are exposed to a bacterial pathogen. At that point, DA neurones are killed by CD8$^+$ T cells and the mice start to develop PD-like motor symptoms. It's like saying: "my teeth and nails are ruined" without specifying that you keep biting your nails. They show up as two distinct events, but are very much correlated and what causes them is you attacking yourself. A solution to this problem could be using one of those disgusting bitter nail polishes that will prevent you from biting your nails. So, going back to PD, we should probably think about something that renders DA neurones inedible to our immune cells. Easier said than done, I guess. Because immunity is not always our friend and has been challenging scientists since the beginning of time, having a clear insight into the role of autoimmunity in Parkinson's will require years and resources. Nevertheless, it can lead to the development of new and efficacious therapies against this and other terrible neurodegenerative diseases, such as Alzheimer's, the above-mentioned ALS, Huntington's and many others, for which a correlation with autoimmune responses has already been found. ■

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GUT TO SLEEP

We sleep what we eat

By Francesca Liva and Raffaele Sarnataro. Francesca is a PhD student in Science of drug and bioactive substances at the University of Pisa, with a background in Human Nutritional Sciences. Raffaele is a DPhil student in Neuroscience at the University of Oxford, with a background in Molecular and Cell Biology.

Sleeping and feeding are two vital behaviours that all animals perform for a substantial amount of their daily time, all through their lives. While feeding serves the metabolic sustenance of the organism, the nature of the vital function of sleep is still obscure.

Despite differences in their characteristics, both sleep and feeding are under homeostatic and circadian control. Sleep and feeding drives are produced and sensed by internal processes, they accumulate over time and once a set threshold is reached, such drives are homeostatically released by the initiation of sleep and feeding behaviours. In addition, oscillations of the circadian clock synchronise many physiological processes, including feeding and sleeping, with the light and dark produced by the Earth's rotation adjusting animal behaviours to the external world [1].

Since sleeping and feeding are both regulated by analogous mechanisms and are key to organisms' physiology, it is plausible to postulate that the two functions interact with each other. Many studies have demonstrated mutual correlations and causalities between nutrition and sleep [2], highlighting the role of an appropriate nutrition for the general psychophysical well-being, priming, triggering and maintenance of sleep [3] **(Figure 1)**. The hormone leptin represents a well-studied example of how homeostatic control can be exerted on appetite via neurohormones. Leptin is produced by cells called adipocytes and suppresses appetite, with its expression being regulated by sleep deprivation [4].

Such studies address questions such as: can the sleep history influence food choices? How can the food that an individual eats affect sleep quality? Which physiological mechanisms and brain circuitries convey such bidirectional information transfer, thus informing behavioural choices?

In this short piece, we will outline established knowledge on the major physiological effects of macro and micronutrients on key neurohormones modulating sleep and will conclude with an example of a scientifically grounded food choice selection that favours sleep quality in humans.

Serotonin and melatonin

Many neuromodulators regulate sleep, but in this work, we will focus on two major and extensively studied molecules regulating relaxation and sleep: melatonin and serotonin.

Serotonin (or 5-HT) is a monoamine neurotransmitter used in the nervous systems of all bilateral animals, where it is biosynthesised from the amino acid L-tryptophan (Trp), and exerts a signalling function in many clades of life **(Figure 2)**.

The production of serotonin requires a sufficient supply of Trp, an essential amino acid that is highly abundant in foods such as: flour, dried fruit, vegetables, dried legumes, dairy foods. Trp is included among the large neutral amino acids (LNAA), a group of amino acids that compete for transport sites across the blood–brain barrier (BBB) through LNAA transporters.

Serotonergic neurons in the central nervous system regulate several behaviours including sleep and appetite. However, 95% of the body's serotonin is found in the enterochromaffin cells of the gastrointestinal tract, where it regulates the peristaltic waves that aid the passage of food [5].

Melatonin is a hormone produced, especially during the night, in the mammalian pineal gland from serotonin, and has been found in almost all clades of life **(Figure 2)**. Its secretion is affected by environmental light. Melatonin synchronises several body functions to circadian rhythms and also operates also as an antioxidant. Some foods contain melatonin, especially plant-derived foods [6] and cow's milk [7]. Melatonin, in the form of a supplement, is absorbed and can be released into the circulation.

Effects of macro and micronutrients on sleep via serotonin and melatonin

The consumption of different types of macro and micronutrients can affect serotonin and melatonin which, in turn, affects the neuronal circuitry and biochemistry regulating mammalian sleep. The main categories of nutrients, and their effects, are described below.

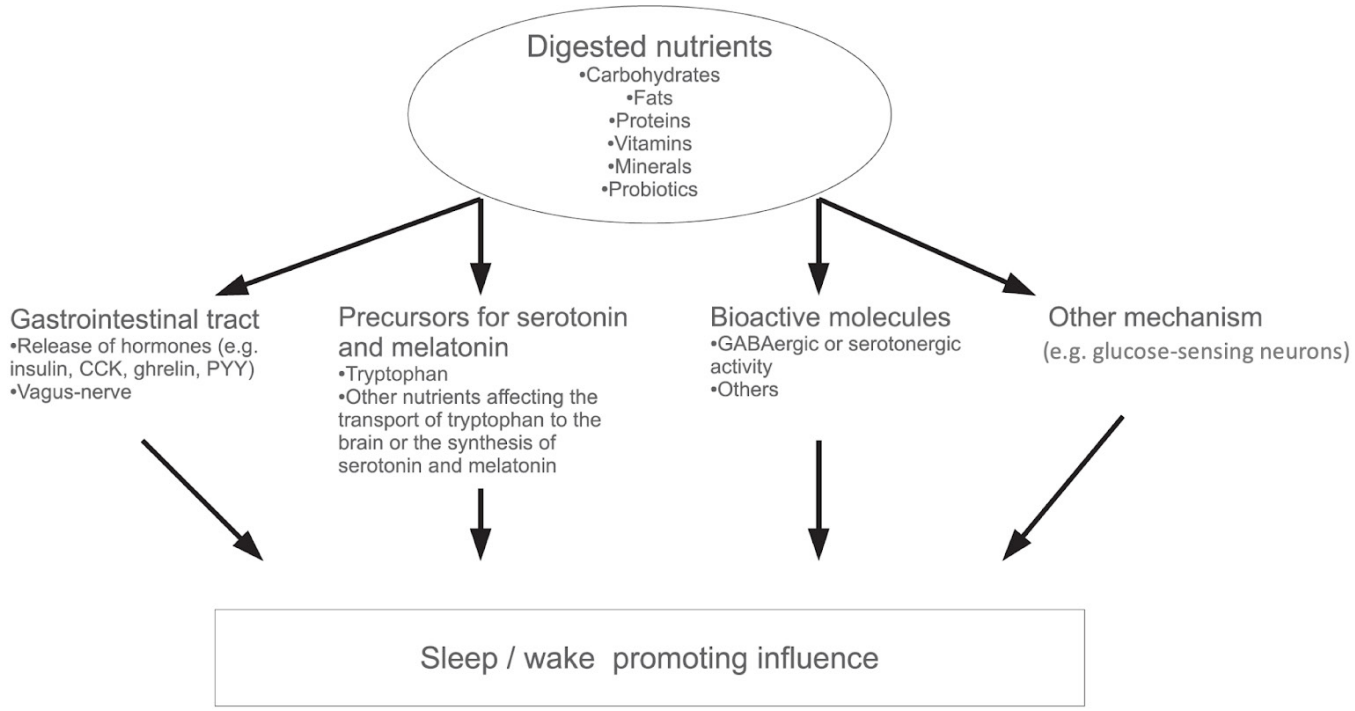

Figure 1. Examples of the pathways through which digested nutrients can affect sleep and wake. Adapted from [27].

Carbohydrates. Carbohydrates promote the bioavailability of Trp, the precursor of serotonin, which has been shown to have effects on human sleepiness, especially in patients with insomnia [8,9]. Indeed, in response to an increase in concentration of glucose in the plasma after a carbohydrate load in the intestinal lumen, insulin is released from the pancreas and promotes the peripheral absorption of LNAA but not of Trp, which is largely bound to plasma albumin. As a result, the Trp/LNAA ratio remains high and thus Trp absorption is favoured at the level of the BBB [9] **(Figure 2)**. In addition to this effect on the central nervous system, carbohydrate-rich meals have the ability to modify "peripheral oscillators", thus affecting human circadian rhythms, measured as changes in internal body temperature and heart rate [10].

Glucose-sensing nuclei (excited or inhibited in seconds or minutes by the surrounding changes in glucose levels) have been identified in different areas of the hippocampus [11]. Serotonin was shown to either excite or inhibit these glucose-sensing neurons in the ventrolateral preoptic nucleus of the hypothalamus, known for its sleep-control functions [12].

Proteins. Trp-rich proteins, such as α-lactalbumin, increase the plasma Trp/LNAA ratio by up to 90%, causing an increase in brain serotonin concentration [13]. In contrast, restriction of the intake of Trp provokes a reduction in the biosynthesis of melatonin [14].

The effect of protein intake on sleep also involves a number of peripheral processes, including the release of gastrin and cholecystokinin, from the cells of the mucosa of the duodenum and jejunum, as well as insulin, glucagon and somatostatin and their interplay in the regulation of peripheral circadian clocks [15,16].

Nucleic acids. Another important role is played by the non-protein nitrogen fraction of the diet: DNA, RNA, nucleotides and their fragments. Apart from adenosine, considered an endogenous sleep-promoting signalling molecule, another key nucleoside is guanosine-5'-monophosphate. It stimulates the secretion of the sleep hormone melatonin through the activation of the secondary messenger cyclic guanosine monophosphate [17]. Nucleotides are naturally present in breast milk, and an improvement in sleep quality has been observed after their oral administration in infants [18].

Vitamins. B vitamins (water-soluble vitamins) are involved in hormonal synthesis. Their deficiency in vitamin B12 in particular, can affect melatonin secretion [19]. Treatments with variable doses of vitamin B12 have been shown to produce beneficial effects on sleep quality [20] and rhythm disorders [21].

Some vitamins promote the availability of Trp for the synthesis of serotonin. Vitamin B3 (also known as niacin) is biosynthesised from Trp through the kynurenine pathway **(Figure 2)**. Researchers speculate that the administration of exogenous niacin causes the accumulation of its derivative nicotinamide adenine dinucleotide. Vitamin B3, in a feedback loop, suppresses the activity of tryptophan 2,3-dioxygenase (TDO), also known as Trp pyrrolase, which is one of the key enzymes in the conversion of Trp to niacin. Therefore, the administration of vitamin B3 reduces the amount of Trp naturally converted to niacin, thus making more Trp available for the synthesis of serotonin and melatonin **(Figure 2)**. Indeed, the administration of nicotinamide to people with insomnia problems improved sleep quality [22].

Vitamin B6, pyridoxine, is needed for the synthesis of serotonin from the precursor Trp. 5-hydroxytryptophan (5-HTP) is converted into serotonin by an enzyme called L-amino acid aromatic decarboxylase (AADC), which is a pyridoxal 5'-phosphate-dependent enzyme and ▶

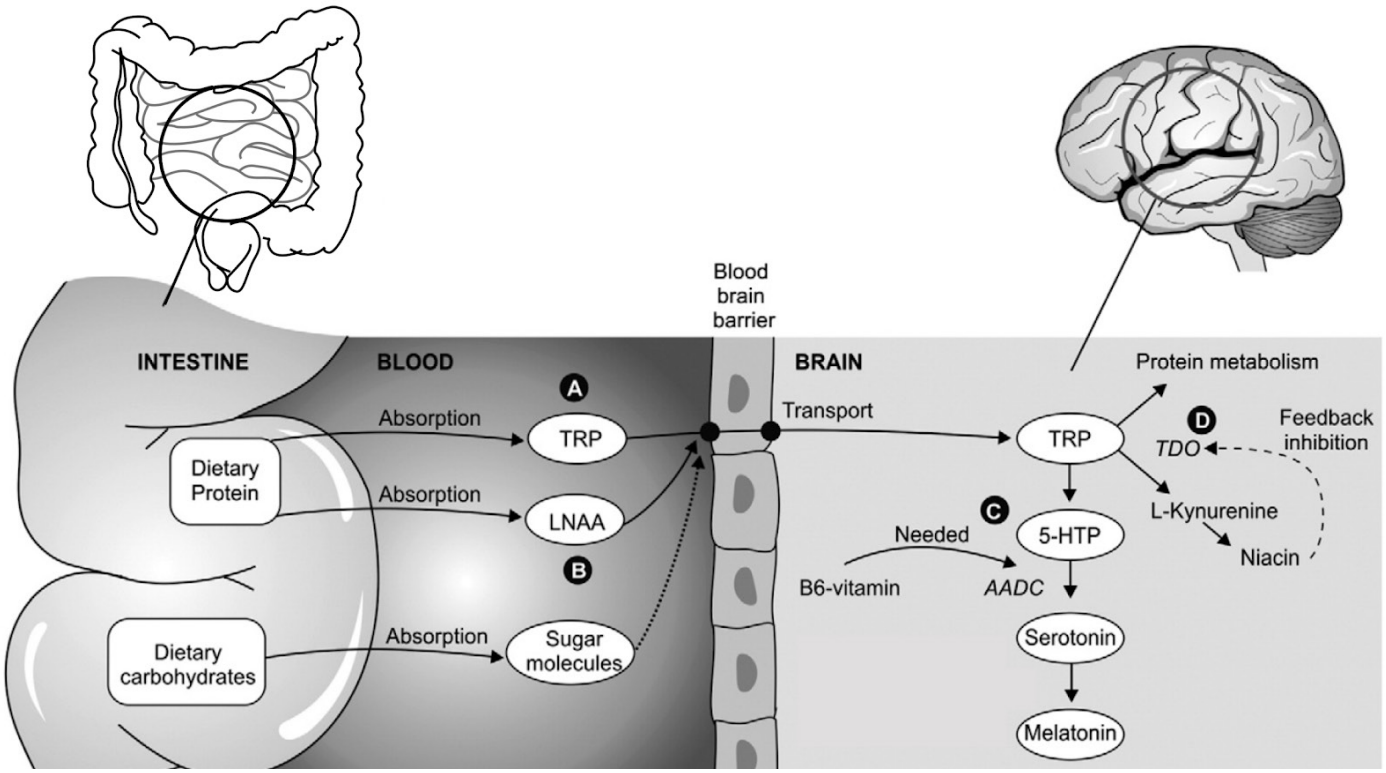

Figure 2. Mechanisms of the influence of dietary components on the synthesis of serotonin and melatonin. Adapted from [27]. (A) Through different pathways, TRP is metabolised in the brain to serotonin, melatonin, and niacin and partly used for protein metabolism. (B) Other dietary components such as carbohydrates and other large neutral amino acids (LNAAs) affect the intensity of TRP in crossing the blood-brain barrier. (C) 5-HTP is converted to serotonin by an enzyme (AADC) requiring pyridoxine (vitamin B6). (D) TDO is an enzyme that catalyses the reaction of TRP to formylkynurenine. Niacin (vitamin B3) inhibits TDO, thus leaving more TRP available to be used in the synthesis of serotonin.

pyridoxine is a precursor for pyridoxal 5'-phosphate (Figure 2).

Mineral salts implicated in neuromuscular relaxation, such as potassium, magnesium, calcium and selenium, positively influence the quality of sleep by regulating the synthesis of serotonin. For example, oral magnesium supplementation has been reported to improve sleep quality [23]. This is believed to be based on the ability of magnesium to improve melatonin secretion from the pineal gland by stimulating the activity of serotonin N-acetyltransferase, a key enzyme in the synthesis of melatonin, beside carrying out its γ-aminobutyric acid (GABA) agonistic activity [24].

"Enemies" of sleep

The cardiovascular system has to "slow down" for effective sleep induction. Indeed, "enemies" of sleep are all the substances promoting the release of adrenaline and norepinephrine, resulting in an excitatory effect (increase of body metabolism, heart rate, blood pressure and the number of respiratory acts).

Foods and drinks that contain **nervine substances** (e.g. theophylline, theobromine, caffeine), such as coffee, tea, chocolate, ginseng and some sugary drinks (e.g. cola), excite the cardiovascular system. They also suppress the production of serotonin and melatonin through still unclarified mechanisms, including their action as adenosine receptor antagonists [25]. A similar excitatory effect is promoted by foods with high **tyramin** content (amine derived from the amino acid tyrosine), such as aged and fermented cheeses.

Other foods are "enemies" of sleep via their interference with the digestive process. Acidic and spicy foods, as well as meals with a heavy content of fat, salt or proteins, promotes a higher production of gastric juices. These foods stay in the stomach longer, thus provoking troubles sleeping. In addition, irritating foods can alter the functions of the stomach, irritate the gastric wall, and in turn hinder sleep by causing heartburn.

Conclusion

Proper nutrition associated with an adequate lifestyle is at the basis of people's psychophysical well-being. It is estimated that sleep-related problems affect 50 to 70 million people of all ages and socioeconomic classes in the US alone [26]. Sleep is also affected by nutrition via neuromodulators, such as serotonin and melatonin. Therefore, acting on sleep, with an appropriate diet, especially at dinner, needs to be taken into consideration in the treatment of sleep disorders, but also for people with normal patterns of sleep.

In conclusion, a dinner that, based on scientific evidences outlined above, promotes sleep, and that is nutritionally balanced, could include:

- A portion of whole grains, such as rice, oats, barley and whole wheat (whole wheat pasta and bread).
- A portion of protein foods in moderate quantity and with low fat content, such as legumes and fish.
- A portion of vegetables, such as pumpkin, asparagus, cabbage, lettuce, spinach, artichokes (rich in mineral salts, such as potassium, magnesium, calcium and selenium).

- As condiment: extra virgin olive oil, aromas such as basil, marjoram, and oregano and seeds (particularly those of sesame, rich in Trp, and those of pumpkin, rich in magnesium). ■

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CIRCADIAN RHYTHM

Small particles going big

By Laura Golusda. Laura Golusda is a PhD student at the Charité – University Medicine Berlin, working in the lab of PD Dr. rer. medic. Anja Kühl on imaging of the extracellular matrix in models of chronic inflammatory bowel diseases.

Paramagnetic iron oxide nanoparticles have become an important alternative for gadolinium-based contrast agents in the field of cellular and molecular imaging, especially in magnetic resonance imaging (MRI). MRI provides in depth anatomical information and is a powerful application in diagnostics and research. For a more informative image, contrast agents are applied to specifically target a tissue of interest or to enhance sensitivity of the imaging process itself [1]. However, toxicity and long-term tissue accumulation of routinely used gadolinium-based contrast agents is currently debated. Paramagnetic iron oxide nanoparticles, which are both biocompatible and biodegradable, could be a valuable alternative [2]. Moreover, they can be used as targeted drug delivery agents, e.g. in cancer therapeutics [3]. The nanoparticles can differ in their hydrodynamic diameter, which can range from 180 nm to less than 10 nm [4], and their surface material can vary from a simple glycine [5] to a more complex carboxy-dextran coating [6]. Size and surface then determine the fate of the nanoparticles in terms of cellular uptake, circulation, cytotoxicity and degradation [7,8].

So-called very small superparamagnetic iron oxide particles (VSOP) developed by the Charité – University Medicine Berlin [9], have already been studied to target inflammation sites in atherosclerotic lesions [10] and inflamed brain endothelial cells [11]. These inflammatory lesions were detectable by MRI. Even though VSOP have been investigated for a few decades, their long-term fate with regard to accumulation and degradation processes still requires further elucidation. In our studies, the application of VSOP in different mouse models of intestinal inflammation mimicking inflammatory bowel diseases (IBD) in humans aims to detect the early onset of inflammation and changes in the extracellular matrix. These animal models allow for studying VSOP accumulation and degradation *in vivo*. Processes associated with long-standing IBD, like fibrosis and stenosis, are associated with a high accumulation of immune cells and extracellular matrix components. It is assumed that VSOP accumulate at the site of inflammation and are phagocytosed by local immune cells, most likely macrophages. MRI allows for the detection of inflamed areas, both in the gut and extraintestinally.

To further investigate persistency and toxicity of VSOP, intercalation of europium (Eu-VSOP) into the nanoparticles [12] allows for detection with conventional fluorescence microscopy [13] or imaging mass cytometry (IMC). IMC is a novel technique which enables the staining and analysis of up to 40 antibodies tagged with isotopes of rare earth metals on a single tissue section by combining techniques of immunohistochemistry and immunocytochemistry [14]. Eu-VSOP are detectable by IMC within tissue sections and the signal can be correlated to immune cell markers allowing for distribution and accumulation analyses. This technique is a useful starting point for understanding the persistence of VSOP in tissues.

In summary, very small superparamagnetic nanoparticles are well on their way to becoming a major player in research, diagnostics and therapeutics. However, their long-term fate needs further examination. ■

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About Charité

The Charité – University Medicine Berlin is one of Europe's largest university hospitals. Originally built in 1710 in response to an outbreak of the bubonic plague, the hospital was subsequently used as a hospice for destitute old people, a workhouse for beggars, and a maternity home for unmarried mothers [1].

In 1727, it was transitioned to a military hospital and educational training centre, and was renamed to Charité by Friedrich Wilhelm I. The hospital continued expanding throughout the 18th century whilst serving as a hospice for the poor, a state hospital, and a teaching facility for military medical personnel. In 1810, the University of Berlin with its medical faculty was established, focusing on training of civilian physicians [1].

Destruction during the Second World War prompted construction of several new buildings in the east German Democratic Republic, and the establishment of the Steglitz Hospital (later renamed Benjamin Franklin Hospital) in the west Federal Republic of Germany [2].

Following the reunification of Germany, and as the result of merger with other medical institutes, the Charité – University Medicine Berlin became one of the main medical centres in Europe.

Since 2017, the University of Oxford and the university hospital, the Charité – University Medicine Berlin has formed a research partnership.

You can read about this partnership in our previous issue of *Phenotype*, in an interview with Professor Alastair Buchan, Pro-Vice-Chancellor (Head of Brexit Strategy).

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ASPIRE TO INSPIRE

Meet the Director of the Kennedy Institute

KENNEDY INSTITUTE OF RHEUMATOLOGY | NDORMS

An interview with Professor Fiona Powrie, FRS, Director of the Kennedy Institute of Rheumatology. Interviewed by Marina Kolesnichenko, Editor-in-Chief.

Photo by Thomas Farnetti, Welcome Trust

Fiona Powrie is the Director of the Kennedy Institute of Rheumatology and Principal Investigator in the Translational Gastroenterology Unit, University of Oxford. Her research interests include characterisation of the interaction between the intestinal microbiota and the host immune system and how this mutualistic relationship breaks down in inflammatory bowel disease. Fiona's work has identified the functional role of regulatory T cells in intestinal homeostasis and shed light on their development and mechanism of action. Her current work seeks to translate findings from model systems into the clinic in inflammatory bowel disease patients. Fiona received the Ita Askonas Award from the European Federation of Immunological Societies for her contribution to immunology in Europe and the Louis-Jeantet Prize for Medicine 2012. She was elected a Fellow of the Royal Society in 2011, EMBO in 2013, the Academy of Medical Sciences in 2014 and the National Academy of Sciences in 2020. Fiona joined the Wellcome Trust's Board of Governors in 2018.

***Phenotype*:** Professor Powrie, thank you for accepting to be interviewed for the section "Aspire to Inspire". Could you please tell us what inspired you to become a scientist?

FP: I was always very curious about how things work, and science offered an opportunity to delve into those questions.

My mother was very ill throughout my childhood with a severe inflammatory disease, and that really motivated me to think about the immune system, and how it's such a positive force – but can also kill you. I was trying to understand the factors that control the immune system, and how they can impact health and disease – so, in the early days, it was a curiosity about the natural world and science, and later it became more focused on medical research.

***Phenotype*:** Do you have anybody in your family who is a scientist?

FP: Nobody at all. I was the first person from my family to go to university, so I did not have too many role models. I went to a comprehensive school in Luton, where it was not particularly fashionable to be doing science. A big thing for me was attending the comprehensive school, where I managed to get some O levels. That moved me on to the next stage to go to a Sixth Form College to do A levels. There I met friends who were interested in further education and had going to university as a goal. I think that your peer group has quite a big influence at that age on how you study, and what you are interested in.

***Phenotype*:** How did your family react to you doing science?

FP: I think they were intrigued. My father was an accountant in the city. For many, many years he wondered when I was going to get a proper job! Doing a PhD, and then a postdoc, and then these fellowships for short periods of time, and always reapplying for your salary – I don't think he really thought I had a proper job!

***Phenotype*:** Were you also working in industry at one point?

FP: I think I've always been interested in a lot of different things, because from the beginning I wanted to understand how we might use science to make an impact on people's lives, particularly in the area of immune-mediated diseases. After my A levels, I went to university to do a biochemistry degree and that degree, at the University of Bath, gave me two 6-month periods in industry or academia. So I went to a pharmaceutical company in Germany, and then I went to a top lab in New York. It was fantastic to work in those different environments and see how science was done. That was in the eighties, and was quite challenging. I left science after my BSc, went into accountancy in the city and worked as an accountant for a year.

***Phenotype*:** When you came back to science, was it always a smooth ride or did you have any obstacles?

FP: Having been in the city and coming back to do a DPhil with Prof Don Mason in Oxford at the Dunn School – I was tremendously lucky to get in there, but Don took a chance, and the combination of him and me worked extremely well. I have been very lucky in the mentors that I have worked with. They helped me to try to deliver my potential, and to follow my interests. They took a chance on me, and they encouraged me to take a chance in the areas I worked in.

I guess the most challenging period was coming back to the UK. I was in America – I had done a postdoc at the DNAX Research Institute – the Director there was Dr Maureen Howard, and there were lots of female scientist PIs. Coming back into the Oxford environment in 1990 was very challenging, and very male-dominated. I had a one-year old baby... I was juggling all those things, whilst trying to maintain the momentum for a career. And at that time for senior fellowships, there was no allowance for maternity leave – you did not even mention it – you just carried on. So that was challenging. But I believed in what I was doing, and I had a good system. It was the beginning of the IBD (inflammatory bowel disease) models, and I was right there. And Oxford is a great place to be of course! You've got to believe in yourself and also have a partner who pitches in and does their share. I am very lucky there – I have the most wonderful partner who supported me during those difficult times. We worked as a team combining both of our careers and caring for our small family. On the other side, being a scientist offers a lot of independence too – meaning you can work at different times. There are lots of positives.

***Phenotype*:** Would you say mentors are important?

FP: Very important. We did not have so many, and now there are many more different sorts of role models. When people come in on the career development track in the Kennedy Institute of Rheumatology, we want to link them up with a mentor who may be inside the Institute, or outside. And just seeing different sorts of people doing well and being successful in a research career, or science in industry, or even advising government – look at Dame Sally Davis for example – these are now great role models of what women can achieve.

***Phenotype*:** What do you think are obstacles that young career scientists face these days?

FP: I think we have to celebrate the things that a science career can offer. But on the other side, it is how we create a positive research culture that enables people to achieve their potential, ensures that there is no bullying, and that optimal management conditions exist from the most senior to junior scientists, including PhD students doing their training. There is a lot to be done to enhance research culture. Overall it is good compared to some workplaces, but we need to make sure that there is zero tolerance for when that is not the case.

The Athena Swan initiative brought real change. If you look at the Kennedy Institute, we have more tenured faculty women than men. We need to give people a path: a lit runway that shows them what is required to be able to progress in academic research.

I think one of the challenges in science now for early career scientists is the structure of our funding – there are not enough opportunities to recognise how postdoctoral fellows contribute to research teams and for early stage scientists transitioning to independence. We have not changed how we fund science for 30 years, but science itself has changed so much. It is not that easy to get a stable job in science, but actually that's the same everywhere now – companies close, and people get moved off jobs. So, I believe that a scientific career is a great career. You can work flexibly; you've got great colleagues. And there are more opportunities outside of academia now than there ever have been. There is still more to do to get rid of unconscious bias and have more diversity, and how we get people up into those really senior positions – I think that that requires bringing together a number of different approaches. One area is networks – knowing who is doing what, to be able to encourage people to apply for these roles. Some people may not even think of it. Somebody encouraged me to apply for the Sidney Truelove Professor of Gastroenterology in Oxford – I never would have done it myself. It is about encouraging people, and taking a chance and thinking outside of the box.

***Phenotype*:** What advice would you give to young people who are pursuing a scientific career?

FP: Keep going! If you really love it, if getting results from experiments excites and motivates you – follow your goals, believe in yourself, take chances. Go for it! ■

5' WITH...

Advice on writing your first grant or fellowship application

An interview with Dr Julia Staykova of *Scriptorium Consulting*.
Interviewed by Marina Kolesnichenko, Editor-in-Chief.

Dr Julia Staykova is a grant consultant at Scriptorium Ltd. She works with researchers in life sciences and natural sciences from leading research institutes across Europe. She supports applications to national funding agencies and European funds including the ERC, Marie Sklodowska Curie Actions, and FET-Open. Julia's research background is in rhetoric and communication skills. You can contact her at julia@scriptoriumconsulting.com.

What mistakes do people make when writing their first grant?

For an early-career researcher, one big mistake to avoid is imitating too closely the writing style of your Principle Investigator (PI). If your PI is experienced and well-known, they can get away with a few imperfections in their writing style that will not be forgiven in a junior grant. Make sure your project is structured very clearly, with every aspect of the research programme explained in meticulous detail. Often, early-career applicants ask me how detailed their descriptions of the work arrangements and methodological programme should be. My answer is always "more detailed than those of your PI - if your PI is established". Why? Because an early-career researcher must prove himself or herself vigorously, whereas a senior researcher, who may be recognised world-wide for inventing or advancing the techniques they propose to use in their grant, does not need to prove themselves to anybody as much. Why would they? Everybody already knows they can use these techniques. For a younger researcher, you need to describe in detail what specifically you plan to do, why you believe this is the optimal approach for any more challenging steps, and how you will manage any risks, should they arise. Unless you explain all this with some level of detail, the feasibility of the proposal remains in question.

Starting is always the hardest. How does one even begin?

Start from the heart of your proposal: the project aims and the central hypothesis or research questions driving your work. Remember that without a hypothesis or question, a project in the sciences is less likely to be funded. Then add the specific objectives, which tend to be between three and five in a smaller-scale grant. Once you have listed the general aims, the hypotheses and specific objectives, the core of your proposal is ready. Then you can add a short paragraph to describe the intended project outcomes and their added value for the field. After this, your next big step is to present the methodological programme step-by-step. Break it down into work packages or specific objectives, so the grant looks well organised – more like a project management plan than an amorphous essay.

For each objective or work package, list the main tasks, the risks and alternatives, and the expected outcomes. Space permitting, you can also mention the timelines, any collaborators or additional personnel involved, and what expertise they bring to the project, as well as the key deliverables. But the vital element is to describe the tasks, risks and intended outcomes.

How is a grant application different from a fellowship?

Not different at all. Often fellowships are funding instruments for a single investigator, whereas grants may involve budgets for hiring more staff and purchasing equipment. But ignoring the vocabulary, the writing principles involved are the same: provide maximum clarity for your reviewers to help them work. Make clear the anticipated knowledge gain and its added value, and be realistic about major risks that may endanger the objectives and the alternative strategies you will employ.

When should I start preparing a grant and when should I write one? Do I really have to? Maybe I will just end up in an institute where they pay all my expenses!

If you have already made the fatal mistake of conducting research at the PhD level, you are stuck for life! The chances are, for the rest of your career as a researcher in both academia and industry, you will have to fight for your share of research funding. You might as well learn how to do it as soon as possible. If you get comfortable with funding provided by your PI or institution, you will miss out on a vital chance to gain some independence. The CV of the applicant is weighted to about 40-50% of the total score in your evaluation, together with the proposal. If you have a slim track record of awards, fellowships or funding obtained, then you are less likely to be funded in the future. Later on in your career, the money will go to those who already have experience managing independent funds. Getting the first grant is always the hardest but once you get your own funding, you start building an independent record. Even if you have more money than you can possibly spend in your current position, apply – because this is how you build up a record of independence for your CV.

Can you provide a brief writing formula?

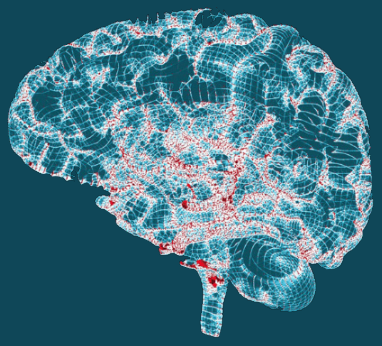
First, your writing formula revolves around answers to the key evaluation criteria: impact and feasibility. Without a clear, concrete discussion of added value, the project will sound uninspiring. Without an honest, detailed discussion of potential risks and how they will be managed, the project will sound like its head is in the clouds. Make sure your feet are firmly planted on the ground – this means thinking about your science in the context of a project management plan, with timelines, limited resources, risks to be managed, and available expertise: your own and that of collaborators. Second, your formula revolves around space distribution. You must have a very clear idea how you will distribute space in your proposal. If I see a ten-page proposal in which the introduction is four pages long, I wonder why so much space was allocated to the background to the main story, and so little space was allocated to the actual story the applicant wishes to tell. The project must provide enough space to address clearly the latest advances and the unmet needs, the objectives and research questions to be explored, and a detailed action plan in the methodological programme, leaving enough space for a final discussion of intended outcomes and their added value for the field. Typically a project ends with a timeline chart, which also takes some space. So, when you finish a first draft, check and double-check that one of these sections is not eating valuable space away from others.

How much should I talk about novelty? Can the reviewers not see for themselves how brilliant and groundbreaking my ideas are?

You know how the joke goes: "if you assume, you will make an ass of you and me". I often hear from applicants "why should I highlight that this step is novel? Isn't it clear to my reviewers that this is very original and ambitious work?", and I always answer: "yes, let's assume things are clear to your expert reviewer. But the expert is typically not engaged at the first stage of the evaluation, when a panel of generalists from your broader field reads all the submissions. To them, it may not be clear at all". Then again, perhaps your expert reviewer also won't find things quite as obvious. Perhaps they are a bit distracted, or burdened with other tasks at the moment. Which they usually are: at any given time, most reviewers have dissertations, journal articles, their own future submissions and your grant piled up on their desk. Help them work. Spell out clear answers to help them evaluate the grant. Make those answers easy to find on the page: don't bury them in page-long paragraphs of continuous text.

Are there internet resources where I can learn more?

If you find them, please let me be the first to know! I find that open-access resources are really thin on the ground. Some scholars run grant-writing blogs as a service to the profession. Some agencies such as the NIH in the US occasionally publish open-access examples of past funded grants. Try searching for examples of grants published online. But best of all, ask your lab colleagues to share their past applications and past evaluation reports. Most people learn from their own mistakes and those of friends and colleagues. The good news is that everybody is in the same boat, with every rejection serving as a building block towards a future success. It is the same as journal submissions: just don't give up on it, and success will be yours eventually. ■



Aducanumab, a fast track to disaster?

By Dr Emma Mee Hayes. Emma was a student in the Department of Clinical Neurosciences at the University of Oxford, and studied the effect of mTOR activation in TAU pathology. Emma is currently a postdoc at the Imperial College London, studying the effect of TAU congregation in proteasome activation.

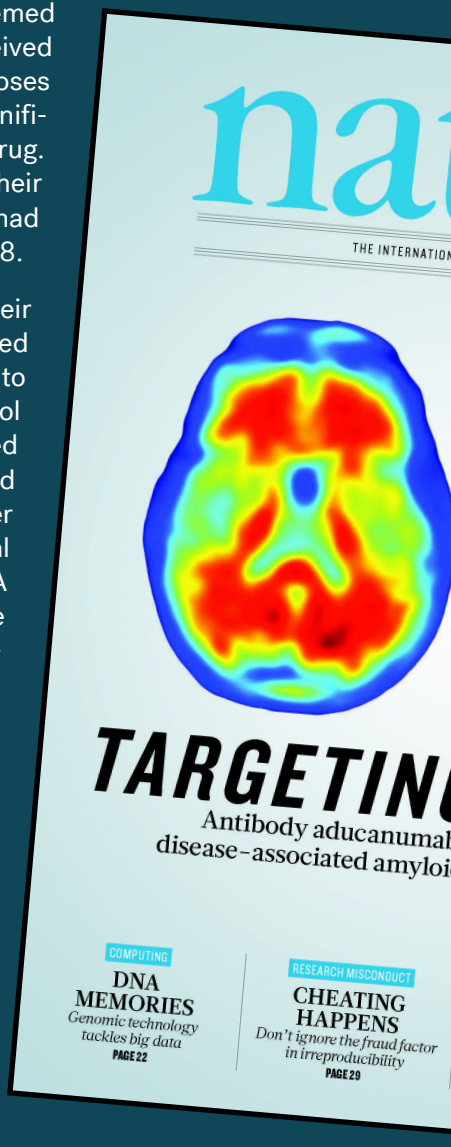
On the 22nd October 2019, Biogen announced that they were applying for regulatory approval for their drug Aducanumab... despite them announcing the previous March that the drug had failed. I – along with most of the field – was fairly shocked at this turn of events but was prepared to give Biogen the benefit of the doubt. However, as more data and information has emerged, I'm less convinced that the risk-benefit of releasing Aducanumab as a therapy is worth it.

Aducanumab is an antibody which targets the aggregated form of the protein Amyloid-β (Aβ) which is one of the main pathological hallmarks of Alzheimer's disease (AD). In a Phase 1 trial, Aducanumab had performed quite well, reducing Aβ burden and slowing cognitive decline (though many state that the cognitive portion of the study was underpowered).

Off the back of this trial, Biogen launched two Phase 3 trials called ENGAGE and EMERGE. One factor taken into consideration with regards to dosing was Amyloid-related imaging abnormalities (ARIA) which is evidence of cerebral edema, a side effect associated with amyloid targeting therapies. Those who are APOE4+ (a known genetic risk factor for AD) were at greatest risk for ARIA. Patients were split roughly 50:50 to low (3 mg/kg for APOE4+, 6 mg/kg for noncarriers) or high (6 mg/kg for APOE4+, 10 mg/kg for noncarriers) doses with patients receiving 14 intravenous doses over 18 months. In the end, the only difference between the trials was that ENGAGE started a month before EMERGE (August versus September 2015).

The two trials ticked along until 21st March 2019 when the results of the interim futility analysis were announced (based on data collected up to December 2018). It appeared that both ENGAGE and EMERGE would miss their primary and secondary endpoints and so the trials were being terminated early. This was yet another blow to the battered Amyloid hypothesis and Biogen suffered a hit as well – their stock dropped 26%.

So how did Aducanumab go from failing to being submitted to the FDA for regulatory approval? After the trials failed, Biogen added the data collected from December 2018 to March 2019 (this increased the amount of patients who had received the 14 doses from 1,748 to 2,066). They deemed anyone who had received 10+ consecutive high doses as having received 'significant exposure' to the drug. This further bumped up their numbers of those who had completed the trial to 3,258.



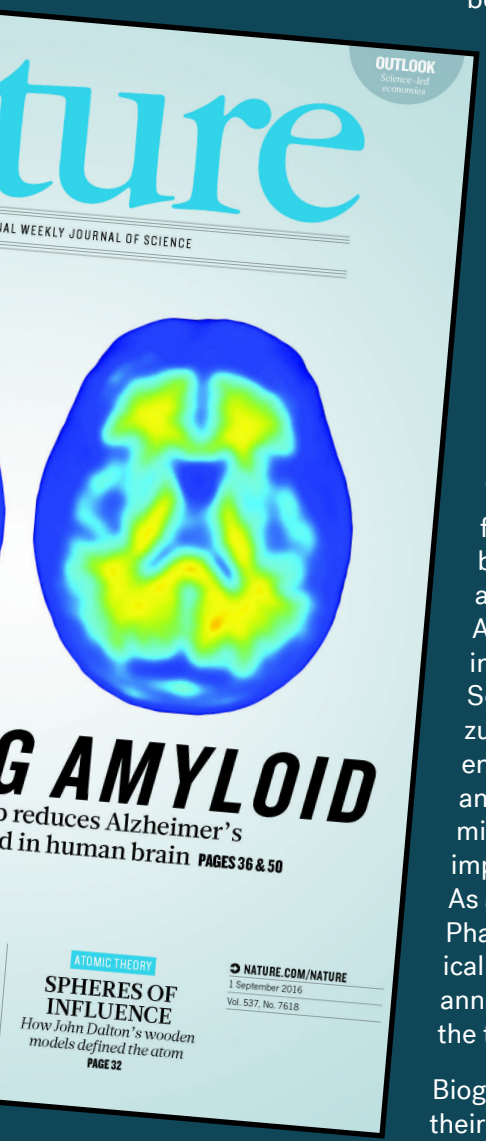
Biogen stated that their interim analysis was flawed as they hadn't taken into account two protocol changes that occurred during the trials with regard to dosage. Data from other amyloid-targeting clinical trials had shown that ARIA was a manageable side effect and thus the restrictions with regard to dosage for APOE4+ patients were relaxed. The net result of these changes was that the amount of patients on the highest Aducanumab dose increased (from 18% to 49% post protocol changes).

EMERGE now looked to be positive with patients declining 23% slower on the primary outcome and showing positive trends on the secondary outcomes. But it was immediately apparent that the data were not clear

cut. The ENGAGE trial appeared to show the opposite result completely, with those on high dose Aducanumab actually trending worse on primary outcomes, though a subgroup analysis of those who received 10+ high doses showed a similar result as EMERGE.

Biogen claims the different results between the trials were due to the different start dates, with more EMERGE patients being affected by the protocol changes (29% of EMERGE patients received 14 consecutive high doses compared to 22% of ENGAGE). They also stated that it was likely that the ENGAGE patients had suffered more interruptions of treatment and/or changes in dosage regimens than EMERGE. Biogen claims that their data meet the FDA criteria of "*substantial evidence of effectiveness*", which is defined as "*one adequate and well-controlled study* [EMERGE] *and additional confirmatory evidence* [Subgroup analysis of ENGAGE]". They had initially planned to submit for regulatory approval in early 2020, though at the time of writing no such claim has been submitted.

At the end of the day, the early termination of the trials has led to a mess of a data set which is difficult to interpret. AD researchers have had mixed opinions from the beginning with regard to Biogen's claims. Though it can't be denied that the AD field desperately want a drug after nearly two decades of failing clinical trials–especially given the money and time sunk into the Amyloid Hypothesis – are these data strong enough to risk it?



This would not be the first time the AD field has been bitten by subgroup analysis. One of the first Aβ antibodies that went into a clinical trial was Solanezumab. Solanezumab missed its primary endpoints but a subgroup analysis of those with mild-AD suggested an improvement in cognition. As a result, Lilly started the Phase 3 EXPEDITION clinical trial in July 2013... and announced the failure of the trial in November 2016.

Biogen appears confident in their data and in their decision to submit for approval. It's hard not to be cynical and

wonder if this is at least in part monetarily motivated. The day that they announced that they would be submitting for approval their stocks rose by 30%. The analyst company GlobalData has predicted that Aducanumab could be worth nearly $5 billion. The FDA could also come under intense pressure to approve Aducanumab. GlobalData analyst Alessio Brunello stated that the FDA is "*unlikely to turn down Aducanumab even if its benefit is modest, given the lack of any therapy that is truly efficacious*".

So should Aducanumab be approved by the FDA? Yes, cognitive tests show some potential benefit, but the data set is so confusing and comes with so many caveats that I personally am not convinced. Antibody therapies are extremely expensive to produce and administer and can be a significant burden on the patient as they will continually have to present themselves at the hospital for intravenous dosing.

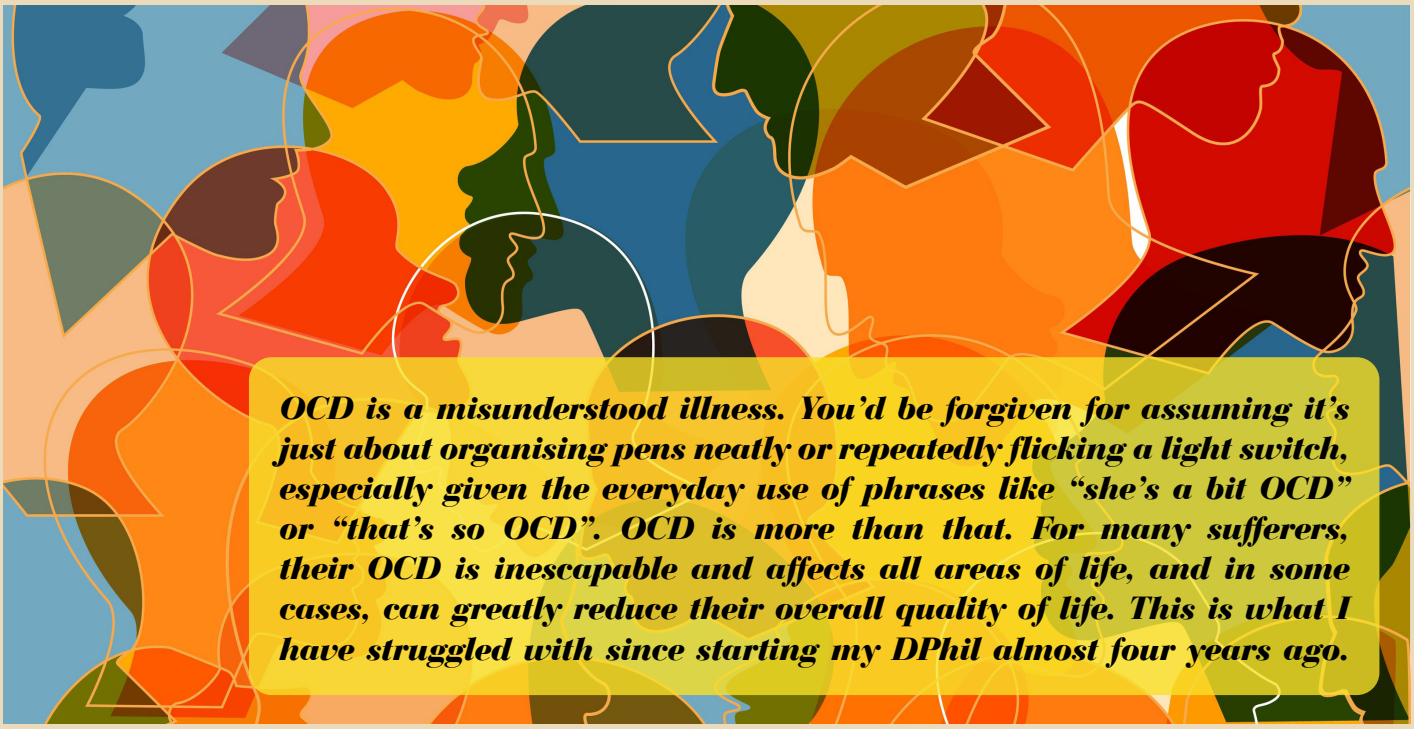
The field as a whole is relatively confident that Aβ is not the main driver of AD but instead triggers TAU pathology which is really what's killing brain cells. Biomarker data did show a reduction in TAU but Biogen only looked at between 30–50 patients. In a trial of over 3000, this isn't enough to draw any concrete conclusions. Maybe Aducanumab had lowered Aβ enough that TAU was also starting to be affected and this is what gave the small cognitive benefit following long term exposure to the drug. However, with the trials being terminated early, this is impossible to confirm.

To me, the most logical option is to conduct another clinical trial specifically looking at the highest dose of Aducanumab for all patients and conduct a more extensive biomarker analysis of TAU. Cognitive benefit may lag behind the biomarker changes and defining this time scale should be a priority and could be incredibly useful information for AD clinical trials as a whole. Biogen will likely balk at this – the original trial was set to run from 2015 to 2022, meaning that it could be nearly 2030 before the drug would hit the clinics. Many will argue that some benefit is better than none, even if it's not a cure. I would appreciate this point if I was convinced that a benefit was guaranteed.

As scientists, we have a responsibility to not allow our wish for a new treatment to cloud our analysis of the facts. Sadly, as much as I'd like to believe in Aducanumab, I am not convinced. ■

Juggling OCD and my DPhil

An anonymous contribution from the University of Oxford.



OCD is a misunderstood illness. You'd be forgiven for assuming it's just about organising pens neatly or repeatedly flicking a light switch, especially given the everyday use of phrases like "she's a bit OCD" or "that's so OCD". OCD is more than that. For many sufferers, their OCD is inescapable and affects all areas of life, and in some cases, can greatly reduce their overall quality of life. This is what I have struggled with since starting my DPhil almost four years ago.

Obsessive-compulsive disorder is a mental illness in which the sufferer experiences an overwhelming desire to carry out a compulsion to counteract an intrusive obsessive thought. The obsession often relates to safety of one's self or that of others around them, and the compulsion may include performing a certain action such as cleaning or checking, repeating a phrase in your head or praying. Performing this compulsion provides short-term relief but actually reinforces the behaviour and therefore obsession, and so the cycle continues. There are multiple types of OCD, and each person's experience is different and greatly personal.

Growing up, I displayed subtle behaviours here and there that I myself dismissed as being "a little bit OCD". It suddenly worsened one month before I started my DPhil at Oxford, when I was bitten by a dog on holiday in Asia. I convinced myself I had rabies and only relaxed once I received treatment while still on holiday. When I started my DPhil, I began struggling with little things like moving my lab book between the office and the lab. I was concerned with contamination of dangerous chemicals in the lab, particularly suspected carcinogens like fixatives. It now occurs to me this was because, during my undergraduate degree, my mother was diagnosed with breast cancer and I was now worried about my exposure to carcinogens. Thankfully my mother recovered but, over time, I became obsessed with the idea that I would develop a chronic health condition too because of my repeated "exposure" to these chemicals, despite my obsession with good safety practice within the lab. The problem worsened when I "realised" indirect contamination could occur via other people. This is not to say they weren't being safe – they were – but crucially it was my perception of safety and risk that changed. I carried hand sanitiser with me everywhere. I began to minimise contact with certain people and things, but gradually this grew into avoiding anyone or anything that had been in indirect contact with the lab – which is essentially everything. The more I performed compulsions to quash the obsessive thoughts, the more severe and frequent the thoughts became – a classic situation for sufferers of OCD to find themselves in. I became isolated and lonely, and as a result my anxiety and depression thrived.

I finally began to look for help in the third year of my DPhil. I first sought solace in online forums for people experiencing similar symptoms. They were helpful to a point, but I became anxious that hearing about other people's obsessions and compulsions would exacerbate my own symptoms. My GP prescribed me with antidepressants (often used to ease symptoms of OCD), and put me on

a waiting list for NHS talking therapy. Next, I told my DPhil supervisor and thankfully he was understanding and supportive. It was he who pointed me towards the University counselling service. The counsellor I spoke with explained the idea of Exposure Response Prevention (ERP) Therapy, in which the patient actively exposes themselves to their problem, and attempts to refrain from performing the corresponding compulsion. However, It wasn't until I was a few sessions in, with a private therapist, that I had my breakthrough. With her help, I was able to place my hands on the pavement outside her house then carry on my day without using sanitiser or washing my hands. I know doing this is nothing to the vast majority of people, but for me, I finally felt I had a chance to be free from my OCD again.

I was ecstatic with the outcome. I now felt I was in control of my life without OCD dictating all my decisions for me. However, this led to new problems. Without OCD "steering my ship", as my therapist put it, I struggled to harness the new-found control I had. This led to some impulsive behaviour and re-evaluation of my life choices, and resulted in the breakup of my long-term relationship. It was also naïve of me to assume I was 'better'. The unfortunate timing of the coronavirus pandemic has reawakened my dormant OCD and demonstrated to me that you are never cured of OCD; it just becomes easier to manage. I am still in the middle of trying to cope with my old obsessions and compulsions that have returned like a familiar friend.

Unfortunately, it is well known that mental illness is all too common in the academic world. My OCD and my DPhil are intrinsically linked, but it does not define my DPhil or make it any less valuable. In some ways, the experience has meant more to me because I've had so many personal challenges I've had to deal with alongside my DPhil. It's difficult to estimate how many people are struggling with OCD that is so interconnected to their job or their workplace, but thankfully I know it is easy to find support when you need it. The best thing to do first is to talk about it. Tell your friends and family, and your supervisor if you are comfortable with it. Talking about it releases a little bit of the burden and, importantly, it will help those around you to make sense of your occasional strange behaviour. You'll find it will make your life that little bit easier. ■

Phenotype alumna

An interview with Dr Sonia Muliyil, former Editor-in-Chief of *Phenotype*. Interviewed by Marina Kolesnichenko, Editor-in-Chief.

Sonia Muliyil served as the Editor in Chief of *Phenotype* from 2019–2020. She now serves as the Editor-in-Chief of *Trends in Genetics* (Cell Press). In Oxford, Sonia was a postdoctoral researcher with expertise in Cell and Developmental biology. She is a recipient of postdoctoral fellowships from the European Molecular Biology Organisation (EMBO) and the Human Frontiers Science Program (HFSP).

Can you tell our readers a bit about your past association with *Phenotype*?

My association with *Phenotype* dates back to May 2016. I started as a copy-editor and then slowly made my way to a section editor followed by Editor-in-Chief. In 2018, I was appointed Editor-in-Chief by Jack Cooper, with whom I served as Co-Editor-in-Chief for the 10th year Anniversary issue. Over the years, the magazine gained popularity and a readership base spanning well beyond the confines of Oxford. For that I need to credit Marina, the present Editor-in-Chief of *Phenotype*. With her help, we could expand the readership of this magazine to Berlin. With our combined issue, we managed to garner an appreciable increase in the social media followers of the magazine coupled with a broadening of its thematic readership. I'm proud of how, over the years, *Phenotype* has provided a medium for various students and postdocs to voice their opinions on various issues of importance.

What made you take the "Big Jump" to a full-time publishing career?

Science writing and communication were more than simple hobbies for me. My interest in science writing played an important part in securing both HFSP and EMBO fellowships for my postdoctoral work. I also enjoyed managing the Newsdesk at the Dunn school of Pathology alongside my postdoctoral work, which further reinforced my passion for all the above. In addition to this, during my postdoc, I realised that managing the workings of Phenotype, in its entirety, excited me more than all my wet lab research experiments. That is when I knew it was time to pay heed to my gut instincts. At the beginning of 2020, I got job offers from a couple of publishing houses, but eventually decided to take on the charge of running and managing *Trends in Genetics* as its Editor-in-Chief. Since then, I've never looked back.

What does your current role entail?

My current job encompasses a number of responsibilities: managing and dedicating quality time to submitted manuscripts; keeping myself abreast of the latest happenings in the field; communicating with authors and reviewers; managing the peer review process; marketing and strategy planning for the journal; attending and organising conferences to keep up with the trends in the field; and commissioning articles on a periodic basis. I believe that many in the scientific community are not that aware of the difficult decisions that editors have to make on a daily basis. I believe this job involves a lot of multi-tasking, trouble-shooting, decision making and networking. It is not simply science reading and writing. Akin to an academic career, I believe you really need to be passionate about the different tasks outlined above. However, having said that, the thrill of reading the latest developments shaping a field and diversifying your knowledge horizons on a daily basis combined with traveling and networking are some of the perks that come with the job!

Who/what has been your greatest source of strength and support?

I believe my close friends and family have always stood by me – come what may. The journey until now hasn't been an easy one; it was full of challenges and obstacles. I would especially credit my husband, who is also my best friend, for being a constant source of inspiration and support. None of this would have been possible without him.

What advice do you have for students and postdocs aspiring for a career in publishing?

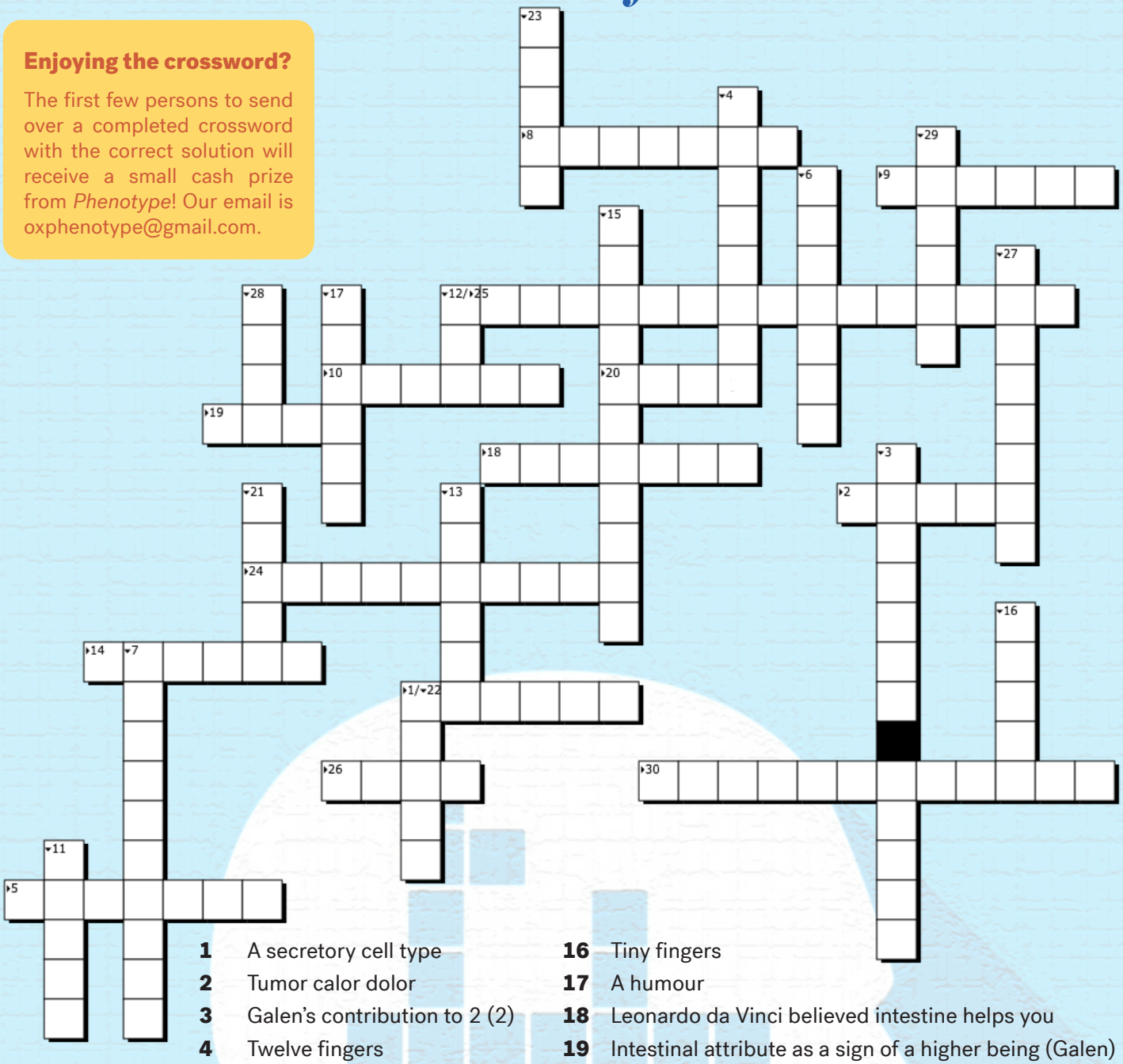
I would say follow your passion with full conviction. Also, make sure to engage in activities and initiatives that bear testimony to the enthusiasm you have for an editing career. Getting involved in initiatives like *Phenotype* can also be a good start! I would also recommend attending networking sessions at various career workshops and talking to full time editors, whenever you get the chance to do so. Most of us are open to questions of any kind. Additionally, several publishing houses also grant graduate students and postdocs the opportunity to take up short internships to help familiarise them with the inner workings of the profession. I would say keep an eye out for such advertisements. For more resources on careers in academia and publishing, you can check out Cell Mentor that frequently brings together voices and opinions of various leaders across the breadth of science. ■

CROSSWORD

Theme: Gut & Inflammation

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.



1 A secretory cell type
2 Tumor calor dolor
3 Galen's contribution to 2 (2)
4 Twelve fingers
5 Colon inflammation
6 A patch (…'s)
7 Affects your weight
8 An opening into the intestine
9 A blind sac
10 Thin
11 Intestine
12 Has its own nervous system
13 A sewer
14 First successful appendectomy
15 Muscular action
16 Tiny fingers
17 A humour
18 Leonardo da Vinci believed intestine helps you
19 Intestinal attribute as a sign of a higher being (Galen)
20 $Lgr5^{+}$
21 Large intestine
22 Named after a sea animal
23 Home of stem cells
24 First described Ileitis terminalis
25 Tract
26 Largest mass of lymphoid tissue in the body (abbrev.)
27 Dubious Victorian weight loss diet
28 Escherichia
29 Scottish delicacy encased in animal stomach
30 Doctor specialising in colon, rectum and anus

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