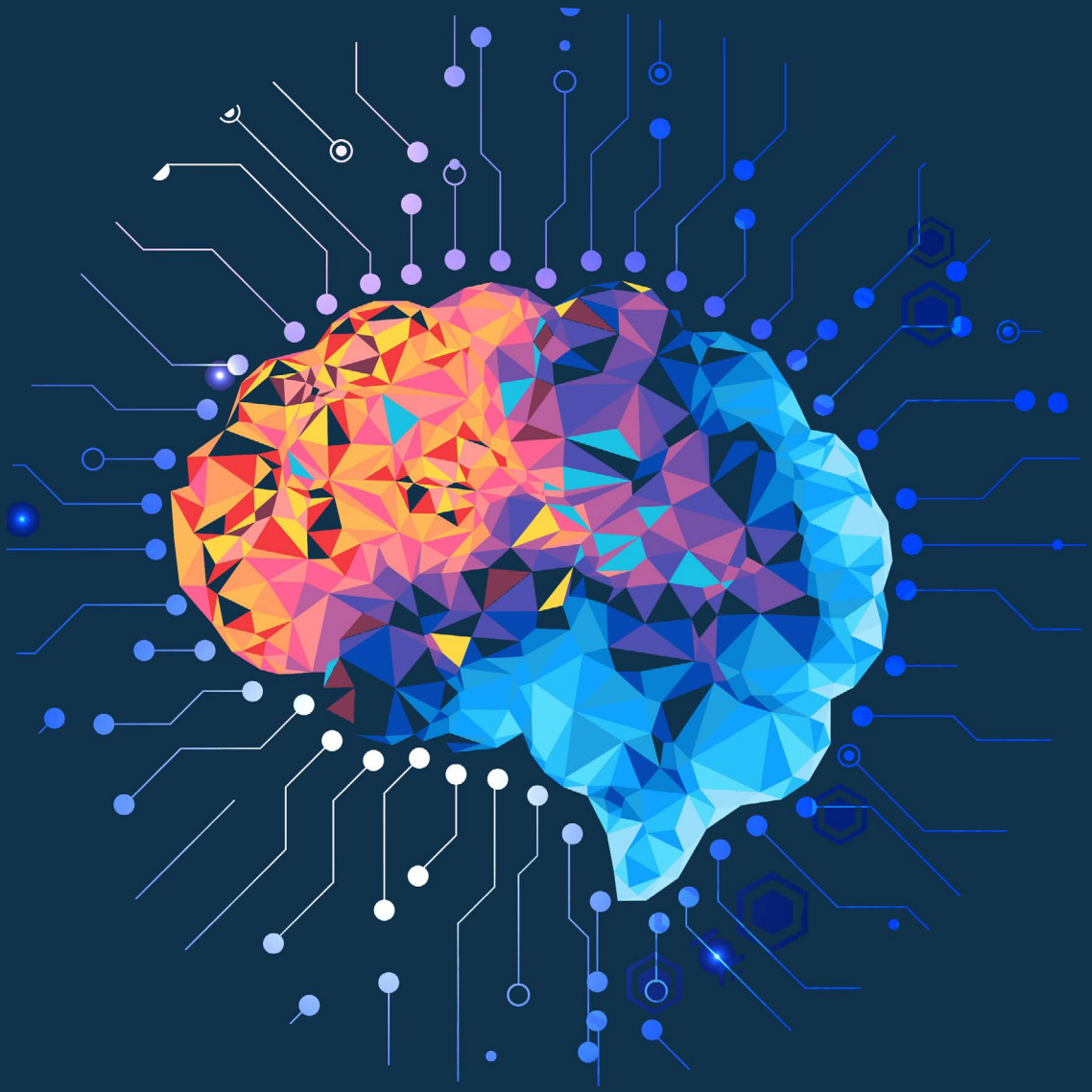
RESEARCHER INSIGHTS ISSUE NO.1

The Neuroscience of Sleep



A report produced by Researcher

Welcome to 'Researcher Insights' – Researcher's brand new collection of content specifically tailored to those working in the life sciences.

Here, you will find insights into the current field, interviews with practitioners and book authors, as well as high-quality contributions from the Researcher community. Each piece is written with our audience in mind, created with or by researchers and experts in the field. Researcher has always helped facilitate the conversation - but now we want to start it.

Our pilot issue will focus on neuroscience, more specifically, *The Neuroscience of Sleep*.

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Why the Neuroscience of Sleep?

Since the dawn of time, sleep has been one of the most fundamental aspects of being alive. Like most animals, humans require sleep to think, to function, and to simply exist. But even though we cannot survive without it, sleep has remained an astounding mystery to science. In the 21st century, we are still trying to understand – why do we need to spend a third of our lives unconscious? Perhaps we're closer to the answer than ever before.

For this first Researcher Insights issue, we reached out to experts in the field of Neuroscience to learn about what makes us go to bed every night, and why that's important. We are excited to share these insights with you. To better understand where our specialists' expertise fit in the real world, we also conducted our own independent investigation. We asked you, our audience, to tell us about your own sleeping habits – and the results of our survey proved to be rather eye-opening (page 12).

An overwhelming 48.02% majority of the people we surveyed were aged 25-35 – working-age adults in their prime, who presumably were at the point of their life where they would be most active and healthy. Despite that, under a third of the respondents (30.98%) reported that, on an average week, 'Never of extremely rarely' it takes them more than 15 minutes to fall asleep at night. The majority of the participants (37.63%) said that it takes them more than 15 minutes to fall asleep multiple times a week while the remaining 31.39% selected 'more often than not' or 'almost every night'. Nearly half (49.27%) of the participants in the survey reported sleeping 5-7 hours a night on average rather than the recommended 7-9 hours, and another 5.82% said they sleep 5 hours or less.

These first results indicated that even though most answers came from relatively young people, evidence of poor sleeping patterns was emerging. Using a 1-7 scale (with 1 being 'not at all' and 7 being 'extremely'), we asked people to rate how tired they feel on a daily basis. Only 37.91% rated their tiredness levels in the range 1-3, with the majority selecting medium values or above. Surprisingly, when we asked participants whether they slept during the day, almost two-thirds reported napping for at least 2 hours a week (73.18%), with equal percentage selecting among the ranges 2-4 hours, 5-7 hours or even 7+ hours a week of naps, presumably to compensate for poor sleep at night. We asked the respondents to choose their top three reasons for poor sleep at night, and the two results that emerged at the top were stress/anxiety (selected by 53.33%) and irregular sleeping patterns (selected by 40.83%).

So, were people aware that sleeping well was important? To find out, we quizzed participants to what extent they agreed with the statements: 'Poor sleep ages me' and 'Poor sleep affects my health'. On the 7-point scale, this resulted in a mean of 4.70 for the first, and 5.13 for the second question, respectively. Only 2.94% selected '1' to confirm they did not think poor sleep impacted their health in any way.

The above survey built a fairly grim picture of people's sleeping habits and how regularly poor sleep, stress and bad routine affected working people's lives. In our first discussion with Dr Alen Juginović, Harvard Medical School (page 6), we explore the impact poor sleep has on human health, as well as how this affects productivity on a grander scale, with some notable evidence highlighting the US as one of the top countries to suffer fiscally as a result of lost zzz's. Alen is an expert in sleep neuroscience and also part of the lab exploring the link between accumulation of reactive oxygen species in the gut, sleep deprivation and lifespan, including developing biomarkers to measure these features. In our conversation with him, we learned about his thoughts on sleep, why sleep is important, but also what, according to current research, could be the cause of the health repercussions associated with poor sleep.

In the second piece outlined in this issue, we spoke to PhD student Mie Andersen, University of Copenhagen, to discuss the *Nature Neuroscience* article she recently co-authored. In an experiment using fibre photometry to track norepinephrine in real time, her colleagues and Mie discovered an unexpected role of this neurotransmitter in sleep - one to do with memory. Fascinated, we raised some questions in our own survey. When asked how much a poor night's sleep affects their memory, only about a third of the participants (31.93%) ranked the effect on the lower side of the scale (1-3) with a mean rating of 4.27. The percentage rating 1-3 dropped to 27.25% when asked about the effects of multiple nights of poor sleep on memory and learning potential, with the mean ranking increasing to 4.59 on the 7-point scale. These results support the idea that sleep impairs memory, but to understand the actual science behind 'why', refer to our chat with Mie, which can be found on page 8.

Our third article in this issue is a contribution from our Researcher community, written by PhD Student Viviana Greco from Cardiff University, where she discusses new directions in studying and understanding how negative emotions are attenuated during REM sleep. In relation to her piece, we asked our audience how irritable they feel if they haven't slept well. About a third, 33.34%, ranked low, selecting 1-3, with a mean of 4.3 on the 7-point scale. In her piece, Viviana reviews some of the methods currently utilised in science for optimising emotional regulation during sleep - find on page 10.

The tools that you'll read about in this report are an exciting step towards the future. In another survey, this time targeting a small sample of 34 neuroscience researchers on a post-doc level or above, the majority (over 61%) had used or were frequently using electroencephalogram (EEG), while 50% reported the same about electromyography (EMG). Only about 12%, however, had used polysomnography (PSG) and 3% had used targeted memory reactivation (TMR) (both of which are discussed in Viviana's article). A modest 17.65% had used fibre photometry - an approach that, as you'll see below, allowed Mie and her colleagues to uncover something incredible about sleep and memory. And if you'd like to learn (with us) about what else is going on in the neuroscience community - the results of our second survey can be found on page 13 where you can learn what methods or equipment are commonly used in the field, what the top challenges faced by academics today are, the most trendy current topics - and more.

Now, to wrap up. In the last three pieces in this report, we turn away from the topic of sleep to highlight the launch of the Focused Ultrasound Foundation in the UK. We converse with Prof Clive Bramham, who has developed nanobodies to study the function of a brain plasticity regulator called Arc. Finally, we reached out to Dr Daniel Z. Liberman - an author of a fantastic book on dopamine, which we especially recommend you look up.

Together, we hope you find the content in this report as useful and enjoyable, as it was for us to put together.

Kristine Lennie

Dr Kristine Lennie
Editor

TOP 5 relevant articles in Neuroscience on Researcher this quarter

1	**'Widespread cortical thinning, excessive glutamate and impaired linguistic functioning in schizophrenia: A cluster analytical approach'** in *Frontiers of Neuroscience* by Liang e tal. (2022)
2	**'Regularly occurring bouts of retinal movements suggest an REM sleep–like state in jumping spiders'** in *Proceedings of the National Academy of Sciences* by Rößler et al. (2022)
3	**'Neurotensin orchestrates valence assignment in the amygdala'** in *Nature* by Li et al. (2022)
4	**'Genetics of the human micorglia regulome refines Alzheimer's disease risk loci'** in *Nature Genetics* by Kosoy et al. (2022)
5	**'Prefrontal feature representations drive memory recall'** in *Nature* by Yadav et al. (2022)

Sleeping for Longevity: How oxidative stress in the gut might explain the effects of sleep on health and lifespan

Dr Kristine Lennie

We've all heard (as well as readily dispensed) the advice to go to bed early, switch off our devices, relax and get our full 8h of beauty sleep to properly recover from a long day. How many of us actually follow this recommendation, is a different matter. After all, sleep is often the first thing to be put on the back burner when life gets busy.

Researchers have long established that sleep is invaluable for our health and cognition. A wide-scoping 2017 meta-analysis by Lowe et al. that included 201 previously published papers and over 1,600 participants, confirmed the negative effects of restricted sleep on sustained attention and executive functioning. A more recent study in Nature (this one by Pesoli et al., 2022) showed that as little as 24 hours of sleep deprivation can make us slower, less accurate in task completion and even affect connectivity in certain brain networks. Beyond focus and reaction time, sleep is also known to assist in memory consolidation, immune response, mental health and mood. What is more, a proper night's rest is important for our physical aptitude. On the sports field, poor quality sleep was shown to increase athletes' risk of injury, impede muscle and trauma recovery, and damage performance (see: review by Singh et al., 2022). The science agrees. We all need to sleep better to do better.

In light of all this, chronic sleep deprivation continues to be a concern. To bring attention to the problem, a cross-country analysis from 2017 by Hafner et al. estimated the continuously rising economic cost of poor sleep for five major world economies. The US ranked at the top, accruing losses of over $400 billion annually according to calculations. Another, later review, from 2019 by Chattu et al. spanning several international studies, highlighted sleep deprivation as a risk factor for a range of morbidities. Things arguably did not get better with the arrival of the COVID-19 outbreak, with some evidence already suggesting that the pandemic lockdowns, anxiety and mental distress and potentially COVID-19 itself had a detrimental impact on the sleeping patterns of vulnerable groups or even people who are in good health (more information in the references on page 19).

So, if sleep is so important, why are we so insistent on trying to stay awake?

Perhaps one of the reasons is that we simply don't understand sleep very well. Despite all the data on the negative impact of sleep deprivation, neuroscience is still looking to uncover many of the mechanisms that underpin sleep's role in health regulation. To find out why, and what makes sleep so difficult to study, we reached out to Dr Alen Juginović, medical doctor and postdoctoral fellow in the Department of Neurobiology at Harvard Medical School, and someone whose current work we wanted to learn more about.

'The brain is very complicated,' says Dr Juginović, 'But sleep is not just about the brain, it's a whole-body experience. And since sleep is vital for health, we cannot safely deprive a human being of sleep for, say, three weeks and experiment on them to see what happens. Instead, to understand sleep, we need to use animal models. For example, fruit flies.'

The humble fruit fly may be small, but it operates using an internal circadian clock that carries surprising similarity to that of mammals. It was the study of the fruit fly's genome that led to an enormous leap in our understanding of the mammalian sleep-wake cycle, with a ground-breaking study that earned Jeffrey C. Hall, Michael Rosbash and Michael W. Young the Nobel Prize in Physiology or Medicine in 2017. The findings centred on the discovery of the so-called PERIOD (PER) gene. The PER gene and its encoded protein PER, were found to maintain levels that oscillate in a 24-hour period and tightly control the internal circadian rhythm of the fruit fly. Mammalian homologues of the same PER protein have been associated with disorders presenting with irregular sleep, cancer vulnerability and long-term memory impairment, edging us closer to our understanding of sleep's role in well-being.

'Fruit flies are great,' agrees Dr Juginović 'but they are much simpler than humans, and so certain aspects of studying this 'full-body experience' that is sleep is not possible. We do also use mice, which are much more complex organisms, but their sleeping patterns are opposite to ours – they are nocturnal - and we don't know exactly how this opposite cycle affects the translational aspect of the data.'

Alen joined Dr Dragana Rogulja's lab in Harvard Medical School in 2020 to take part in studying how sleep quality affects lifespan. We asked him about his current work and the lab's focus – they are presently looking into the relationship between sleep and gut health and have some exciting leads to report.

About two years ago (in 2020), Dr Dragana Rogulja's group published a study showing that fruit flies which were partially deprived of sleep suffered from a dramatically reduced lifespan (up to 60-70% shorter) compared to controls (Vaccaro et al., 2020). These flies presented with substantially increased levels of the chemicals known as Reactive Oxygen Species (ROS) in the gut. ROS is highly reactive and known to cause oxidative distress, which is damaging to cells, proteins, and DNA. To examine if the cause of the reduced lifespan was related to this increase in oxidative stress, Dr Dragana Rogulja's group tested another round of sleep-deprived flies, this time treating them with antioxidants. Shockingly, the antioxidant treatment proved successful, restoring the flies' lifespan almost completely.

> A good laugh and a long sleep are the best cures in the doctor's book
> Irish Proverb

'What we want to see now, for example, is whether oxidative stress occurs in people with insomnia – a condition, which is fairly prevalent,' explains Dr Juginović, who is part of the next stages of this investigation. 'The challenge is to actually detect oxidative stress. In mice and flies, we measure oxidative stress by taking the intestines out of the animal and examining the tissue. This is clearly not feasible with humans. So instead, we are looking for ways to identify oxidative stress by taking easily obtainable clinical samples such as blood, urine, faeces, serum, and identifying biomarkers which indirectly test for oxidative stress in those.'

If oxidative stress is indeed found to be prevalent in people who suffer from insomnia, then given the findings from treating mice and flies, antioxidant treatment may be a feasible option to improve health and quality of life of those patients. 'We are hoping to start human trials in the next 6 months,' says Dr Juginović. 'The data is yet unpublished so I can't mention specifics yet, but we have found a few biomarkers that reliably correlate with oxidative stress in the gut. What is more, these same biomarkers were shown to reduce when we clear oxidative stress – this is very promising!'

The work of Dr Juginović and his colleagues at Dr Dragana Rogulja's lab are one piece of the puzzle of how better sleep might improve our health and extend our lifespan. Much of the data is still work in progress. However, the implications of a successful trial are huge, paving the way to an improved patient care and deeper insight into how sleep deprivation is harmful to us.

Dr Juginović is active in his outreach activities and has been part of a range of projects, including organising 'Nobel Days' in 2019 – an ambitious event, gathering four Nobel laurates to present their award-winning work. He's appeared on YouTube podcasts and given interviews for the Harvard Brain Science Initiative. He's the President of the Organizing Committee & Head of the Program Team for Plexus – an international biomedical conference 23-25th September in Croatia. Despite his busy lifestyle, he still recommends sleeping between 7 and 9h a night, and never less than 6h.

That's certainly something to keep in mind. Sleep is remarkably important, and while we have a way to go to understand it, lessons from the fruit fly tell us not to skip out on those precious zzz.

Dr Dragana Rogulja group's paper:

Vaccaro, A., Kaplan Dor, Y., Nambara, K., Pollina, E. A., Lin, C., Greenberg, M. E., & Rogulja, D. (2020). Sleep Loss Can Cause Death through Accumulation of Reactive Oxygen Species in the Gut. *Cell*, 181(6), 1307-1328.e15. https://doi.org/10.1016/J.CELL.2020.04.049

Other references from this article:

Harvard Brain Science Initiative: https://brain.harvard.edu/
Plexus: https://medx.hr/

Dr Alen Juginović
Photo credit: Dalibor Gabela

Key Article Facts

In 2017 the annual economic cost of poor sleep in the United States was calculated at

$400b

Poor quality of sleep can negatively affect:

- Mental health
- Immune response
- Memory consolidation
- Mood

On the sports field poor quality of sleep was shown to:

- Increase risk of injury
- Impede muscle recovery
- Impede trauma recovery
- Damage performance

Fruit flies partially deprived of sleep suffered a dramatically reduced life span of:

60-70%

Norepinephrine: Shedding light on how the 'fight or flight' hormone affects memory

Dr Kristine Lennie

Norepinephrine – also known as noradrenaline – is both a hormone and a neuromodulator, produced by the locus coeruleus, a small nucleus in the brainstem. Norepinephrine is best known for making us alert, focused and for facilitating the 'fight or flight' response that activates when we are in danger. As such, noradrenaline has generally been assumed to be high when we are awake and low when we aren't, to ensure we are active and vigilant at the right times.

But what if this isn't its only role?

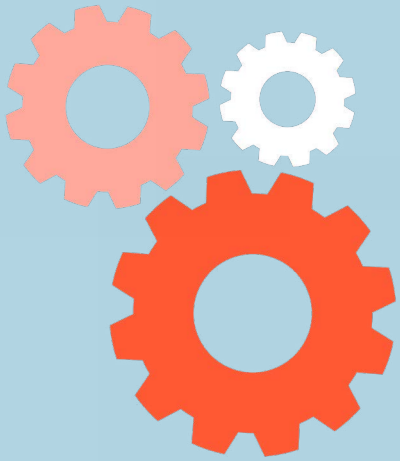
Mie Andersen is a PhD student at the University of Copenhagen, Center for Translational Neuromedicine, where she works in Prof Maiken Nedergaard's lab. She is also the co-first author of a recent article in *Nature Neuroscience* which implicates norepinephrine, the famous 'fight of flight' hormone, as a surprising curator of another process in the brain – memory consolidation during sleep.

'In previous studies where norepinephrine was measured, the method of choice was microdialysis, which is a common way to determine levels of neurotransmitters in the brain. The problem with microdialysis is that it only allows you to take a sample every 20 minutes or so. This is a rather low time-resolution recording – you don't see how chemical levels change in a continuous way - and what microdialysis showed was that norepinephrine was low during REM and non-REM sleep compared to wakefulness. This was to be expected, since norepinephrine is meant to make you 'alert', so scientists weren't really even thinking about norepinephrine's function during sleep. They assumed it was low, so it didn't play a role.' Mie tells me this fit well with publications that the activity of norepinephrine's source, the locus coeruleus, was also low during sleep. So she wasn't looking at norepinephrine at the start of her project – she had a different goal.

'My initial work actually focused on astrocytes,' says Mie. 'Astrocytes are glial (non-neuronal) cells that surround the neurons and maintain their extracellular environment. They exhibit calcium elevations linked to norepinephrine signalling – so I was studying these calcium elevations to find out more about astrocytes during sleep.' Astrocytes are the most abundant cell in the brain and owe their name to their 'star-like' shape. 'My supervisor, Dr Celia Kjærby, had experience from her previous lab in San Francisco with a technique, fiber photometry, which wasn't at all used much at the time. She taught me the technique and we used it to collect calcium measurements from astrocytes and the locus coeruleus, when we noticed something unusual.'

Fiber photometry has a sampling rate of about 1000 Hz (1000 sampling points per second), which is extremely high compared to microdialysis. 'We saw what appeared to be these phasic bursts of the locus coeruleus during sleep. We were a little surprised by this because all the literature so far had said that the locus coeruleus is supposed to be at very low tonic level during sleep, with phasic bursts only linked to arousal. Our results were not supporting that.'

A response to stimuli which is relatively sustained over a period of time can be thought of as 'tonic' – a 'baseline' for the type of activity or brain state - so for instance being more aware or alert can make the tonic level of certain chemicals higher. By contrast, a



> In contrast to the long-believed hypothesis perpetuated in many studies, norepinephrine was not inactive during sleep after all – it was doing something.

phasic firing – or burst – can be thought of as a short-term change. Those can be due to shock responses caused by unexpected or novel stimuli, or could be linked to an unpleasant experience such as a prickle or a burn.

'To understand what was happening with these phasic bursts, we changed the experiment,' says Mie. 'Previously, we had mainly used fiber photometry with calcium indicators to monitor calcium in the astrocytes and locus coeruleus. Once we had observed locus coeruleus activity during sleep, we decided to combine fiber photometry with a novel norepinephrine biosensor developed by Prof Yulong Li's lab, and measure real-time norepinephrine levels across the sleep-wake cycle. We were the first research group to do such a test.'

The new setup of the experiment involved simultaneously tracking the activity of norepinephrine's source (the locus coeruleus) and the neurotransmitter itself. Mice were implanted with electroencephalogram (EEG) and electromyography (EMG) electrodes so they could be monitored during sleep. Biosensors for norepinephrine and calcium were injected using a virus in the prefrontal cortex and the locus coeruleus, respectively. Optic implants were also inserted into these two brain regions. 'With this experimental setup the mice are not restricted or stressed and are able to move freely in their cylinders, where they have food, water, and their nests. This allows for natural sleep,' says Mie. Blue light is sent via the optic fiber to excite the biosensors. If the required chemical species (e.g. norepinephrine) are present, they bind to the biosensors which then emit green light that is collected via the same optic fiber. 'It's an incredibly clever technology, and we were able to measure norepinephrine and calcium in real time for the first time,' Mie goes on to explain the exact meaning of the results.

What they found was remarkable. Rather than maintaining a constant low level, norepinephrine showed slow oscillations – dips of norepinephrine levels, followed by rises and peaks. These peaks were associated with microarousals – brief moments where the brain behaved as if 'awake'. In contrast to the long-believed hypothesis perpetuated in many studies, norepinephrine was not inactive during sleep after all – it was doing something. 'We have a slow norepinephrine descend, followed by an ascend which results in a microarousal. The more prolonged (and therefore deeper) the drop in norepinephrine, the higher its following peak.' Mie shows me what she means with her hands, drawing waves. 'And these bigger drops also allowed for more EEG activity which shows sleep spindle build up. So, we had a clue what norepinephrine was up to.'

Sleep spindles are short bursts of brain activity (sigma waves) which can be detected by EEG and have previously been shown to play a role in memory consolidation. Could it be that the neurotransmitter responsible for making us alert was somehow linked to memory? Prof Maiken Nedergaard's group tested that.

By artificially inducing prolonged norepinephrine dips in mice which had been given a memory task before sleep, the team was able to determine that mice whose norepinephrine waves had been manipulated, performed better than controls – their memory was improved. In fact, the number of micro-arousals - these small moments of wakefulness caused by norepinephrine - that the animals experienced, were indicative of sleep quality and memory consolidation. 'But only indicative,' Mie adds. 'It has to come from within, it has to be your own brain that wakes you up every once in a while. If it's something external, that's not beneficial. The microarousals that you have during sleep are a sign of a normal sleep function. But we don't know why that is. We don't know why we wake up - we know it's because of norepinephrine - but we don't know why norepinephrine waking us up is important.'

'One speculation has to do with what would happen if norepinephrine does not spike up... If norepinephrine continues to decline instead of oscillating, you go into REM sleep, where we don't see the sleep spindles that correspond to memory consolidation anymore. So, we speculate the microarousals help keep you in non-REM sleep for longer. But that's just a speculation.'

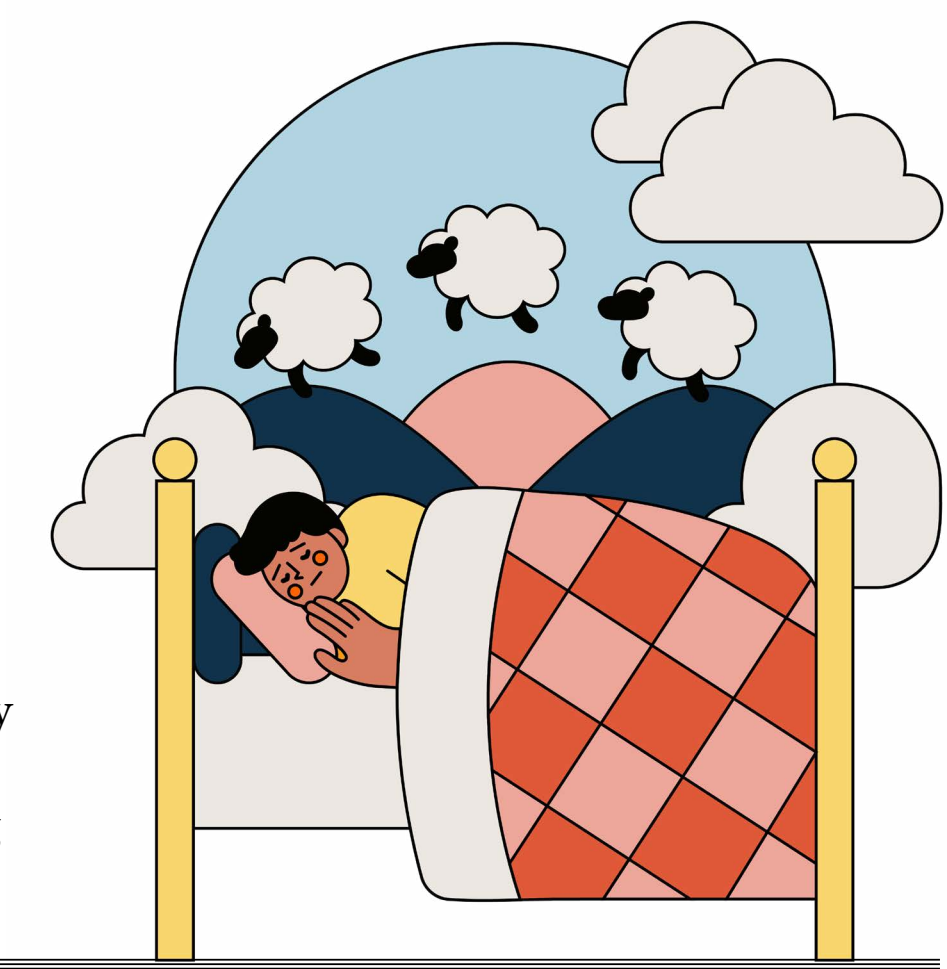
The discovery that norepinephrine is important for memory consolidation can have incredible implications for medicine. A range of drugs, including certain antidepressants, contain norepinephrine reuptake inhibitors – components designed to keep norepinephrine in the tissues for longer – which could impact healthy norepinephrine oscillations and therefore quality of sleep. Conversely, certain drugs can reduce norepinephrine levels to dampen anxiety or promote sleep. Understanding the exact effects of such medications on the norepinephrine's activity could aid clinicians and practitioners in optimising how and when to prescribe such drugs to their patients.

Mie tells me the next steps for her would be something of a return 'to the roots' now that the Nature paper has been completed. To her surprise, in the original study, the phasic bursts observed by locus coeruleus did not reflect matching activity in astrocytes, as Mie would have expected. Instead, the astrocytes were silent. So, she intends to go back and look deeper into what that means – perhaps there would be more insights there to uncover?

Nature Neuroscience paper:

Kjaerby, C., Andersen, M., Hauglund, N., Untiet, V., Dall, C., Sigurdsson, B., Ding, F., Feng, J., Li, Y., Weikop, P., Hirase, H., & Nedergaard, M. (2022). Memory-enhancing properties of sleep depend on the oscillatory amplitude of norepinephrine. Nature Neuroscience 2022 25:8, 25(8), 1059–1070.2022 25:8, 25(8), 1059–1070. https://doi.org/10.1038/s41593-022-01102-9

Mie Andersen
Photo credit: Center for Translational Neuromedicine



Sleeping the Mood Away: Targeted memory reactivation as a new technique for managing emotional responses

Viviana Greco

You have probably experienced first-hand feeling more irritable and short-tempered after a sleepless night. In fact, even a single night of sleep deprivation sets us up to overreact to negative or unpleasant situations. The reason for this lies in the way our brains work.

The brain at a glance: why does sleep impact our mood?

So, what are some of the main actors at play when it comes to emotional regulation? A crucial one is the amygdala. The amygdala is an almond-shaped mass of nuclei, commonly associated with the processing of negative emotional stimuli. This brain structure is strongly connected to the prefrontal cortex, which, as the name suggests, lies at the very front of the brain. The prefrontal cortex is key to decision-making and impulse control, helping us choose the right emotional and behavioural response in a given situation. The integrity of the amygdala-prefrontal cortex connections is essential for adopting appropriate behaviours – but it can also be fragile. A single night of sleep deprivation was shown to disrupt this connectivity, with the amygdala 'taking over' and leaving us to experience and express inappropriate and sometimes intense negative emotions.

While the effects of sleep deprivation on mood are well-known, the specific impact of a 'normal' night of sleep (7-9 hours) on negative emotional experiences has shown mixed results, pointing to either a reduction, preservation or increase of so-called emotional reactivity (the tendency to experience intense, prolonged and frequent emotional responses) after sleep. Emotional reactivity is an interesting phenomenon and to study it, researchers typically first expose participants to images with negative and neutral emotional content, while recording their emotional reactivity through subjective ratings. After a delayed interval where participants are either allowed to sleep in the lab or have to remain awake, they are re-exposed to the same images and their emotional reactivity is recorded again. This method not only allows to differentiate the impact of sleep and wakefulness on the affective tone of emotional responses, but also to examine which specific sleep stages might be involved. The latter is done using a method called polysomnography.

Methods for studying sleep stages and emotions

Polysomnography (PSG) is a combination of electroencephalography (EEG, electrodes attached on the scalp), electromyography (EMG, electrodes placed on the chin) and electrooculography (EOG, electrodes placed below and above the eyes). Polysomnography is also commonly used to diagnose sleep disorders (e.g. narcolepsy or obstructive sleep apnea) by recording brain activity during sleep, including the distinct oscillatory patterns, which are characteristic to each sleep stage. Even though there are disagreements about which sleep stage contributes to changes in emotional reactivity, the majority of the studies point towards Rapid Eye Movement (REM) sleep. Based on these, in 2009, Dr Walker proposed the 'Sleep to remember, sleep to forget' (SRSF) model. The model suggests that REM sleep can provide an 'overnight therapy' for emotional memories where, while the content of the memory is strengthened, the actual emotion is stripped away, making us able to recall the event without being emotionally aroused by the experience.

The relationship between memory and emotion is a fascinating one. One particularly emerging approach for investigating this

> The ‘overnight therapy’ of the SRSF models had worked. This finally confirmed that REM sleep was associated with a reduction of emotional reactivity and was the right candidate for future studies.

relationship is targeted memory reactivation (TMR) – a method that allows researchers to look into the mechanisms of memory reactivation. Awake participants are exposed to learning material paired with sensory cues, such as odours or sounds. The same cues are then re-introduced to the subjects while they sleep. The aim is to trigger memory reactivation in such a way as to selectively improve the quality of certain memories, by using the previously chosen cues.

REM sleep as the key for reducing emotional reactivity

In a 2021 study, Hutchinson and colleagues tested whether reactivation of emotionally arousing memories with TMR was associated with a decrease in emotional reactivity based on specific sleep stages. For this, TMR was carried out during REM versus non-REM sleep using a similar experimental set-up to the one described earlier in this article. Volunteers were split into two groups and asked to rate a set of standardised emotionally negative and neutral picture-sound pairs for emotional arousal. The participants were then ‘wired up’ (fitted with electrodes) for polysomnography recordings throughout the night, and were allowed to sleep. Next, the TMR protocol was delivered: half of the acoustic stimuli previously paired with pictures in the pre-sleep session, were softly replayed during REM sleep in one group, and during non-REM sleep in the other group. In the morning, participants were asked to rate the same picture-sound pairs they were shown before sleep.

REM sleep turned out to be the winner. When TMR was performed during REM, the emotionally-negative picture-sound pairs were rated as less subjectively arousing the next day – the ‘overnight therapy’ of the SRSF models had worked. This finally confirmed that REM sleep was associated with a reduction of emotional reactivity and was the right candidate for future studies.

Future directions in sleep and mood

So, how can we unlock the secret to better managing our mood? The research mentioned above seems to suggest one possible way. TMR, a new, non-invasive technique, could have important implications for anxiety and other emotional disorders. More studies will be needed to optimise the results, but preliminary work with polysomnography and TMR during REM stages of sleep show promise for understanding how to attenuate arousing responses.

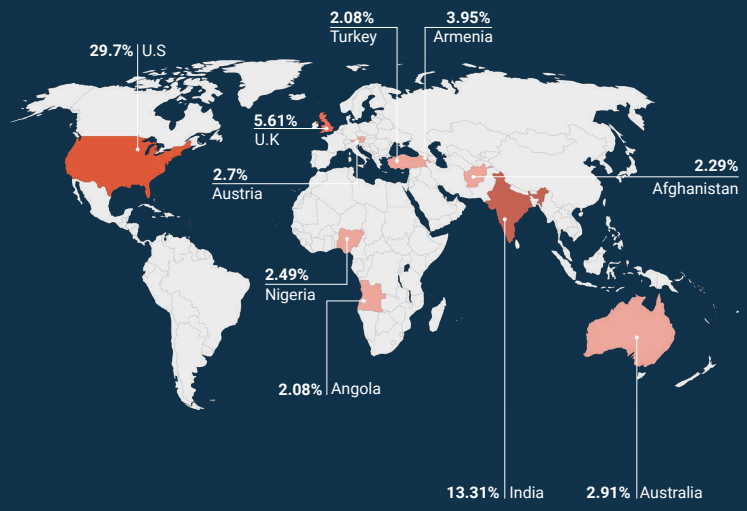
Viviana Greco
Photo credit: Paolo Bono

Key references:

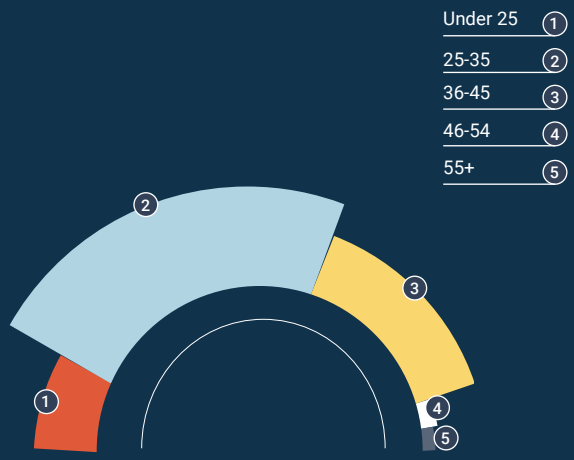
Walker, M. P. (2009). “The Role of Sleep in Cognition and Emotion”. *Annals of the New York Academy of Sciences*, 1156(1), 168–197. https://doi.org/10.1111/J.1749-6632.2009.04416.X

Hutchison, I. C., Pezzoli, S., Tsimpanouli, M. E., Abdellahi, M. E. A., & Lewis, P. A. (2021). “Targeted memory reactivation in REM but not SWS selectively reduces arousal responses”. *Communications Biology* 2021 4:1, 4(1), 1–6. https://doi.org/10.1038/s42003-021-01854-3

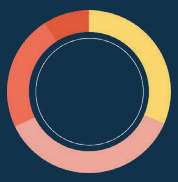
WHICH COUNTRY ARE YOU FROM?



HOW OLD ARE YOU?

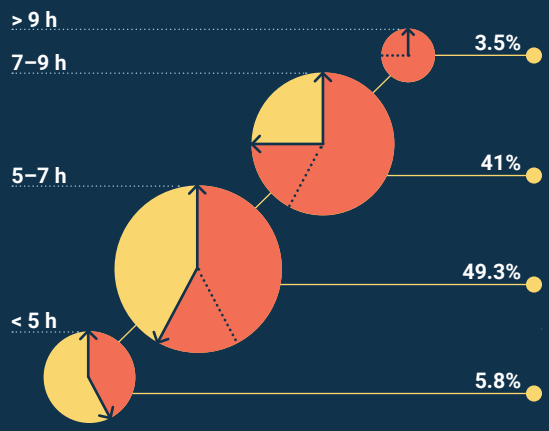


HOW OFTEN DOES IT TAKE YOU MORE THAN 15 MINUTES TO FALL ASLEEP?



31% Never or extremely rarely
37.6% A few times a week
21.4% More often than not
10% Almost every night / every night

ON AVERAGE, HOW MANY HOURS DO YOU SLEEP EACH NIGHT?



HOW MANY HOURS DO YOU NAP PER WEEK?

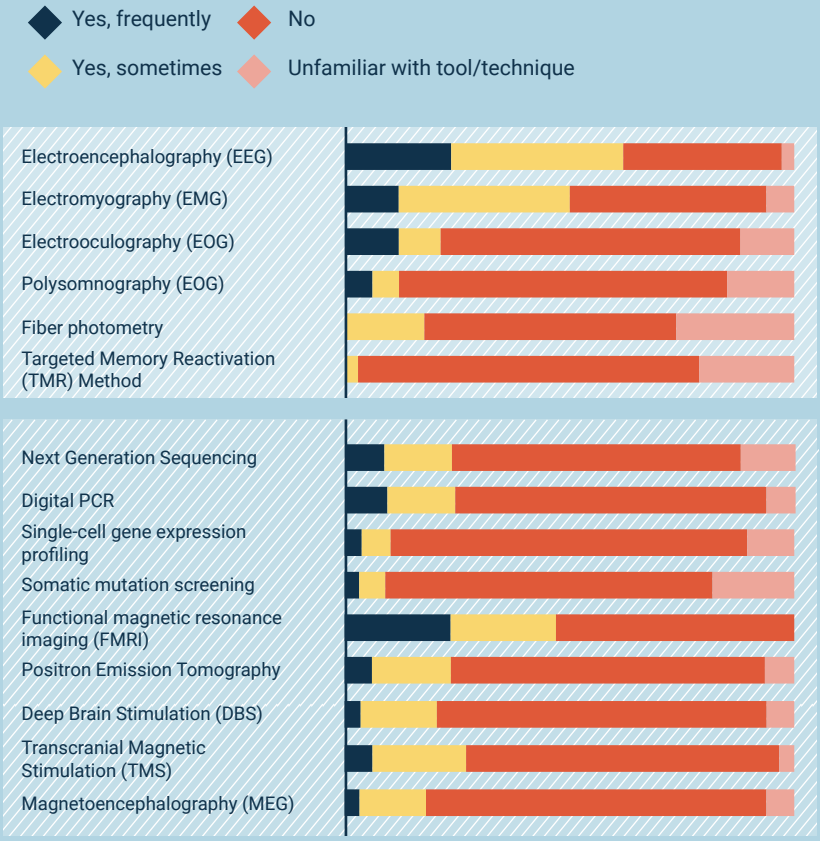
26.8% 0-1 h
27.9% 2-4 h
18.9% 5-7 h
26.4% 7+ h

Sleep in a Nutshell

Sleep is so important. With few exceptions, we're recommended to sleep 7 to 9 hours a night. But how conscientious are we about this advice, what are our reasons, and do we appreciate the potential health repercussions of our actions? A quick glimpse at the sleeping patterns of 481 survey participants quizzed by *Researcher*, uncovered a fair bit about the poor quality of our night routines.

RATE THE FOLLOWING STATEMENTS FROM 'NOT AT ALL' TO 'EXTREMELY'

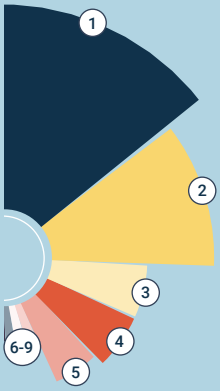
DO YOU KNOW THESE TOOLS/TECHNIQUES?



Insights from Neuroscientists

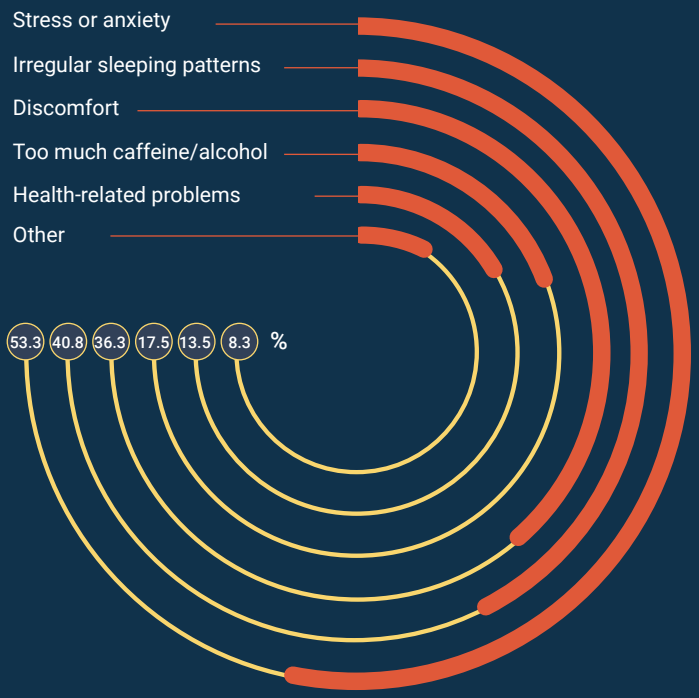
How does the neuroscience academic community overcome the challenges faced by researchers every day? We reached out to 34 academics at a post-doc level or above and asked them about their work and the equipment that helps them succeed. We also wanted to know if they had come across or used any of the tools and techniques that we learned about from the contributors of this report.

WHAT ARE THE GREATEST CHALLENGES YOU FACE AS A RESEARCHER?



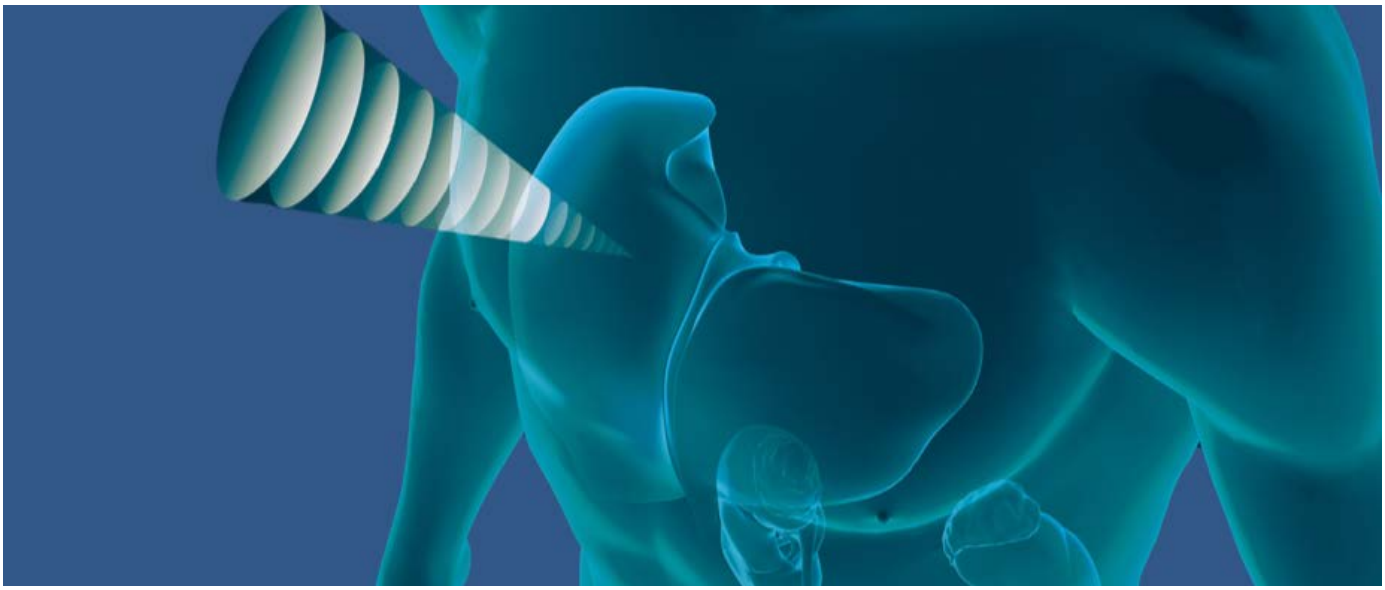
1. **73.5%** Difficulty Finding Or Securing Funding
2. **55.9%** Establishing A Work-Life Balance
3. **29.4%** Difficulty Finding Specialised Researchers In The Field
4. **29.4%** Limited Support
5. **26.5%** Stress
6. **8.8%** Difficulty Accessing Equipment
7. **8.8%** Difficulty Communicating With People Outside Academia
8. **8.8%** Lack Of Motivation
9. **5.9%** Other

WHAT ARE YOUR TOP REASONS FOR HAVING A BAD NIGHT SLEEP?



WHAT ARE THE HOT TOPICS/TRENDS IN NEUROSCIENCE?

1. **AI in Neuroscience**
2. **Gut-brain axis**
3. **Brain implants and nanotechnology**
4. **Mind-body connection**
5. **Sleep and the brain**



Focused Ultrasound Foundation - a New Era of Precision Medicine without Surgery

Dr Kristine Lennie

It sounds like something from a sci-fi film - the idea of a miracle non-invasive treatment that can combat hundreds of devastating diseases: from cancer, to Alzheimer's, and beyond. Unbelievable as it may be though, this technology is already out there - and is promising to revolutionise the way we do (and experience) medicine.

Focused ultrasound (FUS) has existed as a potential for clinical use since the 1950s. It's a method that utilises ultrasonic waves to target cells deep within the human body, with astounding precision. No surgery is required, making the technique safer, faster and less painful, even allowing it to reach tissues previously inaccessible with traditional methods without causing serious collateral damage to vital healthy tissue. In recent years, FUS has shown incredible success in treating patients with Parkinson's, tumours and neuropathic pain.

So, why isn't this technology more widely available?

No matter how promising, an early-stage method of this calibre takes decades to cover the journey from concept to standard of care. Research, approvals, awareness, and bureaucracy at every turn must be overcome to allow FUS to become a commonplace practice - in the meantime, people who need the treatment most would not be able to access it.

> Focused ultrasound today is medicine's best kept secret.

This is what the Focused Ultrasound Foundation (FUSF) is trying to address. Founded by Dr Neal F. Kassell, a former neurosurgeon and co-chair of the neurosurgery department at the University of Virginia, this is an organisation aimed at delivering FUS to patients as fast as possible. By operating several different programmes that fund vital research and educate stakeholders at every level, FUSF strives to deliver treatment to over a million patients per year by 2035. This goal - while ambitious - seems to be on track. From its commencement in 2006, the foundation has assisted in multiple projects to support the growth of FUS, funding first in human clinical trials that ultimately lead to regulatory approvals and working actively towards reimbursement with private payors. The FUSF is rapidly gaining momentum to reach the UK - where its independent sister charity UK Focused Ultrasound Foundation (UKFUSF) has launched its operations this autumn.

By working together with some of the best research institutions in the UK - the Institute of Cancer Research & Royal Marsden, Oxford University, Imperial College London, King's College London and University College London – FUSF and UKFUSF are targeting a range of cancers, including bladder cancer, pancreatic cancer, brain cancer and sarcoma. The foundation's support spans the full spectrum of discovery, from bench research, the development of sophisticated modelling software, small animal cancer research, all the way to clinical trials. Through supporting science, raising awareness and speeding up access to technology, FUSF and UKFUSF are laying the groundwork for better treatment programmes for all British patients.

To learn more about the technology and the foundation, visit the FUSF website where you can find a wealth of useful resources, including what FUS is, how it works, and where you can find treatment sites for a particular condition.

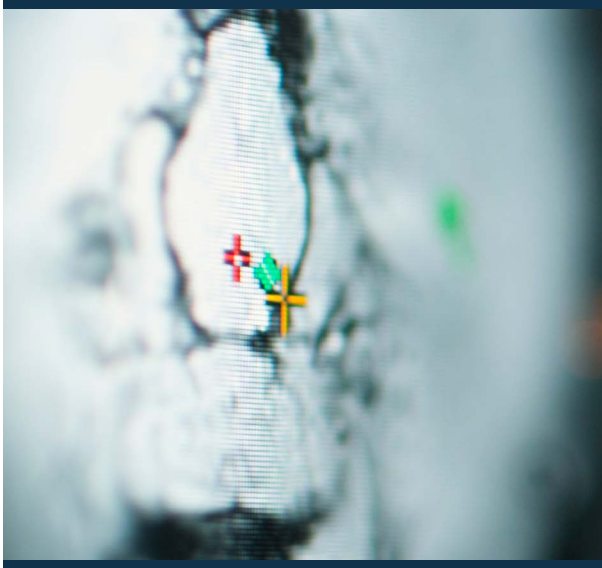
You can also hear about the latest medical research involving focused ultrasound by checking out the 8th International Symposium on Focused Ultrasound hosted by the organisation - the format is hybrid (online and in person).

Key links from this article:

Reference: https://www.fusfoundation.org/ and https://ukfusf.org/ for the UK hub. https://symposium.fusfoundation.org/ for the symposium.

HOW DOES FOCUSED ULTRASOUND WORK?

1. If you imagine a magnifying glass to focus beams of sunlight on a single point to burn a hole in a leaf, focused ultrasound uses an acoustic lens to concentrate multiple intersecting beams of ultrasound on a target deep in the body with extreme precision and accuracy.
2. Where each of the individual beams passes through the tissue, there is no effect. But, at the focal point, the convergence of the multiple beams of focused ultrasound energy results in many important biological effects, creating the possibility of treating a variety of medical disorders.
3. Focused ultrasound treatments can be performed on an outpatient basis, require no incisions, and can result in minimal discomfort and few complications, allowing for rapid recovery.



Credit: UK Focused Ultrasound Foundation

An interview with Prof Clive Bramham on 'Development and validation of Arc nanobodies: new tools for probing Arc dynamics' and function'

Q For the record, please tell us your name, where you work and what you're currently working on.

A My name is Clive Bramham and I'm a Professor of Neuroscience at the University of Bergen in Norway and Head of the Mohn Research Center for the Brain.

Q Could you share what your paper is about?

A My lab works on molecular control of synaptic plasticity. One of the key gene products in brain plasticity - in learning and memory mechanisms and other adaptations that the brain makes - is a protein called Arc (Activity-regulated cytoskeleton-associated protein). Arc is a bit of an enigma, in terms of exactly what it does, exactly how it works at the molecular level. We know that it is critical for memory formation. The animals that don't have the Arc gene can learn but they don't remember. Our paper is about making a new tool for probing Arc function - a nanobody. Nanobodies have fundamentally different properties from antibodies and can be used in different ways.

Q Why is this topic so important and what are the end goals?

A We see Arc as a master regulator of plasticity, because of its multi-functionality. If a connection in your brain gets stronger, Arc is involved, if it gets weaker, Arc is involved, and Arc has other functions as well. So, how do we understand how Arc's function switches? What's controlling it? To do that, we need to get down to the molecular level. The nanobody is a tiny little antibody-like molecule that is ten times smaller [than the antibody] and can bind to a region on Arc. This gives us access to the Arc molecule inside the cell, in the sense that we can use the nanobody to tag the naturally occurring Arc molecule without any genetic modifications or any fluorescent proteins added to the molecule. We can then visualise the behaviour of the Arc protein inside the cell.

When we started out, we injected alpacas with the mutant recombinant Arc protein - this is a dimer. From that, we got these six nanobodies. It turns out that all six of the nanobodies bind to a particular region of the Arc protein that is homologous to the retroviral Gag protein. So, one of the really amazing peculiarities with Arc is that it is able to bind to itself and form a virus-type structure that contains its own RNA, Arc RNA, and is released from cells. Arc's so-called capsid domain has this function and all [of the found] nanobodies bind to that domain. This indicates that they could be used to purify and isolate these Arc capsids and make it possible to see what RNAs are contained within this vehicle of the Arc capsid.

All six nanobodies bind to the capsid domain, but one of them, called H-11, binds to a hydrophobic pocket - a ligand-binding site that is unique to mammals. This was demonstrated in a companion paper with the Petri Kursula lab. In the current paper, we see that nanobody H-11 is able to bind to Arc inside the cell. It's a very strong interaction - so with this particular nanobody we can purify the whole Arc protein. Now, Arc works by binding to other proteins at the same site where H-11 binds, but with H-11 the interaction is much stronger - about 35,000 times stronger than with the naturally occurring compounds. Therefore, H-11 could be useful for function blocking. What we'd like to do now, for instance, is express the nanobody in the neuron and see if it does block Arc function.

> We see Arc as a master regulator of plasticity, because of its multi-functionality

Q **What's the connection between what you're doing right now in this research and actual patient therapies, medical practice and outcomes in the future?**

A There's a lot of interest in the plasticity of the brain. In the context of neurodegenerative disorders, like Alzheimer's disease, in pain states, in drug addiction, recovery from drug addiction, brain trauma and in several of these areas, Arc is implicated.

The thing is that we can't really tackle these problems until we're able to unravel Arc's molecular functions, the different actions of Arc within this cell. It has this multi-functionality and different binding domains, and now we're getting a handle on how these domains are working. Arc has the ability to self-assemble into oligomers – small dimers, tetramers, or much larger structures like capsids that are capable of transmitting RNA between cells. Which one of these self-assembly actions are actually involved in different processes and how exactly? That's what we would like to understand. Nanobodies are a tool for basic research and they are exciting as potentially leading us into therapeutics. Nanobodies are already being used in cancer therapy, and their chemical properties, their stability, their ability to pass through the blood-brain barrier, the fact that you can express them in a neuron, and you could do it in a genetically encoded way, express them in specific cell types and so forth... that's really cool! You can't do that with an antibody, because you have too many working parts, too many pieces!

Q **What's the ultimate goal or dream outcome?**

A We know that there are different types of synapse plasticity - synapses are these small connections between neurons - and they have properties. But what we don't know is how synaptic plasticity is used to alter brain function. We know quite a bit about the firing patterns of neurons because the technology has improved so much that it's possible to record 1000s of neurons in the brain, all at once - for example, when an animal is learning something. But neurons are also quite big, and each neuron may have some 10,000 synapses on them. What we don't understand is that code that's happening at the synaptic level and how changes occur to perform various functions of the brain. We also don't understand how these different types of plasticity change in disease states. I think when we understand these switches on Arc, we'll be in a position to unravel Arc functions in plasticity and brain disorders.

Clive Bramham
Photo credit: Precision Neuroscience Conference

Papers related to this interview:

Ishizuka, Y., Mergiya, T. F., Baldinotti, R., Xu, J., Hallin, E. I., Markússon, S., Kursula, P., & Bramham, C. R. (2022). Development and Validation of Arc Nanobodies: New Tools for Probing Arc Dynamics and Function. Neurochemical Research, 47(9), 2656–2666. https://doi.org/10.1007/s11064-022-03573-5

Other sources: Prof. Clive Bramham's ORCID: 0000-0001-5958-7115

Disclaimer: This is a transcript of a video conversation. You can listen to the recording on Researcher.

Daniel Z. Lieberman: The Molecule of More

Q What do you currently do?

A I'm a psychiatrist, and I've been on the clinical faculty at George Washington University for 26 years.

Q Let's talk about your book 'The Molecule of More'. The topic this book is dopamine. Can you tell us a bit about that and the main story that your book is telling?

A A lot of people have heard of dopamine. They generally think about it as the pleasure molecule. It's the molecule of sex, and drugs, and rock and roll. That's all true, but it doesn't capture the essence of dopamine.

Our brains have developed to have a split between the things that we have, and the things that we do not have. That makes sense from an evolutionary perspective, right? Because if there are things I need, this is going to make a huge difference in whether or not I survive.

The things that we have, we interact with here, in the present moment. The things that we don't have are outside our reach, and so interacting with them is going to be in the future.

The things that we have, are real and tangible. We can see taste, touch, and smell them, meanwhile, we have to imagine the things that we don't have. For example, I might be thinking, 'what am I going to have for lunch today?' Well, that's something that I want, I don't have it, it's in the future, and it's imaginary.

There are a number of brain chemicals that process things in the 'here and now'. However, there's only one brain chemical that processes the things that we don't have, and that's dopamine. There are different dopamine pathways in the brain that do different things, but they are all focused on the future, all on obtaining things that we don't have.

There are two key dopamine pathways in the brain. The dopamine desire pathway makes us want things: it gives us excitement, anticipation, and motivation.

These are all wonderful feelings, but they can also lead us into impulsive behaviours that are not so healthy. There is also another dopamine pathway which involves the more advanced parts of our mind, the prefrontal cortex.

We call that the dopamine control pathway. That's a pathway that looks farther into the future. Sometimes it says 'no' to the dopamine desire pathway. For example, if the dopamine desire pathway says, 'Hey, I would love to have a doughnut', the control pathway says, 'that might be pleasurable now, but in the longer term that's not good for you'. The control pathway makes complicated plans, and it manipulates abstractions: things like mathematics, or abstract concepts like justice. And these two pathways are powerful structures in our brain. In the book, we make the argument that it's because of these dopamine structures, that human beings are so dominant in the animal kingdom.

However, dopamine only works with things that we don't have. As soon as something moves from the possible to the real, the dopamine system is no longer able to process it and it shuts off. So, if we're allowing our lives to be guided by dopaminergic gratification, we can never be satisfied. One example is 'buyer's remorse'. A lot of times, we're so excited when we think about buying something. We may spend hours on the internet researching our options and reading reviews, and then we get the item. Then suddenly, all of that excitement is gone, because dopamine has shut down.

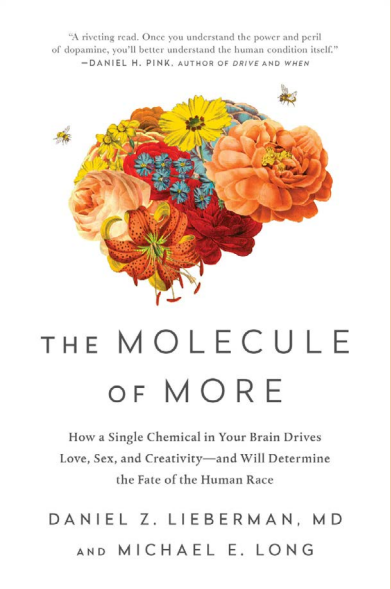
Q So, would you say that there is a very strong correlation between what we understand as fantasy and what we are talking about here as the dopamine pathway?

A Yes, fantasy is one of the ways in which we activate dopamine. Fantasies can be very helpful, they can help us imagine an alternate reality that we might want to make come true. However, fantasy can also be destructive. Such as in an illness called 'maladaptive daydreaming', where people get trapped in their fantasies.

Q Is this an example where the process is malfunctioning a little bit, and not really doing its job but creating problems instead?

A I think that's accurate. Some people are more vulnerable to these kinds of problems: developing maladaptive fantasy disorder or becoming, let's say, a shopaholic, because every time the buzz of buying becomes the boredom of owning, they just buy again. At the same time, I do think that choice plays a role. One of the things that we've heard from people who read the book is that it opened their eyes to what was going on inside their brains, and the ways in which their own circuits were manipulating them.

That enabled [those people] to kind of take a step back and look more objectively and say, well, I have an urge to do this, but I know that that's not really what I want to do.



Q How does the work that you described in the book relates to your everyday job?

A Dopamine is held responsible for many different mental illnesses, that seemed to have nothing in common. Dopamine malfunction is associated with schizophrenia, substance use disorders, and ADHD. What do psychosis, addiction and difficulty concentrating have in common? Now that I have the basis of understanding, it helps me be a more effective clinician.

Q In a clinical sense, how do you think your book benefits the medical practices?

A The book is taking a body of research and putting it together in what we hope is an understandable and entertaining way. I think that the book is most useful to clinicians who are busy taking care of patients, they don't have time to read a dozen journals every single month.

Q What are your current projects now?

A My second book 'Spellbound' is coming on August 23rd. It's about the unconscious mind and its role in people's life and the decisions we make. I approach it from two perspectives - one is neuroscience, and the other is from perspectives of myths, folklore, fairy tales. I also recently started a YouTube channel (DanielZLiebermanMD), where I do short videos about fascinating aspects of brain functioning. I hope people take a moment to check that out.

Q **Is there anything else you'd like to add at the end of this conversation?**

A Dopamine is not the pleasure molecule. It's the molecule of wanting and getting what you don't have and because of that, if you pursue dopaminergic pleasure, you can never experience satisfaction.

Daniel Z Lieberman

Photo credit: https://www.magdalenaphoto.com/

Books:

Molecule of More: https://benbellabooks.com/shop/the-molecule-of-more/

Spellbound: https://benbellabooks.com/shop/spellbound/

References

Access all the references for this issue by scanning the QR code or clicking here.

Contact Us

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Website: https://www.researcher-app.com/

Psst! Our next issue will be on 'AI in Neuroscience', coming up soon, and this time we are including a 'Letter to the Editor' section! With this topic in mind, we are inviting our readers to submit a short letter, telling us about an exciting recent result, finding or fact that you've come across. Drop us a line at the address above!