UNIFORMED SERVICES UNIVERSITY
CENTER FOR THE STUDY OF TRAUMATIC STRESS

+ + + + +

17TH ANNUAL AMYGDALA, STRESS, AND PTSD CONFERENCE

+ + + + +

BRAIN, BEHAVIOR, AND BEING: UNRAVELING STRESS

+ + + + +

APRIL 23, 2024

+ + + + +

This transcript was produced from audio provided by the Henry M. Jackson Foundation for the Advancement of Military Medicine.

P-R-O-C-E-E-D-I-N-G-S

DR. CAPALDI: -- Capaldi, and I have the privilege of being Chair of the Department of Psychiatry here at USU. It really is a tremendous honor to be able to welcome you to the 17th Annual Amygdala Conference. This year, the focus of the conference is on Brain, Behavior, and Being: Unraveling Stress.

For nearly two decades, the Center for the Study of Traumatic Stress, under the leadership of Dr. Bob Ursano, has done an amazing job bringing together world-leading experts here at the Amygdala Conference. And while the format of our conference has changed over the years from an in-person conference now to a virtual forum, its impact and commitment to excellence is really without rival.

The insights that you'll hear today from our experts span from foundational research to practical application. It drives forward our understanding of and the treatment of stress and PTSD.

Today, we look forward to the rich presentations and discussions that challenge us to think about the next milestones in our field. Ask questions like, "What are the cutting-edge research and applications in our discipline?" These questions and more I'm looking forward to exploring with you as we strive to become better clinicians, better researchers, better educators, and ultimately enhancing the care that we provide for our beneficiaries, those who stand in harm's way.

This conference, though virtual, serves as a vital forum for ongoing dialogue that I hope will extend far beyond the session today. You know, the Department of Psychiatry and the Center for the Study of Traumatic Stress are committed to actively engaging our entire uniformed psychiatry community. As a matter of fact, every week, we offer a community-building program. We offer a track that helps us to better understand the operational aspects of psychiatry, helps to promote engagement with the cutting-edge

clinical care resources that are available, and support our community as a whole as we develop into better researchers, better academicians, better educators, and ultimately better leaders within the military health system and beyond. Our community is continually growing, and I encourage you to be part of the group.

Visit our website. Right now on your screen, you'll see a QR code displayed. This QR code will lead you to our external website. This site is really a hub of information and of community activities that are available to you. By clicking on the Connect With Us button, you can join our mailing list, receive monthly newsletters that are filled with valuable information that are delivered straight to your inbox. I promise to not spam. And you can always unsubscribe at the bottom of the newsletter if you don't find it helpful, but you won't because you're going to love it.

Before I conclude, I must acknowledge the tremendous effort put forth by Dr. Naifeh and

the entire Amygdala committee in orchestrating today's agenda. The coordination of our moderators, the speakers, the poster session really represents a significant undertaking, and their dedication deserves recognition. So thank you so much.

As I mentioned before, I hope that this is just the starting point for your engagement with the many educational and community-building opportunities, as well as a host of resources that are available to you from both the Department of Psychiatry and the Center for the Study of Traumatic Stress. Thank you so much for your attention.

I'm now pleased to introduce Dr. Bob Ursano, Director for the Center for the Study of Traumatic Stress, who will offer his opening remarks. Thank you so much.

DR. URSANO: Thank you, Vin, for the warm remarks and also for introducing many people to the Department of Psychiatry. Vin assumed the Chair of the Department this past year, and for

those of you that don't know him, I recommend you get to know him. He is a scholar. He is a leader, and he is making global impact of the Department of Psychiatry, representing both our military community and our broad community that addresses the question of stressors and mental health.

I want to welcome all of you to this meeting, and thank you to the committee, Jamie, Holly, Rachel, and the entire committee on really putting together an outstanding group. It's a pleasure to be here for what represents the 17th time. Some of you will recall that in the fall of last year, we began a piece of what comprises now the Amygdala approach to the world, and it was titled the Brain, Behavior, and Mind Lecture, a tremendous lecture given by Ned Kalin, the distinguished Editor of *The American Journal of Psychiatry*, which is also available on our website.

The Amygdala Conference has evolved over time, but one element that has remained

central, and will, is to span from the cell to the individual to the community. Our goal is to bring together individuals that look through different lenses at how we are people, individuals, and individuals that can have disturbed function, both brain, psychology, and social relationships. The conference today represents this span and is co-sponsored, as Jamie mentioned, by a wonderful group of organizations and departments here at the university, including the newly-founded by our Dean, the Brain and Behavior Hub, which is a touchpoint for reaching all those involved in trying to understand the psychology and brain function associated with being and our disruptions of being.

As we talk today, we will again work from the cell to the community. We'll address who we are, who at times we become, and of course, who we want to be. We will look at disorders. We will look at healthy function. We will try to understand social relationships, a core component

of our health, and perhaps the strongest predictor of our health across all studies ever done.

Another lens of importance within our world at the Center for the Study of Traumatic Stress and, therefore, the Amygdala Conference, which really represents brain, behavior, and mind, is to remember events happen, and we respond to them. That's the core perspective we operate in. PTSD is just one of those. An event happens, and our brain and behavior respond to it. Adjustment disorder, panic disorder, depression—at least early elements of depression—are similarly tied to events in our world. Understanding how an event in the world transduces into our brain function and then into our behavior and mind is what the central task is with the Amygdala Conference.

I often remind people in our own group that one of the pleasures of being in the Amygdala Conference, and in our own work at the Center, is to hear things you know absolutely nothing about

and to leave with perhaps three new words that you can Google to understand. It's a pleasure we don't often take because of our focus on the world in which we are doing most of our work at any given time. But here is the chance to, in fact, enjoy the opportunity to visit a different world. Visit the world. Don't expect to understand all of it, but take away some new piece that allows you a different lens, something new to wonder about, something new to think about.

There's a point at which we will have panels, and in those panels, the corollary to what I said is, remember, there is no stupid question. We once had a wonderful seminar in which we brought together our clinicians with our basic scientists, read a textbook of molecular biology and a textbook of psychology, and realized we had very basic questions to ask each other. And it was such a pleasure to have such discussions in which we engaged, realizing we were learning from each other. I hope that will also occur today for all of you.

It's a marvelous conference and, again, a tribute to the panel to recognize that over nearly 2,000 people have registered for the conference and representing 70 countries. The breadth and reach are truly phenomenal. It is a chance for us to join as a nation and globally to try and understand better our brain, behavior, and mind. I also look forward to and invite you to watch for next year as Jamie, Holly, and the committee and the Center think through perhaps a new tagline for the Amygdala Conference, maybe something related to brain, behavior, and mind. So stay tuned in the next year, you may see 'now-known-as.' I'm glad that you have joined us, and I look forward to the presentations.

Back to you, Jamie.

DR. NAIFEH: Thank you, Dr. Ursano.

Our first speaker today is Dr. Joseph LeDoux. Dr. LeDoux is a university professor and Henry and Lucy Moses Professor of Science at New York University, where he directs the Emotional Brain Institute. His work is focused on the brain

mechanisms of emotion, memory, and consciousness. He is an elected member of the U.S. National Academy of Sciences and the American Academy of Arts and Sciences. We'll now begin Dr. LeDoux's presentation, which is based on his latest book. The presentation is entitled, "The Four Realms of Existence: A Fresh Look at the Science of What and Who We Are."

DR. LEDOUX: So, thank you very much. It's a pleasure to be here. I'll be talking about a new book that I wrote that came out in October. It's called *The Four Realms of Existence*. It takes a fresh look at the science of what and who we are.

So many of us understand that the mental aspect of who we are is embedded in the part of the body known as the brain and, therefore, it is also part of our physical bodily existence. Still, even true believers of the physical nature of the mind sometimes feel as though it possesses some quality or qualities that are lacking in other physical systems within

our body and even within our brain. Just as your mind depends on your brain, your brain, being part of your body, depends on the life-sustaining functions of other components of your body. If your heart stops beating or your lungs collapse, all of your other organs, including your brain, will soon cease to function in a way that is compatible with life. Without bodily life, there is no brain function, and without brain function, there is no mind.

So how, then, out of all of this biological physicality do we each come to exist as a being that knows it was born in the past, knows that it exists now, and knows that it will someday die? The standard approach to such questions about individuality is to focus on psychological notions, like the self or personality. These have long guided philosophical musings as well as scientific theories and research about what it means to be a human being. But there's little agreement about what self and personality refer to and even

whether they refer to real entities as opposed to just being shorthand labels for a variety of psychologically interesting phenomena.

The philosopher David Hume referred to the self as the elusive I. Another philosopher, Shaun Gallagher, said, despite the comfort level of the field to talk about the self, what is usually said is controversial. Daniel Dennett: the self is an illusion. Harry Stack Sullivan, a personality psychologist in the mid-20th century, said, personality is an illusion. Walter Mischel, a personality psychologist, said, personality is a myth. Owen Flanagan, a philosopher, said that just because the self is in our vocabulary does not mean it has any explanatory value or role. And Thomas Metzinger said, no one ever had or was a self. And I say, your self is not a thing; it's a story about who you are.

Scientific discoveries over the last several decades from diverse fields, such as neuroscience, immunology, genetics, and

artificial intelligence have led to new ideas about human beings existing as physical systems. These findings and challenging, cherished assumptions about human nature have resulted in an epistemological vacuum. In no small part, this is because thinking about what and who we are has not advanced significantly beyond traditional ideas, some put forth in ancient times. What if our constructs are inadequate as conceptual hooks on which to hang the empirical findings have been discovered in the name of self and personality? Because these centuries-old notions obscure as much as they reveal, the phenomena might be better served by a new conceptual home, one grounded in contemporary scientific conceptions and empirical research.

A human being can be characterized as a composite of four fundamental, parallel, entwined realms of existence that reflect our evolutionary past and account for our present ways of being. These are biological, neurobiological, cognitive, and conscious. All

four are deep down biological, but the neurobiological realm transcends the mere biological, the cognitive transcends the mere neurobiological, and the conscious transcends the mere cognitive. They coalesce as an ensemble of being, and together, the four realms and the resulting ensemble account for what and who we are, including those aspects of us that fall under the rubrics of self and personality.

Now, the four realms is not about reduction of higher levels to lower ones, something that was popular in the mid-20th century where complex groups, things like social groups and multicellular things, could be reduced to cells, molecules to atoms, and atoms to particles, and so on. That's not what we're talking about here. Instead, we're talking about how symbiotic interactions between the levels sustain the organism. The biological existence enables the neurobiological way of being, but the neurobiological also feeds back to the biological and helps it. The cognitive evolved from the

neurobiological, and it feeds back and helps the neurobiological. And the conscious evolved from the cognitive, and it helps the cognitive.

So, each realm is anatomically permeating and physiologically enabling the level above it, and at the same time, the survival potential of the level below is enhanced by the one above. The realms are somewhat resemblant of components of a Russian doll, but unlike a Russian doll, in which the parts simply stack on top of one another, our component realms are conjoined, integrated, and interdependent. Everything about an individual human being, biologically and psychologically, is subsumed within these entwined, nested, hierarchical realms of existence. A case could be made that society and culture are additional realms, but I believe these are things we do with our cognitive and conscious realms.

It's relatively easy to separate biological beings from non-living matter. If something is alive, it's a biological being.

Deciding which biological beings exist neurobiologically is also straightforward. If an organism has a nervous system, it's a neurobiological being and, by definition, it exists neurobiologically. That narrows things down to animals, or at least most animals, since only animals have a nervous system.

When it comes to the cognitive and conscious realms, things are considerably more complex, since there are no equivalent physical properties that unequivocally identify which organisms are cognitive and conscious beings. We have to rely on behavioral criteria, which can be tricky, since different scientists define cognition and consciousness differently. My goal is less about identifying which animals are cognitive and/or conscious. It's instead about trying to understand how our four realms interact and make humans what and who we are.

So here we see on the bottom left, the brown blob there refers to all organisms, because all organisms are biological organisms or

biological beings. All organisms exist biologically. Some organisms also exist neurobiologically. In other words, they have neuro control. Biological existence is more about metabolism and replication, reproduction. Neurobiological is neuro control of these capacities. All animals have this capacity, but not any other organism has this capacity. Mammals and birds and perhaps some other animals are cognitive animals, cognitive organisms, and they have this capacity by virtue of being able to create mental models of the world. And I'll explain what that is in a minute.

And then some of these are conscious. We know that humans are conscious, but it's a little hard to know whether other animals are conscious. But again, that doesn't really impact what I'm saying here, because what I want to understand with this perspective, this framework, is what it's like to be a human being in terms of having all of these different kinds of realms. So again, there are codependent, entwined realms

where the lower part enhances the one above it, and the one above enhances the one below it.

So, let's start with the biological realm. This is referring to the life-sustaining capacity of bodily functions. So, life can be divided into individual survival and species survival. Individual survival depends on metabolism. Species survival depends on replication or reproduction.

The entwined realms can be thought of in the context of evolution. A biologist, Leo Buss, a number of years ago pointed this out. New organisms not only possess novel features, but also retain the features of the group they diverge from. New or newly-changed features often become the primary target of natural selection going forward. The basic life-sustaining physiological functions that have been repeatedly tested for their survival value tend to change relatively little in the evolution of new species. More often, the changes involve new processes that control the way the organism's

particular kind of body interacts with its environment in supporting metabolism and sustaining life.

So, the new processes come with new body types. You know the difference between a rat and a cat and a dog because they have different body types. They're defining external physiological features, and they do things differently. I mean, let's take the difference between a walrus and a bat and a human. So, the walrus swims away from danger. The bat flies away, and the human walks or runs away from danger. It all depends on the kind of body type that you have, and these are new things that are evolving with new species.

So here, we see kind of the long history of life and its development of new kinds of features. So, at the very bottom, we have unicellular prokaryotic cells. This would be, like, a bacterial cell. It has no sequestered DNA. The DNA is just free-floating inside the cell membrane. From prokaryotes, eukaryotes

evolved, and they differ from prokaryotes in that the DNA is sequestered within a membrane, and they have a metabolic system composed of mitochondria that allow metabolism to take place and replication to take place.

Now, as you get away from single cell eukaryotes, for example, like, a protozoa or an amoeba, those are all single cell, and go to multicellular organisms, you see some are very simple where they have a few cells within their overall structure. But as you get into even more complex ones, it's not just about cells within the structure, but also cells within tissues within the structure. So, all of these are showing how the new evolves from the old. You start with a prokaryotic cell with no sequestered DNA. Then you get sequestered DNA and mitochondria and on and on.

So now we'll go to the neurobiological realm. This is about physiological and behavioral control by a nervous system. Alfred Sherwood Romer, a prominent comparative anatomist

in the mid-20th century, said this: In many respects, the vertebrate organism, whether fish or mammal, is a well-knit structure. But in other respects, there seems to be a somewhat imperfect welding, functionally and structurally, of two distinct beings. One is an external somatic animal, including the flesh and bone of our body, our ability to move in the world externally and interact with the outside world with our body. And the other is an internal visceral animal, basically consisting of the digestive tract and its appendages, which to a considerable degree conducts its own affairs and over which the somatic animal exerts but in incomplete control. So, this is a typical partition of the nervous system into the central and peripheral division. Those are very commonly talked about in neuroscience, and you can take Romer's somatic and visceral components and put them within the central and peripheral nervous system.

But Romer had a different idea. He partitioned it quite differently. He said that,

okay, here on the left, we have the traditional view. The CNS and the PNS contain somatic and visceral functions. But Romer's view is somatic and visceral are the fundamental distinctions within the nervous system, not central and peripheral. And the way he came up with this is that he was able to trace back the history of somatic and visceral functions all the way beyond the beginning of a central nervous system. For example, jellyfish have no central nervous system. They're basically all peripheral nervous system, and yet they have somatic functions that allow them to go through the water and touch things and move away from them and so forth. And they have visceral functions for digestion and other metabolic needs that keep the organism alive. So, it's a revolutionary kind of difference that he's proposing and something I really think is quite important.

So here we can see how this all started, going back all the way to unicellular protozoa. They have visceral functions in their

body, and they have their external body as well. The protozoa move through their environment, single-cell organisms. They don't, like, exactly swim, but they have some flagella that allow them to move around. And when they come into contact with food, they incorporate that from the outside to the inside into the viscera and digest it and so forth. So, they're able to move around, digesting food and avoiding danger. If they run into toxic chemicals in the water, they move away from it, so they have a somatic and a visceral component to their body. Early animals, the somatic and visceral are there because early animals evolved from protozoa. And in addition, they developed a nervous system that could control the somatic and visceral functions of the body.

And then bilateral animals came along. This is about 630 million years ago, a flat worm, like the early animals we're talking about, like jellyfish, that have a top and a bottom but no left-right and front-back. Bilateral animals

have a top and a bottom but also a left and a right and a front and a back. And in the front was a concentration of neurons of what we would now call a brain that is exerting central control over the peripheral nervous system in both the visceral and the somatic domains.

So, through natural selection, the visceral and somatic functions of the body of unicellular protozoan ancestors were carried forward in early animals and their peripheral nervous systems and into the CNS as animals diversified. So, a defining feature of the neurobiological realm in its responses is stimulus-elicited automatic neural activities, talking about sensorimotor integration in relation to reflexes, motor programs, instincts, fixed-action patterns, Pavlovian-conditioned responses, and stimulus-response habit learning. These are all automatic, no cognition required.

Now, something I've worked on for a long time is the amygdala in its role in controlling visceral functions, sort of, for

example, heart rate and blood pressure. And I haven't studied digestion and so forth, but it also is involved in that. And then, on the other side, these somatic functions, like freezing and flight. Now, I didn't realize this when I was making this diagram many years ago that Romer had this exact partition in mind. So, you know, I think the kind of work that we've done on the amygdala has, in effect, verified Romer's hypothesis.

So, one of the points that I emphasize in my work, and have for quite some time now, is these circuits are not fear circuits. They don't make conscious fear. They detect and respond to innate and learned threats with pre-programmed motor reactions. Fear is the conscious awareness of being in harm's way, and we'll talk about that in a little bit more.

So, this is a circuit for stimulus-response learning, for example, let's say, in a reptile. And what you see is that there's stimulus input for the side of food that goes to

the sensory cortex and then to the basal ganglia. The taste of food is then a reinforcing signal that goes back to the basal ganglia, and it stabilizes the sensorimotor connection between sensorimotor neurons and the basal ganglia. And as a result, you then have a stimulus-response habit that you can repeatedly use, again, all without cognition.

Okay. The cognitive realm. What are we talking about here? We're talking about mental models that make predictions based on internal representations. So, my definition of cognition is the capacity to construct mental models of the world and to use these in thinking, planning, deciding, acting, and feeling. Now, the whole idea of mental models largely comes from Kenneth Craik in 1943 in his book, *The Nature of Explanation*. In it, he said, if the organism carries a small-scale model of external reality and of its own possible actions within its head, it's able to try out various alternatives, conclude which is the best of them, react to

future situations before they arise, utilize the knowledge of past events in dealing with the present and the future, and in every way to react in a much fuller, safer, and more competent manner to the emergencies which face it. Now, this is, I think, the clearest definition of a mental model that has ever been proposed.

But there's a twist to it that Nathaniel Daw and colleagues added in the 1990s by borrowing an idea from machine learning, which was in machine learning, you can have model-based or model-free learning. Now, in a machine, it's not cognition. It's just the ability to have more complex kinds of processing as opposed to not having a kind of complex processing and more of a stimulus-response thing in the machine. But in terms of what I've been talking about, the model-based kind of processes that Daw and colleagues were talking about is exactly what the cognitive realm does. In the model-free, the neurobiological realm is what it does. And model-free is using automatic processing of a

stimulus to produce a response. The model-based cognitive realm is using an internal representation to make decisions and predictions and so forth.

Okay. Here's the reptilian basal ganglia capacity again. So, with the emergence or the evolution of mammals, things happened. So, Eric Kandel once noted that the evolution of novel behavioral circuits often involves changes in existing circuits. This is very consistent with Buss's idea that the new evolves from the old. So, in Kandel's work in the Aplysia, an invertebrate organism, he discovered that the circuits for Pavlovian conditioning are very, very similar to the circuits for sensitization. And so, sensitization is non-associative; Pavlovian conditioning is associative. So, it only took a small molecular change in those circuits to allow Pavlovian conditioning to evolve.

So, as Buss said, you start with something that's passed the test of time, and if

you're going to do something new with it, all you have to do is change it a little bit in order to achieve something new. And in mammals, that's what we've got here. We ended up with very complex circuits, not just in a sensorimotor cortex, but a set of medial prefrontal cortical areas and some new areas of the basal ganglia that could integrate body states and reinforcers and all of that stuff in a much more complex form using a mental model.

Okay. The conscious realm, subjective experience. So, is conscious a scientific mystery? Yes, in the sense that the mechanism's underlying biological inheritance or bacterial infections were mysterious before they were figured out. If something is not physical, then it's not a scientific problem. If it's physical, it's a scientific problem that can be potentially solved. So, we don't have to put in a lot of mysterious, oh, consciousness is all this ghostly stuff. We'll never understand it. The scientific pursuit of what it feels like to be

conscious should not be limited by philosophical concerns about such feelings being too hard for science. If you make subjective experiences or what philosophers call qualia non-physical, you are making them non-scientific and setting up the scientific problem in a way that can only lead to failure.

So, William James had this to say once long ago: Our reasonings have not established the non-existence of the soul. They've only proved its superfluity, in other words, its unimportance for scientific purposes. So, this may be the same. This may well be true of dualistic and panpsychic notions about qualia. If you claim that those are non-physical things, that's fine as a philosophical idea. But it's a non-starter as a scientific construct. If it's not physical, then it's not something we can deal with.

So, there are different kinds of ideas about what consciousness is. The most basic is creature consciousness, the condition of being alive, awake, and behaviorally responsive to

environmental stimuli. This exists absolutely in all animals. It's what's missing when a person is in a coma and they're unresponsive to the external world, but this is not the kind of consciousness that deserves the word consciousness in the mental state sense, and that's what I'm more interested in is the mental state consciousness, the capacity to experience the world and one's relationship to it. And that only exists in some animals. We don't have to say which ones they are because, again, I'm more focused here on what's going on in the human brain.

There's another thing called sentience, which may exist more widely in the animal kingdom. It's hard to know because we don't know what's going on in another animal's mind. But there are a lot of different definitions of what sentience is, and I'm not going to be talking about that at all. I'm focusing on this mental state consciousness that we all know we have, this sense that this is your

mind. This is your body. It's not belonging to someone else. So again, it's the capacity to experience the world and one's relationship to it. It only exists in some animals. I'm not going to make a big statement about which animals it exists in. The phone would start ringing or the emails would start flying if I said that because I would offend someone somewhere along the line.

Okay. So, a lot of research and theories about mental state consciousness focus on two brain areas, really, and it's almost always focusing on visual consciousness. You know, the visual system is a favorite system for research and neuroscience because it's been studied so thoroughly, and a lot is known about it. So, in the study of consciousness, Francis Crick and Christof Koch in the 1990s said, well, why don't we start with visual consciousness, since we know so much about the underlying mechanisms of vision? So, I think that was a pretty good idea.

So, here's visual cortex. Here's lateral prefrontal cortex. And the basic idea is if you're a first-order theorist, then visual cortex is all you need to be conscious of a visual stimulus. But if you're a higher-order or a global work-based theorist, you propose that first-order states are not sufficient. You need some kind of prefrontal representation to allow the experience of the stimulus.

Now, this is pretty simplistic, right, for something so complicated as consciousness. So, what I've been trying to do is to add some anatomical substance to all this and kind of rethink how this all might be working. I don't know how many of you in the audience know this, but I did my PhD in the 1970s studying split-brain patients and their conscious experiences, so I've been thinking about consciousness ever since graduate school, and I've written quite a bit about it in my books and review articles over the years. So, it's not something I just decided at this ripe old age I had to jump into and start

fooling around with.

So, my goal here is to suggest a kind of neuroanatomical upgrade to the study of consciousness. So, in real life as opposed to in psychophysics labs where a lot of consciousness research takes place, you know, can you see this dim light, or can you see this dim light? Maybe you need a little broader light to see it. So, in real life as opposed to in psychophysics labs, a conscious experience is typically multimodal. You don't have local vision conscious states in real life. There's all kinds of stuff. There's sights, sounds, all kinds of things going on. So, it involves a variety of lower-order inputs, not just a visual cortex input. Furthermore, the PFC circuitry is far more complex, and its inputs are much more diverse than it's typically acknowledged in the field of consciousness research.

So here we go. On the top right, you see some sensory circuits. We got visual cortex, auditory cortex, somatosensory cortex. They're

all giving sensory inputs to dorsolateral and ventrolateral prefrontal cortex. But in addition, we've got all this memory and conceptual circuitry that is also doing that. So we've got the medial temporal lobe memory systems. They're going to dorsal and ventrolateral prefrontal cortex a bit directly, but they also have some indirect inputs that I'll tell you about later.

In addition, the temporal poles, peri-temporal sulcus, parieto-occipital junction, all of these are highly multimodal brain areas that are either involved in the formation of memories or in the integration of stimuli and other kinds of higher-order states. And those are going to the dorsolateral and ventrolateral prefrontal cortex. There's a lot more than just visual cortex going there.

Now, in addition, though, there are a lot of prefrontal areas that are going there. So we can take what I'll call subgranular prefrontal cortex here. That's areas of prefrontal cortex

that lack a granular layer. And in that group, we have the orbital frontal cortex and the ventromedial. Now, both of these receive information from the medial temporal lobe. So, the medial temporal lobe, hippocampal, perirhinal, entorhinal circuitry, in addition to projecting directly to the dorso- and ventrolateral prefrontal cortex, project to the orbital and ventromedial, which in turn project to the dorso- and ventrolateral prefrontal cortex. And one of the things that the ventromedial does in particular is to unpack schema that are created through the hippocampus and present those schemas as templates in the dorso- and ventrolateral prefrontal cortex that become the kind of foundation with the conscious experience you're having. Anterior cingulate is another one. But in addition, we have these granular prefrontal areas that involve the frontal pole and the dorsomedial prefrontal cortex. So, there's a lot of information that is being integrated in these lateral prefrontal

areas that allow the kind of complex information integration that would be required for a multimodal conscious experience in real life.

So even if this isn't the answer to how consciousness comes about, it's a necessary kind of circuitry that is somewhere in that chain of events that is going to contribute to complex conscious states. So here on the left, we've got these granular prefrontal areas, the dark gray ones, and the APM is the anterior premotor area. DL is the dorsolateral prefrontal; VL, ventrolateral; FPL, frontal pole lateral; OFCL, orbital frontal lateral. And then we've got some granular areas creeping around into the medial prefrontal cortex, like the dorsomedial and the medial frontal pole. Then you have these lighter gray areas, anterior cingulate, prelimbic, orbitofrontal, ventromedial that are dysgranular. Now, the dysgranular make up what's called the medial prefrontal cortex, but only part of it because, you see, there is some dark gray areas there that have granular prefrontal

cortex, and that is a common misconception in the literature where it's often assumed that if it's medial, it's all dysgranular or agranular.

Now, what's interesting about all this is that the dysgranular, agranular, whatever you want to call it, light gray areas are present. If you look on the right side, are present in all mammals. The dorsolateral, ventrolateral areas are present in all primates, and the lateral frontal pole is present only in humans, or at least I should say a component. The black component in the middle of the lateral frontal pole is only a human kind of structure. So, what this suggests is that perhaps the special kinds of features of human consciousness might be related to this lateral frontal pole, whereas the special kinds of primate consciousness might be related to these dorso- and ventrolateral areas, whereas the all-mammal consciousness might be related to these medial prefrontal areas.

Now, this is not proven in any sense. It's just like a hypothesis that gives us

something to think about and something to research. If we knew more about how human consciousness works in the human brain, and if we found that this lateral prefrontal was especially important for certain aspects of higher-level, higher-order kind of human consciousness, it might really help us begin to understand how to think about other animals and their consciousness.

All right. I'm going to skip this because it's getting late. So, what I've been talking about is a multi-state hierarchical framework. This perspective replaces the traditional volley between sensory cortex and the lateral prefrontal cortex with a more complex anatomical arrangement, consisting of a hierarchy of structures, each of which creates different kinds of states that are re-represented or re-described by circuits of the prefrontal cortex and that contribute to mental modeling and conscious experience. I've often talked about this connectivity in relation to the higher-order

theory of consciousness because I'm a fan of that, but it's relevant to any and all theories of mental state consciousness in the brain, I think. So, it's more of a higher-order anatomical framework than a higher-order philosophical framework.

Okay. I want to go back to something that I mentioned earlier, that I did my PhD studying split-brain patients. Now, what we have here is a patient in whom the connections between the left and the right hemispheres have been sectioned in an effort to relieve intractable epilepsy. This was done in the '60s and '70s much more than it's done today, because the medications were not so good back then. But now the medications are better at relieving the epileptic seizures.

But we were interested in the psychology of all this, so we're showing these pictures. And in this case, we've got a snow scene on the left side of the screen and a chicken claw on the right side. Now, everything on the

left side of the screen goes to the right hemisphere; everything on the right side goes to the left hemisphere. Now, the hands then point out to the thing that the hemisphere saw. So, the right hand connected to the left hemisphere saw a chicken claw and pointed to the chicken. The left hand connected to the right hemisphere saw a snow scene and pointed to the shovel. All makes sense.

But now we ask the patient, why did you pick those? Now, when we do that, we're talking to the patient's left hemisphere. That's where the language circuits are in most humans and in this patient in particular. So, the left hemisphere response is, well, I saw a chicken claw, so I pointed to the chicken, and you need a shovel to clean the chicken shed. So, the left hemisphere knew nothing about the snow scene. It simply made up a story that made the chicken make sense with the shovel. And this kind of narration we felt was not something that was like a consequence of the surgery so much. It's not

just a neurological confabulation. We felt it was something that we humans do all the time. We act, and then we justify what we did because we have so many unconscious control systems in our brain that are producing these neurobiological realm responses. And we all kind of believe we have free will, and I know a lot of people who are challenging that concept. But most of us believe we have some kind of free will.

So, it's disturbing if your behaviors are not under your control. And so, the idea that Mike and I had was that one of the things that the human brain has evolved is the ability to tell stories, to generate narrations that make our behaviors, our unconsciously controlled behaviors, make sense. And, you know, one of the things I was particularly interested in was emotional consciousness. And so, when I stopped doing split-brain research, I turned to the study of emotion in rats because there were no good techniques for studying the human brain back then. So that's how I went from consciousness to

emotional behavior in rats, and then from emotional behavior in rats, I was always talking about consciousness in my books and review papers.

So, I hope I've shared some interesting things with you today about the four realms of existence, the biological, neurobiological, cognitive, and conscious, and shown you a little bit about how consciousness might work in the brain. So, thank you very much. It's been a pleasure to be able to deliver this lecture to you.

DR. NAIFEH: Okay. Wonderful, thought-provoking presentation. Thank you, Dr. LeDoux.

Our next speaker today is Dr. Karen Parker. Dr. Parker is Professor and Associate Chair of the Department of Psychiatry and Behavioral Sciences at Stanford University, where she leads the Major Laboratories Steering Committee and directs the Social Neuroscience Research Program. The principal goal of her

research program is to better understand the biology of social functioning across a range of species and to translate these fundamental insights to drive diagnostic and treatment advances for patients with social impairments, with a core focus on autism spectrum disorder. We'll now begin Dr. Parker's presentation, which is titled, "The Role of Neuropeptide Signaling Pathways in Social Impairment."

DR. PARKER: I wanted to begin my talk by thanking the organizers. It's very exciting to be here today. So today I'm going to be talking about a translational autism research roadmap that my lab has created over about the past 10 years, and we'll provide a brief overview of the autism landscape and then talk about the animal model work that we've done with the goal of creating a valid animal model to identify biomarkers of social impairment as well as targets for treatment, and then the work that we've done to translate the findings from this animal model to people with autism. And then,

I'll talk briefly about how we've used this biomarker work to run a first-in-class phase 2A treatment trial to target social symptoms in children with autism.

So, for those of you that are not familiar with autism, autism is a significant public health problem. The autism prevalence rate has increased 417 percent since 2000, and last year, the CDC came out with new statistics. So, in the U.S., autism now impacts one in 36 U.S. children. Autism is comprised of two core behavioral symptoms that are diagnosed based on expert clinical opinion and guided by DSM-5 criteria. And so, these include persistent impairments in social communication and then also the presence of restricted repetitive behaviors. Autism is male-biased in prevalence. It impacts about three to four males to every one female. The social symptoms are widely viewed as the most debilitating feature of autism. Autism is highly heritable, but yet the disease mechanisms remain poorly understood.

One thing I wanted to point out is that if you've seen one child with autism, you've seen one child with autism. So, symptom onset can vary widely, but we do know that by about 24 months of age, autism can be reliably diagnosed. The diagnosis is behavioral, and because the biology of autism is poorly understood, unlike other medical conditions, we don't have a laboratory-based diagnostic test. And so, any child that is showing concerning symptoms needs to see a trained expert. Unfortunately, those experts are in short supply, and so long clinic wait times of 18 months are routinely encountered. And so, the average age of diagnosis for autism in the U.S. is not 24 months, but rather four years. And the mean age of diagnosis is much higher in either rural areas or areas that lack these specialists.

Currently, autism has only behavioral therapies, which are variably effective. They can be extremely expensive, depending on the state of residence of the family. And there are

only two FDA-approved medications, which are two antipsychotic medications that target associated features like irritability, and there are currently no medications approved by the FDA to target core autism features. One thing I want to point out is that this lack of medication options for autism stems from a drug development crisis more generally, particularly for central nervous system or brain disease. So, we know that over 90 percent of central nervous system medications fail, so if they were effective pre-clinically, that they fail in human clinical trials. And 50 percent of these medication failures are attributable to poor animal model selection in the preclinical phase.

So, this suggested when we entered the autism field that we urgently need better animal models, but what constitutes better. And so, what we saw was this opportunity to develop, you know, some of the first valid animal models for autism that would hopefully yield high translational potential. And so, when we started

out in the field of thinking about animal model development for autism, we evaluated various validity criteria. So first, we wanted the onset to be neurodevelopmental, so the symptoms should emerge early in childhood or the developmental period in an animal.

We wanted to establish face validity, which is that there's an outward similarity in appearance between the model's attributes and a patient's symptoms. So, in the case of autism, we would want complex social cognition deficits in a highly social diurnal species, with vision as its primary sensory modality. We also want to establish construct validity so that we would have similarity to the underlying cause of the disease in both the animal model and in the patient population. And historically, animal models for autism had involved models that lack construct validity, so large, gross brain lesions or in the case of peer rearing, taking baby monkeys away from their families and rearing them in isolation. And so, what we wanted was to be

able to establish construct validity, and we then would want homologous, evolutionarily-conserved genes and circuits.

We also want predictive validity. So, we want the model to be able to identify and evaluate drugs for therapeutic safety and efficacy. And there was a cautionary tale of a drug called thalidomide, which was a drug that was supposed to treat morning sickness in pregnant women. It was tested only in rodents, and then it was rolled out into the human market. And it caused significant limb deformities. And when the drug was reevaluated in several species of non-human primates, the toxic effects of thalidomide were revealed. And so, we really want to make sure that our animal model is appropriate for both safety and efficacy, and we also don't want to treat what I'll call neurotypical animals. We want the animal model to have the behavioral or neural features that are shared with the patients, and then we want to deploy the drugs in that manner.

We also want to think about welfare. So, in the case of autism, we want to make sure that our study species has complex social housing and also species-typical enrichment, so that if we are identifying spontaneously occurring social impairments, we want these to be naturally occurring in origin. And to me, as we started out on this path, all of these key criteria pointed to the value of developing a non-human primate model for autism.

So then when we were thinking about points of entry, there's various points of entry when we're constructing an animal model. We can do a genetics-first approach where we could identify evolutionarily-conserved genetic variants. We could do selective breeding. We could also do gene editing, which at the time didn't have sufficient efficiencies to make that a rapid or cost-effective approach. We could also do a behavior-first approach where we try to screen in a large colony for naturally-occurring behavioral features. And then also, either in

parallel with these other approaches or sequentially, we can do biomarker discovery and translation, and so we could then validate some of these biological markers that are shared by the animal model and the patients of interest. And so, we decided to do a behavioral-first approach and then follow this up with this translational biomarker discovery strategy, and I'll outline this in the coming slides.

So, when we started thinking about the way to conceptualize naturally-occurring low sociality, we looked to the human genetic data in autism. And so, although we hear a lot about single-gene causes of autism that are highly penetrant, but they're also very rare, the vast majority of genetic burden in autism is polygenic inherited, meaning that there are a lot of low-penetrant variants that interact to create this autism phenotype.

What was very interesting to me, as we were thinking about how to approach this animal model, was that there was evidence from twin

studies in the general human population that autistic traits are continuously distributed across the general population, and that when we get to the tail of the distribution, that's when we start seeing autism diagnoses. And so, the thinking was that this polygenic-inherited trait burden underlies these phenotypes. And so, the idea for me when we set out was to develop methods to identify and study monkeys at the quantitative extreme of social functioning that would resemble people that are at the quantitative extreme in the general population. And in humans, we diagnose these individuals with autism.

And so, here's our model validation and translation strategy. So, this is called the Social Responsiveness Scale, and this is just for illustrative purposes. So, in humans, you can see that this quantitative autistic trait burden scale, with the further to the right you are, the more autistic trait burden you have. Both in humans and then in rhesus monkeys, you can see that these distributions are similar, and so the

idea was to take an instrument from people and then to reverse translate it to monkeys. And then what we could do is then use this model as a way to identify biomarkers in our monkeys and then forward translate them to patients. And I think I've already showed my hand a bit here that we were going to be studying rhesus monkeys, but I'm going to provide the rationale for this.

So up here, we have an evolutionary map. And you can see that I've worked with new world monkeys before, but that old world monkeys are the closest species of animals, rhesus in particular, that are some of the most recent relatives to humans outside of apes. And they are also a common lab animal. And so, we decided to study rhesus monkeys for the following reasons. They're highly social. They have demonstrated complex social cognition abilities in what we'll call neurotypical animals. They show vision as a primary sensory modality, and a lot of the social cognition impairments in people with autism are focused on vision. So, this was

important to me as we developed this model. There was also evidence over many decades that rhesus monkeys exhibit stable individual differences in social behavior, and there was some initial evidence that animals show these spontaneous, naturally-occurring social deficits that might have relevance to autism.

And so, I like to joke that Stanford wouldn't let me have thousands of monkeys on campus, and so I took the show on the road about 100 miles away to one of the national primate research centers, which is hosted by UC Davis. There, there's over 4,000 rhesus monkeys. Most of them live in these large, outdoor, half-acre field corrals. So, you can see this is a depiction of one of the field corrals. By studying a population that had this many animals, we could hopefully identify more monkeys at this social extreme. And one thing that I really liked was that these animals have really rich social environments and also that they live under ecologically-relevant conditions of mixed

male/female social groups. And we could study all ages, including infants to elderly, across the lifespan.

So, over the years, we've identified three different ways to spot these monkeys with naturally-occurring social impairments, and I had two terrific postdocs that led this work. And what I want to point out here is that all of these measures are highly correlated with one another, even though they use different methods. So, the first method, and this is one that we'll talk a lot about today, is simply focal animal sampling. So, what we can do is use rhesus monkey ethograms where we could quantitatively measure different features of social behavior. But what we found to be the most robust was simply creating what we call the non-social index, the amount of time a monkey spends alone not interacting socially. And we believe this to be fairly important, because rhesus monkeys are so social.

And then I had mentioned this previously, what we did was we took the Social

Responsiveness Scale, which is used to measure social behavior traits in a quantitative way, but also autistic trait burden, and this is a scale that's often used in clinics to identify children for clinical referral for an autism work-up. And we were able to take this scale and be able to modify it for use in rhesus monkeys. And then we had a variety of different laboratory-based tests where animals would come in and do computer-based tasks. So, we had a variety of different assessments, like face recognition, and can animals respond to appropriate social cues? So, if an animal is affiliative, do they respond affiliatively? If an animal behaves aggressively, do they gaze avert, right? So, we had a variety of different laboratory-based tests to be able to think about the type and kind of severity of social impairment. And so going forward, we classified animals as being either naturally low social or socially competent intact or high social animals on this non-social behavior index. So, we'll just call them low

social and high social monkeys going forward.

And so, I'm going to briefly summarize a very large body of past and ongoing work. So, what we were able to document is that these low social monkeys have behavioral features relevant to human autism, and so this was our effort to establish face validity. So, in these low social monkeys, they have a greater burden of autistic-like traits. They show abnormalities in the lab in species typical perception reaction to social stimuli, including face recognition deficits, but not object recognition deficits, which is also consistent with at least some studies in people with autism. When we unobtrusively examine these animals from outside their home corral, they showed impairments in reciprocal social interactions, which is actually a diagnostic feature of ASD.

And in this species, this was characterized by low affiliation and grooming and, interestingly, a decreased initiation but not receipt of pro-social behavior, which is

consistent with one of the theories of autism, which is that at least either all or a subset of individuals with autism lack social motivation. I had a terrific vet student in my lab for a while, and he did a medical record review and was able to show that low social monkeys have greater traumatic injuries and bullying by peers, which worsens with autistic-like trait burden. And the same thing has been shown repeatedly in human autism populations.

And then we were also able to show that there are subtle social information processing deficits in infant monkeys that are about three to four months of age that predict with 100 percent accuracy whether they're going to develop this low social phenotype in adulthood. So, suggesting that this, too, the onset is neurodevelopmental like in people. And then finally, we were able to show that these autistic traits and the low social phenotype is highly heritable, just like in people.

Okay. So, then the next thing we

wanted to ask was how do we biologically detect social deficits? And so, I was thinking, if we could measure biology in fluid that enables direct translation from monkeys to people, right, so things that we can collect in people readily, things like blood or cerebrospinal fluid, as opposed to something like brain tissue. And so most of the biomarker discovery work that had been done in autism was in blood, but it has suffered from poor detection and poor reproducibility.

And so, I became really interested in thinking about autism as a brain disorder, and the fluid that is actually most proximate to the brain is cerebrospinal fluid, which bathes the brain and spinal column. And so, CSF should be more representative of brain biochemistry than blood. And on top of that, there were multiple diseases in neurology where CSF biomarkers were instrumental in being able to diagnose neurological disease, including various forms of dementia, as well as multiple sclerosis. And so

that guided our thinking in terms of how we approach biomarker work in our monkey model.

And so, our first series of studies was looking in monkeys that were two to five years of age, which is characterized by, essentially, early adulthood. And we didn't want to confound our initial studies with development. So, we performed hundreds of unobtrusive behavioral assessments, and we were able to identify these low and high social monkeys. And then we quantified, in this first pass, some targeted CSF and blood-based biomarkers.

And so, in this first pass, we were interested in biomarkers that were either related to mammalian social behavior, had been potentially implicated in autism, potentially through genetics, also neurogenetic syndromes that had high penetrance for a comorbid autism diagnosis. And these included arginine vasopressin and oxytocin. These are two nine-amino acid peptide hormones that have been critical for social functioning in all mammals

studied, as well as two kinase signaling pathways that had been implicated in RASopathies that are related to autism.

And so, when we basically took all of these various measures, and we did a discriminate statistical analysis, with 93 percent accuracy, we could correctly classify low and high social monkeys just based on their biology. But this didn't tell us what are the drivers of this classification. So, then we performed a logistic regression, where we were able to say, well, what are the critical drivers of this classification? And we were able to implicate vasopressin in CSF and two anilides from these kinase pathways that were independently additive in this classification.

So, then what we did was asked, okay, we would expect there to be group differences in a biomarker that was driving these differences. And so, then when we did this further biomarker winnowing strategy, the only marker that was able to differentiate low and high social monkeys on

a group level was vasopressin concentrations in cerebrospinal fluid. And so, it was really important that we replicate this.

And I'm going to just walk you guys through this graph because this has already appeared, but this is probably the cleanest example of it. And then we'll continue to review graphs like this in the human work. So, this is 50 percent probability, and all blue dots above the dash line and all orange dots below the dash line are correctly classified. So, basically knowing just the CSF vasopressin level alone allows us to correctly classify almost every single animal. And this isn't a replication cohort. We again showed a highly statistically significant difference between low and high social monkeys in the vasopressin concentrations.

And then we were able to also show that vasopressin was a robust predictor of time spent in social grooming, which is a behavior that is critical in non-human primates for cementing and maintaining social bonds. And then

finally, as we got more and more interested in thinking of vasopressin as a biomarker of social impairment, we would want to show that it was replicable within individuals. So, on the X-axis here we have individual monkeys and then CSF vasopressin on the Y axis. And we see a large variability across monkeys. We did, I believe, four measurements of vasopressin across a period of time within individuals. And what you can see is in contrast to this large variability, we see within an individual very, very small error bars, suggesting that the vasopressin is a trait-like characteristic, at least in cerebrospinal fluid in this species.

And then a couple other things I wanted to point out was that, you know, oxytocin is a related molecule. And as you can see here and in the subsequent slides, we have not implicated oxytocin in social behavior in this species. But what was interesting is in the very early work, when vasopressin and oxytocin were being investigated in mammalian social behavior,

it was vasopressin that regulated pro-social behavior in male mammals. And this is potentially interesting because it might speak to the sex vulnerability in the prevalence rates that I mentioned earlier in the talk.

So, we have been able to replicate this finding in independent monkey cohorts showing that this is reproducible. And then, interestingly, we have not seen any group differences in vasopressin levels in blood, suggesting that this is brain-specific, and had we only looked in blood, we wouldn't have been able to identify this biomarker. And then, as I mentioned, we've seen no group differences in any of the monkey cohorts in either cerebrospinal fluid or blood levels of oxytocin, suggesting that these findings are specific to vasopressin against the backdrop of a nearly structurally identical molecule.

So, one question I always get is that autism is a highly heritable disorder, and there are now many more than 100-plus autism-

susceptibility genes. Vasopressin is definitely not one of them. And one of the theories about autism is that there are all these autism-susceptibility genes, particularly for this polygenic inherited form of autism, and that maybe what they do is they converge onto several common signaling pathways. And so, one of the questions, and I'm going to raise this and provide a little support for this, although this is something that we're currently investigating in postmortem human brain tissue, so the idea being that if we see this polygenic inherited autistic trait burden in the general human population, is it possible that vasopressin underlies this? And this, of course, would be an extremely difficult study to do in people, but we can actually do this in monkeys using the same reverse translated inventory, right?

So, here's our distribution in rhesus monkeys, and this is actually a biomarker winnowing strategy as well. So, we didn't only just look at vasopressin; we looked at a number

of other biomarkers. And we found vasopressin again, independently, was shown to be the driver. But in this case, the lower your vasopressin levels, the greater your autistic trait-like burden, suggesting that vasopressin could be one of these convergent pathways, and then also suggesting it could be druggable across a variety of individuals.

Okay. So, one of the questions we always got particularly on grant reviews was, who cares? Low social monkeys, it's a non-pathological entity. Maybe this has limited value when we think about autism. And so, the next study that we did was that we partnered with various pediatric clinicians at Stanford and elsewhere to be able to piggyback onto clinical indication for spinal fluid collection. So basically, kids would come into various clinics to have CSF collected as standard of care. And then what we did was we evaluated these samples from kids with autism and kids that did not have autism and had intact social functioning as a

first pass to try to get at this question.

And this is a very small cohort, and again, this is the same graph. So, if we knew your CSF vasopressin concentration, we were able to correctly classify 13 out of 14 kids as having autism or not. So, this was a cohort of seven control kids and seven kids with autism. Again, we were able to replicate this group difference. But the kids with autism, they were sick enough to come in for CSF collection. And so, we were able to partner with Sue Swedo in the NIMH intramural program in one of the largest CSF collections to date.

She was doing work in a large, very well carefully phenotype cohort where the kids were undergoing a research-indicated lumbar puncture, which was being collected not for clinical purposes but for a research study. So, these were kids that were medically healthy. And what we were able to show was that we were again able to replicate this group difference in CSF vasopressin levels, such that kids with autism

had lower CSF vasopressin levels. And we were able to also show that this generalized to females with autism for the first time. And we didn't see, interestingly enough, any differences in oxytocin. And that was fascinating to me, because in female mammals, oxytocin has been more robustly implicated in their social behavior. But in both males and females with autism, what we saw was CSF vasopressin as being reduced.

Because this was such a well phenotyped cohort, we had gold standard research diagnostic inventories on symptom severity, and we were able to show for the first time that CSF vasopressin levels correlated with symptom severity, such that the children that had the lowest CSF vasopressin had the greatest symptom severity. This was specific to the social domain, and vasopressin was unrelated to the other core diagnostic feature, largely restricted repetitive behaviors, suggesting that other biomarkers might be able to be used to increase our ability to detect autism more generally. And

then I just wanted to point out that we have looked in blood in people with autism, and these are children with autism and neurotypical controls. And we've seen no group differences in blood vasopressin levels, again suggesting that this finding is specific to CSF vasopressin level in both our monkey model and in people with autism.

So, the next study that we did was, you know, sort of with an eye toward thinking about vasopressin as being one of these convergent pathways. So, is it possible that this ASD biomarker is evident very early in life, maybe even in the first days to months of life before symptoms first manifest? So, recall at the beginning of my talk that although kids can be diagnosed by about 18 months, autism symptoms are sort of emerging over the first year and a half to two years of life. And so, what we did was, I teamed up with John Constantino, who was at Wash U and is now at Emory. And what we did is he had an archive of newborn CSF samples that

were collected during standard of care. And these are, like the past CSF samples, rule out, meaning that all of the kids that were enrolled in our studies were negative for whatever they were being worked up for. So, in this case, these are newborns that were negative for meningitis, and so it was a relatively healthy population of newborns.

And then, what we were able to do in a quasi-perspective way was to follow these individuals through electronic medical records to see if they received an autism diagnosis in the years to come or if they developed typically, and we followed them for 12 years. And what we were able to show, I think pretty remarkably, was that even before they were showing behavioral symptoms, the low vasopressin levels were already apparent in these newborns for vasopressin, and we saw no difference for oxytocin.

And so, this to me suggests potentially this provocative idea that certainly behaviorally, if we could identify infants that

are at risk for poor developmental outcomes, then we can enact behavioral therapies while kids are still making eye contact, before they begin to develop in an atypical manner where their social learning and social skill acquisition becomes very difficult to overcome. But I think it's tricky, because CSF is very difficult to collect. And although we've never or very rarely seen a relationship between blood and CSF in older individuals, what was pretty interesting was we did a pilot study at Stanford where in neonatal infants that were being evaluated for sepsis, these are all rule-out negative infants, we saw a very tight correlation between blood and CSF vasopressin levels, suggesting that, potentially, blood vasopressin concentration could be used for clinical surveillance, at least in very young children, particularly those that are at familial risk for autism.

Okay. So then just sort of the final part of my talk, vasopressin had really become a pathway of interest for us and to think about

treatment. And so for us, we had several reasons for thinking this might be the case. Vasopressin was a trans-primate biomarker of social impairment. This body of evidence that I've just presented suggested that vasopressin signaling might be impaired in the brains of children with autism. And we knew from some primate work that had been done is that the vasopressin receptor that is usually attributed with the social effects is densely distributed throughout the primate social brain, suggesting that vasopressin administration will target receptors in brain regions that regulate social behavior.

And we knew from a study that was done in people now over 20 years ago that if you give vasopressin intranasally, it penetrates cerebrospinal fluid. And we know from some studies that I haven't presented here that if we give nebulized vasopressin to low social rhesus monkeys, this rescues social abilities in low social monkeys and returns them to neurotypical. So, this body of evidence suggested, does

vasopressin treatment enhance social abilities in people with autism?

And so, I teamed up with Antonio Hardan, who's a child psychiatrist at Stanford and also the director of the autism program here, and we conducted a gold standard blinded randomized placebo control parallel design, where we either treated children with vasopressin for four weeks or treated them with placebo. And our first aim was to just ensure that vasopressin was safe and well-tolerated. And this was assessed by a variety of gold standard monitoring measures, such as study dropout rate, vital signs, a variety of clinical chemistry labs, electrocardiogram, and a clinician-administered to parents side effect questionnaire.

And then we were also really interested in whether vasopressin would improve social abilities in children with autism. And so, our primary outcome measure was this parent report measure, the Social Responsiveness Scale, which is this measure that we've been discussing

repeatedly. And our outcome measure was decided upon jointly between us and the FDA, and partly from our perspective, partly because we knew that vasopressin was related to measures that were obtained from the scale. And, as I mentioned, this was a parent report measure, but what we wanted to do was to see convergent validity in both clinician evaluation as well as child performance on laboratory-based social cognition tests that were computer administered, right, so that we would see improvement by the blinded parents, the blinded clinician, and the child who was unaware of what medication and treatment they had been allocated to.

And so, this was a pilot study, phase 2A, in 30 children with autism, ages six to 12. And we confirmed the expert clinical opinion diagnosis with two autism research diagnostic assessments. And then we assessed safety and tolerability weekly. And then, at the end of the four-week trial, we had parents complete the SRS and then all of the other measures we'll discuss,

I think, in the next, maybe, two slides. And so, fortunately, no vasopressin-treated patient dropped out of this study. There were no differences in adverse events between groups. Here, a few of these are highlighted. And importantly, we saw no significant changes from baseline in a patient's vital signs, clinical labs, or electrocardiogram during vasopressin treatments, suggesting that it was well-tolerated.

And then we were able also to obtain convergent evidence for vasopressin treatment efficacy. We saw that parents thought that their children were improving in social abilities and autistic trait burden. Clinicians agreed on blinded evaluation and also kids as well. So, what we saw was that children were better able to read the mind and the eyes. So, kids are basically asked, here's a picture of an eye region. Which emotion is this person feeling, right? And on vasopressin, across multiple presentations of different sets of male and

female eyes, we saw an improvement. And then also, when presented with still faces of individuals expressing an emotion, they were better able to recognize emotions on vasopressin than on placebo.

So, I'm going to give some summary and some conclusions, and I'll talk a little bit about the future directions for the lab. So, I would like to argue that this monkey model or valid animal models more generally can be indispensable for understanding conserve biological substrates of behavioral functioning, in this case, social functioning. And at this extreme of the social continuum representing autism in humans, these models hold promise for testing new medications with validated endpoints for streamlined translation.

Vasopressin may not be the only drug. I think there will be many, given how clinically heterogeneous autism is. And so, maybe we could use this model to investigate other medications that might be effective for other people. Some

of the limitations are: We did not study repetitive symptoms, and there were no female monkeys studied in this work. We were able to show that cerebrospinal fluid, but not blood vasopressin level, is a robust trans-primate neurochemical marker of social functioning and that CSFA suppressant level could be useful for detecting autism and risk for developing it in neonatal infants.

A couple really important caveats here. The control children were sick enough to require lumbar puncture. It's unethical to enroll neurotypical healthy children in such studies if they're not undergoing lumbar puncture for clinical reasons. And given how incredibly difficult it was to conduct these studies, we didn't have a psychiatric or neurological control group. And so, we're not able to differentiate between whether vasopressin is simply a marker of a brain that's developing atypically or if it's specific to autism. So that's something that we need to follow up on.

And I think that in this initial pilot study, vasopressin was well tolerated, and it improved social abilities in children with autism. It holds promise to be the first medication to treat core features. One limitation was that this was a pilot trial with a very small sample, and it requires replication. And we don't know the mechanism of action, which requires further investigation.

In terms of some ongoing and future research, I just wanted to point out that we are in the final stages of completing the largest single-site medication trial in people with autism to date. So, we are hopefully going to have a readout in the next year on this phase 2B vasopressin trial to be able to replicate and extend our findings from this original pilot trial. If our primary outcome measure is positive, the next step would be to conduct a Phase III trial, likely in a commercial entity, to be able to make this medication affordable and accessible to all who could benefit from it.

And then we've been banking samples from kids in the treatment trial. And in our last treatment trial, although we had an overall positive outcome, there were clearly treatment responders and non-responders. And so, one potential future goal, if this follow-up trial is positive, would be to create iPSC cells and then differentiate them into neurons and then be able to identify in vitro the molecular response to the drug, which could provide, I think, several benefits. One, we would better understand how the medication is working, but it would enable us to study kids who didn't respond to vasopressin, to potentially identify other targets that could be worked up in the monkey model for other medications that could be beneficial to people in this population.

And then I think I mentioned that we had identified these young infants that we know that if we give vasopressin to adult monkeys, that it improved social functioning. But what would happen if we gave vasopressin to neonatal

infants that we already know from their CSF vasopressin, as well as those subtle behavioral processing deficits I mentioned earlier in the talk, if we treat them with vasopressin, could we prevent the formation of social deficits and preserve function? This would also allow us to assess drug safety across development. And then also, we could harness this model to test other medications.

And then finally, I mentioned that the vasopressin level is this robust marker of autism and also risk to develop it in newborn infants. So, we are currently using patient postmortem brain tissue and CSF and blood samples to look at the interrelationships of them and using gene set enrichment analysis, to be able to see what autism susceptibility genes may converge onto vasopressin signaling in the hypothalamus or in CSF or blood. We also have another study underway to assess whether a subset of individuals show medical comorbidities that are indicative of vasopressin insufficiency. So, vasopressin in

the body regulates a variety of different functions. But things like polydipsia, so thirst, frequent urination, frequent water intake could be another marker of vasopressin insufficiency and might suggest those are the kids who could benefit most from vasopressin replacement in terms of targeting their behavioral symptoms.

And then, we are currently in the process of gearing up for a multi-site study to collect CSF and blood in a prospective way from neonatal infants, to follow up on that neonatal infant study. And then, we are also doing some proteomic work to see if we can identify a protein signature of autism in CSF and then potentially see if we can back that out into blood, which would be a more efficient way to make a diagnostic.

Okay. So, I want to finish my talk by thanking the number of people that were really critical to this work, including faculty collaborators, my various current and past

mentees, research assistants and laboratory technicians, patients and families who have heroically participated in our research, as well as the various funding entities that have underwritten this work and made it possible, as well as you for your attention.

DR. NAIFEH: So, we now have a break until 11:15 a.m. Eastern Daylight Time, which is about 32 minutes from now. At that time, we'll begin our live question-and-answer session with Drs. LeDoux and Parker.

In the meantime, we encourage everyone to check out the poster gallery on the conference website, which is filled with wonderful submissions from fellow conference attendees.

As a reminder, you may submit questions at any time before or during the question-and-answer panel using the Q&A function in Zoom. And when you do so, please indicate if the question is for a specific speaker or for both speakers.

Thank you, and we'll see you all after

the break.

(Whereupon, the above-entitled matter went off the record at 10:45 a.m. and resumed at 11:15 a.m.)

DR. NAIFEH: -- Dr. Joseph LeDoux and Dr. Karen Parker. Our moderator for this panel is Colonel Kimberly Kumer, who will help us address as many questions as possible during the allotted time.

Colonel Kumer, welcome. Perhaps you could start by briefly introducing yourself, and then feel free to proceed with asking the questions from attendees.

COL. KUMER: Good morning, Dr. LeDoux, Dr. Parker, and audience. I would like to introduce myself. My name is Kim Kumer. I'm a Colonel in the United States Air Force and an Assistant Professor of Psychiatry here at USUHS. First, I would like to thank both of you for amazing and thought-provoking presentations. It's my pleasure to be the moderator for the morning Q and A session. I have access to the

live feed question bank from the audience, and we'll try to get through as many questions as possible.

So with that being said, let me begin with the first question. This question is for both of you. Who or what was most important to you, and why, for developing your research question?

DR. LEDOUX: Well, I can start, I guess. You know, I have two degrees in Business Administration and Marketing and was not on a track to become a neuroscientist at all. But I happened to take a course that I thought was about the psychology of motivation, but it turned out to be about rat brains and memory. And that professor is the one that first introduced me into the world of neuroscience. His name was Robert Thompson. He had done a stint with Karl Lashley. And from him, I went on to Stony Brook University to study with Mike Gazzaniga, who was my true primary influencer, as he truly introduced me to the world of brain and

consciousness and split-brain patients. And I've been pursuing questions about mind and behavior ever since from the perspective that I had when I was in graduate school.

COL. KUMER: Thank you.

Dr. Parker?

DR. PARKER: I actually just wrote an invited narrative review on my journey to becoming a social neuroscientist. I can put it actually in the Q and A, if you would like, because it actually describes this whole process. As a kid, I grew up as a Bell Labs brat. My dad had a PhD in EE, and he worked at Bell Labs during the heyday of Bell Labs. And so, I grew up going to Bell Labs, seeing him excited about innovation and discovery. And we were able to beta test lots of Bell Labs innovations. And so, I attribute him to me really thinking about becoming a scientist.

And then my aunt has a PhD in Communicative Disorders. And so, I was fortunate to attend college where she was a professor, and

so we spent a lot of time thinking together. But it wasn't until I got into -- and I really thought about becoming a physician. It was very difficult to make that decision, but I was really interested in research. And so, it was taking a series of different classes.

I think my first academic love was evolutionary biology. And then I got very interested in proximate mechanisms and so found social neuroscience that way. My PhD advisor was Terry Lee, and she did work on the biology of social behavior. So, I completed my PhD on oxytocin and vasopressin in voles. And then I came to Stanford as a postdoc and was interested in thinking about animals as model organisms for psychiatric disorders and completed a postdoc with a psychiatrist, Alan Schatzberg, and a basic neuroscientist, David Lyons.

And so, that really put me on the trajectory of thinking about animal models of psychiatric disease. And so, when I started my own lab at Stanford, I had done a lot of work on

stress, vulnerability, and resilience. And I looped back to thinking about oxytocin and vasopressin in the context of autism when I started my faculty position.

COL. KUMER: Great. Yeah, I think it's always interesting what and who inspires us and at what points in our life and how our directions kind of take course from there.

DR. LEDOUX: Yeah.

COL. KUMER: So, thank you both.

DR. LEDOUX: Okay.

COL. KUMER: The next question is for Dr. LeDoux. What was your trajectory from studying split-brain to amygdala to emotional brain? Who or what were turning points?

DR. LEDOUX: Well, I was studying split-brain patients in graduate school and was working with Mike Gazzaniga, who was my mentor. He had done his PhD on split-brain patients a decade earlier. And we were trying to just study a new group of patients that were being operated on at Dartmouth Medical School. We were at Stony

Brook, so it was a pretty short drive up to New England.

And we would drive up in an orange van with a camper trailer behind it that we created a mobile testing lab out of, and we would drive up there and test these patients. And we did all the traditional kinds of things. You put a stimulus to the left hemisphere, and it could talk about it. But if you put the stimulus in the right hemisphere, it can't talk about it. But it was all just reinventing the wheel.

And then we happened to stumble upon a defining observation in a patient, who when we would put information into the right hemisphere and have him produce behaviors by his right hemisphere, we then would ask him, the left hemisphere, to tell us why he did that. And the left hemisphere had no idea why he was doing it because all of the behavioral triggers were from within the right hemisphere. But he always had an explanation for it.

And this was a kind of turning point,

I think both for Mike and certainly for me, where it was kind of like he was confabulating. And neurological patients often confabulate to compensate for their deficit. But our realization was that this is something we do all the time. We make up stories to make our behavior make sense.

A lot of our brain is unconscious, and a lot of our behavior comes out unconsciously. Like, I'm talking to you. I'm not really consciously planning it. It's just kind of I have a template in my head of what we're doing, and it's just kind of coming out. But anyway, these behaviors come out unconsciously, and then in order to maintain our sense of mental unity, we have to figure out, why are we doing that, and what does it mean?

And so, the idea was that we have evolved. The human brain has evolved some kind of mechanism for maintaining mental unity by providing narration or explanations of behaviors that are produced non-consciously. So non-

conscious behaviors, the idea was, produce cognitive dissonance. And by having a narration that reduces the dissonance or explains the behavior, you reduce the dissonance and don't have to really worry about it.

So that's a long way of saying one of the topics that I was interested in in this area was emotional behaviors, because I thought these might be the kinds of behaviors, unconsciously controlled behaviors, that would induce us to have these narratives that would explain why these behaviors are being done.

And at the time, there wasn't much in the way of good methodology for studying emotion in the human brain. So, I decided I would turn to rats, and I would at least be able to study behaviors that are relevant to humans on the assumption that the circuits that control emotional behavior in rats and humans might be similar. So, I did that and chose Pavlovian fear conditioning as my procedure, because it was a simple stimulus and a well-defined response. And

there had been a lot of work on Pavlovian conditioning, of course, but not a lot on the brain mechanisms.

So, within a few years, I and a couple of other people that kind of got involved at the same time, were able to march through the brain and lay out the circuit from the stimulus to the response, with the amygdala being right there in the middle. And so that's how I got to do what I did.

COL. KUMER: Fascinating. Thank you.

The next question is for Dr. Parker. Which came first? Did the monkey show autism-like traits because they were bullied, or were they bullied because they showed these traits? How much of the findings could be attributed to low social rank in these individuals, by virtue of a low-ranking mother?

DR. PARKER: Okay. Yeah. So, for people not familiar with primates, rhesus monkeys are matrilineal, and they have very clear hierarchies. And so that was obviously something

we looked at pretty carefully. And, interestingly, rank is orthogonal to social impairment, so meaning you could be any rank and have any sort of social phenotype. So, rank doesn't drive it. So that's answer one.

I mean, we've got evidence from neonatal human infants as well as monkeys that the vasopressin levels are very low within the first days to months of life. And that's related to the phenotype. I mean, I think from a mechanistic perspective, we can't necessarily tease this out. But in talking, I have a child psychiatrist that I've thought a lot with. And he's a very astute clinician. And his feeling was that a lot of the social anxiety that he sees in high-functioning older patients is due to social rejection and bullying later, right?

So, his working hypothesis is that a lot of people with autism, and particularly subtypes of people with autism, may have an interest in connecting, but they can't read social cues. Their social approach isn't

neurotypical. And so, they get rejected and often teased, right? I've seen this myself that autistic kids often get teased, and kids can be really cruel, right? And so, we can't tease it out, but my thinking is that because these social impairments are present so early in life before bullying would occur, right, I mean, 18-month-olds aren't necessarily bullying each other, that it's likely an emergent property of the social interactions that occur.

COL. KUMER: Great. Thank you.

The next question is for Dr. LeDoux. What does it mean to be agranular, subgranular, dysgranular with regard to the medial prefrontal cortex?

DR. LEDOUX: Well, it's a good question. Most of the granular prefrontal cortex is on the lateral surface of the human brain. But I guess the prefrontal cortex sort of ran out of space at some point. And some of the granular areas that evolved with primates were folded into the medial areas next to the more traditional

medial prefrontal cortical areas that are agranular or dysgranular, depending on your position, but don't have a prominent granular layer.

So, one of the problems, I think, is that when we do research, for example, on large networks and see that there's changes in both the granular and non-granular parts of medial prefrontal cortex, it sometimes is overlooked that the granular prefrontal cortex is a very different kind of cortex. And so, some of the reasons why one might say the default mode network and the attention network might share some overlap is because I think some of the medial granular areas are being conflated. Their presence there is being assumed to be like the other medial areas rather than different kind of cortex. And, you know, the granular thing is important because it underlies a lot of high-level cognition and working memory and so forth.

COL. KUMER: Thank you.

The next question is for Dr. Parker:

Can all types of animals and biological organisms, including fish, snakes, and insects, develop autism?

DR. PARKER: Well, the first thing I would say is I would never say rhesus monkeys have autism, right? So, what I would say is that I think for any neurodevelopmental or neuropsychiatric disorder that we're modeling, we're taking a piece of the phenotype, right? And hopefully we're modeling conserved substrates that are shared between the model organism of interest and the patient population of interest.

And so, there's a couple of different ways to go about creating an animal model. So, one of them is this naturally-occurring variation, whether it's behavior, whether it's some biological substrate. Or you could genetically induce it, right, through gene editing. And so, I think the answer is yes. I think it depends in terms of could we see social impairments in a species non-typical social impairment in any model organism? Yes. Would I

call it autism? No.

COL. KUMER: Great. Thank you.

DR. PARKER: Yeah.

COL. KUMER: The next question is for both of you. Do children with autism show impaired fear response? For example, what is the role of social relatedness and normal fear responsiveness?

Dr. LeDoux, do you want to go first?

DR. LEDOUX: What was the last part? What was the role of what?

COL. KUMER: What is the role of social relatedness in normal fear responsiveness?

DR. LEDOUX: You know, I think those questions are really better answered by Karen. I'm not an autism person, so I think she should take it.

COL. KUMER: Okay.

DR. PARKER: Yeah. And I'll say this isn't exactly my area of expertise, either. And I'm not a clinician, right? I always like to tell people that. I mean, 80 percent of kids

with autism have anxiety disorders, right? And again, Joe is, I think much better positioned to disentangle fear, anxiety, stress, right? They're not the same thing, necessarily, right? But they're related.

But there are kids with autism who don't show fear. So, for instance, there are kids that don't have an appreciation of water. And so, drowning is actually one of the leading causes of death in kids with autism. So, there isn't this sort of appreciation of you could drown if you go into the water, especially if you can't swim, running into the street and not having an awareness that there's a car coming towards you. But I think the tricky part is that can also be confounded by intellectual disability, right? So, is it a lack of fear, or is it that you have pervasive intellectual disability, and you aren't able to sort of weigh risks and understand that the speeding car is coming straight at you?

DR. LEDOUX: Practically all medical

disorders have some kind of anxiety associated with them, you know? Because whether it's heart problems or GI problems or whatever, you're going to worry about it. And so, if you're an autistic child, I assume to the extent that you're capable of conceptualizing all this, you'll worry about it. And even if you can't conceptualize it, then you might still have behavioral and physiological symptoms that trouble you even if you're not experiencing the fear and anxiety itself.

COL. KUMER: Thank you. The next question is for Dr. LeDoux.

How does consciousness work between right awareness and left storytelling, rationalizing hemispheres?

DR. LEDOUX: Well, I think we shouldn't attribute too much to the hemisphere itself. I mean, the things that you've mentioned, rationalizing, and what was the first one?

COL. KUMER: Let's see. Storytelling.

DR. LEDOUX: Storytelling. Well, I

mean, we tell stories, and we rationalize verbally, but we can also do some of that non-verbally. If you're watching a movie, the storyline is as important as the script. So, it's not like these things are easily separated.

In split-brain patients, you have the luxury, I would say, of being able to see some separation between what the two hemispheres do. But I think the old idea of, you know, the left brain, right brain, one is artistic, one is intellectual and all that, it was just sort of pop psychology. There are differences between the hemisphere, but they reflect specific kinds of regions that are different, not whole hemispheres that are different. At least that's my perspective. I mean, I haven't worked in this area in 40 or 50 years, so I haven't followed it that carefully.

COL. KUMER: Okay. Thank you.

The next question is for Dr. Parker: Does your research indicate whether vasopressin might be more effective when given to children at

an early age, versus adolescence or even adulthood?

DR. PARKER: Yeah. I mean, it's an excellent question. You know, at least with behavioral therapy, which is one of the only things that we have in the toolkit, the earlier it's inactive, the more likely it is to benefit a child. So, I mean, the conventional wisdom would suggest that the earlier we could intervene, the better. We don't have data on that.

We have a pilot trial that was a Phase 2A trial of vasopressin where we did this in six-to-12-year-olds. And we just closed the largest, single-site medication trial to date for autism of 108 children. And that was in ages six to 17. And so, we'll be better powered to look at age in that trial. And I think if the trial is positive, obviously doing an early intervention trial would be an obvious next step to see if we could have even more robust responses.

COL. KUMER: Great. Dr. Parker, a

follow-up question: Is autism developed in the womb or after birth?

DR. PARKER: I mean, that's a tricky question. I don't think that anybody has the answer to that. I mean, we know from a study that we did that vasopressin levels in CSF are low, essentially within the first few days of life, right? So, we're already seeing a biological readout that's predictive of a later autism diagnosis. But I think as with anything, these things are very difficult to untangle, like Joe said, right? That's why we have animal models because you can't do really rigorous experimental work in people for a variety of different reasons. So, we sort of rely on epidemiological studies and go to the animal models to think more about mechanism.

COL. KUMER: Great.

The next question is for both of you. Both of you are truly biologists. Many of us do not often think of evolution and various preserved and not preserved behaviors in

neurobiology.

Do you have thoughts on the importance of this element of thinking in your work and what healthcare providers may need to be more alert to?

Dr. Ledoux, I cannot hear you.

DR. LEDOUX: How's that? Can you hear me?

COL. KUMER: There we go. Yes.

DR. LEDOUX: Okay.

COL. KUMER: Thank you.

DR. LEDOUX: Obviously, evolution plays an important role in all aspects of biology, and all of psychology depends on biology to a certain extent. So, I'm not sure how important it is for clinicians to be thinking too much in terms of evolution. I think scientists need to provide clinicians with information about evolution so that it maybe provides a perspective. But I'm not sure that there's a lot of advantage to try and come up with an evolutionary theory of, say, I don't know, how to

manage a patient. But maybe I'm wrong.

Karen, what do you think?

DR. PARKER: Yeah. I think I have a couple answers for that. So, I think one, thinking to the extent that we think about animal models and streamline translation to patients. Modeling homologous circuits is, I think, important because we're more likely to translate the findings. I think that when you design tests for an animal model, you want to think about the environment the animal evolved in, right? So, Joe is doing a test in a rodent. He'll exploit a test that a rodent might naturally do in the wild. And I think about that in a monkey. So, if I'm going to be thinking about a test I'm going to do, I'm going to think about something that the monkey would be able to perform based on what I know about how they behave in the wild and how they evolved.

One thing that I think is interesting is we think a lot about neurodevelopmental and neuropsychiatric disorders as impairments, sort

of in this very ableist way. And one thing that's really important to think about is when we see neurodevelopmental or neuropsychiatric disorders that are highly heritable, and if autism impacts one to two percent of the world's population, one of the questions that comes up is why do we see this? Is there stabilizing selection involved, for example? Are some of these traits maintained in the population for a reason?

And so, there is a whole movement within psychiatry to think about individual variation as being part of the natural human experience and having a strength-based approach to these disorders. And so, the hyperfocus that people with autism have can be used for, say, coding, and it has been wildly successful. Neurotypical people can't sit and stare at something for 15 hours without getting bored, without getting distracted. But there are people with autism, especially if this is something that is of strong importance to them, where they can do a much better job than a neurotypical person,

right?

And so, what I would like to think about is some of these traits are maintained in the population because they do have a selective advantage. And so, there's a colleague in my department, Lawrence Fung, who has this really fantastic initiative. He's a faculty member in the division I run, and it's all about neurodiversity. And so, anyone who's interested in that, I strongly suggest checking out Lawrence's work, because I think it will soon provide many breakthroughs.

DR. LEDOUX: I would like to follow up on something. I have it in my lecture. I didn't talk too much about the amygdala because I was talking about this new book that I've written. But I do want to say something about, for example, we sometimes overattribute things to the amygdala.

For example, let's just take the idea of the amygdala being some kind of defensive circuit that helps you detect danger. And this

gets back to the evolution point. So, the amygdala evolves as a predatory defense system, or those parts of the amygdala that deal with this evolve as a predatory defense system or circuit. But there are many ways to be in danger. For example, let's say you're stuck on a mountaintop, and you've dropped your backpack off. So, you have no clothing to keep warm or food to eat or water to drink.

You can now become fearful or anxious because of all of these things. You're going to dehydrate. You're going to starve. You're going to freeze to death. All of those are fears or anxieties about what's going to happen to you. But they're not about necessarily a predator. I mean, you can add to that. There could be a predator that's going to come along and kill you as well.

But there are many, many ways to be afraid and anxious. And it's not all about predation. And I don't know. Some of you may know my views of fear, that it's not coming out

of the amygdala, but it's more of a cognitive interpretation of the situation we find ourselves in, whereas the more behavioral and physiological responses are clearly highly encoded evolutionarily. But let's not say that everything that is dangerous is going to be processed by the amygdala.

COL. KUMER: Great. Thank you.

Before I ask the next question, I wanted to ask about a time check.

DR. NAIFEH: Yeah. Yeah. Yeah. I hate to interrupt. We have time for a couple more questions. So, let's maybe do two more questions. Thanks.

COL. KUMER: Great. Thank you so much.

The next question is for Dr. Parker. Social relatedness is a critical overall function to health, particularly in our areas of suicide prevention.

Do you have thoughts on acute treatment to improve social responsiveness?

DR. PARKER: I think it would be irresponsible for me to answer that question. I'm not a clinician, but I do think that suicide is a very large problem within the autism community, right? There was a big study that just came out that kids as young as eight are contemplating suicide because of their lack of social connectedness. So, I will certainly leave it to the clinicians, particularly behavioral therapists, to think and react to that. But I agree that it's a very large problem.

COL. KUMER: Great.

The next question is for both of you. What areas of the brain are you targeting for postmortem brain studies?

DR. LEDOUX: Oh, that's easy. I'm not doing any postmortem brain studies. I've closed my lab, so I'm not doing research anymore. Awesome.

But before we leave, I just want to thank you and say that the questions have been very good. It's not always the case in something

like this. So, congratulations on that.

COL. KUMER: Thank you.

Dr. Parker, any other comments?

DR. PARKER: Yeah. I think maybe some of the questions and even in the ones I peeked at in the Q and A, this is the foundation of the work, and we haven't done any of the mechanistic work yet, right? So, we do have some funding from the DoD, actually, to do some work in hypothalamic postmortem tissue. The vasopressin gene does not come up as a high confidence autism susceptibility gene, right?

And so, our working hypothesis is that vasopressin is one of these common pathways that autism susceptibility genes converge onto. And so, we can do work with gene set enrichment analysis to ask the question of, do autism susceptibility genes within postmortem tissue from people with and without autism actually drive vasopressin gene expression, and what we see in, say, vasopressin and CSF in blood. So, stay tuned. That's going to be one of the next

papers the lab will put out.

DR. LEDOUX: I see a note in the chat, a quick question I'm going to answer. Yes, the Amygdalas still play from time to time.

COL. KUMER: Great. Thank you both so much.

DR. PARKER: Yeah. Thank you.

DR. NAIFEH: That is unfortunately all the time we have to answer questions. Thank you so much to Drs. LeDoux and Parker. It was wonderful to have you join us and share your remarkable expertise. Your presentations and discussion were enlightening.

Also, thank you to our moderator, Kimberly Kumer.

We'll take a break now, reconvening at 12:45 Eastern Daylight Time, which is in approximately 57 minutes. We hope everyone will use this as an opportunity to review the posters.

(Whereupon, the above-entitled matter went off the record at 11:45 a.m. and resumed at 12:45 p.m.)

DR. NAIFEH: Okay. Well, welcome back. As a reminder, we're going to have another Q and A session this afternoon with our three afternoon speakers. You can submit questions for these last three speakers at any time using the Q and A feature at the bottom of the Zoom window. To begin the second half of the day, we are excited to share with you a presentation by Dr. Diego Pizzagalli.

Dr. Pizzagalli is the founding director of the Center for Depression, Anxiety, and Stress Research at McLean Hospital, as well as the Director of the McLean Imaging Center and the Director of Research for the Division of Depression and Anxiety. He is a Professor of Psychiatry at Harvard Medical School and the Center Director for the Silvio O. Conte Center for Basic Translational Mental Health Research, focused on the neurobiology of and novel treatment targets for depression and anxiety.

We'll now begin Dr. Pizzagalli's presentation, which is titled, "Cross-Species

Investigations of Anhedonia and Stress-Related Phenotypes: Implications for Treatment Development and Stratification."

DR. PIZZAGALLI: Well, thank you so much, James, for the very kind introduction. It's a real pleasure to be here today and have an opportunity to share some of our work focused on anhedonia and stress-related phenotypes. I hope to show you the potential implications of this work in terms of treatment development and certification.

Before I start with the presentation, here are my disclosures. Everything I'll be showing today has been funded by NIMH, but I've received consultancy fees. And also, I'll be showing data derived from the use of a task, the probabilistic reward task, which has been licensed through Neumora Therapeutics. So, these are my disclosures for today.

So, this is the roadmap for today's talk. I'll very briefly emphasize the heterogeneity of depression and how studying

anatomic behaviors, in particular, reward learning, might be a fruitful avenue to improve our understanding of depression and its treatment. Given also the focus of the symposium, I will also emphasize the role of stress in the etiology and pathophysiology of depression.

But then in the second part of my talk, I will use some of this information to hopefully convince you that studying these types of phenotypes in depression also could be useful to guide treatment selection. Of course, personalized treatment remains imperative in psychiatry, and I'll be showing data derived from the EMBARC study in which we have identified biomarkers that potentially may be used to eventually guide treatment selection. And I'll end, then, with some potential implications in terms of patient stratification and personalized treatment in depression.

So off we go with the first part of my presentation. I've long been interested in

studying anhedonia for a variety of reasons. The first reason is that from a clinical perspective, we know that anhedonia is associated with a host of negative clinical outcomes. The presence of anhedonia has been found to predict depression, years later has been linked to poor outcome and also chronicity.

Critically, we also know that anhedonia and lack of motivation are currently quite poorly addressed by first-line treatments. This could be pharmacology. It could be psychotherapy, such as cognitive behavioral therapy, or even neurostimulation. And in fact, the presence of anhedonia at the beginning of any type of treatment actually has been linked to poor outcomes. So, from a clinical perspective, it's really a very important kind of clinical construct that deserve our attention.

The second reason that I've long been interested in anhedonia is that I felt, especially when I started my research program over 20 years ago, that I might have some precise

hypothesis about the pathophysiology that might be implicated in this important construct. And that probably comes as no surprise to many people in the audience that focusing on the mesolimbic dopaminergic pathways may be a fruitful avenue to study this important construct in depression.

This system originates from the ventral tegmental area and projects to both the ventral as well as dorsal striatum regions, such as the nucleus accumbens or the putamen and the caudate and then project to the prefrontal cortex. And what we know based on decades of preclinical research is that this system is incredibly important for learning from our actions, reinforcement learning. But it's also very important for incentive motivation. And I'll get back to this later. This system also has been found to be very sensitive to the effect of stress, in particular uncontrollable and chronic stressors. And so coupled with the clinical imperative of understanding anhedonia, as well as trying to understand the

pathophysiology of anhedonia, a long time ago, we embarked on a journey to basically really focus on this important phenotype.

Now, when we started this work decades ago, we were also very interested in assessing objectively some of these anhedonic phenotypes. And actually, almost 20 years ago, we developed a task that we coined the probabilistic reward task, which allowed us to objectively assess a core component of anhedonic phenotypes, which is people's ability to respond to and to learn from reward.

The way this task works in an abstract way, I will show you in a second a more concrete example, is that we present one of two stimuli in every trial. And we ask participants or animals to make a decision, whether a Stimulus A or Stimulus B had been presented. As you will see in a second from the next slides, the stimuli are actually remarkably similar to each other, so it's a rather difficult discrimination. And critically, and unbeknownst to participants, what

we're doing during the task is that we are disproportionately rewarding one stimulus over the other. So, the stimulus is ambiguous. The subject is not really sure what stimulus has been presented. And by presenting three times more frequently reward for one stimulus over the other, we can basically elicit the development of a so-called response bias or a preference for the stimulus that has been rewarded more frequently in previous trials.

This is something that has been widely described. Rats, mice, pigeons, non-human primates, and humans all show this behavior. Under normal circumstances, their behavior will be biased toward the stimulus that has been paired with more reward in previous trials. This is actually how the study, how the task works. We have developed a version to be used in humans, as well as a version to be used in rodents. Although, as you will see in a second, we have also progressed quite heavily with respect to preclinical testing.

But in humans, the task actually looks like this; a participant sits in front of a computer. We present a facial stimulus, first without a mouth, and then a mouth is presented very briefly for 100 milliseconds. And the subject's task is to say whether the short or the long mouth had been presented. As you can see here on the screen, the difference between the two stimuli is remarkably small, so again, it's a rather difficult discrimination. And subjects are asked to press a key if they thought that the short mouth had been presented or a different key if the long mouth had been presented. And again, we provide, in this case, monetary reward to correct responses. But critically, we use a three-to-one ratio, where basically, correct identification for one stimulus, let's say the short mouth, is rewarded three times more frequently compared to correct identification of the other stimulus.

In rats, in a first incarnation of this task, what we did is that we trained animals

to associate one lever with one tone duration, a second lever with a different tone duration. After they learned this discrimination, the tone duration was made even closer to each other. And the rat's task was basically to determine whether a short or long tone had been presented. And instead of presenting monetary reward, we gave them nice food pellets. But again, also in an identical way as in humans, there was also in rodents, this three-to-one ratio where one stimulus, let's say the short tone, was rewarded more frequently compared to the other stimulus.

And I wanted to spend just one second to explain how we calculate our main variable of interest, response bias, which again, captures the participant's or subject's ability to modulate behavior as a function of rewards. We use a signal detection theory to derive this variable. And as you can see from this formula, a subject has a high response bias when they basically are very good in correctly classifying the rich stimulus, the one that is paired with

more reward, and in fact tend to misclassify the other stimulus as being the rich, right? Because their behavior has been shaped toward the rich stimulus.

And when we use this paradigm and this formula in humans, our data from healthy controls, they basically developed a nice response bias that typically tends to increase over time. We engage them in three different blocks. And again, not surprisingly, if we look at accuracy, what you see is that accuracy for the rich stimulus goes up across the task, and accuracy for the lean stimulus goes down across time; again, because their behavior has been shaped toward the rich stimulus. And to our delight, different strains of rats actually performed very similarly to humans. You can see how they developed a very nice response bias over time. And their performance in terms of accuracy was, again, remarkably close to what we see in humans, with accuracy for the rich stimulus going up linearly, and accuracy for the lean stimulus

going down.

I should say that this was, again, the very first kind of version of the rodent task, but these days we are using exclusively different technology. In fact, we use touchscreen technology, and we can actually train rats, mice, or non-human primates with this technology very rapidly. And in fact, actually, we're now using also virtual stimuli, where the animals need to decide by pressing these virtual levers, whether a long or short line was presented. It takes us about 15 to 20 sessions to train mice or rats to do this task, a bit faster in non-human primates. And now, actually, we don't even have a confound, that we were using the auditory modality in rats and visual modality in humans. Now across species we're using the visual modality.

And I don't have time to review this data in detail, but suffice to say that in terms of our validation, we have found that if you provide challenges, pharmacological challenges that we know increase dopamine, such as by

providing the amphetamine for example, we see those dependent increases in response bias. And if we use compounds such as scopolamine or ketamine, which of course have shown rapid antidepressant responses in humans, we also see that the response bias is boosted. But critically, we have also seen that in positive controls, the conditions where we give oxycodone or PCP, we see no effects on response bias. So not all compounds that we have evaluated modulate response bias, but critically, especially those that are known to modulate dopamine, have shown antidepressant properties in other studies.

And so, when we use this paradigm in humans, these were our first findings in unmedicated individuals with major depressive disorders. As you can see, our healthy controls very quickly developed a robust response bias, and unmedicated individuals with MDD showed an overall blunted response bias in this task.

Critically, we and others have replicated this finding in independent samples.

In this particular study done with collaborators in Belgium, we found that the presence of low response bias predicted MDD chronicity in an inpatient sample. And also critically, which I think it's important to appreciate, is that, although we see overall group effects when we compare MDD subjects to healthy controls, follow-up analysis and studies have shown that this behavior is really driven exclusively by patients who report anhedonia, loss of pleasure, in their daily life, or in the so-called melancholy subtype of depression; again, showing nice internal consistency, if you wish.

And so, then, in other studies later on, we wondered whether this behavior might be present potentially after people developed their first onset of depression, or potentially whether this type of subtle abnormality might be present even after remission, or potentially even after the very first onset of depression.

And this is an interesting question, an important question, because, typically, if you

follow the anhedonia literature, many people believe that anhedonia and depression may be a bit more state-related, right, that after people remit, their anhedonic tone might be restored. Whereas anhedonia is typically conceptualized as being more trait-like in schizophrenia, for example.

Well, what we found was actually quite surprising. When we studied truly remitted individuals, these were not people that just came out from a depressive episode, but these were people that had had more than a year remission from their depression and actually were not different from healthy controls in their self-reported assessment of anhedonia by using clinical scales. And yet in our task, they still showed quite a strong blunting.

And we have been very interested also in ongoing study to evaluate whether the presence of this blunting despite actually, again, self-reported anhedonia being restored, so to speak, whether this type of behavioral blunting might

predict future episodes. And we have several studies that are currently evaluating this.

But strikingly, also, what we found was that if we study 12 to 14 year old offspring of individuals with depression, with the offspring without ever having any depression themselves, we found that actually, so-called high-risk individuals, so basically children of a parent with depression, also actually showed significantly reduced response bias in our task. So collectively, this data suggests that difficulty learning from reward may be a potential trait, like vulnerability of depression, that in fact might precede the very first onset of a major depressive episode.

I've kind of hinted that potentially, this phenotype, this difficulty to learn from reward, might be associated with dopaminergic dysfunction. This was one of the kind of heuristic or one of the key working hypotheses that we have evaluated in my lab over the years. Well, if that's the case, we wondered whether

using pharmacological challenges that, based on the preclinical literature, we know should affect the dopaminergic signaling, whether using these challenges might actually modulate response bias in our task.

And in a first early study testing this type of hypothesis, what we decided to do in humans and then later in rats was to give single, very low doses of pramipexole. Pramipexole is a D2/D3 agonist. It's FDA approved for the treatment of Parkinson's, and at high doses has been used in Europe and in other countries, continents, has been used to treat depression and anhedonia.

But very interestingly, when used at very low doses, these D2/D3 agonists have paradoxical effects. That is, they have been shown, again, in rodents especially, to preferentially activate presynaptic autoreceptors, which of course are inhibitory in nature and have been found to basically decrease neural firing in the ventral tegmental area and

nucleus accumbens and also decrease dopaminergic outflow in these regions.

And what we found in these studies is that in humans, this was a between-subjects design, healthy controls receiving a placebo, which are shown here in gray, again, showed a very nice increase in response bias over the course of the task, whereas those healthy controls receiving pramipexole were remarkably blunted in their ability to learn from rewards, and in some respect showed a pattern similar to what we had seen in MDD subjects. We were able, again, to kind of replicate or to show this effect in rats, where here we use the within-subjects design, and the rats developed a very nice, robust response bias when receiving saline, but they were blunted when receiving pramipexole. Again, these types of findings can be explained by postulating a decreased dopaminergic signaling via presynaptic autoreceptor activation.

What about if we use the opposite approach or if we give compounds that we know

based on the clinical studies and also human studies, increase dopaminergic signaling, especially in the striatum? Well, in humans, we did a study several years ago in which we gave nicotine patches. Nicotine, among other things, increases dopaminergic signaling in the striatum. And basically, we saw that non-smokers receiving 40 milligrams of nicotine showed a strongly-boosted response bias in the task compared to when they received placebo. It was a within-subjects design, crossover design. And we basically saw very similar effect, but this time using amphetamine in rats, where the animals showed potentially the response bias when receiving amphetamine over saline. Again, a potential indication that by boosting dopaminergic signaling we are able to modulate this behavior.

And we've also shown in a variety of studies that when we expose individuals to psychosocial stressors, in this case healthy controls, or a much more severe stressor in rats,

in this case three days of social defeat, again, across species, we see correspondence in that the animals or the subjects actually are less able to learn from reward when exposed to, in this case, a laboratory stressor, which was not particularly stressful, but still somewhat stressful, but a much more blunted response bias in rats exposed to chronic social defeat.

Critically, obviously, in animals, we can then look at potential molecular abnormality associated with this behavior. And what we found in these early studies in rats was that chronic social defeat actually increased nociceptin/orphanin FQ mRNA levels in the nucleus accumbens and also decrease Fos in the VTA, so basically blunted activation in the VTA, and we saw potentiation of these nociceptin/orphanin FQs.

And what was actually quite striking in this particular study in rats, we saw a negative correlation between the level of the peptide, as well as level of the receptors in

both the cingulate as well as the VTA, being inversely related to reward learning or response bias. So, animals that showed the highest levels of either the peptide or the receptors in these different regions were the ones actually showing the most blunted ability to learn from reward. And we have ongoing studies, both our Conte Center, as well as another one in which we're investigating the role of nociceptin/orphanin FQ in the pathophysiology of MDD and stress-related disorders.

And finally, what we have seen is that this ability to learn from reward has interesting features. First of all, if you look even among healthy controls and you look at the distribution of their ability to learn from reward, it follows a relatively normal distribution. In larger sample, we have actually seen quite a remarkable kind of bell shape. But critically, also, this ability to learn from reward that we have found correlates with molecular markers of the dopaminergic system. So, in this particular

study, we used a PET tracer called Carbon-11 altropane, which as I'll explain later in my talk, it's an exquisite tracer to study dopamine transporter.

And what we found in healthy controls, there was a negative association between level of altropane binding potential and reward learning. So, individuals that had higher levels of dopamine transporter binding, that is supposedly their dopamine is re-uptaken very quickly into the synaptic cleft, actually had relatively lower ability to learn from reward. Whereas healthy controls at that lower level of dopamine binding potential, and supposedly their dopamine can linger a bit longer in the presynaptic cleft, were actually the ones showing the greatest ability to learn from reward. So, a really kind of interesting observation that this behavioral marker seems to be associated with molecular markers of dopaminergic functioning.

And also in the same study, we saw that, again, the ability to learn from reward,

which is plotted here on the X-axis, showed a very nice correlation with functional connectivity between the bilateral accumbens and this region in the ventral medial PFC. So, in this analysis, we basically seeded the bilateral nucleus accumbens, and we asked the question, which regions show functional coupling with the accumbens? And such coupling actually is associated with reward learning. And the answer was that it was in this region of the ventral medial prefrontal cortex. Such as healthy controls who show more coupling between these two key nodes of the brain reward system also were the ones that two to three weeks earlier showed actually higher ability to learn from reward. Again, just to kind of set the stage for some of the later findings that I'll be showing where basically, this type of behavior seems to be associated with important markers of the brain reward system, in this case, coupling between the accumbens and the ventral medial PFC.

Okay. But one of the key questions

that we asked in a series of studies was what are the potential neuromechanisms associated with this blunting that I've been showing? And for that, we turn to different types of paradigms, including using functional MRIs. In an early study, we used the monetary incentive delay task, which you might be familiar with. But in a nutshell, we were able to basically image brain activation while individuals are waiting or anticipating a potential reward or penalty or no incentive trials and also their brain activation when they receive a reward or a penalty or no incentive, a neutral outcome.

And what we saw in this first study was that both during the anticipation of reward, as well as the receipts of reward, and in fact, actually specifically for reward but not penalties, individuals with depression were less able to activate the striatum both during anticipation as well as the consumption of reward. And this expansion emerged in the putamen, in the accumbens, and the caudate. And

this was actually in a sample of unmedicated individuals with MDD. We have replicated these findings using additional paradigms.

But a key question that we ask is that based on some of the evidence I just showed you, whether this type of blunting might be associated, again, with dopaminergic dysfunction. And if that were the case, we argued, what if we give a manipulation, a pharmacological challenge, that now, actually, based on the preclinical literature, we believe will actually increase dopaminergic signaling. And the approach that we took was exactly the opposite as what we did in the pramipexole study; that is, we decided to use small doses of D2/D3 antagonist, with the idea that these low doses would actually preferentially block, now, the presynaptic auto receptors and accordingly remove inhibition on the presynaptic cells and actually increase dopaminergic signaling.

These types of findings have been shown abundantly in the preclinical literature

where, again, single low doses of D2/D3 antagonists, including amisulpride, have been found to increase dopaminergic synthesis and release in the accumbens and have been found to have prohedonic effects. And in humans, there was a study that actually came out before ours, in which by giving low doses of amisulpride, they were able to increase reward-related ventral striatal activation. This is, again, in healthy controls. But critically, in various European countries, low doses of amisulpride, typically 50 milligrams per day, have long been used as a potential antidepressant, especially when anhedonia or fatigue are present.

And just to give you an idea, again, amisulpride is a D2/D3 antagonist, which is approved in the U.S. for schizophrenia. But the starting dose for schizophrenia is 400 milligrams. And so, again, with 50 milligrams, you are well below that dose. And again, these types of findings have been explained in the literature by invoking presynaptic autoreceptor

blockade.

So, this is just a graph showing the pharmacokinetics of amisulpride based on a study published in the literature. And based on this data, what we decided to do was to administer the MID test, the monitor incentive delay test, in conjunction with the fMRI during this first peak. And then we also gave additional tasks outside the scanner at the second peak. But I'll show you, for the sake of time, only the results of the fMRI study.

So, we had about 90 individuals. Everybody was unmedicated. It was a between-subjects design. Half of the MDD subjects received amisulpride; again, single low doses. The other half received placebo, and the same for the healthy controls. And these are our findings. We were stunned that basically, our hypotheses were completely met in these particular studies. What we found was that individuals with MDD receiving amisulpride basically were indistinguishable from healthy

controls receiving placebo in their report-related activation in both the dorsal and ventral striatum. So, those patients receiving placebo were, again, blunted with respect to healthy controls, especially in the accumbens. But those receiving amisulpride were basically indistinguishable.

So again, this was not a treatment study. We gave single low doses. But this was a proof of mechanism study, right? We wanted to evaluate whether having a challenge that supposedly increased the dopaminergic signaling might be sufficient for increasing reward-related activation. And this was the case. But even in these studies by showing that, again, dopaminergic challenge rescued some of this deficit, it's also not showing directly the potential presence of dopaminergic abnormality in depression.

And to test that, we turned, again, to positron emission tomography. And we decided to test the hypothesis that perhaps if indeed MDD is

linked to a low level of dopaminergic transmission, especially in the striatum, what we should be seeing is actually a reduction in dopamine transporter. Because many studies have shown, mostly in preclinical models, that conditions that are associated with low levels of dopaminergic signaling lead to compensatory downregulation of the dopamine transporter, with the idea that, again, there is no level of dopamine available, and the brain tries to compensate by reducing this mechanism that is basically responsible for a reuptake in dopamine back to the presynaptic cell.

And when we designed our one study, there was actually very minimal postmortem evidence that this might be the case. There was actually quite compelling evidence that animal models relevant to depression, all of which involve exposing animals to chronic stress, were associated with reduced dopamine transporter levels in the accumbens or mesolimbic pathways. And there was also evidence from rats that have

been bred to have increased vulnerability to depression that basically are characterized by reduced dopamine transporter. But critically, when we started the study, the in vivo human imaging evidence was really very inconsistent. But the reason, I believe, is that prior study had used tracers that were not particularly selective for dopamine transporter.

And so, to address this limitation, we elected to use, again, this tracer called Carbon-11 altropane, which was developed at MGH to study Parkinson's, which has really some exquisite property in terms of study in the dopamine transporter because it is very selective to the dopamine transporter over this returning transporter. And you see very, very strong uptake in the entire striatum. And what we found was the following.

First of all, we used the origin of interest analysis where we anatomically trace the accumbens, the putamen, and the caudate in every subject. We co-varied age because there is a

quite strong negative correlation between age and dopamine transporter binding. And what we found was that, indeed, across all the striatal region, but specifically in the putamen, we saw that depression was associated with reduction in dopamine transporter binding, as hypothesized. We also saw blunting in the ventral tegmental area. And interestingly, we also found that there was a negative correlation between the level of dopamine transporter in both the putamen and the VTA and the number of lifetime major depressive episodes.

So, individuals that had five of more episodes of depression had the lowest level of dopamine transporter binding. Because this was a cross-sectional study, we don't know whether what you're seeing here is the cumulative effect of depression, especially potentially untreated depression, or whether having a low level of dopamine transporter may be a premorbid mark of chronicity. We don't know.

But I should say that we are about to

submit a paper where we basically have replicated this finding in an independent sample, so we have some independent replication. But also, I should say that in this first paper that was published in 2019, because again, there was such inconsistent evidence in the imaging literature in depression, we also had an opportunity to study the same abnormality using postmortem tissues. This was tissue obtained from the putamen in 15 individuals that died by suicide, actually, and were compared to 15 healthy controls that died by natural causes or accidents. And basically what we found was that confirming the PET finding, we had evidence of a reduction of the dopamine transporter, especially in the more mature form of the dopamine transporter. And we also saw reduction in tyrosine hydroxylase. So, we were pleased to see that basically across modalities and kind of approach, postmortem versus positron emission tomography, we saw concordance in findings, both pointing to downregulation of the dopamine

transporter in the striatum.

So, I also wanted to spend some time just to mention, given the focus of this conference on stress, obviously, spend a couple of minutes talking about the potential role of stress in the etiology and pathophysiology of depression. So first of all, what we know is that when you look at weeks or months preceding a major depressive episode, many studies, including prospective studies, have found that people that go on and develop a major depressive episode actually report much more frequently the occurrence of severe life stress in their lives, right? And this is a well-established finding. And in fact, meta-analyses have shown that about 80 percent of first episodes of depression are immediately preceded by severe life stress.

We also note that this link might be moderated also by the presence of childhood maltreatment or abuse, which is a very potent risk factor for MDD. We know that events that are characterized by a loss of control, by a

feeling of being trapped in a very difficult situation, and potentially a situation where we feel humiliation, these are the three key ingredients that make a stressor particularly depressogenic.

And finally, this is something that really has always intrigued me, is that studies have shown that with increasing number of episodes, the link between stress and recurrence becomes increasingly weaker. That is, again, many first episodes of depression are immediately preceded by severe life stress. But individuals that have many recurrences in their life, their new recurrence of depression often emerges, if you wish, out of the blue without any objective, severe stressors. So, we see this uncoupling between stress and depression with increasing number of episodes.

And so, a couple of years ago, we decided to look into these findings. This was kind of inspired by, if you wish, the kindling hypothesis that Robert Post and others have

postulated, that potentially with increasing number of episodes there might be neurobiological sequelae that remain and then actually increase the risk for future depression that will arise even when not exposed to stressors. And basically, what we found in this particular study was that in a relatively large sample of an individual with MDD, all are medicated, is that a number of lifetime episodes was actually associated with decrease in volume in both the medial prefrontal cortex as well as the hippocampus. So, people that had more lifetime episodes had smaller hippocampi and medial PFC. And these were actually our predicted regions because, of course, these two regions are prominently implicated in inhibiting stress responses. And so, we felt that it made sense that potentially dysregulation in these areas might be associated with an increasing number of lifetime episodes.

And again, I mentioned that early adversity has been linked with increased risk for

later depression. The question is why? Well, based on some of the data that I just showed you today, you might appreciate that we felt in our studies and hypotheses that potentially this link might be mediated by the emergence of anhedonic phenotypes and dysfunction in the mesolimbic dopaminergic pathways. And in fact, in several studies over the years, we found that, for example, the exposure to early life adversity, very early in life, was actually associated with a reduced ability to recruit the pallidum, the left pallidum specifically, while anticipating potential reward but not loss of no incentive. So, evidence that being exposed to early life adversity was actually associated 20 years later in this particular sample with blunting in the ability to recruit the pallidum while anticipating rewards.

And in another study, we found that experience of childhood sexual abuse was specifically associated with a reduced ability to learn from positive but not negative outcomes

using a reinforcement learning task. So, we had evidence, again, in both studies that this finding just seems to be really specific to the reward component, so to speak. In this particular study, we saw no differences between the groups in either anticipating a potential loss or in learning from a negative outcome. But again, going back to our rodent models where we can actually investigate more mechanistically some of this effect, we also found evidence that exposure to early life adversity actually induces enduring anhedonia phenotypes.

In this particular study what we did was to expose animals to the limited bed nesting procedure that was developed by Tallie Baram and others at UC Irvine. And basically, what you have in this situation is that you provide insufficient material to build a nest. And the mother basically has a very fragmented kind of behavior or care of the pups. And this behavior has been linked to anhedonic phenotypes in Dr. Baram's work and other labs. And basically, what

we found by using our rodent version of the probabilistic reward task using touchscreen technology was indeed that, in different cohorts of rats exposed to this early life adversity, we saw a strong reduction in response bias; so again, suggesting that exposure to fragmented care was actually associated then with an adult phenotype that was prominently characterized by anhedonia.

And I'll end by just highlighting how some of these markers, which again I've showed you, are associated with both functional as well as molecular markers of the dopaminergic system and potentially might be used to guide treatment selection. And again, these are findings from the EMBARC study, which was a large, multi-site biomarker study where over 300 patients with depression or phenotype quite deeply using a variety of approaches.

And this was the design of the study. Basically, the study had two phases. This was a treatment study. Again, over 260 people with MRI

data and also behavioral data, they were randomized to sertraline, an SSRI, or placebo in the first phase for eight weeks. People that received sertraline responded, stayed on sertraline for eight more weeks. Those actually who did not respond to sertraline were crossed over to bupropion, which is an atypical antidepressant, which has been found to increase, among other things, dopamine signaling. And those patients receiving placebo stayed on placebo for eight more weeks, whereas those who did not respond to placebo were crossed over to sertraline.

And the key question that we asked is whether behavioral as well as neural markers collected at baseline predicted responses to bupropion after failing sertraline. We felt this was an important question because, of course, often people that start on antidepressant treatment will start on an SSRI. But we asked whether there are individuals who actually might benefit from an atypical antidepressant over

sertraline.

And so, what we found was that our two markers that we had hypothesized in the grant to be associated with responsive bupropion, which was reward learning as well as connectivity in the reward system, indeed selectively, specifically predicted response to bupropion after failing eight weeks of sertraline. But critically, what we found was that it was in the opposite direction that we had hypothesized. Initially, we had hypothesized when we wrote the grant that people that have these anhedonic phenotypes will preferentially benefit from the typical antidepressants, bupropion. But in fact, we found that people that basically are depressed, clinically we don't see differences from non-responders at baseline in terms of depression severity, for example, and so on. But those actually who had a more normative ability to learn from reward in our tests ended up responding very well to bupropion after failing on SSRI.

And similarly, what we found when we looked at resting-state functional connectivity, we again see that in the bilateral accumbens. And we asked the questions where in the brain there is connectivity between the accumbens and this level of connectivity is associated with differential response to treatment, we found that also here, more normative connectivity between the accumbens and the rostral ACC at baseline predicted response to bupropion 16 weeks later after failing sertraline. And actually, with respect to sertraline, this was a kind of a dissociation, right, because in this case higher connectivity predicted non-response to sertraline. So, it seems that, in fact, a more normative brain reward system, as measured by both our behavioral probe as well as functional connectivity, this is resting-state functional connectivity, might be necessary to respond to bupropion after failing sertraline.

But based on these studies, and also because we found in other publications other

markets that were specific to sertraline, what we are currently doing in a study funded by the Wellcome Leap, and we hope to finish recruitment very soon, is that we are collecting MRI data and engaging participants in our probabilistic reward task, and we give also other scales. And basically, we analyze the data immediately, and our goal is to run, actually stratify, basically 60 individuals that have the bupropion markers to either receive the intended treatment or sertraline and vice versa for individuals that have the sertraline markers.

So, we are collecting the data. We analyze it immediately. We feed the data in an algorithm. And our biostatistician provides information to the pharmacy. We are blind, but people basically receive either their intended or non-intended treatment. And the hypothesis, of course, is that those individuals receiving their intended treatment will respond much, much better compared to the other. And again, we hope to finish this study in the next six months or so.

So what I wanted to do today is to show that reward learning is, I believe, an important phenotype of depression that we can study across species. And I think it's biologically supported. We have found that it is associated with individual differences in electrophysiological, functional, as well as molecular markers of the brain reward system. So, it might be an interesting kind of surrogate marker to probe the brain reward system. We have found that this marker is reduced in individuals with MDD, but specifically those who report anhedonia or melancholia. And again, there is preliminary evidence that this marker could be used to guide treatment selections and we have this ongoing prospective study to test this hypothesis.

And finally, I've shown you that there is preclinical and clinical evidence from our lab but also other labs suggesting that, potentially, exposure to early adversity and severe stressors might increase the risk for depression by

inducing anhedonic phenotypes and by dysregulating the dopaminergic signaling in the mesolimbic pathways and potentially also upregulating nociceptin/orphanin FQ pathways. And so, our current work is particularly geared at testing mechanistically some of these hypotheses, again, mostly in cross-species studies, integrating approaches in rodents, non-human primates, and in humans.

And I'll end by acknowledging many, many people, too many to mention, but several people listed here have played key roles in different modalities, including our postmortem work with Sabina Berretta and Gustavo Turecki, my preclinical collaborators. And also, I would like to thank our funding, in particular NIMH for funding most of our works. And I would like also again to thank the organizers for having me today and to all of you for your attention.

DR. NAIFEH: Thank you, Dr. Pizzagalli. A very impressive work and presentation. That was great.

Our next presenter is Dr. Leanne Williams. Dr. Williams is the inaugural Vincent V.C. Woo Professor of Psychiatry and Behavioral Sciences and Associate Chair of Translational Neuroscience at Stanford University School of Medicine. She is the founding director of the Stanford Center for Precision Mental Health and Wellness, and of the Stanford PanLab for Personalized and Translational Neuroscience. She holds a joint appointment as Director of the Precision Medicine Core at Palo Alto VA Medical Center, Mental Illness Research, Education, and Clinical Center. She has developed a first of its kind technology to identify neuroscience-based biotypes for depression and anxiety. Her biotype approach integrates advanced neuroimaging and data sciences. And her treatment studies use biotypes to personalize and tailor interventions to promote wellness.

We'll now begin Dr. Williams' presentation, which is titled, "Precision Mental Health: Using Amygdala Circuit Measures to

Diagnose Subtypes and Inform Treatment."

DR. WILLIAMS: Thank you so much to Co-Chairs Dr. Holly Mash and Dr. Jamie Naifeh for the kind invitation to present at the 2024 Amygdala, Stress, and PTSD Conference. Thank you also to Dr. Robert Ursano, Director of the Center for the Study of Traumatic Stress, and to Dr. Vincent Capaldi, Chair of the Department of Psychiatry at the Uniformed Services University of the Health Sciences.

Today I will talk to you about the amygdala in the context of precision mental health. As we all know, in our current diagnostic approach, we rely on broad categories to define disorders, such as PTSD and overlapping disorders, such as major depressive disorder. And these categories are defined by collections of symptoms that occur together, and they are described in our diagnostic and statistical manual, the DSM. And of course, the DSM is very useful for reliable communication between clinicians.

However, because it's a broad category and assumes typically that an individual represents the average, it does not necessarily focus on what individual treatment should we consider for each individual person. So, with the most incredibly dedicated clinicians and our current evidence-based approach, we still lack a means to personalize treatments. And typically, our response rates hover around 30 to 40 percent over the longer term.

So, the goal of the precision approach is to move the average approach into a more individualized approach. And an intermediate step toward making that possible is to think about identifying more homogenous subtypes that can have a basis in how our brain functions. And this is where the focus on the amygdala comes in. So, we can envision a situation in which, within the diagnostic category, we have a means to assess details of what defines each individual person's functioning and anchor that in the insights from neuroscience, the insights about

human brain function, and use those insights to complement our clinical information.

So here today we are focusing on the amygdala. And we've seen in the accumulated evidence to date that that does identify a coherent group of individuals, specifically with PTSD, but also spanning across other disorders, such as major depressive disorder. And the goal is by having information about not only the diagnostic and clinical information, but also a way to measure underlying amygdala activity, we can personalize and tailor treatments to boost the number of people who respond and who stay well, and to at least think about doubling that response rate. And this vision for mental health is aligned with the priorities of our Veterans Administration and with Congress.

So, we can think of the Scott Hannon Initiative for Precision Mental Health, which now directs the VA, to focus on precision mental health. And under this initiative, known as SHIPMH, there's a specific focus on including

measures of brain function; so, brain function listed here or highlighted here in the blue, and also brain function and structure. And the directive highlights specific measurement modalities, such as functional magnetic resonance imaging and EEG. In this act, you'll also see how there's a highlight on integrating functional MRI with other measures that we acquire, such as the Million Vets genetic program.

Within the recent White House report on mental health, there was also a focus on advancing precision mental health. And here, the focus was similarly on including measures of brain structure and function or activity and integrating those with other measures, such as behavioral measures and genetics, and moving us towards an individual or subgroup level. And this White House report was released in February last year, 2023.

So, today I focus specifically on the brain imaging or the brain circuit modality and specifically on brain function. And I'll talk a

little more about how that focus on brain circuit measures is thinking about deriving information from functional magnetic resonance imaging, in particular.

If we delve more into this idea of stratification on identifying subgroups, here I'm showing eight types that I've identified based on disruptions in the large-scale circuits of the human brain: circuits that help us reflect on our thoughts and our future, circuits that help us respond to negative and positive emotion and to regulate those emotions, and circuits that help us plan ahead and engage in executive functions. So, these are eight that specifically I've characterized for depression, but also have a transdiagnostic application in PTSD. And the one we are focusing on today I've called the threat dysregulation biotype. It specifically involves a disruption in the amygdala. And we'll delve into that a little more in a moment.

This biotyping or subtyping approach is, in this case, we are focusing on brain

circuits, as I've mentioned. But necessarily, as we refine this approach, we would want to link to other measurement modalities, including those mentioned in the SHIPMH directive, genetic measures, behavioral measures, life events, traumatic experiences, and our self-reported clinical information. And then the goal from there is to use this information to think about how do we tailor or personalize treatments going back to the overall goal of precision mental health.

Let's now zoom into this negative affect circuit. It is known by several names. I refer to it as negative affect circuit to link to the NIMH's research domain criteria, which refers to a negative affect or negative valence system. It's also known as the amygdala prefrontal circuit or limbic prefrontal. But key to today's focus is that it's anchored in the amygdala.

So here is the left and right amygdala and connections to prefrontal regions; so, for example, subgenual anterior cingulate and the

medial prefrontal cortex. And through accumulated research and excellent work across many labs, we know that there is a connection between the amygdala and these prefrontal regions, and that typically, we need the prefrontal region to help regulate the amygdala. And this is how we develop the basis of understanding for the mechanisms by which stress and trauma disorders develop and why it may be difficult to regulate the amygdala if it becomes overactive in a prolonged way.

So, looking at evidence that gives us a basis of that mechanistic understanding, we know from several studies that amygdala activity is often heightened in individuals experiencing PTSD. And the figure here is from the seminal work of Scott Rauch in biological psychiatry. And what he highlighted is that, interestingly, the amygdala shown here is heightened in response to threat stimuli. So that's trauma-relevant stimuli but also generic, for example, facial emotion stimuli. The amygdala is heightened even

in the absence of conscious awareness. And so here you see individuals who are not experiencing PTSD and then the heightened, a major activity of individuals who are experiencing PTSD.

And this observation was incredibly important, because it can help us understand why it is difficult to suppress or to regulate threat-related, fear-related PTSD symptoms. If the amygdala is overactive, even in the absence of conscious awareness, then it's difficult to access by conscious processing through willpower and effort, and it's difficult also to describe in words. So that leads us to think about what kind of approaches may help us regulate overactive amygdala in PTSD.

Expanding this finding in my work with Richard Bryant, we've shown that the medial prefrontal cortex, which is part of that frontal region of the circuit within negative affect that connects to the amygdala, that medial prefrontal cortex is also heightened in its activity in PTSD when responding to threat stimuli without

conscious awareness. So here we are using a similar paradigm to Scott Rauch, but we've looked at these frontal regions. And here you can see the amygdala is overactive in individuals experiencing PTSD. This is for non-conscious facial emotion threat stimuli, but also these medial prefrontal regions are overactive. So this, again, suggests that we need ways to harness the regulation of the overactive amygdala.

So that's a segue to saying, let's think about what do we know about existing treatments and how they're informed by knowing about how the amygdala is overactive and how we might be able to target prefrontal circuitry to help regulate the amygdala or to directly target the amygdala. I will focus first on behavioral and pharmacological treatments. And then I will move to some emerging evidence for exploratory therapeutics.

So here, turning to behavioral therapies, there has been very important work on

cognitive behavior therapy, cognitive processing therapy, also known as trauma-focused CBT. And here I'm highlighting work, again that I've undertaken with Richard Bryant, in which we asked this question of whether trauma-focused cognitive therapy could enhance that prefrontal control or that prefrontal regulation of amygdala activity in PTSD.

And in this sample, we were studying civilians with PTSD who were survivors of fatalities or major disasters, such as the bushfires in Australia. And here we found a fascinating result, in which we did see that trauma-focused therapy, or cognitive processing therapy, can help enhance the prefrontal control of the amygdala. So, what we did here was we imaged individuals with PTSD, and then they undertook a structured trauma-focused CBT program, and then we reassessed their symptoms. So, what we saw was improvement in symptoms on the Y-axis here, measured by the caps, was greater when there was a reduction in the

amygdala. So here we're talking about the top left quadrant here, so more reduction in amygdala with higher symptom improvement. So, this finding indicates that using a trauma-focused therapy to engage prefrontal circuitry can help with the regulation of the amygdala.

Interestingly, SSRIs or antidepressants have also been found to increase the capacity for prefrontal control of amygdala activity. And this figure shows a finding for the SSRI paroxetine. And here you see prefrontal activity in PTSD prior to treatment. And then post-treatment you see the increase in prefrontal activity. And this is showing that paroxetine is serving to increase that prefrontal activity and also that symptoms were improved.

In this case, it was a distinct task in which participants were asked to actively try and regulate their emotion. And so, it leads to the possibility that there may be future studies that could delve into what are the mechanisms by which SSRIs such as paroxetine might help enhance

that prefrontal control and boost symptoms. Because we don't know why in this case the effect is having this subsequent impact on improving prefrontal activity. We also need studies that pinpoint which individuals specifically are benefiting from their SSRIs and producing or having the effect of their prefrontal activity being increased.

In this slide, I show a different angle on the effect of SSRIs and also SNRIs that target both serotonin and norepinephrine. And in this case, it's evidence that the amygdala may also serve as a predictor of who responds to a treatment and who may not. And that is another piece of evidence for thinking about how we might personalize treatments. So, in this case, it was a study that I led called iSPOT-D, and it was focused on depression. But the findings are broadly applicable to PTSD and other disorders because we are focusing on amygdala activity and not specifically the diagnosis.

So, in this case, we are finding that

too much amygdala activity is predictive of poor response, particularly for an antidepressant that does target both serotonin and norepinephrine, in this case venlafaxine. Whereas a relatively intact level of amygdala activity predicted better response to SSRIs. And this model was reasonably accurate. So, these two predictive models had 75 to 81 percent accuracy with an internal cross-validation. What's interesting about this is it opens up the possibility of using measures such as amygdala activity to inform how we choose a treatment for each individual. For example, if we see an individual has an overactive amygdala in response to threat, we could consider ruling out SNRIs such as venlafaxine, which are sometimes used for PTSD, and then we might prioritize trauma-focused therapy or some of the exploratory therapeutics I'll talk about later.

As I mentioned earlier on, when we are thinking about developing measures based on brain circuits, in the future we would want to add other

information that helps us refine our understanding of how these circuits function, how they impact experience of trauma, how they might interact with experiences of trauma, and how they might ultimately produce the symptoms we observe. This example is where we included in one study in PNAS measures of early-life trauma, so this was taking into account had individuals experienced significant levels of early-life trauma. This is prior to the age of 18. And we are looking at experiences of abuse, experiences of being in war zones, and experiences of bullying.

And in this case, we found that if we included these measures with amygdala activity, the accuracy of predicting who is likely to respond and remit to SSRIs and SNRIs was boosted; so, it highlights that having these multiple measures may be important. Currently, clinically, we do have access to a life history. If we add the brain information, we have the potential to pinpoint, with reasonable accuracy, who might benefit and who may not from these types

of medications.

Interestingly, in another study, we found that behavior therapy can also help modify or reduce amygdala activity. And in this case, again, it was with depression, but broadly holds for PTSD because of the focus on the amygdala. In this study, we looked at a form of problem-solving therapy, which is also broadly used in the VA and for PTSD. And what we found was that, compared to usual care, this form of behavioral therapy actually reduced the amygdala. We observed this after eight weeks, and we found that there was a twofold increase in positive clinical outcomes. And in this case, it was a randomized controlled trial, so we looked at those who are randomized to the behavioral therapy versus usual care according to the provider. So, this is very promising for, again, saying, could we use pre-treatment predictors to identify who is likely to benefit from these forms of behavioral therapy and others, and then be able to measure that change in amygdala

response, knowing that it does have an impact on the clinical outcomes.

I'm turning now to a new area of exploratory therapeutics, and this is one that I went into as I would say a healthy skeptic, given there are so many different implications and considerations. What I'm sharing with you is mechanistic work on using MDMA to understand what happens to the brain. And why this is relevant here, of course, is that MDMA combined with cognitive processing therapy is being considered for a new therapeutic for difficult-to-treat PTSD or complex PTSD.

In this study, I was not looking at MDMA as a therapy. Well, at least I wasn't looking at it directly as a therapy but as a precision medicine or precision mental health approach to understand what are the acute changes in the brain? How does this approach affect the amygdala? And could we gather information that helps us inform who might benefit from MDMA with assisted therapy in the future? And the idea of

that is if we are considering these exploratory approaches, we may not want to be considering a one-size-fits-all. We may be not only considering who is clinically eligible, but can we consider using biomarkers or measures of brain function to help inform treatment selection.

So big shout out to the team that made this possible and the support that made it possible. And now I'll jump into the design. So, in this case, it was a detailed, within-subjects, randomized, repeated measures design. At baseline, we were acquiring scans to assess the amygdala, and this is using the same task that we use with PTSD. We also gathered clinical information, and we gathered behavioral information.

So, we gathered, for example, measures of how individuals responded to the faces in terms of their reaction times. And we can do this in the non-conscious condition. So, we show the faces in an implicit condition, and it allows us to find out if there are implicit biases,

meaning does reaction time speed up or slow down when these faces are presented in an implicit task. And we found that implicit priming does correlate with amygdala activity in the non-conscious condition.

Two days later, we ran the placebo condition, which was matched to the active dose. The pills looked exactly the same, and we scanned 40 minutes after that placebo dose. And then in two sessions, which were randomized and blinded, we delivered an 80-milligram dose and a 120-milligram dose and, again, scanned 40 minutes after. The individuals in this study did not meet diagnostic criteria because of the design of the study and because we are not directly giving therapy-assisted treatment. However, we did find, as you'll see, that there were some symptomatic experiences within the healthy range.

This is the scanning set up, and one of the issues to consider was how will the experience be once people have an active dose and they are inside the scanner during the experience

of the MDMA. And that was something we paid careful attention to because we were unsure exactly how that would go. What was to our pleasant surprise, the participants told us that it was a very much a positive experience, and this was the majority feedback.

So, these are quotes from the participants. They told us the MRI actually felt secure and snuggly. It felt like a cocoon. It felt like foam around them. It felt insulating. It felt supportive. So, to our, as I said, pleasant surprise, it seems like the structure of the scanning environment and the regularity of the scan sounds may actually provide something that feels not only cozy but also safe for when these experiences are happening.

So, to go back to the circuit that we were assessing in this study, it was the same. The non-conscious evoked amygdala and prefrontal regions within the negative affect circuit in response to the non-consciously presented emotion face stimuli, and we focused on stimuli depicting

threat. And here you'll see two regions that are highlighted in our findings.

What was interesting is that we could take our sample and split it, even though it's a relatively small sample, so right now, 17 participants, and we are continuing the second half of the study to achieve 40 subjects total. But we did find a relatively even split at the baseline of individuals who had high amygdala reactivity to threat and individuals who had intact or low reactivity. And in a moment, I'll talk about the method we used to get that individualized amygdala quantification. So, this gave us a way at the baseline to subtype our individuals, and what we found when we then looked at other measures is that subtyping was very important to understanding the sample and then, subsequently, the effects of the MDMA.

So here, we can look at early-life stress or early-life trauma, and the number of experiences, this is all in our standard deviations, of early-life trauma events was much

higher in the high amygdala group, even though, as I mentioned, these are not individuals who meet diagnostic criteria. The low amygdala group had a relative absence of life trauma. The high amygdala group also had more PTSD symptoms, albeit not meeting criteria compared to the lower group, and they also had more of this bias measure on the behavioral test. So, they were faster in responding to threats, suggesting a kind of hypervigilance for threat, even when it's in an implicit task, again, compared to the low amygdala group.

And when we look at the effects of the MDMA, you'll see that they were specific to the high reactivity group. So, when we compare the high to low amygdala group, we see this specific pattern significantly different in the high amygdala group. So, the 120-milligram dose compared to placebo was associated with a significant reduction in that amygdala activity. So, at that point, which was either 10 or 20 days later, but the effect was observed during the

dosing session, the amygdala was reduced in the high reactivity group back into the healthy baseline to the point that they did not differ from the other group. We saw a trend for this also for the 80-milligram dose, but it was most apparent for the 120. So, in the next phase of the study where we'll be recruiting more participants, we are going to expand this study and also refine the recruitment so we can enrich further these high, medium, and low activity groups to test whether this holds in a larger sample.

The other interesting finding was that not only did the amygdala reduce, but the connectivity between the amygdala and the prefrontal region, particularly the subgenual ACC, was enhanced, so it suggests that there is boosting of the regulation of the amygdala. And you see these findings here in plot version. So here, this is the pre-post-reduction in amygdala activity, is a pre-post-increase in the subgenual activity, and here the increase in the

connectivity.

Interestingly, also, these findings are consistent with Robin Carhart-Harris's findings for the amygdala, in which he also sees MDMA may help to regulate or suppress overactive amygdala activity. When we were looking at symptoms, as I mentioned, these are not diagnostic, but in the subclinical range, we did find that there was an improvement, so a greater change in anxiety and fear in the high amygdala group as well. So, this is the amount of change or improvement. So, you can see higher symptoms at pre-dose and then reduction post-dose.

What I wanted to share now is some of the individual experiences that were reported by our participants. One other aspect of the study we hadn't expected is just how rich and detailed would be the feedback from our participants, especially given how much was happening during the dosing session. So, some of the feedback was after they had done the scan, during the monitoring session, and some was day after.

And here we see feedback from a participant who had high amygdala reactivity. And it's an example that is consistent across several of the participants with high amygdala activity, where they explain this sense of feeling kind of removed from their negative experiences or topics that gave them anxiety and able to process them, which is consistent with the goal of the therapeutic use of this compound. They describe a deeper understanding of how their trauma changes their brain. And this particular participant was motivated to reach out to their family, particularly their father, and reconnect after a long estrangement. They talked about things clicking into place, feeling more accepted, and experiences of their defenses being lowered or feeling safer. And here, you'll see this description of not feeling so overwhelmed and being able to have some sense of control that they didn't have before.

To highlight, though, this is not like a kind of party-type experience of the MDMA. In

this case, they're saying it's not fun but very introspective. And we do wonder if the scan or a space like that is helping boost that introspection, meaning the setting may be important here, just like it is in the therapeutic session.

By contrast, the participants with the lower amygdala activity and the lower stress and the lower PTSD symptoms, they typically reported a different experience. They did experience feeling a sense of acceptance, forgiveness, and gratitude and ability to connect, and also this lower sense of feeling defensive. They also felt, in general, more positive experiences of empathy and a state of feeling connected. But this one quote is more the kind of sense of it being an enjoyable rather than a processing experience. So, these results from this mechanistic study suggest that for individuals with PTSD and the higher amygdala, that is where there may be particularly a therapeutic effect. That is not the typical experience of MDMA for

recreational purposes but for the introspection and the ability to process the trauma.

There are other directions that we could consider going, and there are many new directions that we could think of informed by advances also in the field of depression. But here is one example. So, this is now moving away from threat stimuli and thinking about positive stimuli. And there's increasing evidence that in PTSD, similarly to depression, there is a flattening of positive emotional responses. It may not be in everyone. It may be, again, in a particular subtype, but of kind of blunting of positive emotion.

And in this study, we found that, for positive emotion faces, there was a reduction rather than an increase in amygdala activity, and there was also a reduction in the reward center of the brain, the ventral striatum. And this was importantly associated with symptoms of emotional numbing. And this kind of finding opens up the opportunity for considering treatments we may not

currently always think of, and the idea that having access to circuit measures may help us select different treatment options. For example, behavioral activation therapy has been found to boost striatal activity, ventral striatal activity. There are some selective new treatments being developed that specifically target the ventral striatum as well, and may have transdiagnostic applications. So, given that there is also an interplay between the ventral striatum and the amygdala, this may be an interesting area to explore in future studies.

In what I'm proposing today, this moving toward a more precision approach, the application of precision mental health, and studies that advance precision mental health, we necessarily are thinking about moving beyond averages to subject-level understanding and subject-level measures. And that's something I touched on right at the beginning. We already use subject-level measures in assessing PTSD and assessing other disorders. For example, we use

neuropsychological tests, and we have a way of providing someone with a profile of their performance on neuropsychological tests.

For functional brain imaging, we have not had access to these individualized test scores. Typically, in our studies, we create a group average, and we use that to infer what might be going on for individuals. And this is a reflection of the history of development of imaging technology. What I've been excited to work on in the past few years is developing a method by which we can individualize the quantification of fMRI data and quantify it similarly to what we do for neuropsychology, and generate subject-level scores that are also presented at standard deviations.

So let me walk you through this example. In this figure here, which was published in *Biological Psychiatry*, I describe six different circuits. They're the six different colors here. And the orange one is the one we've been talking about today, amygdala and

prefrontal. There are two forms of this orange one, because I've also looked at in response to sad, which I didn't talk about today. And in the process of standardizing the regions that define these circuits, I created a set of masks. These are ways to define the regions within fMRI data. And I defined each region based on the Neurosynth database and then refined it with a series of quality assessment steps.

So, what that led to was a total of just over 90 regions that are used to define each circuit and that are broadly consistent with the consensus in the field. So, for example, the circuit we're talking about today is the left and right amygdala and this ACC frontal region. Clearly, there are many other regions involved, but this is a proof of concept for how we can apply individualized imaging and then expand with other regions. What the second row shows is the quantification of these circuits. So, I quantify for the one we are talking about today, amount of activation in response to stimuli in beta

estimates. Similarly, the connectivity as a function of the task, as beta parameter estimates from psychophysiological interaction analysis, and then those measures are expressed relative to a healthy reference dataset to generate standard deviation scores. And then on the last row, it's showing that those scores can then be summed into an average, or they can be looked at for the individual region, such as the amygdala region.

So, this is a method that helps us now run prospective studies or prospective trials where we want to know, does someone have an overactive amygdala at the baseline, or do they not. Of course, there's also a need to continually refine this approach and these biotypes based on regions, such as the amygdala. So, circling back to what I mentioned at the beginning, we would want to add in more of the other important areas of measurement, not only life events and behavior, but genetics and other fluid biomarkers and expand to multiple other treatments.

But today, I hope that I've given you an overview of how we are thinking about precision mental health, focusing on this amygdala-based biotype, and how that might help us inform more personalized treatments. And I thank you very much again for the kind invitation to present, and I really look forward to your questions and invite your feedback. Thank you so much.

DR. NAIFEH: Thank you, Dr. Williams. Fascinating research and very well-presented.

We now have a break until 2:45 p.m., Eastern Daylight Time, which is about 18, 19 minutes from now. At that time, we'll begin our last presentation of the day. As a reminder, you can use the Q and A feature in Zoom to submit questions to our speakers at any time before or during the Q and A session. And please visit the poster gallery on the conference website to learn about the research being done by other conference attendees. Thank you, and we'll see you again after the break.

(Whereupon, the above-entitled matter went off the record at 2:15 p.m. and resumed at 2:45 p.m.)

DR. NAIFEH: Welcome back. Our final presenter today is Dr. Sandro Galea. Dr. Galea is Dean and Robert A. Knox Professor at Boston University School of Public Health. He has published extensively in the peer-reviewed literature about the social causes of health, mental health, and trauma. He has documented the consequences of mass trauma and conflict worldwide, including the September 11 attacks, Hurricane Katrina, conflicts in Sub-Saharan Africa, and the American wars in Iraq and Afghanistan. He is an elected member of the National Academy of Medicine. He currently serves as Chair of the Boston Public Health Commission Board of Health. We'll now begin Dr. Galea's presentation, which is titled, "Structures, Systems, and History and the Consequences of Trauma in the Present Day."

DR. GALEA: Thank you for having me

join today. I'm going to talk about the consequences of trauma, which I realize many people who are listening to this are experts in their own particular areas. But I'm going to try to talk about this within the context of the structures that generate trauma and how we need to understand these structures in order to understand the consequences of trauma. So, the structure of my talk, broadly speaking, is I want to frame why I think when you think about trauma and why thinking about the ubiquity of trauma is important. Then I want to move into some key points, three key points that I think we need to take when we think about the consequences of trauma. And then I want to frame our understanding of trauma within these consequences using some mathematical examples. So that's broadly my outline, and let me then jump in.

I'll start by making the point that trauma, violence as one particular sub-example, are nearly ubiquitous. If one were to take a hypothetical population, we know from many

studies that more than 90 percent of the population experience a trauma at some point in their lives. And that is extraordinarily high for an experience that is a risk factor for adverse health, adverse mental health, and as we'll get to in a second, adverse physical health. There are, in fact, very few risk factors that are actually remotely this high.

If you try to think of it by way of illustration, for example, you take something like cigarette smoking, which we talk about a lot, as a risk factor for poor physical health. Well, the prevalence of cigarette smoking in this country is now in the 15, 20 percent range versus 90 percent for trauma. And in high-risk populations, populations like military populations and some urban populations, give about a 50 percent experience of trauma even in the past year.

The best studies have been done worldwide. Obviously, we have quite imperfect data about the prevalence of exposure to

traumatic events in countries. But the dark red are populations where more than 70 percent of population have exposure to traumatic events, and you can see the U.S. is one of those countries. And the other way to look at this is the number of traumatic events experienced per person. And what you see is that in the U.S., we actually have among the highest number of traumatic events experienced per person.

Just to give a bit of a sense of the scale of the issue of traumatic events, it's very hard to actually get a full burden of traumatic events worldwide, but something that is a proxy, perhaps, is injuries. You see that injuries are about 50 percent more than the number of cases of HIV/AIDS, TB, and malaria cumulatively worldwide.

These obviously come at an enormous cost, this is, again, very hard to estimate the cost of trauma worldwide. But a cost of containment of violence, as one example, is high in many countries, but broadly speaking, in the U.S., again, we're among the highest expenditure

countries. But containment of violence is more than 75 times of all foreign aid expenditure. And what this analysis does from a focus on economics and peace, if violence containments were reduced by 15 percent, we save $1.4 trillion in the world. It just gives a sense of how ubiquitous this problem is and I think why it's important to understand the structures that generate trauma, given the ubiquity of this problem.

And just to make one last point about the importance of the ubiquity of this problem, this is a slide from the most costly medical conditions in the United States, combining both civilian populations, that's in the darker green, and the active-duty military populations in the lighter green. And what you see, mental disorders have the highest cost of all medical conditions in the U.S., but trauma-related disorders, as were separated out in the study, are the third. So, if you add trauma-related disorders and mental disorders, the two being

quite linked, as people know, obviously we end up with a substantially great cost.

And the reason why trauma and mental disorders are so expensive, of course, is because they're both highly prevalent, as I discussed a second ago, and because they happen early in life and they are persistent. So, you end up with a lot of people acquiring these disorders and that persisting with them throughout their life, resulting in billions of dollars. So, the point of the first bullet, really, is to make the point that we're dealing with ubiquitous exposure, that trauma is a ubiquitous exposure.

Now, let's move on to the second point, which is I want to talk about the consequences of trauma. And I really only want to make three points about the consequences of trauma. The purpose of this talk is not to do a comprehensive presentation about the full range of phenotypes that emerge from trauma, but rather to draw three general points about the consequences of trauma.

The first point is that trauma ends up having long-tail health consequences of traumatic events, that when you have traumatic events, the health consequences, mental health consequences, physical health consequences, tend to last for a long time, which, of course, this builds on my previous point about the ubiquity of these traumatic events.

I'll show you this in a couple of ways. This is the prevalence of lifetime post-traumatic stress disorder by cumulative childhood risks. So, this is in a cohort. This is from a paper by Karestan Koenen and colleagues from the (inaudible) cohort, showing that you have more childhood risk, going from left to right. You have greater prevalence of lifetime PTSD. So risk in childhood results in post-traumatic stress, then throughout life.

I'll show you another study. This is one which our group had done. This study looked at conflict in a county in Liberia. This is in Nimba County, which is in northeastern Liberia.

And Nimba County experienced substantial conflict around 1990. This was part of the civil war in Liberia where Nimba County was a county where a lot of warriors passed through on their way to the capital of Liberia, Monrovia. And what you have here is the circles you're looking at are areas of documented conflict. And if you look carefully at the legend on the bottom right, you'll see dates there. These are dates of when conflict happened.

The reason I'm showing you this slide is because we did a population-based survey of Nimba County, and we assessed the mental health of communities in Nimba County 20 years after the passing of conflict. And what you see here is the little dots are all the villages assessed. The big dots are the villages assessed, which had a higher burden of post-traumatic stress disorder. And what you see is a similar pattern of post-traumatic stress disorder compared to where there were the battles, the skirmishes.

So, 25 years after a traumatic event,

you end up with this shadow of these traumatic events that last in terms of greater burden of post-traumatic stress in these villages. And of course, that reflects the long tail of the traumatic event experiences themselves among those who experienced them, but also that these traumatic events result in enormous social and economic disruption, which then result in more traumatic events, more violence events, and greater risk of the consequences of traumatic events for those who continue living in the villages.

Another way of looking at it from another place in conflict, this was from a study we had done in Ethiopia. And we looked here in an area of conflict in Ethiopia and looking at number of traumatic events experienced. So, this looks at women and men. On the X-axis, you have number of traumatic events experienced, and then the Y axis, you have a risk of depression. And what you see is more traumatic events experienced, more depression. There's nothing

surprising there.

But what we did in the study was we then wanted to assess what happens to the children of the moms who experience traumatic events. And this slide looks at depression and not depression among the moms. The black line are moms who are depressed; the white line are moms who are not depressed. And here, we're looking at a child's personal social development.

As you go on the X-axis, you have the child's getting older, so the child's development is getting more. But what you see in particular is that for all time points, except for one, there's a statistically significant difference between the development of the child and mom's depression. So, moms who experience more traumatic events are more likely to have depression. Moms with depression are more likely to have children who have delayed social development, which of course, has its own full range of consequences throughout the child's life.

Another way of looking at this is from a study that we had done looking at children of mothers who experienced traumatic events. And the way to read this graph is on the X-axis, you have number of post-traumatic stress symptoms which the mother had, and the green bars look at number of symptoms that the child had of post-traumatic stress, if children experience traumas. And what you see is the darker the green, the more number of symptoms in the child. So, as you go from left to right, which means mothers having more PTS symptoms, you see there's more dark green, which means the child, if the child experienced a traumatic event, that the child themselves then has more post-traumatic stress symptoms. Now, of course, the etiology of this is complicated. It probably involves some combination of genetic forces, including things like epigenetic forces, but also enormous behavioral influences in terms of different behavior of the moms and different conditions in which the child grew up.

And just to make this point one last time with one other study; this is children of mothers who experienced abuse and the initiation of smoking and higher levels of smoking consumption. So, you go from left to right. We have mothers who experienced childhood abuse, and then we're looking at their children. And the way to look at their children is the black bar are early initiators with high consumption of cigarettes. So, what you see as we go from left to right with moms who experienced more abuse, their children are more likely to be early initiators of smoking and to smoke a lot of cigarettes.

So, I think Point A, which is sometimes lost when we think about trauma, and I think it's critical, is that the consequence of traumas are deep and long-lasting, which builds a little bit on the point I was making earlier about the ubiquity and the enormous cost incurred by traumatic events. Point B is that it's really hard to separate traumatic events from the

context in which traumatic events happen. And this, of course, now I'm starting to build slowly to some of the comments I want to make about structures and how they shape traumas and their consequences. I'll start by showing an example from Hurricane Katrina, which people know, but will remember almost 20 years ago was devastating in the Southern United States, in Louisiana and Mississippi. And it is impossible to understand the consequences of Hurricane Katrina without understanding the context in which it happened.

It's impossible to understand that without understanding that the areas that were mostly affected by Hurricane Katrina were areas which were marked by substantial poverty and deprivation. When you look at, for example, post-traumatic stress after Hurricane Katrina from a study that we had done, what you see here, this is a survival curve of people with post-traumatic stress. Everybody in this graph had post-traumatic stress, and then over time, you're seeing remission.

But what you see is this separation of the curve where people with higher stressors are much more slow to remit than people with lower stressors. People with lower stressors are remitting much more rapidly than people with higher stress. And these stressors are not trauma, at least by our DSM definition. These are problems with putting children in school, problems looking after parents, problems with spousal problems, challenges at the job. And those stressors accentuate the course of the traumatic event.

Another way to think about context and the ineluctable influence of context is from a study that we had done after hurricanes that hit also about 20 years ago that hit Florida. There was a time when you may remember that there were Hurricanes Jeanne and Ivan, Charley. Four hurricanes hit Florida at the same time. You can see where those hurricanes were on the left.

And we did a study looking at Florida counties where we separated out the counties by

counties which had high crime and low crime. So, we took these counties which were affected by the hurricane marked here. And then we looked at the relationship between the best documented thing. It looks like that polymorphism, the serotonin transporter gene, and its association with post-traumatic stress. And what you see is you have this classic effect modification crossover between the serotonin transporter gene and the likelihood of PTSD.

And what you actually see is that there is a different role of the long/long versus short/short genotype in counties where there was high exposure to county-level crime versus low exposure to county-level crime. And the high crime rate, by the way, is the triangles, and the low crime rate is the diamonds. So, the triangles, you have the long/long genotype is protective against PTSD, while in the low-crime-rate neighborhoods, it is a risk factor. What this suggests, of course, is that the genotype is not in and of itself a risk factor, but rather

it's sensitizing to local conditions. And in fact, the only way to understand the role of the genotype is to understand those local conditions. So, it makes it clear, as I hope my previous Katrina example also makes clear, that you cannot understand the consequences of these traumatic events without also understanding context.

One other example now taken from the military cohort, some work that we have done over the years, this looks at trajectory of depression symptoms among soldiers. And what you have is the purple line are soldiers who in a cohort over many years continue to have depression. The green line at the bottom are soldiers who had very little depression, and then the blue line is increasing depression, red line decreasing depression. All I want you to see is that the key factors that characterize the purple line and the blue line versus the other lines are childhood adversity, having substantial number of lifetime stressors, number of lifetime traumatic events, that it's actually almost impossible to

separate out these soldiers without looking at childhood adversity and ongoing stressors.

So although when we tend to think of our women and men in uniform, we tend to think about the traumas that they experience in the military context, study after study teaches that the military context is only part of the exposure that matters, that, in fact, the exposure that matters just as much, if not more, are their childhood conditions, their exposure to stressors that surround the military experience, which means the context is impossible to separate from the actual traumatic event experience itself.

And just to show one more study. This was a study from Israel and from the Israel-Palestine scenario, that's obviously, when I'm recording this today, which is in March of 2024, it's a highly-contested area with a war ongoing, particularly in Gaza. But there's a study that was done before then. And what we looked at was we looked at post-traumatic stress among citizens of Israel. And this was among Israeli Jews and

Arabs. And what we found is a substantially higher, three times higher, greater risk of post-traumatic stress among Arabs than Jews. These are all citizens of Israel.

When we then did a number of studies to try to explain this greater likelihood of PTSD among Jews and Arabs, we were able to reduce the risk quite a bit when we took into account all sorts of demographics: sex, age, income, education, religiosity, also took into account documented direct exploratory trauma, took into account threat from conflict, took into account economic loss, psychosocial loss, traumatic growth, and social support. And at the end of the day, in the best specified model we could come up with, Arabs, Israeli Arabs, still had 90 percent greater odds of post-traumatic stress than did Israeli Jews.

What this speaks to is the fact that there are unmeasured elements of context that we are actually not capturing in the study. And I think that really captures some of the profound

differences in living conditions between Israeli Arabs and Israeli Jews, which are all embedded in context, right? So, what we're seeing here is context is almost inextricable from understanding the consequence of traumatic events, in much the same way as context intersects with traumatic events, to result in health consequences that last over the long term. So those are Points 1 and 2.

And then the third point is that when we think about this, we need to think about this being more than mental health. A lot of us who are in this meeting, our work is about mental health, certainly my work is predominantly about mental health. But it's important to recognize that the consequences of traumatic events, the long-term consequence of traumatic events, and the ineluctable role of context in shaping traumatic events is more than just about mental health. This is from a Global Loss Status on Violence Prevention report, I think did a nice job of talking about the verticals: physical

health, mental health, sexual reproductive health, chronic disease, talking about the full range of disorders that are affected by trauma and violence.

Just to show a couple of studies on this, this is from one study looking at association with a whole range of physical health conditions, looking at traumatic events. You go from left to right, you go from one traumatic event to five-plus traumatic events. And you have conditions like arthritis, back pain, neck pain, heart disease, high blood pressure, asthma, diabetes, et cetera. All you need to see here is if you go from left to right, you see essentially in all these conditions this increase in the odds ratio showing a greater risk of all these conditions when you have more traumatic events.

This is from a study that looked at, over time, the body mass index. So you look at people over time, and you have a number of curves. The diamond curve, which is the bottom curve, were people with no traumatic events. And then

when you go up, you have trauma and no symptoms, trauma and some symptoms, trauma and many symptoms. And what you see is that over time, people who have trauma and multiple post-traumatic stress symptoms end up having higher BMI consistently. And of course, higher BMI, more obesity, is associated with a full range of non-communicable diseases.

And one last way of showing this from another study that looked at the cumulative incidence of type 2 diabetes stratified by post-traumatic stress disorder, on the X-axis here, you have age, so people getting older. And you see more diabetes, which is entirely consistent with what we know about the pathology of disease. But then you have these different lines. The black line, which is the bottom line, are people who had no traumatic event. The red line are people who had trauma with no symptoms. And then you go into one or three symptoms, four or five symptoms, six or seven symptoms. So, the course, which is over age, there is greater risk of having

type 2 diabetes is consistent for everybody, but when you've had trauma, and when you've had traumatic event experiences, and we have post-traumatic stress symptoms, your slope is steeper. You're more likely to have type 2 diabetes, which is consistent, of course, with everything else I'm saying here.

So, I think as we set up this talk trying to understand the context of trauma, the structures that shape trauma, and the consequences of those contexts and structures, we need to understand that trauma has deep, long-tail, long-term consequences, that those consequences are inextricable from understanding the context in which traumatic events happen and that these consequences are deep and lasting and involve both mental health and physical health.

Now, let's move on to structures. So, what are the structures that shape all this? Well, the structures that shape this fundamentally are features of the world. They're around us. Now, it doesn't take much to explain

to this audience that it is the world around us that makes it more or less likely that we're going to be exposed to a violent event. We're going to be exposed to a traumatic event.

In fact, most analyses that look at the determinants of health, and this is broadly speaking, the determinants of health here, it looks at clinical signs and improvement, not just the systems' improvement, are very clear that while healthcare, medicine at the bottom part of this infographic person's feet, accounts for 10 to 20 percent of health, most health is accounted for by our behaviors, our physical environment, our education, our jobs, our family, social support, our income, our community stability.

And, of course, these forces all influence our risk of trauma and violence: community safety, whether we're able to have the income to protect ourselves, whether we have a job that keeps us out of trouble, the kind of physical environment we live in, whether or not we're engaged in dangerous sexual activity or

alcohol use. All of that goes to and contributes to the risk of traumatic event, which based on my setup is actually inextricable from this kind of context, has long-lasting consequences, and affects mental and physical health all around, which means that it is impossible to think about the consequences of trauma and try to understand the etiologic determinants of trauma without also understanding the conditions of the world around us, without understanding whether or not people are stably housed, whether or not they actually are living in good neighborhoods, whether or not they have poverty, whether they're isolated and homeless.

And when you look at the data that I showed you earlier, you see that these elements of context infiltrate everything about the consequences of trauma. Now, I know I showed you this with some empiric examples, but as I move to the latter third of my talk, I want to now show you some of this with a couple of mathematically-modeled illustrations. And I want to talk a

little bit about what is the role of context in mathematically determining the risk of the consequences of traumatic events.

So, here's what I'm going to do. I'm going to model a world where I'm going to focus on depression. I'm going to focus specifically on a mood disorder. I could do the exact same math with, say, a stress-related disorder, with post-traumatic stress, but I'm here focusing on depression. And I'm going to say, look, let us say that the depression is caused by two things alone, genes and environments, and that the genes and environments happen together. And you need both the gene and an environment to create depression. And I'm simplifying the world here. I'm saying there's one gene, and it's a depressogenic gene. And there's one bad environment. It is a depressogenic environment.

So those two together, when you have the gene and environment together, they result in depression. Now, obviously everybody realizes, I'm grossly oversimplifying the world, but

hopefully, you realize that it is a useful simplification because ultimately, it is some combination of biology and context that shapes the consequences of traumatic events. So, I think we're actually getting at a model that can helpfully explain the world. So, in this example, what I'm going to do is I'm going to keep genetic influence the same. I'm going to have a population, not change their genetic influence, but I'm going to vary the traumatic environmental influence. I'm going to vary the traumatogenic environment that people are in.

So schematically, here's my population. There's a population of people, and this is going to stay the same throughout my two examples, that these people marked in the dark gray have the depressogenic gene. That's not going to change. So, the pattern which you see there with the dark gray is always going to be the same. These people have the depressogenic gene, that one gene, that if in the right environment will become depression. The

environment is green. So, this is the traumatogenic environment. That's green.

So, the schema, the math behind this, depression is marked in red, that if an individual has both the gene and the traumatogenic environment here, marked, you can see the gene in the gray dots, just so you can see it behind the green. So, you have the gene with the traumatogenic environment, and that becomes depression. So that's the math. So now, let's do some math and see how much do genes and environments contribute to this mood disorder as a consequence of these traumatic events.

Scenario 1. Scenario 1, we are in a highly traumatogenic environment. Perhaps we are in a combat zone where essentially everybody is in this green environment. Now, remember, green is a traumatogenic environment. Now, remember that underlying this there's a certain pattern of people who have the depressogenic gene. That's the same pattern now with the black dots behind it that I showed you earlier. So, I have not

changed that pattern.

Well, the people with the depressogenic gene now all are in a traumatogenic environment. The two together become red, which means they all have depression. That means when you do the math, you can do the relative risk of depression given the gene or the population at triple-risk proportion for depression given the gene. The top number is 300-something. More importantly, the PARP, which is population-attributable risk proportion, which is the proportion of depression that's attributable to gene is 1, which means 100 percent. Why is that? Well, everybody with the depressogenic gene got depression. You can see that right here, right? So, it's 100 percent, which means the proportion of depression attributable to gene is 100 percent. It's everybody. So that's Scenario 1.

Now let's move to Scenario 2, where the genetic architecture remains the same, same pattern of people who are in the dark gray. But now, only very few people are actually in the

traumatogenic environment. That's the green. Well, now, only two of these figures actually have the gene and the environment. Those two together become red. Those two become depression. So, when you do the same math, the relative risk of depression given the gene is 1.7, and the PARP, population-attributable proportion, is 40 percent, which is only 40 percent of depression is attributable to gene, because many people with the gene, what you see on the left, they never got depression because they were not in the traumatogenic environment.

So, what does this mean? Why is this? Well, when causes happen together, here we're talking about the gene and the traumatic environment, what determines how much one of the causes matters is the other factor, which means our estimates of the role that genes play in shaping our risk of mood anxiety disorder, the second traumatic event, depends entirely on the context within which this happened. This, of course, means that we cannot simply ignore the

traumatic environment within which population lives and within which health happens.

So, context is an inextricable part of our analysis of the consequences of traumatic events, that we simply cannot even forge ahead in looking at genetic influences, say, without also understanding consequences and context. So hopefully, you now all understand why I started off by showing you the data and the evidence about how context is inextricable from trauma when you're thinking about those consequences and also about the ubiquity of those traumatic events, and how they have effects over the life course. Because now you realize that, actually, inquiry into the determinants of the consequences of traumatic events is virtually meaningless without actually also thinking about context. This is why we have spent a lot of money on GWAS and mGWAS studies looking at genetic variants associated with the consequences of traumatic events.

Here's a quote from a mGWAS paper that's saying, genetic variants detected by GWAS

typically explain only a small fraction of total family or twin-based heritability. The reason for that is because of context, which is this other missing variable, that all these studies have as a feature. And this is not a criticism of these studies. This is simply a way of understanding these studies and to understand that we simply cannot understand genetic or individual determinants of the consequences of traumatic events without also understanding consequences.

You know, there's a metaphor for this, and there's actually a bias in epidemiology, in my field, that we use the term lamppost bias. So, lamppost bias goes something like this, which is you have a man who's crawling around under the lamppost looking at the ground for something, and another man comes up to him and says, what are you doing here?

And the first man says, well, I'm looking for my keys.

So, the second man says, I'll help

you.

And they both are rolling around looking for the keys. After a while, when no keys are found, the second man says, well, we can't find your keys. Did you lose them here?

And the first man says, no, I didn't lose them here. I lost him over there.

And the second man says, well, then why are we looking here?

And the first man says, we're looking here, because here, there's light.

Now, the point of this metaphor is that yes, there's light here, but of course, you're not going to find your keys because your keys are not here. I go back to then thinking about the consequences of traumatic events. That determination is not going to help us, because unless we take into account the other factors, the keys simply are not under where the light is, which means we need to move the light. We need to get another light, so we can actually find where the keys are.

Similarly, we simply cannot understand the consequences of these traumatic events unless we actually also understand the structures and the context within which these traumatic events happen, unless we understand the conditions of people's lives that ultimately shape their experience of traumatic events. And the point I'm trying to make in this whole talk is that it's non-discretionary to consider these contextual forces. Yeah, I think in scholarship and research about the consequences of traumatic events, it's not difficult to appreciate that context matters. It's not difficult to appreciate that these structures matter, but I think not infrequently, we can say, well, yes, but it's sort of complicated. It's sort of difficult, so we're not going to pay attention to it. But the point is actually not paying attention to it means we're not going to find the right answer, in a similar way to saying, well, we're only going to look for the keys under the lamppost because that's where the light is.

So, I want to conclude, and I want to conclude by talking about what does this mean for interventions, because I know a number of people in this room are interested in intervening. And while I'm making an argument that is relevant to research, one might say, well, what does this mean to me if I'm interested in interventions? And I want to make the point that all of this matters to interventions just as well.

So, I go back to the world for a second. This is a world. And I'm going to say that the blue people have experienced less trauma; the red people have experienced more trauma, rather than saying no trauma and trauma, recognizing the ubiquity of trauma. I'm just dichotomizing for the sake of ease that the people in red experienced more trauma; people in blue experienced less trauma. That's the world.

Now, we know that both people with and without trauma can get disease. By disease here, let's just focus on a mood/anxiety disorder as a direct result of traumatic event. And those are

marked with Xs. Now, the red are more likely to have a mood/anxiety disorder related to trauma. The blue are less likely because they have less trauma. But they both get some extent of disease, right? Now, we can take this and map it out like this as a population. So, if you take trauma experienced on the X-axis, we have some people who are on the far right, more trauma experience. Some people on the left, those are the lucky few who have less trauma experience. And most of us are somewhere in the middle, where we have certain number of traumatic event experiences in our lives.

Now, we could approach the world from a perspective where we say we're going to isolate and focus only on people with a lot of traumatic event experience. So, we identify those at high risk, and then we work only on those people. And we work to reduce their risk, so we try to make those people better by removing their traumatic experience. What we're doing effectively when we do that is we are in the population focusing on

the red people, the people with more traumatic event experience. So, we are indeed helping, and we're helping those people with the X's. But what we're missing is all those people, all those people who also are in blue, who also had traumatic event experience.

There is a different way of looking at this. And this is the way where we make thinking about context and structures an ineluctable part of what we do in research and intervention. That approach takes this curve, takes the number of people with traumatic event experiences, some more, some less, and tries to shift the whole curve to the left by making the environment less traumatogenic, if you go back to my empiric example, by reducing the number of green figures in that figure. What does that approach do? Well, that approach looks at the whole population, and what it does is it reduces number of reds, and that reduces number of X's in the whole population.

I'm now going to wrap up. I think

this can be captured in a number of ways. Here's a quote from Dr. Ruth Shim, who says that to effectively prepare to face public health crisis, she's talking about consequence of trauma, healthcare practitioners, policymakers must commit to addressing the social determinants of health and mental health. And the term social determinants is a term that you've all heard that is related to what I'm talking about here when I talk about history structures and conditions that shape trauma. But the point, and hopefully I've made that point effectively in my talk, is that thinking about those conditions, thinking about these contexts and circumstances, is inextricable with understanding the consequence of traumatic events and that doing one without the other fundamentally has us not understanding the full picture.

I'll conclude with just a couple of thoughts. I do think it's critical that we understand what we know and what we don't know and why we know it. This is just a couple of

slides that tries to make this point. The figure on the left is from a paper review that we had done looking at behavioral consequence of terrorism, the meta-analysis. And what I want you to see there, I know it's small, is you can see the imbalance between the black bar and the gray bar. The gray bar is number of studies; the black bar is number of events. And what you see is there is a deep imbalance between where the gray bar and the black bar is, which means most of our studies are actually in places where the problem is least.

In fact, on the right, what I have is number of deaths from violence in high-income countries and low and middle-income countries, you see much more in the darker pink, which is in low and middle-income countries than the high-income countries, although the vast majority of our studies come from high-income countries. So sometimes when I look at the literature and I try to think comprehensively about what we should know about context, structures, history that

shape traumatic events, I feel a little bit like this. I feel like yes, we're looking at the picture, but hopefully, as you're all doing as you're looking at the picture, you're looking at the picture saying, what is this?

Well, what this is, of course, is part of the whole picture. And the whole picture now is much more clear, but we actually don't have the whole picture. What we have is only a very small part of the picture. And I think in order to get the whole picture, we need to have a much better understanding of the full set of contexts, circumstances, histories, and structures, including from a range of countries around the world, to be able to understand better the determinants of trauma, to be able to intervene to mitigate its consequences.

I'm going to stop here. I'll simply point out this, actually. I recently published a book, which is on the left. And there's a blog which I do regularly, which is in the middle, and the two codes, QR codes, the first one on the

left will get you to the book quickly, and the one on the right gets you to the blog quickly, in case people are interested in following up on some of my thoughts, evolving thoughts, about health and its determinants, all of which is really framed within the context of trying to understand context, structures, history that shape health.

Thank you for inviting me here. Thank you for being in this room, and I look forward to opportunity for question and answer and a conversation. Thank you.

DR. NAIFEH: Thank you, Dr. Galea, for providing that very important perspective on trauma and for presenting it so well.

We'll take one more break until 3:45 p.m. Eastern Daylight Time, which is about 20, 21 minutes from now, and then we will return for our second question-and-answer panel with our last three speakers. Hopefully, you've had an opportunity to check out our poster gallery on the conference website.

We had some great submissions this year, voting to identify two winners for the best clinical poster and one winner for the best preclinical poster. In alphabetical order, our poster winners are Basarkod and colleagues' "Right Amygdala Volume Predicts Future PTSD Severity in Preadolescent Children Exposed to Trauma"; Smith and colleagues' "A Novel Animal Behavioral Model for Assessing Arousal and Anxiety States: Implications for Post-Traumatic Stress Disorder"; and Spangler and colleagues' "Stress Biomarkers and In-Session Exposure to Nightmare Content: Results From a Pilot Trial of Nightmare Deconstruction of Reprocessing."

Congratulations to our winners, and we'll see you again after the break.

(Whereupon, the above-entitled matter went off the record at 3:30 p.m. and resumed at 3:45 p.m.)

DR. NAIFEH: We're joined now by Dr. Diego Pizzagalli, Dr. Leanne Williams, and Dr. Sandro Galea. Our moderator for this panel is

Major Elle Cleaves.

Major Cleaves, welcome. Please briefly introduce yourself and then feel free to proceed with asking the questions from our attendees.

MAJ. CLEAVES: Hello. I am a Major in the United States Air Force, and I am an Assistant Professor of Psychiatry at Uniformed Services University. I'm really happy to be here to moderate, and I thought all three of your presentations were extremely interesting. So, I'll get started with asking questions from the audience. The first question is for Dr. Pizzagalli, Dr. Williams, and Dr. Galea.

Dr. LeDoux this morning spoke of the perhaps most human, e.g., conscious self-activity as telling stories as one way in which we bring together a conflicting aspect of experience, including split-brain studies in which stories are how the patient rationalizes different experience on the right and left side of the body.

Do you see

storytelling/narration/developing meaning as affecting any of the areas you study: reward responsiveness, circuits, communities, ZIP Code, and context?

DR. PIZZAGALLI: I'll maybe start, get us started. Again, thanks so much for having us. This has been a wonderful program. I'll say in my particular area, I've emphasized how uncontrollable stressors can be particularly depressogenic. If one considers preclinical models that are relevant to depression, they almost invariably involve exposing animals to uncontrollable stressors. So, I've long been interested in the perception of control or perceived stress.

And in the literature also, there are findings, kind of both sides of the coin, so to speak, that is, when people basically perceive the situation or the stressor as being, you know, uncontrollable or their copings, basically, are overwhelmed by the situation, often, we see negative consequences with respect to depression.

We and others also have done studies actually looking at the other side, so to speak, at how people, their brain reward systems react to the perception of control. That is, when we perform actions that, let's say, lead to a reward, for example.

People like Mauricio Delgado and others have shown that when people basically make an action that leads to a reward, that that reward actually activates the brain reward system much more strongly compared to a situation where a computer decides whether you will get a reward or not.

So, I think that humans are particularly attuned to trying to basically perceive control over situations and lack of the (inaudible) of them can increase the risk for negative consequences; so potentially an interesting feature that may have some relationship to what Dr. LeDoux was telling us today.

MAJ. CLEAVES: Thank you.

Dr. Williams or Dr. Galea?

DR. WILLIAMS: I'm happy to jump in with some thoughts, and also, Major Cleaves, thank you for inviting us to this panel and to the whole program, which is excellent, and to be here with wonderful colleagues.

What comes to my mind is thinking about amygdala activation and in relation to the idea of narratives and particularly when you're able to verbalize the experience of stressors, so touching on what Dr. Pizzagalli was highlighting, the kind of uncontrollable, negative stresses. As I understand it in how the human brain functions, at the point where you can verbalize those stresses or talk about the narratives around them, you are engaging cortical regions of the brain linked to the amygdala. So, it's the more indirect pathway, and it gives you, potentially, access to being able to regulate them, as opposed to when the amygdala is activated by stress through a direct pathway that is very rapid in its actions and automatic and is

outside our conscious awareness, typically, so not able to be accessed for verbally understanding what's going on. It's not related to brain regions that are engaged when we have to develop a narrative.

So, I think of those two pathways as really important for thinking about the implications, then, of therapies. If you have a therapy in which you are using a verbal or behavioral framework to describe stresses, then you're kind of engaging that cortical, indirect pathway to regulate the amygdala.

Yeah. So maybe I could stop there and ask you if Dr. Galea has more thoughts.

DR. GALEA: I will pass it. I'll move on to the next question.

Thank you, Major Cleaves. I'll echo Dr. Williams' comment. Thank you for inviting us.

MAJ. CLEAVES: Okay. The next question is for Dr. Williams. What are the major obstacles to developing neurocircuits as

individual measures for precision health?

DR. WILLIAMS: Wonderful question. There are a number of obstacles which I consider as kind of challenges to overcome. One is clearly that the brain is extraordinarily complex. So, one question is, how can you identify regions or connections between regions of the brain that are tractable for measuring a more kind of tractable subset? And that's something I presented in my talk and think about in many studies. Are there regions, such as the amygdala, and how it connects to prefrontal regions that are fairly well-documented in animal studies and human studies, and for which there's reasonable consensus that give us a Step 1 to being able to measure information at an individual level? Then there are multiple measurement issues, like how reliable is a measure, like functional MRI, if we repeat it? These are similar challenges that we saw in the development of neuropsychology and other fields, so I think it's just a case of approaching them a step at a time and tackling

them.

A related issue, though, is that in our field, we don't tend to use the same measures across studies. And so, it's difficult to build up a pool of data and results from which we can test what is specific and reproducible. We tend to use different approaches in different studies, and that's something I think the field is tackling actively right now, to say, well, how can we use some common imaging measures, common imaging analysis approaches to really look at what does stack up sufficiently to be measured in the individual level?

MAJ. CLEAVES: Thank you.

The next question is for Dr. Galea. Contexts, like community, family, and ZIP Code, matter.

What are examples of our best contextual interventions, and why don't we use them?

DR. GALEA: Yeah. Thank you. It's a good question. I mean, there's a whole range of

interventions that tackle contextual factors, and sometimes I'm asked, which are the best interventions? And the answer is, well, they all might matter in different contexts. Efforts that improve education, improve housing, create safer neighborhoods. These are all efforts that are both reducing risk of trauma and stressors and injury, but also increasing resilience and ability for people to have physiological bounce-back.

We do use them. We often don't think of them as being directly nested within our discipline, but it is a conceptual shift that has us thinking about interventions that improve context. There are also interventions that reduce the consequences of stressors and traumas, and they are as much interventions as our cognitive behavioral interventions, as our neuropharmacological interventions.

So, when we need to think about the pathophysiology, and we think about the generation of dysregulation, which we tend to

call psychiatric disorder, for example, that pathophysiology is not just within the body, not just endogenous, but also exogenous. It also comes from the world around us, from our behavior, our interactions, and our context around us. Similarly, interventions can be at all those levels, the world around us, all the way through and inside the body. And I think when we think that way, it expands our lens. It expands our potential both for understanding etiology and thinking about where we can intervene.

MAJ. CLEAVES: Thank you.

The next question is for Dr. Pizzagalli. Reward responsiveness is a compelling construct. Another construct often related to depression and suicide risk is optimism.

Do you have thoughts on how these may relate? Can we model optimism or loss of it in animals?

DR. PIZZAGALLI: That's a great

question. So clearly, optimism is a very important construct in depression. There are many studies looking at the protective factors that optimism can have, especially when we face very challenging situations. I'm aware of a very recent study, one by Emma Robinson at University of Bristol and others that actually have started to develop tests for rodents that actually probing potential optimistic biases or pessimistic biases in rodents. They're rather complex, but actually have been found to show very interesting predictive validity, including, actually, most recently in a recent paper in *Science Translational Medicine*, looking at the effect of psilocybin and other psychedelics, I believe also ketamine, on modulating some of these optimistic biases in animals. So yeah, it's a growing literature.

And one important comment I'll do, as I tried also to convey during my talk, I think partially, the integration of preclinical and clinical research has been so challenging, first

of all because humans are not rodents, obviously. We are much more complex, and as very eloquently explained by Dr. Galea also, obviously, context matters and so on, right? But also, in our field often, people working in animals or in humans are using vastly different approaches. So, I think that there is value in potentially using paradigms and tests that can be done across species, with the hope that translation, then, may hopefully be more successful.

MAJ. CLEAVES: Thank you.

The next question is for Dr. Williams. Are the identified neural circuits you spoke of seen in other primates or lower animals?

DR. WILLIAMS: That's another great question. Some of them are. So, there's not a homology for all of them. Let's see. The subcortical regions, amygdala, for example, ventral striatum that Dr. Pizzagalli studied so much, we see them homologous in other species. We see some aspects of the cortical regions, like we can identify prefrontal regions in primates.

But when we get into the complexity of the connectivity defining something like, say, the default mode, I think that's still different points of view about to what extent do we observe a circuit involved in self-reflective functions in rodents and primates? I know there's kind of views on both sides, but what I suggest it boils down to, we clearly have a lot of complexity in terms of the cortical development connections in humans that underlie some distinctive functions.

MAJ. CLEAVES: Thank you.

The next question is for Dr. Galea. Do you have thoughts on how best to prepare for our next pandemic to protect vulnerable communities/ZIP Codes?

DR. GALEA: Yeah. It's an excellent question. You know, I think the pandemic, as I've written many times, didn't create anything, but it highlighted underlying vulnerabilities, and insofar as we were living in vulnerable contexts, the pandemic made it very clear. I mean, we saw enormous clarity of association

between vulnerable neighborhoods, vulnerable ZIP Codes, and adverse outcomes of the pandemic, both in terms of physical health and in terms of mental health.

The biggest finding, in terms there's now a large body of literature about mental health consequences of the pandemic, was that people who had fewer assets were the ones who suffered most, but quite a bit, by like fourfold difference. There's been quite a bit of awareness of mental health or mental health during the pandemic, but the media conversation is almost like, poor mental health is something that occurred mostly to well-off people. It's not the case. The burden of poor mental health fell on people with fewer assets, with a fourfold gap among people with fewer assets than those with more assets, more assets being financial assets, physical assets, social assets, material assets, protected us.

So, the pandemic really showed us what we've always known. I think in mental health,

and particularly in the context of trauma and stressors, I think there's been confusion about this. But I think there's plenty of literature now to show that underlying vulnerability predisposes one to adverse mental health consequences of traumas and stressors substantially, and it is people with greater burden of that vulnerability that experience it.

You know, one classic example of this, I think, is something which is often lost in the conversation when we talk about military mental health. We've done work that has shown that the people in the military who have adverse mental health are those who have actually had traumas and stressors outside the military. Yes, there are military traumas. Yes, there are military stressors, but the burden of trauma and stressors is often before military engagement that is consistently associated with poor mental health among those in the military.

MAJ. CLEAVES: Thank you.

The next question is for Dr.

Pizzagalli. What do you think about using psychedelics to treat depression and anhedonia?

DR. PIZZAGALLI: Yeah. Excellent question. So, I think that many of us are following this literature. There is actually quite a robust literature using ketamine, for example, to treat especially treatment-resistant depression. People have shown, I think quite convincingly, that it can have rapid antidepressant responses, including the anti-anhedonic response, and also can acutely reduce suicidal ideation. So, I think that that literature is robust. Of course, many of us, it's also very nicely highlighted by Dr. Williams, are trying to understand for whom these type of approaches may be best. And so, I'm sure that we'll all see a lot of work in coming years looking at trying to personalize because, of course, they can be rather complex treatments.

With respect to psilocybin, for example, or other psychedelics, again, I've been following this literature. I feel that these

studies are difficult to do, difficult to interpret often. It's virtually impossible to have a true placebo control or to blind. Many of these studies, including the early studies, especially with a new imaging component for obvious and reasonable reasons, were actually recruiting participants often that had prior experience with psychedelics, and so there are potential expectancy biases, I believe.

So, there are methodically several important kinds of issues with this literature. I find personally the pharmacology very interesting. They clearly act on different types of receptors that are localized in different part of the brain. So, I think that there is a very interesting neuroscientific literature. The clinical literature is a bit more challenging, and I guess we'll need to do very rigorous studies trying to have the best possible control arms.

And I think it's safe to say that we have all read, especially in the press, quite hyped-up interpretation. There was just enormous

interest in this, and I feel that we in the scientific community and also clinical community will find an equilibrium at some point when, again, additional studies have been performed. I think they have a lot of promise, but the studies need to be done.

MAJ. CLEAVES: Thank you.

The next question is for Dr. Williams. Can amygdala reactivity be inherited from a parent with a history of early childhood abuse and trauma and history of a diagnosis of PTSD?

DR. WILLIAMS: That's a really interesting question. I will ask for other thoughts from my colleagues, if they have them. I don't know the direct evidence for that. I know there is a reasonable heritability on both the volume of the amygdala and the activation. There's certainly a kind of longstanding literature on the effect of genetic variance on the amygdala and how they're also interacting with early-life trauma. I don't know directly about inheriting from a parent with PTSD.

So I don't know if that's something that you're familiar with, Dr. Pizzagalli?

DR. PIZZAGALLI: Yeah. With respect to, if you wish, epigenetic transmission or so in humans, I'm not familiar with any studies. Of course, there was a literature a while back looking at trauma-unexposed co-twins. Roger Pitman and others in the VA system had done some important studies. It was actually not specifically related to the amygdala, but more to hippocampal volume, so certainly showing that there is heritability. But I'm not familiar with any studies that have linked specifically, especially epigenetic effects in humans. But very fascinating --

DR. WILLIAMS: It is.

DR. PIZZAGALLI: -- question. In preclinical models, people have shown, obviously, epigenetic effects, including transmission from one generation to the other, especially, again, when using early-life adversity in rodents.

DR. WILLIAMS: You prompted one other

thought on that is in a twin study, we have seen the heritability of the activation of the amygdala in response to threat stimuli, so they're kind of corresponding to the volume. In one other interesting study, it wasn't specifically sons and daughters of parents with PTSD, but more broadly depression, anxiety, and stress disorders. In that case, what was interesting, tying back to our earlier discussion, is there was a heightened amygdala activity in the sons and daughters, even in the absence of overt illness. So, they were sort of slightly elevated, but the difference when we followed them up was for those where they had the elevated amygdala activity and they had well-developed regulation, like, problem-solving capacity and skills, they actually didn't develop the illness compared to others who didn't have that kind of contextual processing as much. So, it's not a direct heritability, but it could indicate how there's a kind of vulnerability that isn't expressed in certain contexts and may be in

others.

MAJ. CLEAVES: Thank you.

The next question is for Dr. Galea. Are there community-level interventions that can assist in lowering suicide risk in active-duty soldiers?

DR. GALEA: Yeah. It's a very, very tough question. It's an excellent question. Suicide risk has been a very difficult epidemiologic question, really, for a century. Suicide is one of the very few causes of mortality that has budged very little over the past century. The data are pretty clear, I think, that one of the biggest protective factors against suicide risk is supports, particularly social supports, also financial supports and material supports, but supports.

We have done studies that show that unit support, unit cohesion, particularly support in transition from military to civilian life, are among the biggest protective factors against anxiety and mood disorders as well as suicide

risk. It is difficult to implement, but creating supportive environments for our military and for veterans is probably the single biggest thing that we could do to mitigate suicide risk.

MAJ. CLEAVES: Thank you.

The next question is for Dr. Pizzagalli. Do you have any thoughts on the potential transdiagnostic role of anhedonia and explaining the clinical overlap/comorbidity in depression/anxiety?

DR. PIZZAGALLI: Yeah. I should have actually emphasized this. Obviously, my talk was very much focused on anhedonia in depression, but I clearly see anhedonia as being an important transdiagnostic construct. We clearly see it in many people with PTSD, substance-use disorders, in psychosis, and so on. So Dr. Williams mentioned the RDoC in one of her comments. It's really something that, again, in a similar way, cut across diagnostic entities.

What we and others have been discovering, which I think could be quite

interesting and hopefully clinically relevant is that, for example, despite the fact that anhedonia might be important for psychosis or PTSD and depression, there is emerging evidence that perhaps they are different, so to speak. So, one example is, as shown today in this type of, what we call, implicit reinforcement learning test that we have been using, we clearly see this regulation of blunting in individuals with depression, especially if they have anhedonia.

And interestingly enough, people with psychosis, for example, and sometimes people with anxiety disorder don't show dysfunction in our test. People with psychosis, however, show very clear dysfunction in more explicit forms of reinforcement learning. That is especially when they need to use their working memory, probably more PFC, prefrontal cortex-based type of processes. So even with clinical scales, you often see very little differences between DSM diagnoses.

If you look with more granularity and

potential with more precisions, you may be able to see that one disorder is associated with a type of dysfunction that is not present in the other category. And so, the big question is whether this type of approach also might allow you to identify subgroups of patients that, from a neurobiological perspective, are a bit more homogeneous, right? And based on this information, we might be able to make some informed decisions about what we believe might be the best way to treat them.

These are all empirical questions that the field will need to answer, but the bottom line is that I believe that anhedonia is important across diagnoses. It's potentially instantiated or manifest in different way across disorders, and potentially, this information is helpful to help by making more informed treatment selection.

DR. NAIFEH: Major Cleaves, we've got a couple of minutes left. Maybe time for two more questions?

MAJ. CLEAVES: Sounds good.

The next question is for Dr. Williams. Which neural circuits may be related to or are socially responsive?

DR. WILLIAMS: Also an excellent question. I mean, you could argue all of them, potentially. The ones that specifically are implicated in responding to social input would be the one that Dr. Pizzagalli is talking about, the reward circuit, positive affect because one source of reward is social reward, social interaction, kind of a sense of purpose and motivation. You would then have the negative-affect circuit that involves the amygdala being responsive to sources of social input that may be perceived as negative or even potentially threatening and aversive.

And the one called the salient circuit is kind of orienting to sources of internal and external stimuli that are salient, so that would also be potential social cues. And one thing that's kind of been a theme across the work I've

done is how salient facial emotions are in our social interaction. And that's, at least as a kind of speculation, one thing that really contributed to the difficulties during the pandemic, that we weren't having the same amount of social interaction and the dynamic engagement around that.

MAJ. CLEAVES: Thank you.

And the last question is for Dr. Galea. You have done much work in disasters. Recently, there are many episodes of mass gun violence.

Other than limiting weapons in safe storage, are there community-level interventions to address the epidemic of mass shootings?

DR. GALEA: I think there's a whole literature about community-level interventions that could work. There are efforts at better screening, at reducing availability of guns to people who have shown themselves to be dangerous, at creating safer weapons themselves. I think there's a whole range of community-level

interventions that could be explored. And I'm very grateful to the questioner because, of course, the literature on gun violence has been very poor in documenting the mental health consequences of gun violence, both among those injured by guns, which by the way, for every person who dies by guns, about three people are injured and live with those injuries, and they're very severe injuries much of the time, and also their communities.

Our team just recently did a review, which is coming out soon, about the community mental health burden of interpersonal gun violence, and I think there's a greater appreciation of the mental health burden of gun violence. So, I think it lends itself very well to thinking about contextual efforts to mitigate the mental health consequences of gun violence.

MAJ. CLEAVES: Okay. Thank you so much to all three presenters.

DR. NAIFEH: Yes. I wish we had more time for questions. But we are grateful to Drs.

Pizzagalli, Williams, and Galea for being here, to share your deep knowledge and thoughtful responses to our questions. It was so nice of you to join us today.

Also, thank you to our moderator, Major Cleaves.

Before turning it back over to Dr. Ursano, I will ask Dr. Rachel Shor to provide some additional guidance on receiving continuing education credits.

Dr. Shor?

DR. SHOR: Thank you so much.

And just echoing, thank you again to our esteemed speakers for these really wonderful presentations and to everyone who was able to attend today's conference. So, continuing education for this conference is available for physicians and psychologists through the American Psychiatric Association. So, for those of you who are interested in claiming continuing education credits, please complete the evaluation and credit-claim form that I'll be emailing out

after the completion of today's conference. And this form will include a link and an invitation code to access the evaluation, and that will allow you to then claim the CE credits. Of course, though, if you have any questions, whether it's tech-related, accessing, or completing the form, please don't hesitate to reach out to me. My contact has been in all of the registration emails. It's rachel.shor.ctr@usuhs.edu.

And actually, Allie, would you mind dropping my email in the chat as well?

And I believe there was also that evaluation form with the credit-claim instructions was also just dropped in the chat, so you should be able to access that directly, although I will be emailing it out to all of today's participants. So, thank you again, and please feel free to reach out if you have any questions about continuing ed.

DR. NAIFEH: Thank you, Dr. Shor.

Now I'll turn it back over to Dr.

Ursano for some final comments.

Dr. Ursano?

DR. URSANO: Thank you, Jamie.

Not much to say other than to applaud on a marvelous day. I am always impressed by the span in which the conference covers, and I much appreciate our speakers' wonderful example this afternoon of Dr. Galea, Dr. Williams, Dr. Pizzagalli, in stretching with us across questions that they may not often gain. But in fact, we much appreciate your responding to things that you may not often be asked, but they allow us to think with you from perspectives that may be somewhat different.

I have often commented about for myself, I went through medical school with a 3 x 5 card in my pocket, and what I learned was a lot of medicine was about dictionary and looking up a word you didn't know. That's how I made it through medical school, how many things went on my 3 x 5 card and how many things I looked up that evening. That's how I experience the

pleasure of this type of conference, and needless to say, I've filled my 3 x 5 card.

I look forward to Googling tonight and continuing to go from what has been a wonderful discussion from health to illness. I think protozoa got mentioned this morning, as well as primates, as well as humans, from neurocircuits to pharmacologic interactions to community interventions. It's just such a wonderful dialogue that we so rarely get to engage in. Even in cocktail parties, usually, you move away if the conversation isn't one you know about. This is one in which we stay engaged when we don't know what's happening, with the hope of learning more.

Thank you, Jamie and the whole team, and thank you, speakers.

DR. NAIFEH: Thank you, Dr. Ursano.

And one last thank you to the speakers, to the Center for the Study of Traumatic Stress and our other sponsors, and to the planning committee for the conference and our

colleagues at the Center for Deployment Psychology, who helped us put this on.

And thank you to all of you for attending. We hope you'll join us again for future events, so please watch for those announcements. Take care, and we'll see you next time. Bye.

(Whereupon, the above-entitled matter was concluded.)