Why we still don't understand sleep, and why it matters

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One of my first jobs was to keep a lookout for lions. There are some occupations that are not suitable for someone with untreated narcolepsy and this is probably one of them. I was 22, a recent zoology graduate studying meerkats in the Kalahari desert in South Africa. We worked in pairs, one of us on foot, walking with meerkats, the other in the jeep scanning the horizon for signs of leonine danger. On many occasions, I awoke with the imprint of the steering wheel on my forehead, realising that meerkats and colleague had wandered out of sight. I would look for signs of life and, as the panic grew, signs of death. I can tell this story now only because nobody got eaten.

I have not always been like this. For the first 20 years of my life, I had a healthy relationship with sleep. Shortly after my 21st birthday, though, I began to experience symptoms of narcolepsy, a rare but not-so-rare disorder thought to affect around one in 2,500 people. If people know one thing about narcolepsy, it's that it involves frequent bouts of uncontrollable sleepiness. This is true, but the condition is so much more disabling, often accompanied by cataplexy (where a strong emotion causes loss of muscle tone and a ragdoll-like collapse), trippy dreams, sleep paralysis, frightening hallucinations and, paradoxically, fractured night-time sleep. There is no cure. Yet.

In the Kalahari, back in 1995, I was new to these symptoms. I had little sense of the incalculable toll that fighting a never-ending battle against sleep (with defeat the inevitable outcome) would take on mind, body and soul. I was not alone. Few family doctors had heard of the disorder, let

alone encountered a patient. Some neurologists knew what to look for, but many did not. Not even sleep specialists could explain why this disorder would suddenly strike, with peak onset at around 15 years of age.

A lot has changed in 20 years. There is now overwhelming evidence that by far the most common cause of narcolepsy is an autoimmune attack, where the body's immune system mishandles an upper respiratory infection and mistakenly wipes out the estimated 30,000 neurons in the centre of the brain.

In an organ of up to 100 billion cells, this might not sound like too much to worry about. But these are no ordinary cells. They are found in the hypothalamus, a small, evolutionarily ancient and unbelievably important structure that helps regulate many of the body's basic operations, including the daily see-saw between wakefulness and sleep. The cells in question are also the only ones in the brain that express the orexins (also known as hypocretins). This pair of related peptides – short chains of amino acids – were completely unknown at the time of my diagnosis in 1995.

The story of their discovery, beginning in the 1970s, is a brilliant tale of chance and luck, imagination and foresight, risk and rivalry, and involves a colony of narcoleptic Doberman pinschers to boot. It might even be the perfect illustration of how science works.

Yet while there are drugs that can help manage the worst of the symptoms of narcolepsy, none of these comes close to repairing the underlying brain damage. It is remarkable that a lack of two chemicals results in such a bewildering constellation of symptoms. The answer to my problems appears to be simple – I just need to get the orexins (or something similar) back inside my brain. So why am I still waiting?

In April 1972, a toy poodle in Canada produced a litter of four. Eager families were quick to snap up the cute puppies, but one of them, a silver-grey female called Monique, soon developed what her owners described as "drop attacks" when she tried to play. These did not look like sleep; they were mostly partial paralyses: her hind legs would go weak, her bottom would slump to the floor and her eyes would become still and glass-like. At other times, particularly when fed, Monique would be struck by a full-blown attack.

When vets at the University of Saskatchewan observed Monique, they suspected these were bouts of cataplexy, and hence figured this might be a case of narcolepsy with accompanying cataplexy. As luck would have it, Monique's diagnosis coincided with the arrival of a peculiar circular from William Dement, a sleep specialist at Stanford University in California. He was on the lookout for narcoleptic dogs. The Saskatchewan vets wrote back to him immediately. With Monique's owners persuaded to relinquish their pet, all that was needed was to figure out a way to get her to California.

I met Dement, now 89, to find out what he remembers about those early years. He retired several years ago, but still lives in a leafy neighbourhood on the edge of the Stanford campus. His office is a large, shed-like structure attached to the main house and not unlike a Scout hut.

The walls are wood-clad and covered with framed posters, photographs and miscellaneous memorabilia from an illustrious career in sleep medicine. Dement's desk is a picture of organised chaos. Among all this is a water pistol. I ask him why. "It's for when students fall asleep in class," he explains, referring to an incredibly popular lecture series on sleep and dreams he instigated in the early 1970s.

In 1973, Dement approached Western Airlines to see if they could fly

Monique down from Saskatchewan to San Francisco. They had a strict 'no sick dogs' policy. "It's not a sick dog. It's a dog with a brain abnormality," he told them. "It's an animal model of an important illness." Eventually, with some political lobbying, Dement succeeded in persuading the airline to help. Once in San Francisco, Monique quickly became something of a celebrity.

"Monique is very likely to collapse when she's eating something she especially likes, or when she smells a new flower outside, or romps around," Dement's colleague Merrill Mitler told the Associated Press for a story that ran in dozens of newspapers across the USA. "We hope to discover exactly where in the brain the dysfunction occurs that causes narcolepsy," Mitler had told the newspapers soon after Monique's arrival at Stanford. "This could be the first step towards developing a cure."

Mitler is now a forensic examiner based in Washington, DC, specialising in litigation arising from fatigue-related accidents. I ask him if the story of the discovery of narcolepsy is really as good as it appears. "In a word, yes," he says. "In the Seventies, we didn't know what we didn't know about narcolepsy." There is simply no way anyone could have anticipated how profitable the research into Monique and other dogs would turn out to be. The plan at that stage, he admits, was simply to use the animals to test new drugs that might improve treatment of the symptoms and to carry out autopsies in case there were some obvious physical changes to the brain.

Word began to spread, and soon Dement and Mitler were looking after Monique alongside several other narcoleptic dogs, including a Chihuahua–terrier cross, a wire-haired griffon, a Malamute, Labrador retrievers and Doberman pinschers. The fact that narcolepsy appeared to be more common in some breeds than others suggested there could be some kind of genetic basis to the disorder. Then came the breakthrough: a litter of around seven Doberman puppies, all of them with narcolepsy

and cataplexy. "Within 24 hours or less we saw the first of the litter and then the last of the litter all collapse," says Mitler. "There was a large group of us at Stanford and we collectively had our chins on the floor."

It turned out that in Labradors and Dobermans, the disorder was inherited. Dement made the decision to focus on Dobermans and, by the end of the 1970s, he was the proud custodian of a large colony and had established that narcolepsy in this breed was caused by the transmission of a single recessive gene. By the 1980s, methods of genetic analysis had advanced just enough to contemplate an effort to hunt down the defective Doberman gene.

I can never reconstruct the combination of factors that led to the onset of my own narcolepsy, but the stage was set at the moment of my conception in 1972, at around the time of Monique's birth in Saskatchewan. My one-cell self inherited a particular version of a gene (known as HLA-DQB1*0602) that forms part of a set that helps the immune system distinguish friend from foe. HLA-DQB1*0602 is pretty common – around one in four people in Europe boasts a copy – but it plays a key role in many cases of narcolepsy, and is present in 98 per cent of those with narcolepsy and cataplexy.

On top of this genetic background, there may have been some bad timing too. People with narcolepsy are slightly but significantly more likely to be born in March (as, indeed, I was). This so-called 'birth effect' is seen in other autoimmune disorders and is probably explained by a seasonally variable infection at a particular moment in development. In the case of narcolepsy, it seems that those of us born in March are just a little bit more vulnerable than others.

While other infections during my childhood, hormonal fluctuations and emotional stress may also have played a part, it was in late 1993 that I probably encountered a key pathogen – an influenza virus or

Streptococcus perhaps. It was this that took me to an autoimmune tipping point and resulted in the rapid dismantling of my orexin system. In short, most cases of narcolepsy are probably the result of an unfortunate combination of events that create the perfect immunological storm.

Around this time, the Doberman project in Stanford was on the verge of unravelling the genetic basis of narcolepsy in this breed. The man tasked with hunting down the mutation responsible was Emmanuel Mignot, who subsequently succeeded Dement as director of the Stanford Center for Sleep Sciences and Medicine. We meet in his office there, joined by Watson, a narcoleptic Chihuahua he adopted a few years ago. "It's such a silly breed," he says, holding down Watson's ears to prevent them from burning, then setting him on the floor. "Not one I would ever have chosen myself."

At first, Watson is wary of me, keeping his distance and growling. When I get down to his eye level, he yaps and jumps in at me, then out, pretending he is fiercer than he is. I can empathise, even across the gulf that separates his species from mine. I know about the excessive daytime sleepiness. I know about the cataplexy, how it feels to have emotions short a neurological circuit in the brainstem and cause a muscular collapse (just as occurs in the rapid eye movement, or REM, stage of sleep, when most dreaming takes place). I wonder if Watson suffers the total terror of sleep paralysis and the supernatural hallucinations that often accompany it.

As he looks back at me, his eyelids close and open with a dullness I recognise. He turns, daintily steps into his basket and curls up for the rest of the interview.

Back in the 1980s, the idea of locating the gene for canine narcolepsy was off-the-scale ambitious. Breeding narcoleptic Dobermans is harder

than it sounds, as the afflicted tend to topple over mid-coitus, temporarily paralysed by a cataplectic thrill (a so-called 'orgasmolepsy' that can occur in humans too). This impracticality aside, there was also the task of locating a gene whose sequence was not known, in a genome that was, at the time, a no man's land. "Most people said I was crazy," says Mignot. In a sense, they were right, because it took him more than a decade, hundreds of dogs and over $1 million. And he was nearly beaten to it.

In January 1998, after more than a decade of painstaking mapping, and just as Mignot's team was closing in on the gene, a young neuroscientist called Luis de Lecea at the Scripps Research Institute, San Diego, and colleagues published a paper describing two novel brain peptides. They gave them the name 'hypocretins' – an elision of hypothalamus (where they were found) and secretin (a gut hormone with a similar structure). They appeared to be chemical messengers acting exclusively inside the brain.

Just weeks later, a team led by Masashi Yanagisawa at the University of Texas independently described the exact same peptides, though called them 'orexins' and added the structure of their receptors into the bargain. They speculated that the interaction of these proteins with their receptors might have something to do with regulating feeding behaviour. "We didn't even think about sleep at all," admits Yanagisawa, now director of the International Institute for Integrative Sleep Medicine at the University of Tsukuba in Japan.

Back at Stanford, Mignot heard about the two papers, but there was no reason to imagine this new pathway had anything to do with narcolepsy or sleep. By the spring of 1999, however, he and his team had worked out that the recessive mutation had to lie in one of two genes. One was expressed in the foreskin. "It didn't look like a candidate for narcolepsy," says Mignot. The smart money was on the other gene, which encoded

one of the two orexin receptors. When he got wind that Yanagisawa had engineered a mouse lacking orexins that slept in a manner characteristic of narcolepsy, the race was on.

Within weeks, Mignot and his team had submitted a paper to the journal *Cell*, revealing a defect in the gene encoding one of the orexin receptors. "This result identifies hypocretins [orexins] as major sleep-modulating neurotransmitters and opens novel potential therapeutic approaches for narcoleptic patients," they wrote. Kahlua – one of a litter of Dobermans all named after alcoholic beverages – lay sprawled across the cover of the issue. Yanagisawa and colleagues added their experimental evidence to the mix just two weeks later, also in *Cell*.

Under normal circumstances, a chemical messenger and its receptor work a lot like a key and lock. A key (the messenger) fits into a lock (its receptor) to open a door (cause a change within the target cell). In the case of Mignot's Dobermans, a massive mutation had effectively jammed the lock of the orexin receptor, rendering the orexin useless.

Whether it's the lock that doesn't work, as in this case, or that the keys are missing, as they were in Yanagisawa's mice, the upshot is the same. The door won't open. The orexin system is broken. In human narcolepsy, there are many ways to break the orexin system. Occasionally, a brain tumour or head trauma is sufficient to do the damage. In most cases, however, narcolepsy is caused by the series of unfortunate events outlined above.

The orexin neurons are a very big deal, and not just for those like me who've lost them. Present in every major class of vertebrate, they have to be doing something seriously important. When de Lecea first described the orexins in 1998, he was in his mid-20s and had only recently moved from Barcelona in Spain to San Diego. In 2006, he made the move from there to Stanford to be closer to the sleep action. "To be honest, I

thought we'd understand the system much better at this point than we actually do," he says.

But we have found out a lot, particularly thanks to optogenetics, a technique de Lecea helped pioneer. By deploying a virus, a promoter and a gene found in blue-green algae, it is possible to render a particular population of neurons sensitive to light.

To illustrate this wizardry, de Lecea brings up a video on his laptop. There is a mouse in a cage that has been engineered so its orexin neurons will fire in response to light. There is a thin fibre-optic cable running into its brain. "The mouse is asleep," he says, waves of electrical activity characteristic of deep sleep spooling across an inset video at the top of the screen. The optic cable comes alive, a pulse of bluish light flashing for precisely ten seconds. The light-sensitive orexin neurons release their neuropeptides and, all of a sudden, the mouse wakes up. When the light goes off, it falls asleep as rapidly as it awoke.

There can be few more striking illustrations of the power of the orexins than this. Completely unexpectedly, I feel my tear ducts tingling and for a split-second I almost envy the mouse.

Using optogenetics and other methods, de Lecea has been able to show that the orexins have a powerful effect on many important neurological networks. In some settings, they act like neurotransmitters, crossing gaps in neurons to activate target neurons that release a chemical called norepinephrine throughout the brain's cortex.

In other settings, the orexins act more like hormones, working further afield in the brain. This is how orexins influence other brain chemicals, including dopamine (essential for the processing of reward, in planning and for motivation), serotonin (strongly associated with mood and implicated in depression) and histamine (an important alerting signal).

"In most other neural networks, there are parallel and multiple layers of security," says de Lecea, so if something isn't working properly, there are systems that can step in and pick up the slack. In the case of the orexins, however, there appears to be little or no backup at all. So, manipulating this system produces the kind of clear-cut response that scientists can work with. "It is a brilliant model for understanding neural networks more generally," says de Lecea.

What we now know about orexins also helps explain why losing just a few tens of thousands of cells should result in a disabling, multi-symptomatic disorder like narcolepsy – something that messes with wakefulness and sleep, body temperature, metabolism, feeding, motivation and mood. These proteins are giving us a privileged insight into how the human brain does what it does.

All this makes the orexin story sound like the archetypal double helix-like tale of scientific discovery, the perfect illustration of how science works. There's an underlying puzzle (narcolepsy), an origin story (Monique), foresight (Dement), ambition (Mignot), technological developments (genetics), a photogenic animal (Dobermans), a race (with Yanagisawa), it looks like science (optogenetics) and there's a still-higher purpose (sleep and the brain).

It is elements like these that can transform everyday scientific events into a compelling cultural narrative, says Stephen Casper, a historian of neurology at Clarkson University in New York. "It has all the ingredients of something that I think physiologists and neurologists in the early part of the 20th century were looking for and hoping they would find, something that would bring together heredity, biochemistry, biophysics, neurology and psychology."

But there is a pattern in biomedical research of niche disorders opening up promising avenues of research that never end up helping the patients

themselves, Casper adds. The narrative around narcolepsy has something missing, he says: "A good story should have a clear happy ending."

We are still waiting for that happy ending. Even if I could get my hands on a vial of orexin-A or orexin-B, how would it get into my brain? Swallowed in solution, the enzymes in my gut would make short shrift of it, plucking off the amino acids like beads off a necklace. Injected into muscle or the bloodstream, not enough would make it through the blood–brain barrier. There have been some experiments on a nasal delivery, suggesting that sniffing orexins may be a way to smuggle some of them into the hypothalamus via the olfactory nerve, but there has been relatively little investment in this approach.

This does not mean that the pharmaceutical industry has ignored the discovery of the orexin pathway. Far from it. Within just 15 years of the Cell publication by Mignot and colleagues that linked a loss of orexin to narcolepsy, Merck had received US Food and Drug Administration (FDA) approval for suvorexant (or Belsomra as it's known in the trade), a small molecule capable of getting through the blood–brain barrier and blocking orexin receptors.

A drug that promoted sleepiness was not the application that most people with narcolepsy were looking for. By preventing the orexins from binding to their receptors, Belsomra effectively creates an acute case of narcolepsy, but where the fog, ideally, will have started to lift by the morning.

Sleeping pills commonly used to treat insomnia tend to work by depressing the central nervous system as a whole, says Paul Coleman, a medicinal chemist who works at Merck's laboratories at West Point, Philadelphia, and who was instrumental in the development of Belsomra. "What's so exciting about Belsomra is that it is very selective for blocking wakefulness, so it doesn't affect the systems that control

balance, memory and cognition," he says.

In his career, Coleman has developed drugs to treat a range of different infections, illnesses and disorders, but the orexin system stands out. "Narcolepsy has given us a thread we can pull on to unravel a lot about what underlies the systems that govern wakefulness and sleep," he says.

"Wakefulness is a pretty central process for everybody, whether you are a healthy person or have narcolepsy or insomnia. It's the most exciting thing I've had a chance to work on." The applications of Belsomra may be wider still, with clinical trials proposed to investigate its potential to help shift workers sleep during the hours of daylight, improve the sleep of Alzheimer's patients, help those suffering from post-traumatic stress disorder, combat drug addiction and ease human panic disorder.

I am delighted to see these developments, but the millions of us with narcolepsy are still hoping for a drug that could work in the brain to rouse rather than silence the orexin system.

This has been a long-term project for Masashi Yanagisawa, who was in the race with Mignot to link the orexins with narcolepsy 20 years ago. But designing and synthesising a compound that will make it through the gut intact, that has what it takes to find its way from blood to brain, and that boasts the perfect configuration to activate one or both of the orexin receptors is "a very, very high challenge" he says, one that is "significantly" greater than finding a compound to interfere with the receptor as Belsomra does.

Earlier this year, Yanagisawa and his colleagues published data on the most potent such compound to date, a small molecule called YNT-185. Injections of this molecule into narcoleptic mice significantly improves their wakefulness and cataplexy and reduces the abundance of the REM stage of sleep in which most dreaming occurs (one of the characteristics

of narcolepsy). This, says Yanagisawa, is a "proof of concept". Although the affinity of YNT-185 (how strongly it binds to the orexin receptor) is not great enough to warrant a clinical trial, Yanagisawa's team has already hit upon several other potential candidates. "The best one is almost 1,000 times stronger than YNT-185," he says.

While the symptoms of narcolepsy can vary wildly from one person to the next, the underlying pathology – the absence of orexins – is still the same. "If this compound works, it'll work for all those patients," he says. "In that sense, it's a relatively simple clinical trial compared to many other disorders."

A still more futuristic avenue involves stem cells. Sergiu Paşca has the office next to Emmanuel Mignot at Stanford and in 2015, he and his colleagues developed a way to take induced pluripotent stem cells (fashioned from skin cells) and direct them towards a new life as brain cells. "You can use this system to derive various brain regions and like a Lego game, assemble them to form circuits in a dish," he says.

Recently, his lab has developed methods to do something similar for people with narcolepsy, starting with a skin cell and ending up with a fully functional orexin neuron. In theory, it should be possible to transplant this into the brains of people with narcolepsy and restore some of the function. This is, however, not something to be taken lightly. For a start, the cells themselves are unlikely to be exactly the same as orexin cells, inserting a needle into the brain is not a risk-free exercise, and there's always the possibility that the immune system might make another assault on the transplanted cells.

So, will the tale of the orexins really have a happy ending? The translation of basic research into the clinic is notoriously difficult and expensive, says Casper. (The cost of the current best available treatment for narcolepsy – sodium oxybate, or Xyrem – is such that it is not

routinely available for adults in England, even though it could transform the lives of many.)

There is a widespread perception that narcolepsy is a rare disorder with a small market, so any pharmaceutical research and development in this area would be unlikely to reap a significant return. This ignores the fact that narcolepsy is probably undiagnosed in many people, and that someone who develops narcolepsy in their teens and lives into their 80s would need some 25,000 doses over their lifetime.

Even more compellingly perhaps, the orchestrating role that the orexins play in the brain suggests the market for such a drug would go far beyond narcolepsy. Something that tickled up the orexins would be useful for any condition where excessive daytime sleepiness is an issue, not to mention the myriad other situations where low levels of these messengers may play a role, including obesity, depression, post-traumatic stress disorder and dementia.

There is, I believe, one other reason why this story has not yet reached its conclusion. For too long, sleep has been undervalued, seen as an inconvenient distraction from wakefulness. With this mindset, research into the neuroscience of sleep does not seem like it should be a priority. Nothing could be further from the truth. There is now abundant evidence that poor sleep can have devastating consequences for physical, mental and psychological health. Sleep is not incidental. It is fundamental, a matter of serious public health. Investing in sleep research is not just about the few with demonstrable sleep disorders. It is about everyone.

More information: The hypocretins: Hypothalamus-specific peptides with neuroexcitatory activity *PNAS*. 1998 Jan 6; 95(1): 322–327. www.ncbi.nlm.nih.gov/pmc/articles/PMC18213/

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