# **CortexCast S2E6 - Wired for Care: Neural Circuits with Dr. Johannes Kohl.**

**Introduction:** Hello, It's Ritika, Katie and Neddy and you're listening to, the Cortex-Cast. In this episode, I'm joined by neuroscientist Doctor Johannes Cole, who's a group leader at the Francis Crick Institute in London. Jonny's lab investigates the brain circuitry behind instinctive behaviours like parenting and mice, and how those circuits adapt across sex, hormonal state and experience.

**Neddy** – So your 2018 nature paper showed that galanin expressing neurons in the medial preoptic area coordinate different aspects of parental behaviour in mice. What first drew your attention to those neurons, and why choose parenting as a behaviour to study?

**Jonny** – It's a very good question. After my PhD, which I did in flies, I'm studying sex differences in pheromone processing in Drosophila. I was really drawn to a system in which I could link neural circuits with behaviour a bit better, and for that the mouse seemed to be the perfect sort of spot. And, um, I wasn't thinking about parenting at all, but, um, in at a conference in Toronto, I saw Catherine Dulux talk on the discovery of these parenting relevant, um, neurons that you mentioned in the public area, the MPOA. And I was I was blown away. I thought, it's so cool that you can have very complex behaviour, such as parenting that is, uh, orchestrated by the small population of neurons. And I thought that was a really sort of interesting, um, problem, how such a small population could orchestrate such complex behaviour. And I thought that's worth, you know, studying for a few years of my life.

**Neddy** **[Voiceover Bridge]**– whilst parenting was the entry point, it's the circuitry beneath it that drives Johnny's work. What makes instinctive behaviours both stable and flexible, and could these hardwired circuits be just as plastic as the rest of the brain? It's questions like these that push Johnny's work far beyond parenting itself.

**Jonny** – we use parental behaviour as a model a lot, but we don't. I mean, it sounds horrible, but we don't fundamentally care about parental behaviour in mice. We use it as a model to understand bigger questions. And the bigger questions are, for instance, how do circuits for instinctive behaviour such as parenting, how do such circuits balance their incredibly, their incredible robustness and with plasticity at the same time? And so you have a behaviour that needs to be robustly performed, but it needs to be flexible enough to be, you know, state appropriate and state dependent. So how is that how is that balanced? Another big question that that we that we're hoping to address using this model is, um, whether certain parts of the brain, certain circuits are more plastic than others. Because again, there's this sort of this thinking that certain parts of the brain, such as hippocampus, cortex and cerebellum, they are the the learning parts, they're highly plastic. And others such as hypothalamus and brainstem, they are just this stupid circuit boards. But the closer you look at these subcortical hypothalamic circuits, The more you realize the design plasticity mechanisms and operate there as well. But the question is whether these subcortical and hypothalamic circuits, which drive these robust behaviours, whether they are actually equally plastic as their cortical, um, counterparts. And that is um, of not very well, um, understood because these, you know, these fields of cognitive neuroscience on the one hand and affective neuroscience on the other hand, um, don't really overlap as much. So we're hoping to, to bridge this gap.

**Neddy** – It seems like parenting isn't just a set of triggered actions, but rather more like brain state. The animal enters. How does the brain transition out of this state or adapt when parenting demands change over time?

**Jonny** – That's a very good question. What you said is exactly how we think about it now. We think that these neurons, these neurons, these neurons, that their primary role is to basically produce a parenting state. And then what follows is basically the behaviour that is released when an animal in that state meets the appropriate stimulus. So in this case it's not just a simple reflex that when you stimulate these neurons in absence of a target, that you get parental behaviour in thin air. So that's how we think about this now. How did I get out of this state? Well, I guess it depends on how they get into the state in the first place. So I guess there are several routes to get into a high parental state. The hormonal route is the most robust one, as I as I mentioned. So you can become pregnant, you can give birth and that leads that is associated with these dramatic hormonal changes which require certain parts of parenting circuits. And we know at least two parts of parenting circuits that are rewired during these transitions. One is the MPOA galanin neurons themselves during pregnancy. But then there's also neurons in the primary auditory cortex, which are sensitive to vocalizations, which are remodelled by action of oxytocin and at the time of giving birth, which then increases the sensitivity to this stimuli. Now, in this case, I guess the expectation would be that the system goes back to baseline levels once these hormonal changes go back to baseline as well. But what we actually find in the pregnancy situation is that this remodelling is actually quite long lasting, and it persists even after these hormonal changes have gone back to baseline. We don't know whether the same thing happens in the primary auditory cortex, but that really suggests that this this experience of pregnancy has long lasting effects on the on the female brain. The other the other way to to become highly parental is an experience driven process. So so people have known for a while that when you repeatedly expose virgin female mice which are normally low parental or not parental, when you repeatedly expose these animals to pups, they become highly parental too. But it's relatively unknown. And how long lasting this improvement in parenting is. So once we find out, we can then look at the underlying plasticity mechanisms. But the assumption is that those sort of learned, I guess behavioural responses would be a bit more fragile as the hormonally induced ones. That's my. That's my guess anyway.

**Neddy** – Do you think there's an upper limit to how plastic or reconfigurable these circuits are in adults?

**Jonny** – Yeah, we can only, um, you know, approach this, uh, this question from a behaviour perspective at the moment. But if you look at the behaviour performance, we are really hitting a ceiling effect here. So these animals are extremely parental, uh, once they are pregnant and once they've become parents. Um, but you know, which which makes me think, okay, so what's what's happening during a normal, um, um, gestation and parturition? This really, uh, brings this circuit to maximum, um, performance. Um, the question is whether, for instance, um, you know, successive pregnancies have a cumulative effect, you know, so because we see that the performance goes down after parturition again, um, over time. But what if you have a mouse that has been, um, a mom, you know, um, twice or three times, you know, does she then basically just, you know, reach a steady state at an extremely high level. Um, that's that's an interesting question that we that we generally answer for.

**Neddy [Voiceover Bridge]** – So far, we've talked about why parenting is such a powerful model for studying the brain and how these circuits aren't just fixed or instinctive, but surprisingly flexible, but beyond plasticity. Johnny's work also reveals something else – Structure – In this next part, we get into how parenting behaviour is wired across the brain and how surprisingly modular that wiring turned out to be.

**Neddy** – So your work has mapped out a core parenting circuit, showing that galanin in the medial preoptic area receive inputs from over twenty brain areas and drive behaviour like grooming, crouching, and pup retrieval. Did you expect parenting to be that modular?

**Jonny** – Uh, not at all. You know, this project started by first using anatomical tracing techniques to map out the circuitry. And I remember when I did my first rabies tracing experiment. So the first experiment to map the direct, and synaptic inputs into these parenting controlling neurons. I was actually quite shocked because because it was just this, this, this, you know, enormous number of input areas into these neurons. And I thought, like, okay, this is a level of complexity that I won't be able to make sense of. And the same happened when I traced the outputs of these neurons. And you get a roughly the same number of output. So it took two years of further anatomical tracing to actually make sense of this circuit, before I then started to produce functional investigations or so photometry, optogenetics to to really tease apart. But I think it was really important to first and carefully characterize the anatomy of, of, of these circuits before we could make sense of their function.

**Neddy** – Did anything surprise you in how coordinated or fragmented these outputs were?

**Jonny** – Yeah, absolutely. I mean, I think we were maybe in this circuit quite lucky in that it's organized in this very, um, this very modular way. So you have these protection defined subpopulation of neurons that each receive inputs from all of the input areas. But I guess it could easily have been a different sort of circuit architecture in which you have these branch protections, you know, and once you have an architecture like that, you have a handling. Handling. This is very, very difficult because you could you could imagine that each protection contributes to many different aspects of the behaviour. But I guess we got lucky and we found that, you know, that we have these discrete pools that control different aspects of parental behaviours. Is that so? So maternal, motivational, hormonal and so on. So I was surprised by how clean the and it was segregated at this level of the circuit. Yeah.

**Neddy [Voiceover Bridge]** – We've just seen how parental behaviour maps onto distinct circuit modules. Now we look at how sex and hormonal state influences parental behaviour, and how examining both males and females could unlock new insights into plasticity, bias, and the deep logic of brain circuits.

**Neddy** – So is the use of female models in your lab driven by practical needs in parenting research, or by a desire to push the field towards new perspectives on variability and modelling.

**Jonny** – So I guess it really started out as a practical need, because I guess, um, parental behaviour is so much more, um, robust in females. And, you know, we know a lot more about the circuit basis of parental behaviour in females, arguably. But now I realize that it also has these nice effects. Um, um, you know, for comparing, um, males and females and for considering sex as a biological variable, which I think is increasingly recognized to be, um, a crucial, um, dimension. Um, and so for us, you know, that's always something that's, that's, that's on our mind when we design projects. And I think it's highly, you know, useful, um, in the hormonal context as well, because, uh, you know, I guess hormones are an area where males are incredibly, um, understudied because and as a consequence, um, people tend to believe that hormones are sort of a female thing, you know? But I guess behaviours, um, and neural processes are subject to hormonal regulation in females, but not really in males. And I think, you know, our work will probably contribute to dispelling some of those myths as well.

**Neddy** – So, like, how do hormonal states like pregnancy or lactation affect experimental design, and how do you handle that variability in your own work?

**Jonny** – Once you have a good handle on these, on these processes and, you know, I mean, we do our best. Um, you can actually turn this sort of bug or this source of variability. You can turn it into a feature, you know, you can use it to explore, um, how, um, different states affect neuronal processing. Arguably, if you, if you just, you know, um, you know, let's say use males and just rely on the notion that, you know, they don't have these sort of hormonal states, which is almost certainly not true. Um, you will actually have a higher variability. That's what people they see. If you look at, um, um, behavioural variability in studies. It's actually typically higher in males. And one reason, I think, is that there are certain state variables that we don't control for, such as hierarchy. I guess in females, because of, you know, because what we know about hormones, we are always forced to to really, you know, stratify our data set according to, let's say, eastern time state and so on. But I think it's a practice that would be generally very useful in neuroscience.

**Neddy** – You said that hormonal studies in mice are very much understudied. If you were to go about doing that, how would you approach that?

**Jonny** – A very simple. I'm a very simple approach would be that every time we study something in females, let's say we try to understand where estrogen are expressed in the brain. We just also throw in a few male ones, you know, the comparison. And whenever we've done that, we were actually surprised to find that the patterns look very, very, very similar. This is same with prolactin for instance, which for a long time, because of its name and its supposed role in lactation, I mean, it has a very profound role in lactation. But I guess the question was then why prolactin exists in males and why prolactin receptors exist in the male brain? We know because males don't lactate. So why would you have this hormone there? So I guess, you know, a lot of these patterns are they are a bit sexually dimorphic, but only quantitatively. But you still have all of these factors in males as well. Which begs the question of what they do there. And so so actually I'm addressing this is very simple conceptually. It just means that you have a larger cohort sizes. But I think that's how we will go about in doing this. So in a very hypothesis free but also dogma free way, we just compare males and females in our stems and see where it takes us.

**Neddy** – Parental roles vary a lot across species, but in mice you've shown that the core medial preoptic area circuit exists in both sexes. So what actually shifts in male brains to bring out parental care.

**Jonny** – Another very good question. So I guess when, um, in our, 2018, um, paper where we mapped the functional architecture of these circuits, we were actually quite surprised to find that at least at this mesoscale level. So when you trace from hundreds of neurons, um, thousands of neurons, um, the overall anatomy of parenting circuits in males and females is almost completely the same. So it's only when you dig deeper and you look at higher resolution, you find some quantitative differences between males and females. But in general, the circuits between fathers, between mothers and also virgin females were extremely similar. And the same was the case for the um activity signatures in um, in these neurons, um, during parental behavior. So that, that really, um, you know, and that's why I guess, you know, um, we, we think that, um, by and large, these circuits are non dimorphic, but how you get to robust parental behavior might be very different between males and females and females. You obviously have pregnancy. is pregnancy, associated with hormonal changes and parturition associated to hormonal changes in males. We don't actually know how it works at all. So. So males undergo this very dramatic change between being infanticidal as virgins and between um, between being, um, parental as fathers. So this so-called post-mating switch. So they have um they again, as in females, it takes about three weeks for them to undergo this dramatic behavioral switch. It's called a so-called post-mating switch. And it depends on a combination of um, ejaculation and being co-housed with a pregnant female. But fundamentally, we don't know, uh, what the underlying mechanisms are and also what, what these plasticity mechanisms are that act over this extended period of about three weeks. So people have suggested it could be, um, you know, neurogenesis, it could be hormonal changes, it could be just other forms or unknown forms of plasticity. But fundamentally, we don't know. And it's very intriguing. Um, question.

**Neddy** – So one of these studies showed that activating the medial preoptic area in virgin male mice suppresses pup directed aggression. Um, does this suggest that parenting and aggression circuits are in competition? And if so, what tips the balance?

**Jonny** – Yes, it's a very good question. Um, so there was the, um, work by Herbert Wu, um, in, in the lab in twenty fourteen who showed that indeed, that activating these neurons and suppresses pup directed aggression in males and also, um, unlocks elements of pop directed care and pup grooming, for instance. Now, as you said, this suggests that there is some competition between pup directed aggression and pup directed care circuits. And recently actually violin. Dualin’s lab at NYU, um, has shown that there are these two opposing populations estrogen receptor one expressing neurons in the mpoa and estrogen receptor expressing neurons in the bed nucleus of the stria terminalis Bnst, which I've shown this mutual inhibition and Circuits. So in in virgin females. So they selected a mouse strain in which virgin females are also infanticidal to get maximum contrast between the behaviour of a virgin female mouse and a mother, and they found that the activity of these bnst esr1 neurons was high in virgin females and infanticidal virgin females, suppressing the activity of these neurons, which are pro parental parenting promoting. And then when these mice became mothers, this was inverted. So now, uh, the activity of these mpoa esr1 neurons was elevated, thus suppressing dispensed, um, infanticide promoting esr1 neurons. So you have this, uh, basically this, um, you know, gestation dependent, um, you know, um, balance, uh, between between those, um, two populations of neurons. But importantly, we don't know what actually triggers this switch, No. These neurons are all being estrogen sensitive in principle and suggested it will be. It will be these rising hormone levels or the rising levels of estrogen estradiol during pregnancy. But that's still very much unknown

**Neddy [Voiceover Bridge]** – in this last segment. Jenny and I talk about the tools his lab uses and why the best methods start with asking the right questions.

**Neddy** – You use projection specific optogenetics to dissect the function of individual outputs, but you also use fiber photometry, tracing, and intersectional genetics. What drives your decision to use one method over another when asking specific questions?

**Jonny** – Um, we are very pragmatic in general. I've been very pragmatic about methods, and we in the lab now, we're very pragmatic. We try to use, um, you know, sort of the, the simplest method that can give us an answer, um, which means that we need to establish quite a few methods for any given projects. But luckily, we're in a place, um, such as the Crick, where we have a lot of resources and a lot of amazing colleagues from which we can learn. But it's usually, yeah, it's always a question that's first, you know. So, um, and we want to use techniques not for the sake of the technique, but to really answer a specific question. And you typically, you know, the easiest, the simplest one that can give you an answer. For instance, photometry I guess, doesn't give you a cellular resolution. But in this case you don't need cellular resolution and was easy to implement. So that's what we did. Uh, nowadays you could probably use many scopes, but that would give you an enormous data set that's way harder to interpret and gives you lots of data that's not required to address the primary question.

**Neddy** – So your lab works across such a wide range from viral tools to behaviour. What would you say is the hardest part of training people to think fluently across those scales?

**Jonny** – Oooh, very good question. Um, I maybe the hardest part is to wean yourself, um, of this, uh, methods first thinking, because I guess in neuroscience we're very spoiled for a choice of methods. And it's a very methods heavy field. But what I observe, and I observe this in myself as an undergrad as well, that it's it's very easy to think about the methods first. You know, like I often I talk to people and they say, oh, I want to do neuropixels recordings in behavior. But then when you ask them, you know, why do you want to do that? It's actually often clear that they think they need or they need to like doing these kind of things, because that's what everyone else is. You know, there's a certain, um, hype cycle in neuroscience. And, um, but but what's more important, I think, is to ask good questions and to ask good biological questions. And that's the hard part. And that's what no one ever teaches you. So, so that's, that's that's one thing that I try to emphasize is, um, with trainees. And let's think about the questions first and what kind of methods we need to to address those questions. Because methods come and methods go. But it's discoveries. It's concepts that really live on. So I really try to emphasize that, that kind of thinking in the lab. But luckily we are again. We are, we are, we are privileged and we are in a place where we can, uh, we can use different types of methods to address these questions. Not everyone has this sort of luxury. I'm aware of that. But, you know, if you if you have that luxury, you know, it would be foolish not to use it.

**Neddy [Voiceover Bridge]** – Now that we've covered some of the core ideas behind Johnny's work, I wanted to wrap up this episode by asking what advice Jonny had for young scientists, especially those navigating a field as fast moving and methods heavy as neuroscience.

**Neddy** – So last time we mentioned the importance of asking good questions rather than just using the flashiest methods. When you're designing an experiment, how do you know when a question is worth asking?

**Jonny** – Ahhhah! When do you know whether you got a good question? Um, I mean, it's a gut feeling, so. So, you know, when you're onto something interesting, when you feel like, okay, I could, I could easily dedicate the next few years of my, you know, you know, professional life to this to this question, you know, and then also talking to people you can often gauge, um, you know, the, the interest level. You know, you can you can gauge whether what, what you're, what you're talking about is actually, um, broadly exciting. And, you know, obviously that shouldn't be, um, the predominating factor, but but as a Pi, you also need to recruit people that are excited to work on, on on your questions, you know, so it's an important, um, factor nevertheless, that, that you can sort of communicate a certain excitement about about this question. Yeah. So it's often, it's often quite clear, you know, when you start talking to people whether you're onto something or not, you know, and sometimes you might be excited about something but can't quite communicate it well. And then it takes a few iterations. Um, but again, then it's really useful to have, um, colleagues that are both, um, critical but also open, you know, as a sounding board for some of your ideas. Yeah. So again, that's that's my strategy. You know, you just create lots of ideas and then you, then you talk to, um, you know, talk to people that you trust. Um, uh, and see what what what comes back.

**Neddy** – What experimental skills or tools do you think are most valuable for early career researchers to prioritize if they wanna work across molecular, cellular and systems levels?

**Jonny** – Um, I would say very broadly that the type of experimental skills, uh, or methods that you, um, that you, that you master doesn't really matter as much. But increasingly, being able to, uh, work with high dimensional and complex data sets is, uh, of an advantage. But in a way, um, what those data sets are, is, is, is almost secondary. But I guess I think the, um, the ability to work with those type of data, huge data sets and not being intimidated by that kind of data, that's, um, that's quite valuable. And, and then it doesn't really matter whether you're working with a proteomics data set or a single cell RNA sequencing data set, or a multi-electrode neural activity recording data sets. Um, then my opinion doesn't matter, because a lot of these, um, you know, dimensionality reduction techniques that we now use to make sense of those data, um, are quite similar, if you think about it, between those, between those types of data. So I in general, I'm, I'm not to, you know, you know, I'm too fussed about, you know, the types of techniques, um, you know, that that people know as long as there's a certain openness, um, to, to learning, um, you know, new ways of, of understanding and doing the data.

**Neddy** – From your experience, how did you approach beginning to learn how to handle large data sets?

**Jonny** – Um, I guess bit by bit, you know, I guess I sort of at some point accepted that I am always going to be a bit incompetent, you know, uh, and that the goal is to reduce that incompetence, uh, you know, and to basically chip away at those, those problems. But I guess in neuroscience, it's an interesting field because we are basically doing a lot of things for which we're not trained. So we are basically a bit of engineers. We are a bit of data scientists, a bit of molecular biologists, but nothing quite properly because we're trying to to bridge all of those, um, those, those fields. So it's important to basically have, um, um, colleagues around you that, that know these things and, um, and then just have a sanity check, um, in there. But otherwise there's there's no, there's no really, um, there's no silver bullet here. It's just like, um, trying things and then having some kind of quality control mechanism, um, in, in form of, you know, um, friends and colleagues. I think, I think that that's been sort of my, my approach.

**Neddy** – You currently as a PI (Principle Investigator) are hiring people. What exactly do you look for when you are hiring someone new? What exactly makes you say yes - this is the person to handle this project.

**Jonny** – I guess there's a few different dimensions. There's, uh, you know, uh, one dimension is, uh, enthusiasm, you know, so people have to be enthusiastic about science and about, you know, working with us. You know, I look whether people have actually done their homework, that they know that what we have done and that they're interested in what we are doing and where we are potentially going, that that basically put some thoughts into into this process. Another dimension is basically, um, you know, whether, um, people are, um, persevering. I guess, you know, science is tough. Um, a lot of lot of times things don't work out, you know, uh, so it's important to, um, you know, to appreciate that and to show some degree of perseverance, you know, of, um, you know, just, you know, not not not being bogged down by, um, you know, by things not, not working out. So I think that's, that's quite important as well. And another dimension is that there are, um, good team players. We have a medium sized lab. Um, I think we have a superpower. And that superpower is collaboration. So we have a very collaborative lab atmosphere. People help each other out and it's in our field is very important because we want to work on ambitious questions that require investigations at many different levels of investigation, ranging from, let's say, molecular and cellular, all the way to behavioral. And no individual can ever master all of those techniques that are involved to addressing involved in addressing those those questions to the degree that we want to address them. And that really then requires some collaboration between specialists in the lab. And I think that's something that we do very well. But, um, you know, it works well because, um, everyone in the lab has a say, um, as, as to who gets, um, hired. And so it's a very democratic process, and we all work well together.

**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.