Aaron Van Dorn (00:07):

Welcome to AJP Audio for April 2023. I'm Aaron Van Dorn. In this month's episode, we're joined by Dr. David Amaral, a distinguished professor as the Department of Psychiatry and Behavioral Sciences in the Mind Institute at the University of California Davis. Dr. Amaral was a co-author on an overview paper from the April 2023 issue of the American Journal of Psychiatry, looking at the use of animal models and other forms of translational neuroscience in the investigation of autism spectrum disorder. Afterwards, I'll speak with Dr. Ned Kalin, AJP editor-in-chief about the rest of the April issue. Dr. Amaral, many animals are used as models for different aspects of human neurology and research regarding autism spectrum disorder. From simpler animals like in environment nematodes and fruit flies to more complex vertebrates like zebra fish and rodents, as well as those more closely related to humans such as non-human primates. What can be gained by looking for similarity to animals as different as a fruit fly and a human when researching something that's specific but diffuses autism spectrum disorder?

Dr. David Amaral (00:56):

Well, before I answer that question, it's important to start with some basics. The diagnostic features of autism include first altered social interactions and secondly, repetitive behaviors or circumscribed interests. It's important to note that they're likely to be many ideologies of autism, but virtually all appear to occur in the prenatal period. Autism is also a genetically heterogeneous disorder with currently nearly 200 genes that are known to increase the likelihood of an autism diagnosis, and the predicted number may be as high as 1000. So the field is still trying to identify clear causes of autism and how those causes impact the structure and function of the nervous system. Various mechanistic hypotheses have been proposed for the causes of autism. These range from hyper conductivity versus hypo conductivity of brain systems to imbalances of excitatory and inflammatory influences in the nervous system. Since the patterns of conductivity and even transmitter systems are conserved across species, many of these mechanisms can be explored in species ranging from drosophila to humans.

Aaron Van Dorn (02:14):

So what are the advantages and disadvantages of conducting research in simpler, more complex organisms when investigating human neurology?

Dr. David Amaral (02:21):

There are a number of model systems that are currently being used to understand the biological underpinnings of autism. Because of the shorter lifespan of simpler organisms, examining the impact of causal factors on phenotype can be explored more quickly and cheaply. Simpler organisms have, also have higher throughput for confirming that genetic mutations may produce a phenotype that is consistent with autism. So for example, zebrafish have a lifespan of 10 to 12 weeks. They have 26,000 protein coating genes, and at least one obvious ortholog has been identified for about 70% of human genes. So using this information, one can investigate whether a proposed autism risk gene produces a phenotype that is consistent with what's seen in human autism. So one example is megalencephaly or large brains. We and others have shown that about 15% of boys with autism have brains that are enlarged disproportionate to their body size.

(03:34):

While we know some genes that lead to this phenotype, it's not clear that we understand all of the genes that cause both autism and megalencephaly. And this is something where when we have a candidate, for example, from an individual person who has megalencephaly and autism with a particular genetic variant, we can take that information and then try and model it in zebrafish to see whether it

consistently produces megalencephaly and other features of autism. So we can sort of reverse engineer from genes that are implicated in humans back into zebrafish, and then see if a phenotype is produced and then look at the underpinnings at a cellular and a molecular basis for those changes. We similarly can look at changes in neuronal structure and connectivity, which can be modeled in simpler animals as well. So again, the fundamental of basic mechanisms that might be at the heart of autism can be evaluated in a number of simpler organisms, both effectively, efficiently and less expensively than could be done in mammalian model systems.

Aaron Van Dorn (04:52):

What goes into determining which animals might be good candidates for research in human neurology? You mentioned a couple of things for simpler animals such as zebrafish and nematodes are being shorter life cycles, simpler neurological structures and the relative ease of working with them. But do you go about identifying aspects that make those animals desirable from a research standpoint? Are you looking for something that is for animals that will have a better characteristics that more closely align with humans and then trying to find animals that will be suitable for research?

Dr. David Amaral (05:18):

Right. Yeah. So you need to select the animal model appropriate for the question that you want to answer. As I mentioned previously for looking at basic neurobiological alterations at the synaptic level, simpler systems are appropriate, but when you actually want to start studying neural systems and behavior that mimic autism, you then have to turn to higher organisms such as the rodent and the non-human primate. Much of my work has been in the non-human primate as a model for autistic individuals, and we selected the non-human primate for much of our work for a number of reasons. One is that the parts of the brain that we think are most likely to be altered leading to autistic symptoms are areas of the frontal lobe that even mammals such as the mouse and rat really don't have, and the parts of the frontal lobe that mice and rats do have, have very significant differences from those in the non-human primate.

(06:29):

So from our perspective, anatomically the non-human primate is probably the best model system for understanding the human brain alterations that are associated with autism. Similarly, behaviorally, the non-human primate is most appropriate because non-human primates, unlike mice, have very sophisticated social hierarchies, social organization systems so that rhesus monkeys, for example, have sophisticated what are called natural lines. There are social orders where there are dominant females and subordinate females. They communicate social intention with each other using both facial expressions, vocalizations and body postures. Much like humans do. All of these things make them a much more appropriate behaviorally relevant model for human autism where things like appreciating facial expressions is one of the characteristics that are difficult for an autistic individual.

(07:37):

So again, the precise animal model that you want to select needs to be determined by what questions you're trying to answer. Of course, they're practical issues as well. So as I mentioned earlier, some of the simpler model systems have a much faster throughput. They're less expensive working with non-human primates, of course, because of the sophistication of non-human primates and also because of the cost, you generally use a smaller numbers of animals. So you have to design experiments that take those features into consideration.

Aaron Van Dorn (08:17):

Basic research brings us a greater understanding of the structure and function of the brain, but how does that get translated into treatments and approaches that will be of interest to clinicians? Has this research resulted in clinical treatments for autism spectrum disorder?

Dr. David Amaral (08:28):

Well, at this point we're still trying to understand a lot of the phenomenology of autism, and I think number one, we understand some of the neural systems that are involved in autism. To a certain extent, we have information about the social brain that has come largely from studies of animal models, although a human imaging has also contributed to that as well. We also know a substantial amount about the parts of the brain that have been implicated in some of the co-occurring conditions. So one of the things I failed to mention when we were talking about the basics of autism is that in addition to the core features of autism, there are a whole host of co-occurring conditions that can affect some individuals, some autistic individuals. So for example, anxiety is found in about 70% of people with autism. Intellectual disabilities found in about 35% of autistic individuals. Autistic individuals often have a much higher prevalence of gastrointestinal problems.

(09:37):

And so for example, with anxiety, the animal research has led us to the understanding that a structure called the amygdala is probably highly related to the emergence of anxiety. And it turns out that both in animal studies as well as in human studies, the amygdala has become a focus of autism research. I would say that at this point we are still at too early of a stage, both at understanding human autism and understanding the animal models that have been developed concerning autism to move anything to clinical treatments. But that I think is the hope for the future.

Aaron Van Dorn (10:18):

What are the alternatives to the use of animals as a model for the human brain in research?

Dr. David Amaral (10:21):

So one I think, one of the exciting developments over the last decade has been the production of induced pluripotent stem cells, which that can then produce neurons and glial cells. And these are stem cells that are produced either from blood or fibroblast taken from an individual that has a particular disorder like autism and induced pluripotent stem cells, I think could go a long way, particularly as they now have evolved to being able to produce not just simply two-dimensional cultures, but three-dimensional organoids that are in essence little mini brains that have interconnectivity. And so again, these can be used to study neuronal structure. They can be used to look at SNAP tech organization and they have the advantage of having the genetic architecture, the known genetic architecture of an individual that has autism.

(11:23):

They of course do have limitations. They don't behave and they tend to have limited circuitry characteristics, although even that now is becoming studied in ways in which how they integrate into the brain and how they may communicate with other brain regions are being carried out by taking IPSC cells and organoids that are a derived from humans and implanting them into the brains of rodents, for example, and seeing how the organoids then interact and communicate and survive in the environment of their rodent brain. So I think that this is an area for the future that may contribute highly to our understanding of autism and hopefully would be a strategy that may reduce the number of animals that would be needed.

Aaron Van Dorn (12:19):

Speaking of the future, what promise do you see from this research going forward?

Dr. David Amaral (12:22):

So the use of animal models in translational research is just one component of a multidisciplinary approach. All that has the goal to treating the disabilities associated with autism. As I mentioned, I think we're still at an early stage of understanding the phenomenology of autism. We don't quite understand how it develops, how it changes over time in human autistic individuals, but we're getting a clearer picture of both in humans, and as we understand more of the neural systems altered in autism, we can explore ways in animal models to optimize the functioning of these systems. In some cases, we may ultimately be able to carry this out prenatally and prevent some of the deleterious impacts of genetic or environmental challenges to optimal brain development.

(13:17):

So one example of this is Angelman syndrome, which is a neurodevelopmental disorder that we know the gene defect that produces this disorder. It produces a phenotype that has really serious challenges such as profound intellectual disability, oftentimes epilepsy, lack of language, and this is a genetic disorder that can be detected prenatally. And there are now a variety of strategies using gene therapy strategies that have proven successful in animal models. And I believe that as we take this work from the mouse where it's been shown to be effective to other animal models, perhaps the non-human primate and show that it's safe and effective, this would be a therapy where it could, when detected prenatally, be applied and produce an outcome that would remove the deleterious effects of this unknown genetic alteration.

(14:26):

When I mentioned this, I want to make it clear that the goal of this type of research is not to eliminate autism or in Angelman syndrome, which is often associated with autism. It's a good example of where the goal is to eliminate or mitigate the deleterious consequences of a condition like Angelman or autism. And again, autism as I mentioned earlier, is associated with a variety of co-occurring conditions. Epilepsy is one of them. So I think where we're all heading is trying to understand how these environmental and genetic impacts alter brain function, and then through using strategies first tested in animals, determined a safe and effective way of removing these deleterious effects and producing an individual that'll have a much higher quality of life.

Aaron Van Dorn (15:21):

Dr. Amaral, thank you for taking the time to speak with us today.

Dr. David Amaral (15:23):

You're very welcome. I enjoyed it. Thank you.

Aaron Van Dorn (15:26):

Up next, Dr. Ned Kalin. Dr. Kalin, welcome back to AJP Audio for April 2023.

Dr. Ned Kalin (15:30):

Thank you, Aaron.

Aaron Van Dorn (15:31):

The April issue is a wide-ranging one, taking in issues of genetics, neuroimaging and behavioral neuroscience studies. I spoke earlier with Dr. David Amaral, one of the authors of an overview paper looking at the use of animal models and other forms of translational neuroscience in the study of autism spectrum disorder. What can you tell us about that paper?

Dr. Ned Kalin (15:46):

So first of all, this issue is a really interesting issue because it does bring together various approaches to help us better understand the underpinnings of psychiatric illnesses and ultimately help us develop new treatments. The overview by Dr. Amaral from UC, Davis is an outstanding piece. And in this piece, he and his co-author look at how to help us understand behavioral neuroscience ways to better get at mechanisms underlying autism symptoms, its ideology and ultimately to think about how we can develop new treatments. And here he talks about how we can use animal models and also how we can use some in vitro models including organoids or stem cells, but mostly focusing on animal models. And this really highlights the critical importance of using preclinical research along with clinical research to better understand and come up with new ideas about treatment for illnesses across the board. But in this particular case in relation to autism, which as you know is an illness that affects so many people in ways that can be considerably impactful.

Aaron Van Dorn (16:58):

Next up, we have a paper from Kato and colleagues looking at the use of positron and emission tomography.

Dr. Ned Kalin (17:02):

Yeah, so this is an interesting paper as well. This continues on the autism theme, and in this study, the investigators used brain imaging and in this case used what we call positron admission tomography to attempt to quantitate the amount of a particular mitochondrial enzyme in males, adult males that had autism who were not on other medications and not currently being treated with other medications. This is a unique study because the way that the authors went about this was to use a novel compound that was radioactively labeled with fluorinated F18, and then use this to visualize binding to this particular enzyme that's in the electron transport chain complex, and then to try to understand what may be different in brains of individuals with autism.

(17:53):

Now, the reason they're interested in mitochondria is that mitochondria operations have been implicated in various psychiatric illnesses across the board. And what we learned way back in our medical school or college physiology classes is that mitochondria are the energy, major energy sources and powerhouses of the cell where ATP is transformed into ADP and energy is released. And in that regard, they wanted to investigate this hypothesis that mitochondria function may be altered in individual autism.

(18:25):

What they found was is that in one brain region, the anterior single cortex, which is a region that's really interesting in relation to various illnesses, there was actually a significant reduction in binding of this novel ligand, which implicates this particular mitochondrial enzyme as being down regulated or individuals having less of it. So it's an early study, it's an relatively small sample, but it's a unique study because it's done in vivo, it's not done in postmortem tissue to look at mitochondrial function. And the early, very early indication is that there may be some alteration in this particular brain region, the

interesting cortex in relation to this particular enzyme that has to do with energy transport and energy generation.

Aaron Van Dorn (19:12):

Zwicker and colleagues, took a look at polygenic scores and major mood and psychotic disorders.

Dr. Ned Kalin (19:17):

So this also is an interesting study, and now we're getting into a bit more clinical type of research. This is a study that was done from eight different samples, actually. And the idea here was to look at offspring of parents that had, the parents had depression, recurrent depression or schizophrenia or bipolar disorder, and to look in the offspring longitudinally over time to see the likelihood of them developing these types of disorders and also measuring genes or developing the polygenic risk score in the offspring and asking the question, to what extent does the polygenic risk score add to the predictive value of what we know from family history in relation to offspring's likelihood of developing these disorders? It's well known that these disorders run in families. It's well known that they're heritable. And so it wasn't surprising that the investigators found that if you had a family history of a mood or psychotic disorder, your risk or hazard ratio of developing one of these disorders, the child's risk was about 2.8 fold.

(20:26):

So that's generally consistent. It was also found that when they looked at the polygenic risk scores in the children, that they too predicted the likelihood of developing these types of problems, but frankly, they were not as strong predictors as the family history. And what they found was is that, and they did polygenic risk scores for a variety of different illnesses. So they calculated it for neuroticism, schizophrenia, depression, ADHD, and what we call the P factor, which is a broad dimensional psychopathology factor. So they looked at all these different polygenic risk scores and all of them were related to a greater likelihood of developing these illnesses in offspring.

(21:05):

And interestingly enough, they looked at a polygenic risk score for wellbeing and found that that actually somewhat reduced the risk of developing these disorders as well. But when they controlled for family history, what they found was is that only two of the polygenic risk scores were independently predictive, and that was the one for neuroticism being independently predictive of family history, increasing the risk of developing these problems in offspring and also the wellbeing polygenic risk score was independently associated with a reduction in the likelihood of developing these types of problems. So the bottom line here is that this is an expansion of our understanding of the familial transmission of psychotic and disorders and depression from the standpoint of understanding not only how family history predicts or relates to the increased risk, but how the offsprings polygenic risk score does that and gets to the idea that right now the polygenic risk scores in general don't add a lot to family history. However, when we think about the polygenic risk scores for neuroticism and polygenic risk scores for wellbeing, they do add a bit to the prediction with family history.

Aaron Van Dorn (22:14):

Linking back to the paper from Amaral and colleagues, Kim and colleagues have a paper looking at a rodent study looking at interior insula associated social novelty recognition.

Dr. Ned Kalin (22:22):

So this is, now we're going to a preclinical study. And again, what I like about this issue is that we're ranging all the way from preclinical studies in mice to novel imaging studies in humans, to looking at large samples in humans as well for these genetic and family associations. In this particular study, the investigators used a mouse model to understand the extent to which the anterior insula is involved in social cognition and novel social recognition. This is broadly important because alterations and social processes and socialization and social cognition are common across psychiatric illnesses and for sure is one of the hallmark symptoms of autism. Also is associated, like I said, with a variety of other types of problems that we deal with in psychiatry. So it's sort of a global issue and important that way, or a generic issue. The investigators are interested in the anterior singular cortex because it has been implicated in some types of social processes and social functioning.

(23:24):

It's also been implicated from the standpoint of auditor function in relation to a various psychiatric illnesses and in general, and traditionally, the anterior insular cortex has been associated with introceptive processing or the ability to modulate internal cues and signals that we all have both physiologically and emotionally. So it's an interesting region. And what the investigators did was that they first manipulated this region by lesioning it and basically killing some of the brain cells in that region, the neurons, and they showed that this had an impact on social recognition, novel social recognition in the mice. And then they went on to investigate a interesting pathway called the retinoic acid pathway by doing a variety of neurochemical and molecular and genetic manipulations, which I won't go into, but they basically showed that this retinoic acid signaling pathway was important also in this novel social recognition process.

(24:21):

This is, as I mentioned, it's an interesting pathway because it's very much involved with fetal neural development, and it's also involved later in life in the regulation of various genes and also in neuroplasticity from the standpoint of synaptic plasticity. So they've done this study that first implicated the region by doing a lesion study, then delved more deeply and tried to understand the involvement of this retinoic acid pathway and were able to show that retinoic acid signaling is important in relation to altered social recognition processes that are mediated in the anterior insula neurons. And then finally, they also looked at oxytocin in this regard. They were interested in oxytocin because oxytocin has been implicated in a variety of social processes, attachment and bonding. And here they found that oxytocin was important in these processes, but it wasn't oxytocin per se in the anterior insula, but rather oxytocin in the brainstem modulating serotonin neurons that then actually project to and modulate function in the anterior insula.

(25:26):

So taken together, these findings support a couple of things. One is that neurons in the anterior insula, especially neurons that are excitatory, are involved in social recognition processes and mice. Secondly, that the retinoic acid pathway appears to be important. And thirdly, that oxytocin by modulating serotonin input to this region also is important. Taken together, these findings provide some insights into both adaptive and maladaptive social cognition processes. The extent to which they're applicable to primates and humans remains to be determined. We have to keep in context of these are mouse studies, but yet provide interesting leads in relation to how we might think about the neurochemical systems and the brain regions that may be involved.

Aaron Van Dorn (26:14):

Zeng and colleagues have a paper looking at genetic associations between stress related disorders and autoimmune disease. What can you tell us about it?

Dr. Ned Kalin (26:21):

This is an interesting study because we have known clinically, and there are a number of studies that have linked a greater risk of having psychiatric problems if you have an autoimmune disorder, and also if you have a psychiatric illness, a greater likelihood of developing an autoimmune disorder. So this linkage has been known, and what this study does is basically further develops our understanding of the heritability of the co-occurrence of these conditions within individuals and also the shared genetics that may be underlying this. And so this was done in an extremely large sample from Sweden. It looked at over four million individuals and looked at the presence of stress related problems such as PTSD, acute stress disorder and adjustment disorders. And they also looked at the co-occurrence of 36 different autoimmune disorders. And the first finding that they came up with was that if you have an autoimmune disorder, your risk of developing a stress related disorder is about, the odds ratio is about 1.6 or 1.7.

(27:27):

So there's an increased likelihood of developing a stress related disorder if you have an autoimmune disorder, which is what I just talked about and what we've already known. But by having this large sample and being able to look at the family tree, they were able to look at the pattern throughout the family tree of individuals that had both an autoimmune disease and a stress-related disorder. And this allows us then to get at the question of whether or not the co-occurrence of these illnesses within an individual tends to be heritable. And what they did find was that yes, it was likely that this is heritable as the more related individuals were in the family tree, the more likely it was that they had the co-occurrence of both a stress related disorder and an autoimmune disorder. And then finally, they used polygenic risk scores from another sample of about 400,000 individuals, less than that, a little bit less than that from the United Kingdom Biobank.

(28:20):

And what they found was is that there is some shared genetics between the risk for autoimmune disorders and the risk for stress related disorders. Now, it wasn't a lot of genetics that were shared, but there was some shared genetics across both of these illnesses. So the bottom line here is that are the association that we've seen in the clinic between individuals having autoimmune problems and stress related psychiatric problems. We now know from this study to some extent that that co-occurrence is related to heritability and that there are some genes that are shared across these two illnesses that may confer the risk for one individual to have both of them. And we have really nice editorial here by Dr. Andy Miller from Emory and co-author by Dr. Elizabeth Binder from the Max Planck Institute, and also one of the deputy editors of AJP, where they more deeply discuss the mechanisms that underlie the linkage between autoimmune disorders and stress related psychopathology. And really interestingly point to the use of new types of drug targets, targeted autoimmune processes to treat some subsets of patients with stress related psychiatric disorders.

Aaron Van Dorn (29:33):

Dr. Kalin, thanks once again for Joining us.

Dr. Ned Kalin (29:35):

Thank you.

Aaron Van Dorn (29:36):