Dr Raffaele Sarnataro on snoozing fruitflies and cruising through PhDs

Summary

In this episode with Dr Sarnataro, we explore techniques used during his recent PhD to investigate the mitochondrial dynamics in neurons of sleeping fruitflies. Raffaele also offers advice on how to make the most of an Oxford PhD position.

SUMMARY KEYWORDS

sleep, neurons, animals, drosophila, behavior, control, eureka moments, mitochondria, flies, people, neuroscience, studies, readout, lab, science, imaging, understanding, experiences

Hello, it's Ritika, Katy and Neddy, and you're listening to the CortexCast.

Ritika

In today's episode, we dive deep into the world of sleep. We speak to Dr. Raffaele Sarnataro, a postdoctoral researcher, whose very recent DPhil or PhD research focused on the mitochondrial dynamics in sleep active neurons in fruitflies. Thank you for joining us today!

Raffaele

So thanks a lot for asking me to participate in this episode. I'm Raffaele Sarnataro, a postdoctoral research scientist here at the University of Oxford in the lab of Dr Gero Meisenbock. I've completed my DPhil in Neuroscience in the same lab a few months ago. I'm Italian and I was educated at the University of Pisa in Scuola Normale Superiore, where I got my Bachelor of Science in Biotechnology and Master of Science in Molecular and Cell Biology. So I'm a molecular biologist by background. Then I did some experiences abroad, I was for some time at the Harvard Medical School, I did research there, also in Molecular Biology. And then I moved to Neuroscience. So I came to Oxford, where I did my MSc in Neuroscience. The programme continues with the DPhil in Neuroscience. And here I am!

Ritika

That's fantastic. So you've been an Oxford as a Master's student, a DPhil student, or in other words, a PhD student, and now as a postdoc. How's the journey been for you at each stage?

Raffaele

It has been really fun. Of course there's been COVID in between - so very peculiar, also, for this reason. Of course, Oxford is amazing, because I mean, it's a place that is ready for international people to come. And is a very welcoming environment, there is all the infrastructure ready for integrating a new comer into the community. There's colleges - especially if you're a student, colleges are super welcoming. So I really made lots of friends in my first years in Oxford, then, of course, I mean, starting from a master in neuroscience to a DPhil, and then a postdoc, these are very different positions. But I felt that the biggest transition was within each of these stages in a way. Because really, in the Master's, for example, you start from a point where it's mostly courses, and then you switch to doing more research, and your daily life really changes. If you're already doing research during your Master's - and then you continue you're your

DPhil – it is basically the continuation of the same style. And during the DPhil itself, I felt there was, you know, more maturity that comes over time and experience in seniority. So that to a point where when you transition to becoming a postdoc, there's not really any difference anymore, but I'm very different now to what I was five years ago, so I felt most of the transitions are actually within each of these stages.

Ritika

I see. And in this journey, how did you come to study sleep?

Raffaele

So yeah, I find it a super interesting topic, because first of all, it is a basic behavioural state that all the animals display, in a way or another. And I find it interesting just because understanding a basic behavior, is interesting by itself, and why such a basic behavior has been kept by evolution – I think is one of the biggest mysteries that life science have. We all know that when one night we are sleep deprived, then on the following day, we work very poorly. So understanding, first of all, why we need to sleep and what's the essential function that sleep has, it's still unknown in the scientific community. There are many theories, but still a lot needs to be done and proven. And the way we, in our lab, we approach the problem is to understand how sleep is controlled. And we believe that understanding how sleep is controlled will tell us more about what are the essential variables that are linked to the function of sleep. We know that there are centers and neurons in the brain that have a key control over the implementation of sleep, our lab has looked at this under many different angles. The way I approach the problem is looking both at what's happening within these neurons in terms of the molecular machinery that is engaged by sleep loss in these neurons and how they respond in a cell autonomous way to loss of sleep, sleep deprivation and different states of deprivation in a way. And also what are the circuit dynamics of these neurons? And how do they receive inputs and outputs? So that's in broad terms, and I think that we had some interesting findings in both ways. Definitely I can say that rearrangement in the mitochondrial machinery is something that happens in these neurons depending on the sleep pressure again, and these neurons have very interesting network dynamics and there are different modes through which these dynamics can transmit information about sleep need to the whole brain. And I think the reason why I feel understanding sleep is interesting is not only because it's, it's a big mystery, but also because more and more in the population, people are suffering of sleep problems. I mean there are different reports and the sleep problems is a broad term, but up to half of the world population experiences some sort of sleep problems, which have consequences, in almost virtually any pathology. And yeah, so I think understanding how sleep is controlled will help us understanding how we can control sleep, and improve it and fix it when needed.

Ritika

Certainly, what I also find super fascinating is how there is such a diversity in nature in terms of how different animals in different environments sleep, isn't it?

Raffaele

Yeah, as we said, sleep is, is universal, but it varies a lot between species: the amount of sleep, the distribution of sleep within one day and also over a month, we know that it's very different. We know that the animals completely forgo sleep for months when they are migrating for example, or when they're looking for partners or when they're hungry. So that's extremely interesting. There are animals

that display different kinds of camouflages when they're sleeping. People say it's, you know, cuttlefish or, or the octopus. Some people say that's, you know, somehow there it's a reflection of their dreaming. But again, that's very interesting, because we know that when we are in REM sleep, which is the phase where we dream the most, our body is paralyzed. And whether this is true or not for all the animal species, it's very interesting. Also, why do we dream in the first place? Is it that because we need to go through the experiences of the day, in a way or another, and having our body paralyzed, is a way for us to avoid moving in response to those stimuli that we perceive, for example. Whether this is conserved or not in other species, I feel it's something unknown. And it will be interesting to know, and this will tell us about, for example, the nature of the essential need for sleep, whether, for example, replaying the experience that we had in a day, in one way or another is a fundamental function of sleep, or is one of the many accessory functions of sleep that came afterwards. So yeah, I think there is value in studying sleeping many different animals. There are animals that have unihemispherical sleep; there are animals that, for example, jellyfish for example have different ways of pulsating, when they are asleep or not. So I think it's such a beautiful behavior, but there's so many ways of manifesting that, that understanding what it's in common across all of them will be, I think, extremely powerful.

Ritika

And therefore, we still do not know what sleep is.

Raffaele

Yeah, yeah. Well, we know what sleep does we – know a lot about what it does. Definitely, definitely. And also, the definition of sleep is also very interesting. Yeah!

Ritika

It's so multifaceted. And I feel like sometimes we forget to acknowledge that, because we, as scientists looking at one particular readout, get fixated on just that. It's, it's a qualm of science, we can't do anything about it. But it's very important to acknowledge that there are several other things that happen, could happen, and lead to, and result from sleep.

Raffaele

Exactly! Some behavioral readouts might be a specific adaptation of a specific ecological niche. And they are very solid within those animals, but may not be, you know, the common ancestor feature of asleep, or one of them.

Ritika

True, and also in those terms, the results we find in our laboratory conditions may actually be very different from what's happening in nature. What are your views on that?

Raffaele

Yeah, that's absolutely true. I mean, the animals that we use in the labs, maybe rats, flies or any other animal, are absolutely different from the wild animals. And of course, I mean, even just the fact that we keep animals in a very fixed light:dark cycle is absolutely different from what these animals will experience in wildlife. And the same is true for the availability of food. And even just those two things are two parameters that we know affect sleep a lot. So I think more and more there are attempts and

many studies that they look at sleep in animals when they are in their normal ecological niches. And I think this is definitely one of the ways forward for understanding sleep better. Of course, it's difficult on the technical point of view, and variation in the data is definitely higher, but I think there is definitely value in that – so in more naturalistic settings.

Ritika

Fantastic. Now moving on, could we talk a little bit about how you approached and arrived at the idea of looking at mitochondrial dynamics during sleep?

Raffaele

That's a very interesting question. Thank you. Multiple things actually, I started with an unbiased approach – so I did some transcriptomics on these neurons, and mitochondrial genes popped up. And this combined with the fact that our lab previously showed already that mitochondria, and reactive oxygen species produced in the mitochondria of the sleep controlling neurons involved in the control of sleep, brought me to study this even more. I think one of the cool things about looking at mitochondria in our system is that we can isolate neurons that are responsible for controlling (sleep) behavior, which is a homeostatic behavior and it's something to do with bio-energy utilization – the consumption of energy and the uptake of calories differs between when we are awake and when we are asleep. But I think just looking broadly at the whole brain isn't enough, because there are many processes that are going on while I was sleeping. And probably one of the keys is to be able to isolate neurons that are responsible for given homeostatic behavior that depends on the bio-energetical state of the animal, and looking only in those neurons at the mitochondria are involved. I think this was what at least for me, I think it's one of the was one of the keys that brought me to finding something interesting into the mitochondria of these neurons. And of course, this is combined with the fact that we have now you know, the ability to look through amazing imaging resources, have technologies like single cell sequencing that help us finding interesting results in unbiased ways. And also, working with a system of *Drosophila* helps because we can isolate neurons in a very specific way in a genetically targeted fashion. So I think the combination of these, it's, it's quite powerful.

Ritika

That's very interesting. Your model organism for this bio-energetics oriented neuronal exploration was the marvelous fruitfly. Now, in my limited reading of fly literature in the context of sleep, I've come across the recording argument that using reduction in locomotion as the sole readout of sleep, may not be completely reliable. What are your thoughts on this? And could you maybe tell us a bit about how sleep in flies is scientifically validated in recent research?

Raffaele

And it is a question I get asked a lot about, first of all, whether *Drosophila* sleep at all - actually, if they have a brain - that's always the first question. And they have they do have a brain like 10 to the 5th neurons more or less, and they have complex behaviors. So I think understanding any behavior in behaving animal is a big challenge. So I think starting with sleep, I think already we know a lot about sexual behavior, for example, and memory from studies that come from flies and sleep is just another behavior. And as I said that I think there is value in understanding any behavior decomposing any behavior in its components in any animal and with *Drosophila*, we have the advantage of having, you

know, almost 100 years of genetics done on those and toolkits of any sort. So definitely there is value in studying flies. There is a famous quote from Seymour Benzer, which is one of the fathers of behavioural neurogenetics in flies, to Francis Crick, who said – “do not underestimate flies, they can do more than you can do. For example, can you fly away and land upside down on the ceiling?” So they definitely do things that we can’t. But yeah, I think the general understanding sleep is controlled by two processes. The circadian clock and the sleep homeostat. And we know that the studies done in *Drosophila*, on the circadian clock recently have been incredibly valuable. The Nobel Prize in 2017 was awarded exactly on this. And so it's natural to study also the other components of the controllers of sleep in the same animal. It’s true that the way we define sleep in flies is maybe rudimental, is only by locomotion. But of course, I mean, it would be surprising if *Drosophila* wasn't sleeping, it will be the only animal that doesn't sleep. So I think that there is clearly room for improvement in the definition the operative definition of sleeping flies, but as this is not the only thing that happens: there are electrical signatures of sleep, broadly in the whole brain and in specific neurons in Drosophila. And I think the big question, the big challenge is really to find good ways to increase the throughput of recording these in flies. We now do head fixed flies, and we can’t do freely moving animals. So this really much limits to throughput of using electrical signatures for defining sleep in this animal. So I think this is something that can be improved and technology will bring us there. But there is definitely a great advantage in using different models for studying sleep. What are the components, how sleep need is accumulated, integrated, what are the thresholds, how the threshold for going to sleep are set in a given animal – I think that will be valuable, and there's chances that this can apply to other animals. And even if was this was not that they will be interesting a set of discoveries even just understand how such an important behavior is implemented in the brain of any animal.

Ritika

That was fantastic.

Raffaele

Did that convince you?

Ritika

Yeah! Thank you! Sure. It's really cool that now we can do so much with an organism that is so tiny. Could you maybe talk a bit about the amazing technology that makes all of this possible?

Raffaele

Yeah, so I've been lucky enough, because in my PhD, I could use very different techniques, which is something that is possible in our lab, and I'm very grateful for having done very different experiments during my PhD. Just to list some of the techniques I've used there, there is a single cell transcriptomics, transgenic animals, behavioural studies, molecular biology, imaging – confocal imaging and two photon imaging. Definitely single cell transcriptomics is one is a relatively new technique. So transcriptomics is just a way to study the whole content, RNA content of a cell or tissue, or a given sample; and single cell is a new embodiment of this technology. You can look at the content of RNA in each cell of your sample, which is powerful and has been generating a lot of data and hypothesis in any field in life science, really. Nowadays, there's also spatial transcriptomics available, which is even more powerful, because it not only gives you a readout per se, but also keeps the anatomy of your sample. So you can also have some

spatial information, which is powerful; I think it's really the future. Transgenic animals – definitely, we can manipulate animals by inserting genes, and making them express in different tissues different times, for example, and you can generate animals like that. And with *Drosophila*, this is something that is generally routine, there are big repositories around the world of transgenic animals, we made some ourselves for example, we use some that other people made. And that's a very useful way to manipulate, really the molecular machinery of a given tissue. And confocal microscopy is really now the gold standard for looking at tissues. Brain tissues, for example, and I think gives a very high like, it's a good compromise of a high throughput technology, but also a decent signal to noise ratio and resolution and clarity of the image. And I've done some two-photon imaging. It's basically a technology for allowing deeper penetration of photons into a tissue. So this way you can image deeper down a brain, for example. And since you can image deeper down in the tissue, you can also keep the animal alive and look at what's happening within the brain of the animal while the animal is behaving, for example. And it's a very useful technique. Now, it's almost standard in any big neuroscience lab. This really allows us to perform experiments that can really correlate, within the same animal, with these within the same individual behavior and manipulation of all different sorts to an accurate fashion. And I think it's one of the technologies that I mean, people should really start learning about when they are in high school.

We can use light in neuroscience, both for imaging, so for getting a readout from our system, and for controlling our system. So that's something that my supervisor, Gero Meisenbock, worked a lot on. So he was the first one to develop the technology of optogenetics, which is now again, standard almost in any any big neuroscience project in a way. What does optogenetics mean? In a simple way, it's a way to control a molecule with light. The most used kind of optogenetics are ion channels that are in the membrane of neurons that can be switched with light. We know ion channels allow changing the voltage of neurons in a way that drives their activity. And this way, we can control the electrical activity of neurons using light. And when this was explained to me in university, this was extremely fascinating. And so this was applied there in the paper of Tonegawa, where they implanted the visual memories using optogenetics. And at the same time the concept of optogenetics was introduced to me. And I find this extremely elegant, because these are proteins that are from algae or bacteria. And you're implanting them into another animal, and using them to control behavior. So it's really using biology to understand the biology. So it's extremely elegant and open so many ways in understanding how even a behavioral or cognitive function is controlled. So that lecture was one of the major drivers for me to switch from cell and molecular biology to neuroscience in a way. So I find it very fascinating. And the other way round is also very interesting, because you can collect photons from a sample, for example expressing a fluorescent protein, either a green fluorescent protein, or any modification of it that can also change intensity, depending on for example, the activity of the neuron. And you can get a lot of information from that and two-photon imaging or confocal imaging or just wait to collect photons. So we are really now in a stage in neuroscience, where it's possible to write and read from a neuronal sample using very little invasive techniques.

Ritika

Okay, great. Thank you so much. I never thought someone could explain these things so easily. But that was fantastic. Technology allows us to look at a problem through so many different lenses. This makes me think, did you have any particularly special or eureka moments during this whole DPhil journey?

Raffaele

Yeah, I mean, I'm still at the beginning of my academic career, hopefully. But there have been a couple of eureka moments, if you will. I mean, when I when we talk about these eureka moments, I always remember a lecture from a professor in my university in Pisa.So this was a lecture towards the end of his academic career, and he said that there are generally few eureka moments that you can count on the fingers of one hand, maybe, but they are totally worth the journey. And me, of course, no, I've had some little ones. For example, the first time I observed mitochondria of neurons in sleep deprived animals, in my units of interest – I saw them, they were quite different from what they used to see. Or, for example, when we were predicting some neurons to have some strong connection, or a different or a specific kind. And then thanks to the *Drosophila* connectome being available, we looked into it. And then in ours, we found that exactly the prediction that we had on the connectome, which was extremely cool. Or another example is when, so we had some behavioral experiments done with a certain animal in a specific set of neurons, and we tried to use the same manipulations in another line that you know, overlaps more or less with the neurons that we wanted. And we saw exactly the same pattern. So this was done by other people. So that was extremely beautiful, because we knew that the manipulations were done in two different ways; but still, they both gave exactly the same results, I think, you know, there is more eureka moments, but then when everything aligns so perfectly, it's easy to see the beauty. I look forward to more eureka moments.

Ritika

You're DPhil work is a really robust and impressive body of research, and it uses so many different cool techniques. As a new and shiny DPhil student myself, I just have to ask, did you plan on this while you were writing your project proposal? Or is this something that you discover for yourself as you go along the journey?

Raffaele

Yeah, I mean, the experiences of PhDs are very, you know, very valuable and individual. I've been lucky because first of all, I work with Drosophila, which allows you to do lots of different experiments with little planning compared to working with mammals, for example, that you can explore more and the same time I've been with working in a lab where different techniques are used at the same time, and you can learn them or a lot of them and use them. So I was lucky in this point of view. But the other thing is that I started working with hypothesis generating techniques, which is single cell transcriptomics. And a lot of my follow up work comes directly from this. But in general what I did in last years was completely unplanned, is completely different from what I wrote in my PhD proposal. I mean, it's same topic in a way. But the findings are, I was completely unaware and completely unexpected. And also the directions are completely unexpected. And that's something that they found very cool. I liked it a lot. I like the fact that one can explore different ways, and then just focus on those that look the most promising. But that's not always the case. So and I think each PhD, even if it's more planned, that works well, it's absolutely satisfying. So as I said, this, it's hard to generalize comments on how PhD projects should be developed, I think there is a good component of, first of all, luck, and being in the right environment. But also, one of the cool things of the PhD is that one has the opportunity of exploring, but also one should be able to put to completion a project. And I think these two components are present, I think if they're both present in a PhD, that's good enough – if we were just to put completion to a project with this will be very technical work and, you know, decrease the fun of doing science, for sure, and definitely will not improve our independence as scientists, so and the more we continue on, the more independent we

need to be. So the more we continue in our career, the less the projects are going to be well defined from the beginning. So that's just completing a project isn't enough, I feel. And I think exploring just for the sake of exploring or getting lost in exploration is also very dangerous, because one has to be able to tell a story in the end. So yeah, I think these are the two components that are important for a PhD.

Ritika

That makes sense. Can you tell me what would your primary advice to any prospective and incoming students be?

Raffaele

I think we already know the research is frustrating, most of the times. We are international people, we also move from other countries with all the stresses that are related to that. So I think that first thing is to find something that will really keep your mood up for next 3, 4, 5 years of your time, which is not easy, I guess. And of course, another important thing is to find an environment, both in workplace and outside the workplace that is that is nice and friendly. So I will definitely advise to chat with the people in the lab that you're looking at, for example, to see what's the impression, to see if they're happy with the environment. And of course, if you have the possibility also of being in a place that gives you motivation, that's not boring. That's why I feel lucky to be in Oxford, because it's a very vibrant scene, there's a lot of international people, there's always something going on. And that really helps because it is, you know, it helps to have something outside of the lab that goes well anyway.

Ritika

To end this podcast, and in hindsight, is there any advice you would give to your younger self?

Raffaele

In terms of science, for sure, the biggest advice is to label things perfectly, properly and backup everything many times. This is what I will tell to the first year PhD student – Raffaele. And I also tell to my students sometimes when to always assume that one is totally stupid, because we're always fighting with ourselves in the future, they will have completely forgotten what we were talking about what we were recording, which date we did the experiments, and so on and so forth. So always assume that the future self is totally stupid. That's the best approach, I think to complete projects. And then definitely in general to how to approach academic life is definitely to not to be shy and to write to people, to contact people, to not be worried about professors' responses, or whether it is any sort of application. I think that's something that at least I learned over time. Even just the opportunity of doing internships abroad. It's something that I did and I learnt late. And you know, if I will go back, I will redo it. I was very focused on the courses on the classes on the, on the grades, but I think especially in, you know, in this environment, you really learn a lot by doing. And so that definitely something if I could, if I had the opportunity, I will do more. And in the end for international students, definitely, I will advise to find something passionate to you, and not be afraid because it's worth just for the experience itself or going abroad. And I think one of the good, cool advantages that we have as scientists is that science is international. And we have the opportunity of talking to people that come from all over the world on topics that are interesting to us, moving all over the world for conferences or for working somewhere else, and as we are very young. So I yeah, there are many drawbacks in science, but I think this is really a privilege that we have in our job. So yeah, so take advantage of it.

Ritika

That’s brilliant!

Neddy

We've now come to the end of this episode. In summary of Raffaele’s interview, we've covered the importance of sleep, and how studies using *Drosophila* have aided in contributing to the understanding of sleep, which was highlighted in Raffaele's doctoral research. Furthermore, it was truly inspiring to see how Raffaele's journey developed from a young masters and DPhil student to now being a postdoctoral research scientist here at Oxford. I have no doubt that there'll be more to hear in the near future, from Raffaele's research on the molecular nature and cellular biology of sleep control.

Katy

Thanks for listening in on our conversation today. We hope you enjoyed it as much as we did. Please keep an eye on our social media to find our next one.