Experiments Show Pathologies Emerge in the Developing Brain

What to Look For: Anxiety & Depression in Childhood

MAGAZINE

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Reducing Medication Impact to Improve Cognition in Schizophrenia

PRESIDENT'S LETTER

This September issue of *Brain & Behavior Magazine* showcases the impact that BBRF's strong commitment to funding basic research is having in the field of neuropsychiatry.

Our **SCIENCE IN PROGRESS** story demonstrates the clinical impact of knowledge gained since 2003 and 2006, when BBRF awarded Young Investigator grants to Dr. Gregory Light. Dr. Light, whose aim has been to identify ways of overcoming cognitive impairments that are a core symptom of schizophrenia, succeeded in finding a reliable biomarker of these impairments which became a standard tool in subsequent research. Recently, he and one of his former mentees, Dr. Yash B. Joshi, a 2018 BBRF Young Investigator, have studied how medications taken by schizophrenia patients might act to accentuate cognitive difficulties. In some patients, especially those taking multiple medications, the impact on the neurotransmitter acetylcholine was found to be considerable. This is an important insight since the cholinergic system plays a key role in supporting cognitive function. Our story explains why these grantees suggest that in some patients, medication trade-offs to reduce total anticholinergic medication burden could help boost cognitive function.

The **PATHWAYS TO THE FUTURE** story follows pathbreaking technology research by two distinguished BBRF grantees, Dr. Sergiu Pasca and Dr. Fred Gage. They have been among the leaders in harnessing stem-cell technology to grow unlimited numbers of human brain cells in the laboratory. In their most recent innovation, they have transplanted assemblies of these cells, called "organoids," into living animal brains, where they make connections and begin to function. This is making possible unprecedented experiments to reveal early pathologies in human brain illnesses, particularly those like schizophrenia and autism with developmental origins, and provides a unique test-bed for assessing new therapeutics.

Our **ADVICE ON MENTAL HEALTH** story captures the essence of a fascinating and informative conversation I had with Dr. Joan Luby, one of the world's foremost experts on depression and anxiety in early childhood. Our focus was: "Warning Signs & What to Look For: Anxiety and Depression in Childhood," and it's our hope that teachers and parents, especially, will find the information useful.

This issue also highlights our 2023 winners and honorable mentions of the annual BBRF Klerman and Freedman Prizes. We also feature recent news on treatments for psychiatric conditions in our **THERAPY UPDATE** and important research advances that are moving the field forward in **RECENT RESEARCH DISCOVERIES**.

I thank you for being an important part of the BBRF community. Together, we will continue to fund innovative and impactful research that will pave the way forward for scientific advancements that are making a difference in the lives of those living with mental illness.

Sincerely,

Jeffrey Borenstein, M.D.

100% percent of every dollar donated for research is invested in our research grants. Our operating expenses and this magazine are covered by separate foundation grants.

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In Schizophrenia, Reducing the Impact of Medications on One Neurotransmitter System Could Yield Gains in Cognition

Research provides "a new lens through which we can view the care of our patients"

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IN BRIEF
Many medications prescribed for schizophrenia patients can indirectly impact cognitive functioning by acting to suppress the brain's cholinergic system. The combined anticholinergic impact of all medications taken by a patient can work to significantly intensify core symptoms of cognitive dysfunction. Medication trade-offs to reduce total anticholinergic burden could help patients, especially those with the highest burden.

Several important insights have recently combined to provide "a new lens" on improving how people with schizophrenia are cared for. This new perspective is arguably important not only for all schizophrenia patients but for all medical professionals who prescribe medications for them.

One of the insights has its origins in BBRF Young Investigator grants awarded in 2003 and 2006 to **Gregory A. Light, Ph.D.** A second insight comes from a question asked a few years ago by one of Dr. Light's former mentees at the University of California, San Diego, **Yash B. Joshi, M.D., Ph.D.**, who received a BBRF Young Investigator grant in 2018.

In 2021, Dr. Joshi was the lead author of a study in the *American Journal of Psychiatry* published by a team whose senior member was Dr. Light and included eight other past BBRF grantees. The paper called to the attention of both psychiatrists and the broader medical community questions about how medicines are selected for schizophrenia patients and what the cumulative impact of these medicines might be upon one of the core symptoms of the illness: problems with the brain's cognitive machinery.

THE PROBLEM OF COMORBIDITY

One bit of background provides context for the new insights of Drs. Light and Joshi: it has been known for many years that comorbidity is a major problem in caring for people with schizophrenia. Comorbidity refers to other conditions which can co-occur with schizophrenia. These include conditions affecting the brain and behavior, as well as illnesses occurring in the rest of the body. "Medical" comorbidities of schizophrenia include cardiovascular, pulmonary, neurological, and endocrine (hormone system) diseases. Regarding psychiatric comorbidities, depression occurs in about 50% of schizophrenia patients, and as many as half of these individuals also have a lifetime diagnosis of substance abuse. Anxiety, OCD, and panic disorder also co-occur with schizophrenia with a frequency that significantly exceeds their occurrence in the general population.

Because comorbidity is common in schizophrenia, it comes as no surprise that many patients are prescribed multiple medications. The recent insights of Drs. Light and Joshi suggest the wisdom of identifying in a comprehensive way the total complement of prescribed medications in each patient and assessing how those medications together may affect the patient's overall condition. The problem, in other words, includes medications patients are prescribed for both psychiatric and "medical" reasons.

Drs. Light and Joshi have focused in particular on how the total complement of medicines prescribed for patients affects cognition. Cognitive impairment is a core symptom and a key disabling feature of schizophrenia. There's a large literature documenting significant difficulties in attention, learning, memory, executive functioning, and social cognition (the ability to understand and successfully communicate with other people) in those living with schizophrenia and related disorders.

The antipsychotic medicines that most patients take to control symptoms such as hallucinations and delusions do not reduce cognitive impairment. And, as Drs. Light and Joshi point out, cognitive impairments are a core symptom of schizophrenia and contribute significantly to the difficulty patients have in functioning in society. It's related directly to limited skill acquisition, lower educational attainment, and reduced quality of life.

Cognitive impairments are a core symptom of schizophrenia and contribute significantly to the difficulty patients have in functioning in society.

What do cognitive impairments have to do with medications? Dr. Joshi thought that medications might have quite a bit of impact, specifically in the area of cognition. The root of this thought is not controversial. Many studies have linked cognitive difficulties to problems with the brain's cholinergic system. Dr. Joshi's concern was that many medications prescribed for schizophrenia patients, from antipsychotics to antidepressants to some medicines for comorbid bodily ailments, could indirectly impact cognitive functioning by acting on the brain's cholinergic system.

THE CHOLINERGIC SYSTEM IN THE BRAIN

The "cholinergic system" refers to signaling by the neurotransmitter acetylcholine, which is important for neurons throughout the body. In the brain, regions in which acetylcholine is active play an important role in learning, memory, stress response, and broadly, in cognitive functioning. Unlike some neurotransmitting chemicals, acetylcholine does not typically shut neurons on or off. It is rather a neuromodulator—in Dr. Joshi's words, it acts more like the volume switch on a radio rather than the on-off switch.

Dr. Joshi was not the only doctor or scientist who knew that many medicines act to impede the operation of the cholinergic system—that they have "anticholinergic" properties. What gnawed at him was: how would such medicines affect cognition in a schizophrenia patient, assuming that the patient already had core symptoms impairing cognitive function? When patients take multiple medications, several of which had some known anticholinergic effect, might the combined anticholinergic impact of all the medications, regardless of their potential benefits on other individual symptoms that are being targeted, tend to intensify the cognitive problems that all schizophrenia patients have?

The total "anticholingeric burden" of medications can intensify cognitive problems schizophrenia patients have.

Drs. Joshi and Light had another concern in mind. Among the medicines with anticholinergic impact that schizophrenia patients might be taking, there is a class that is explicitly designed to have anti-cholinergic action. These medicines are often prescribed to patients with motor-system side effects such as tardive dyskinesia and dystonia. Anticholinergic medicines help to control such symptoms, which ironically can be side-effects of the antipsychotic medicines that patients need to take to control their hallucinations and delusions.

In their 2021 paper in the *American Journal of Psychiatry*, Drs. Joshi, Light and colleagues described the potential utility of calculating the total "anticholinergic burden" (ACB) of medications prescribed for chronic schizophrenia patients, using a sample of 1,120 individuals with known medication histories, 58% of whom lived in board-and-care or transitional living programs. The average age of participants was 46; nearly 70% were male; the average participant had

been diagnosed with schizophrenia at age 22 and took a single antipsychotic medicine. One-third of participants also took an antidepressant medicine and/or other medicines, including mood stabilizers, anti-anxiety agents such as benzodiazepines, or anticholinergic medicines (e.g., benztropine, diphenhydramine, trihexyphenidyl, hydroxyzine) to control antipsychotic motor side effects like involuntary movements.

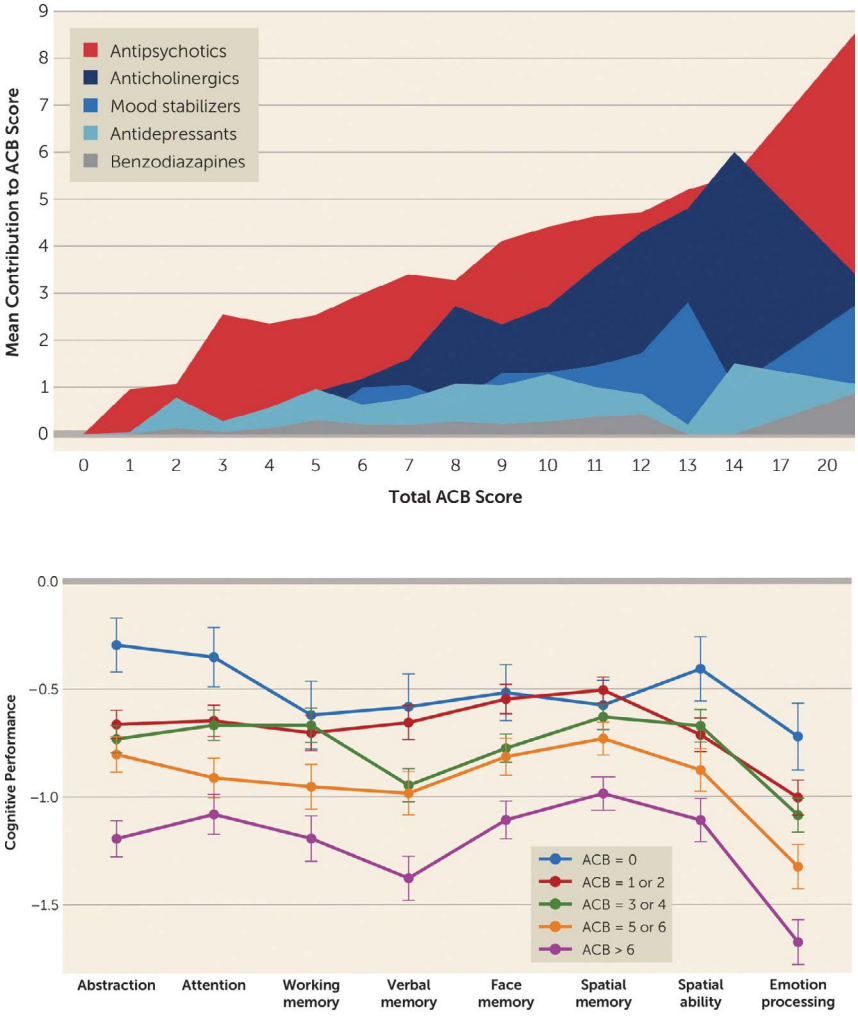
Guided by previously established research protocols, the researchers assigned each prescribed medicine a numerical score, rating it on a scale from having no anticholinergic effect (0) to having a high effect (3). The study rated participants with a combined medication score of 3 or greater to have a "high" anticholinergic burden. In a prior study of *healthy* older adults, the team noted, scores of 3 or greater for 3 years or more were associated with a 50% increase in the odds of developing dementia over the study's 11-year duration. What impact might ACB have in schizophrenia patients, who have underlying cognitive impairments?

"We found that many patients [in our study] have medication regimens with high anticholinergic burden, with an average score of 3.8," the team reported. Overall, 63% of the 1,120 participants had a score of at least 3, and one-fourth had a very high score of 6 or greater. A few had scores as high as 15 to 20. Since participants in the study were not included if they had major medical issues, it's likely the results probably underestimate the total anticholinergic burden in patients living in the community, many of whom do have medical comorbidities and likely have additional anticholinergic burden from medications prescribed to treat those conditions.

Cognitive deficits make it hard for patients with schizophrenia and other psychotic disorders to accurately detect changes in verbal pitch, or whether somebody's facial expression conveys humor, anger, sarcasm, frustration, sadness, etc. This directly affects the ability to function effectively in society.

Consistent with findings in the prior study of healthy older adults, this study in schizophrenia patients by Drs. Joshi, Light and colleagues found that "anticholinergic burden was significantly associated with generalized impairments in cognitive functioning." Antipsychotic medicines contributed more than half of the total anticholinergic burden, they said, with other medicines accounting for the remainder. There was no appreciable difference between first- and second-generation antipsychotics; most medicines in both categories had anticholinergic effects, although of varying magnitude.

Drs. Joshi and Light stress that their results point to the total score—total anticholinergic burden—as being the



TOP: Relative contribution of various types of medications typically taken by schizophrenia patients to anticholinergic medication burden (ACB), as measured by total ACB score. BOTTOM: Patients with low ACB scores (the blue line corresponds with zero burden) are less impacted than those with high and very high ACB scores (green, orange, purple lines, in order of increasing burden).

key factor in contributing to risk for cognitive impairments, as opposed to any particular medication or medications considered individually. This is a delicate and crucial point. They say it is important that their results be understood in the proper context: they are working "to optimize outcomes" in chronic schizophrenia patients. And, they stress, "psychotropic medications, especially antipsychotics, are critically important in [treating] schizophrenia, have substantially improved the lives and outcomes for countless patients living with the illness, and represent an essential staple of comprehensive treatment."

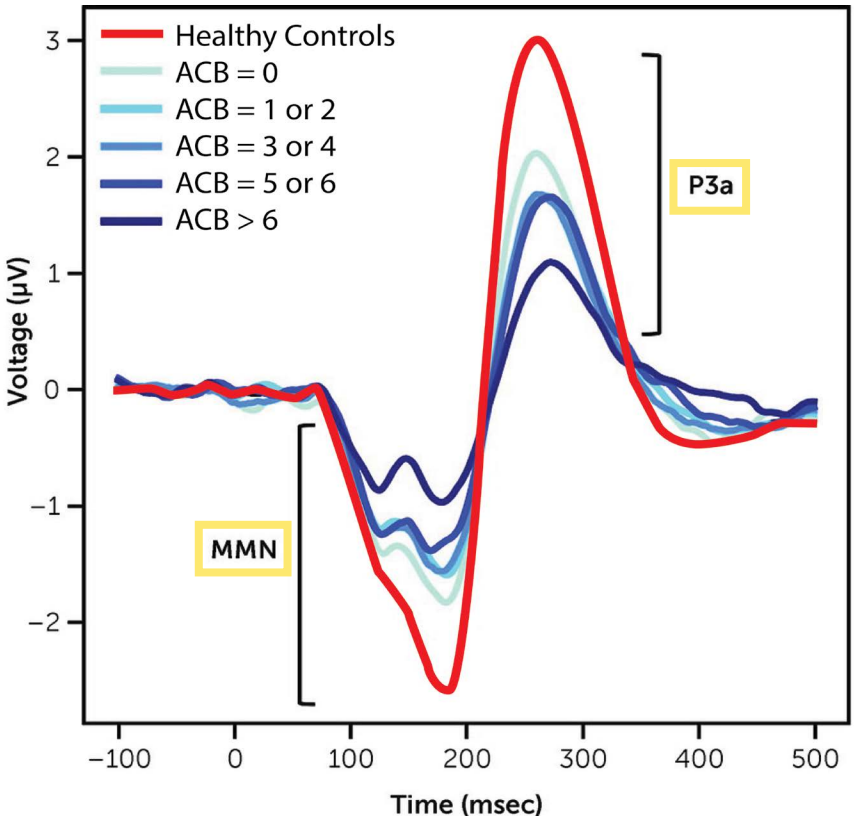
"We are definitely not anti-medication," Dr. Light says. "We want to keep people functioning at their best, keep them out of the streets, out of jails and prisons, and functioning better in their community."

This guiding passion of both doctors and their colleagues inspired additional research leading to their second paper in the *American Journal of Psychiatry*, which appeared earlier this year. If it now seemed reasonable to consider using the total anticholinergic burden score of each patient to help inform which medicines to prescribe, it would be important to provide an objective measure of cognitive impairment that is specifically associated with total ACB score. Can the impact of ACB on cognitive function in patients be quantified in terms of patients' ability to function? The answer turned out to be "yes."

MEASURING THE IMPACT ON COGNITION

Dr. Light's early-career research provided a plausible candidate, or a pair of them, to objectively measure functional impact. Called MMN and P3a, they are features of EEG (electroencephalogram) readouts of brain activity. When he received his first BBRF grant, Dr. Light was searching for ways in which cognition might be better understood in patients with schizophrenia by looking at how electrical waves measured by EEG are linked to cognition, symptoms, or functioning.

MMN, or "mismatch negativity," tests the brain's ability to detect subtle changes in an otherwise repetitive background of sounds after an "oddball" occurs. People with a normally functioning brain can automatically detect an auditory oddball—say, a single rising tone in a long series of descending ones, or a long sound in a series of short ones. Normally, the brain makes these discriminations routinely and unconsciously, and can do so from our earliest days out of the womb. With his first BBRF grants, Dr. Light showed that schizophrenia patients had *reduced* MMN responses—the "oddball" tones were heard, but the brain responses to those tones were not well differentiated from responses to the other tones. Perhaps more important, he discovered that patients with the lowest responses in the MMN test had the greatest impairments in real-world, daily functioning.



The MMN and P3a indicators of cognitive function, derived from EEG tests, are muted in schizophrenia patients when compared with the responses of "healthy controls" (red line). This effect is even more pronounced in patients with high anticholinergic burden (ACB) scores. In this graph, those patients with the highest ACB medication scores (black line) have the most muted responses and thus the greatest functional deficits in cognitive performance.

The MMN indicator as measured with EEG turned out to be highly influential in research, in part because the measurement was obtained with absolutely no effort on the part of the study subject. "It can be measured in sleeping babies, in children, and adults with neuropsychiatric disorders," says Dr. Light. "What it tells you is how the brain processes environmental sounds; how it uses that information to determine whether a sound is pertinent [or not], whether it requires you to act in response to it." It became an objective biomarker in schizophrenia research and treatment development when Dr. Light and others showed that in patients, the MMN response over frontal brain regions was blunted. And it turned out, he explains, that lowered MMN response "is related to how well patients navigate through life, how they function in real-world settings." P3a is another biomarker of cognitive function that is also muted in schizophrenia patients. It can be seen in the same EEG readout that displays the MMN response. "MMN occurs about 1/20 of a second before the P3a response, and like MMN, P3a is instant, immediate, and everybody has one."

In their 2023 paper, Drs. Joshi, Light and colleagues wanted to know if the total anticholinergic medication burden of schizophrenia patients had any impact on the MMN and P3a biomarkers. Both were thought to be remarkably stable and insensitive to such factors as changes in medications. The team would test whether chronic schizophrenia patients with high total ACB scores differed in their MMN and P3a responses compared to patients with lower scores. 555 patients from the earlier study were used, all having fully documented ACB scores and EEG data. In a modification of the standard ACB scale, the investigators considered those patients with total ACB scores of 1 or 2 to be "low," 3 or 4 to be "moderate," 5 or 6 to be "high," and 6 and over "very high."

‘Knowing a patient’s total anticholinergic medication burden makes it possible to contemplate making medication trade-offs.’

Contrary to the prior supposition that MMN and P3a were stable in schizophrenia, the team found that patients with higher ACB scores had even lower MMN and P3a responses relative to schizophrenia patients with lower total ACB scores. In fact, they discovered, a patient’s ACB score predicted what their MMN and P3a response would be.

How large was the effect? The team concluded that having a very high ACB score, i.e., 6 or greater—which pertains to roughly 1 patient in 4—“may uniquely attenuate” the two biomarkers that reflect “the earliest stages of core information processing necessary for most higher-order cognitive functions.”

There are several important implications of this finding. One concerns the search for medicines that might boost cognitive function. It will take years and cost billions to develop such drugs. In the meantime, actions can be taken now that might potentially improve cognitive function in chronic schizophrenia patients.

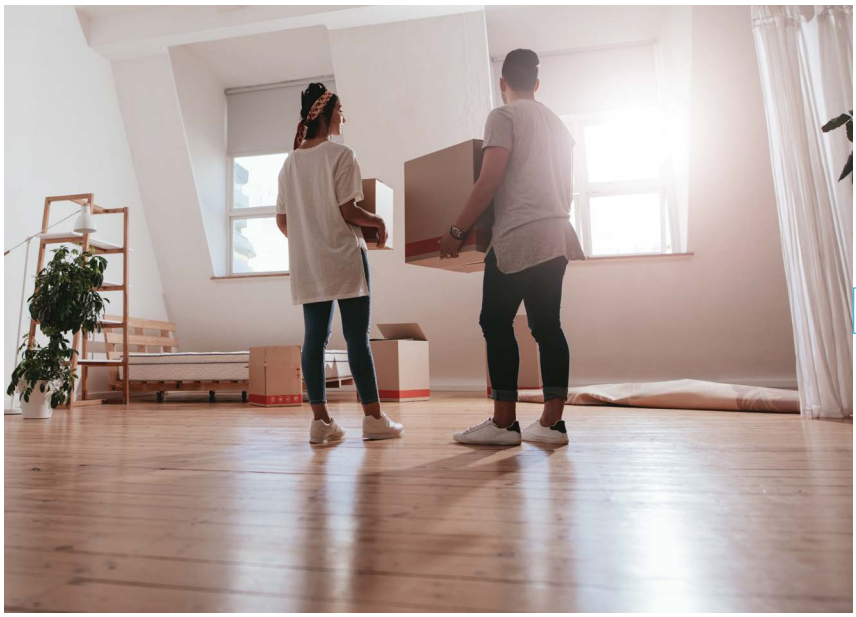
MAKING MEDICATION TRADE-OFFS

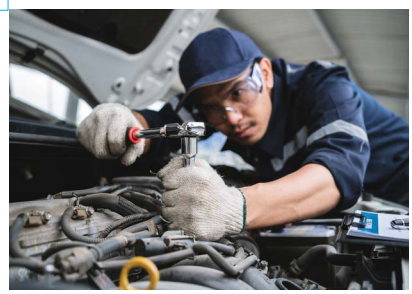
The new data shows that patients with the highest ACB scores have the most muted MMN and P3a responses. This raises a crucial question. If patients with high ACB scores have significantly more muted MMN and P3a responses, and if these muted responses correlate with greater functional impairment due to cognitive dysfunction, then might one try to improve cognitive performance in patients by trying to lower their total ACB score? This could be done, at least in theory, by making trade-offs: swapping out medications with greater anticholinergic impact for ones that have lesser impact.

Medication swaps are conceivable but will not be easy in many cases. Some patients and their families are adamant about the effectiveness of specific medicines—for example, anticholinergics in helping to control motor dysfunctions; or a specific antipsychotic in helping to control the patient’s hallucinations and delusions. Coming up with a medication regimen is typically the result of many trial-and-error experiments. Finding a regimen that meets important treatment goals and to which the patient will adhere is no small thing.

“What Yash [Joshi]’s work has so nicely illustrated,” Dr. Light says, “is that when you look into the overall anticholinergic medication burden you can contemplate making trade-offs. You can think about whether a specific medicine you might want to swap out might be the thing that is enabling this patient to be somewhat functional. But it could also mean swapping out some of the medications people are taking for problems unrelated to schizophrenia with equally effective medicines with lower anticholinergic burden. The idea is that we should now be thinking carefully about piling on more burden in patients who already have a very high burden. What Yash’s work suggests is that there are pathways for providing better, more integrated care, even if it is just a little better in some cases.”

Dr. Joshi, who in addition to his research actively cares for





Reductions in cognitive deficits are likely to pay dividends in terms of the ability of schizophrenia patients to function in society—for instance, to live independently and to hold jobs.

schizophrenia patients at the VA Hospital in San Diego, says, "This is what I do in the clinic all the time. At the VA, I take care of a group of veterans who are uniquely vulnerable and require a high degree of mental and medical health care. Most have treatment-refractory symptoms. What if we had the ability to improve their cognitive functioning, even if it's just a little bit?"

If such a thing were possible, would it be worth the effort? Both Drs. Light and Joshi think it may be well worth it in many cases. "Greg [Light] published a paper in 2017 showing us that those little 'squiggles' in the EEG readout have cascading effects to disability. What that means is, even a small change in MMN and P3a has outsized and additive impacts on cognitive symptoms, and ultimately, disability." This, he notes, "is the subject of our next paper."

Dr. Light says "This goes back to our early research made possible with my early BBRF grants. We began to speculate, beginning in EEG studies with healthy subjects, that if someone can automatically detect subtle changes in the environment, they will be better able to read another person's facial gestures, body language changes, and function better." These are the very abilities impaired in schizophrenia and reflected in the MMN and P3a indicators, and even more in those with high ACB scores. "A lot of communication is nonverbal, and it's subtle. If you can accurately detect changes in pitch, or whether somebody is trying to convey humor, anger, sarcasm, frustration, sadness, you're going to function more effectively in the real world."

Drs. Light and Joshi think it makes sense for doctors to "take responsibility for the entire medication regimen in every patient," as Dr. Joshi puts it, "and to consider it through the prism of the total anticholinergic burden that person has." In Dr. Light's view, "it's one lens, a new lens, through which we can view the care of our patients." It's a lens that is commonly applied today in the care of healthy, older adults, to minimize risk of dementia and cognitive impairment. In schizophrenia patients, it is not widely considered, but easily could be: it's a calculation that involves simple addition, made with existing tools.

"It might end up helping people just enough that they can function a little bit better," Dr. Light says. And, the recent paper suggests, all the more for those with very high anticholinergic burden. ❖ **PETER TARR**

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Carlos A. Zarate, Jr., M.D.
Jon-Kar Zubieta, M.D., Ph.D.

Stanford's Sergiu P. Pasca, M.D., a two-time BBRF grantee, is among the pioneers who have harnessed stem-cell technology to study schizophrenia and autism.

'Brains Within Brains': Organoid Experiments Show How Pathologies Emerge in the Developing Brain

IN BRIEF

Pioneering grantees have harnessed stem-cell technology to grow unlimited numbers of human brain cells in the laboratory. In their most recent innovation, they have transplanted assemblies of these cells, called "organoids," into living animal brains, where they make connections and begin to function. This is making possible unprecedented experiments to reveal pathologies in human brain illnesses, particularly those like schizophrenia and autism with developmental origins, and provides a unique test-bed for assessing new therapeutics.

Those who study illnesses rooted in the early brain like schizophrenia and autism spectrum disorders face an obstacle that most medical researchers don't. While they have ample access to patients, they have no access at all to living, functioning tissue of the organ in which pathology is presumed to be centered.

2017 BBRF Independent Investigator and 2012 Young Investigator **Sergiu P. Pasca, M.D.**, of Stanford University, explains the situation that prevailed at the very beginning of his career, in the early 2000s: "I'm a physician by training, and my interest has always been in understanding the biology of neuropsychiatric disorders. I found it very frustrating to try to do this research without having access to brain tissue from patients."

Dr. Pasca is well known as a key innovator of a technology that addresses this seemingly insoluble problem. The solution, as this story will explain, builds upon powerful insights about stem cells, sometimes referred to as "the mothers of all cells." Dr. Pasca and other investigators have figured out ways to grow unlimited numbers of human brain cells in the laboratory, and, in their most recent innovation, to transplant assemblies of these cells, called "organoids," into the brains of living animals, where they make connections and begin to function. These transplanted organoids

become, in effect, brains within brains—segments of the human brain living within a fully functional animal brain. This is making possible unprecedented experiments to reveal pathologies in human brain illnesses, particularly those with developmental origins, and provides a unique test-bed for assessing new therapeutics.

In the past there have been many meaningful efforts to cope with the problem of access to the living human brain. Collections of postmortem human brains have been assembled and archived for research, thanks to the great generosity of families whose loved ones lived and died with mental illness. These collections have supported many important studies, but such research can by definition only go so far. Some of the central questions of biological research—showing how complex living systems function in real time and how they change over time—need to be explored in living, functioning brains.

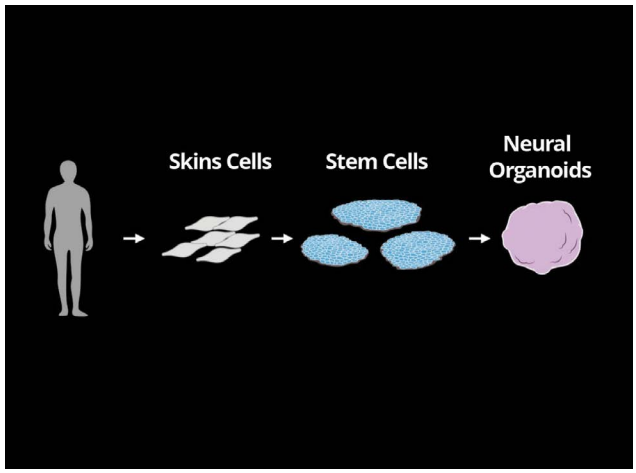
Brain scans and other non-invasive technologies like EEGs (electroencephalograms) that reveal function in the living human brain have also been powerful tools for researchers. But these technologies also have their limits—as do animal models of human disorders. It's possible to observe behaviors in animals that resemble those seen in some human psychiatric illnesses. But again, there are limits: no mouse or rat can ever be said to have schizophrenia, bipolar disorder, or autism. These are uniquely human disorders, defined by changes in human perception and behavior.

TURNING TIME BACKWARD

In 2006, a seminal discovery was made by Dr. Shinya Yamanaka, of Japan, which brought him the Nobel Prize six years later. Dr. Yamanaka was interested in stem cells, particularly pluripotent stem cells, which at the beginning of life populate the embryo. These precursors are capable of developing into all of the cell types that make up the adult organism. As the weeks pass, stem cells give rise to specialized cells that form the organs of the body. Before Yamanaka, this journey from immature to specialized cell was assumed to be unidirectional—once specialization occurred, there was no way for a cell to return to an early, pluripotent stage.

Through trial and error, Yamanaka identified a set of just a few genes whose activation in cells effectively acted like a time machine—the cells turned back into pluripotent stem-like cells. This worked first in mouse cells, but soon was shown to be just as effective in human cells.

It was now possible, in other words, to sample mature cells from an organism—humans included—and return them to a pluripotent state. In the lab, these pluripotent cells, grown in culture dishes, could then be induced to *re-develop* as any of a variety of specialized cell types. Something as innocuous as a skin cell, which can be sampled painlessly from any individual, could be returned to a stem-cell-like state in the lab, and then reprogrammed to redevelop as,



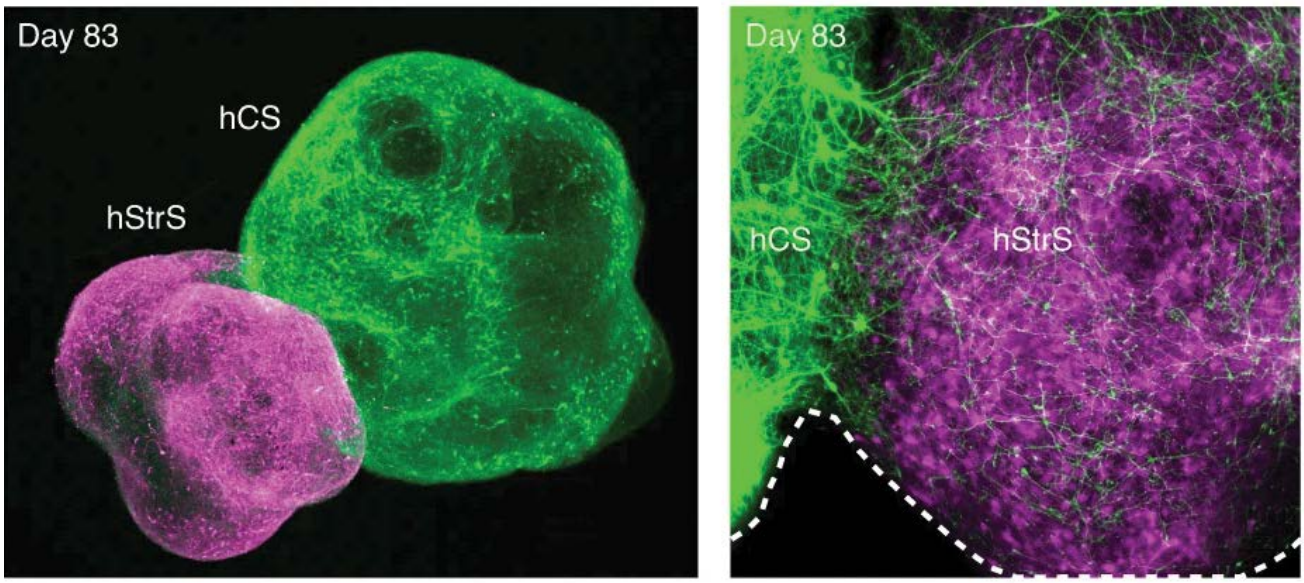
LEFT: brain organoids growing in a culture dish. RIGHT: Something as innocuous as a skin cell, sampled painlessly from a person, can be returned to a stem-cell-like state in the lab, then reprogrammed to redevelop as say, a neuron. It's a way to generate an unlimited supply of living brain tissue.

say, a neuron. There was now a path to generating an unlimited supply of living brain tissue.

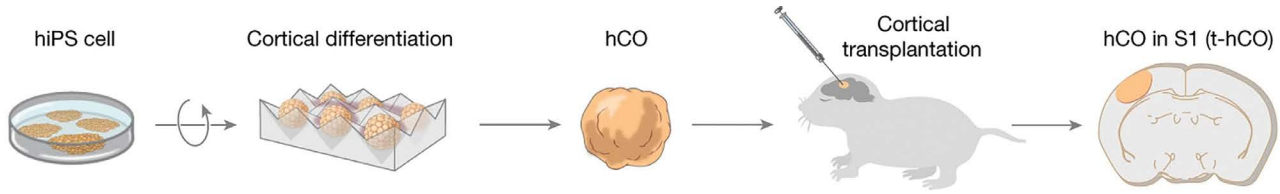
This game-changing technology made possible research that Dr. Pasca and so many other neuroscientists wanted to perform. It had an imposing name: "induced pluripotent stem cell" technology, or iPS. By the time Dr. Pasca was awarded a Young Investigator grant by BBRF in 2012, he had already developed some of the first models with iPS cells by generating neurons in a dish from patients with a form of autism caused by a genetic mutation.

It took some time to make good on the promise of iPS technology. "We were able to make these beautiful cultures at the bottom of a dish, cultures of neurons," Dr. Pasca remembers. "And we found that we could easily keep them for 10 weeks or so, if we fed them." That was exciting. "But we found that this could only go so far. We could not, with this set up, really recapitulate later, key stages of brain development." In the emerging human fetal brain, for example, it takes more than 20 weeks to generate all the neuronal types found in the human cerebral cortex—something that iPS technology in its early version was not able to sustain.

Dr. Pasca had an idea. His cortical neurons generated with stem cell technology were laying at the bottom of the culture dish; why not try to grow them so that they were suspended in three-dimensional space? A special coating applied to culture plates made the cells lift off the surface and float. These neurons were more dynamic. They formed balls of cells that self-assembled and could be kept alive indefinitely. Each ball started with about 10,000 cells but could grow to contain several million. "We've maintained them for as long as 800 or 900 days," Dr. Pasca says.



To model multi-region brain complexity, Dr. Pasca's team built the first assembloids—organoid cultures that fused together. Here, an organoid based on cortical cells (green) fuses with one based on cells from the striatum (purple). RIGHT: connections from the cortical cells can be clearly seen in the striatal part of the assembloid.



To understand the biology of psychiatric disorders in a dynamic context, Drs. Pasca, Gage and others have grown human brain organoids in the lab and then transplanted them into the living rodent brain. At far right, note the position of the "graft" in the rodent cortex.

ORGANOIDS DERIVED FROM PATIENTS' CELLS

In a series of papers, Dr. Pasca and colleagues showed that over long periods of time, these balls of brain cells, called organoids, "will develop at pretty much the same pace as they would in the living context." Remarkably, "after nine months of keeping them in a dish, they transitioned to a postnatal signature. This transition in signature from fetal to postnatal occurs at about 280 days and tells us there's an intrinsic clock, a maturation clock, built into these cells."

What made these early organoids potentially so powerful was the fact that they could be generated from skin cells harmlessly sampled from any person—including people with psychiatric (or other) illnesses. Dr. Pasca and others were especially eager to create organoids derived from cells sampled from patients with disorders like schizophrenia and autism thought to have roots in early development, when the fetal brain is just emerging.

In organoids based on cells sampled from patients, every cell has the genome of the patient-donor. If this donor has genetic mutations linked with high risk for disease pathology, then a novel kind of experiment becomes possible. One can watch these cells from their earliest days as they develop and begin to manifest pathologies caused (at least in part) by their risk-related variant genes.

In an important paper in *Nature Medicine* in 2020, Dr. Pasca and colleagues provided a vivid example of how stem cell-based technology could help reveal pathological mechanisms in neurodevelopmental illnesses. The subject was an illness called 22q11.2 deletion syndrome, which is associated with schizophrenia and autism spectrum disorder. It's caused by a chromosomal deletion of about 60 genes.

His team generated organoids composed of cells reprogrammed to redevelop as cortical neurons—the cells that populate the brain's cerebral cortex. In organoids derived from over a dozen patients, neurons showed deficits in how they "fire," as well as in how they handle ions of calcium, which help regulate voltage in cells. This was evidence of at least one of the pathologies in 22q11.2 deletion syndrome that likely relates to its devastating impact on patients.

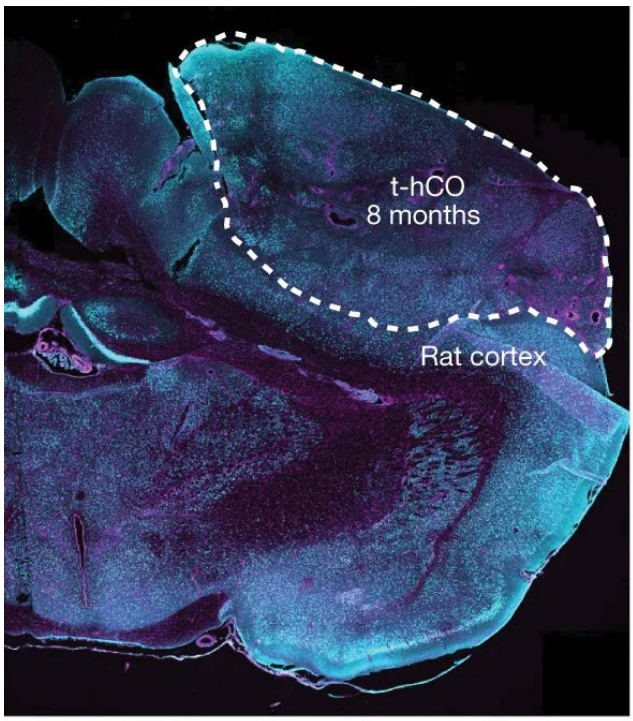
FROM LAB DISH TO LIVING BRAIN

Dr. Pasca's early brain organoids showed signs, albeit partial, that they marked the transition from prenatal to postnatal. But, he notes, "these cells in the organoid were modeling just one brain region—the cortex." To model multi-region brain complexity "we built the first assembloids."

Assembloids are combinations of three-dimensional organoid cultures that represent different regions of the brain. Cells of the cortex, including neurons and helper cells called astrocytes, formed organoids that were combined with organoids composed of cells found in the striatum, or, in other experiments, the spinal cord. "We started putting more brain regions together and looking at the connections between them."

There was much more to do. "Even with these models, and even being able to maintain these cultures for hundreds of days, we realized that there were still some properties of the cells that we were not capturing in the dish. For instance, neurons still do not grow in culture as large as they are in the actual human brain. They do not become fully mature."

There were also questions about their functional properties. "If we wanted to understand the biology of psychiatric disorders, we had to find a way to enable human neurons to influence circuits in the context of the living brain," Dr. Pasca says. "That's why, about 8 years ago, we started playing with the idea of transplantation—the possibility of



Eight months post-transplantation, the human brain organoid (t-hCO) was nine times its original volume, occupying a third of the host rat's cortex yet not interfering with its function, but rather, integrating with it.

transplanting intact three-dimensional cell cultures directly into the rodent brain."

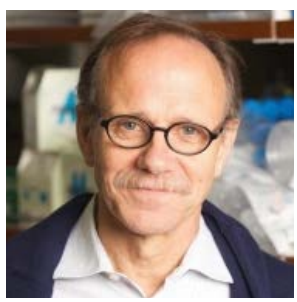
In 2018, a team led by BBRF Scientific Council member and 2013 Distinguished Investigator **Fred "Rusty" Gage, Ph.D.**, of the Salk Institute for Biological Studies—for decades, a pioneer in stem cell-related technologies to study the brain—published a paper in *Nature Biotechnology* introducing a method of transplanting human brain organoids into the adult mouse brain. These "grafts" were observed to generate a variety of cell types which matured in the rodent brain environment. Remarkably, the team observed connectivity develop between the human brain-cell graft and the rodent brain host, and the formation of synapses between neurons in each that appeared to generate connectivity that could affect the animals' activity.

In 2022, Dr. Pasca and colleagues reported in *Nature* on experiments in which they grafted intact human-derived cortical organoids into the brains of rats that had just been born. The hope was that the organoids "would actually grow and become a unit within the rat's cortex, and in a very specific position." The targeted location was the rat's somatosensory cortex, which was easy to access. It was also targeted because this part of the cortex receives abundant input from the thalamus, a kind of relay station, where inputs arrive from the rat's whiskers, the animal's primary source of sensory information.

"We did the transplants in the first week after the rats were born, when the animal's brain circuits are still forming." The results were "remarkable," Dr. Pasca says. Within 8 months, the transplanted organoids grew to nine times their pre-transplantation volume [see illustration, left], and, as revealed by MRI, came to occupy about one-third of a hemisphere of the rat brain. Not only were the transplanted neurons larger; they also formed more complex branching connections with other brain cells than did neurons grown in the lab. The rodent hosts receiving the organoid transplants steadily supplied the human neurons with nutrients and electrical inputs, a measure of their successful integration.

This was no stunt. The team went on to conduct experiments demonstrating that the human neurons within the rat brain began to respond to inputs the rats were receiving from their whiskers. In other words, the human cells were integrating *functionally* and could receive sensory stimulation. And in what might be their most consequential success, the team engrafted cortical organoids derived from cells donated by patients with Timothy Syndrome, a disorder that shares many clinical features with autism spectrum disorders. These organoids developed and integrated with the host brain in ways that clearly revealed pathologies consistent with the illness. [see illustrations, facing page]

Beyond making it possible to observe the origins of pathology, the technology creates new opportunities to test potential therapeutics for developmental disorders.



Fred "Rusty" Gage, Ph.D.

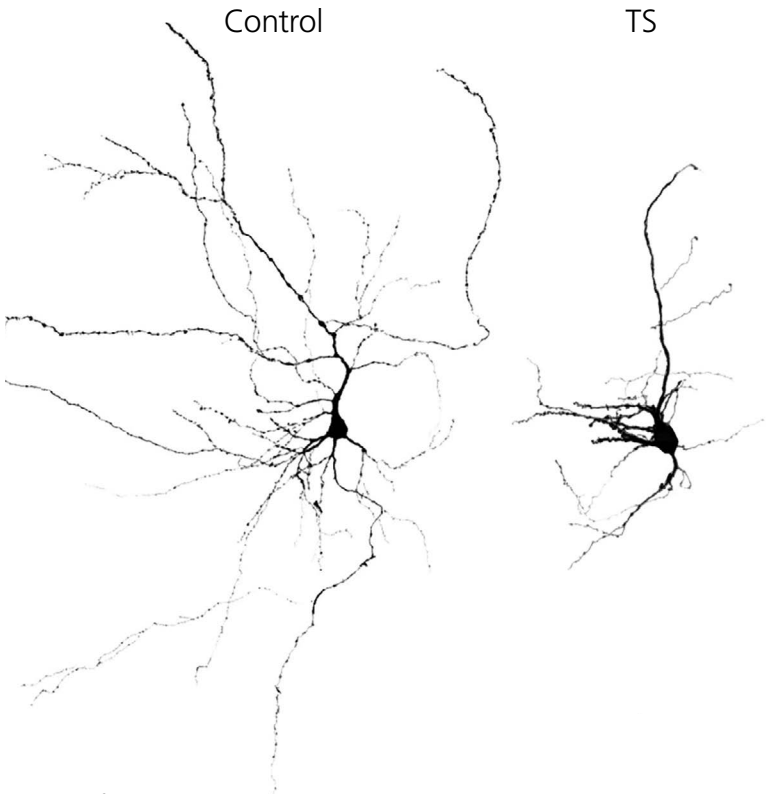
Simon T. Schäfer, Ph.D.

"Very often," Dr. Pasca says, "animal models don't recapitulate them well enough to gauge the impact of a newly developed drug." But testing candidate drugs in animals with highly integrated patient-derived organoids might be particularly helpful in determining a drug's impact on the pathologies that emerge in the organoids. In the last year, Dr. Pasca has created a new team within his lab dedicated to developing therapeutics, including one, he says, that appears to have promise in a neurodevelopmental disorder with genetic roots.

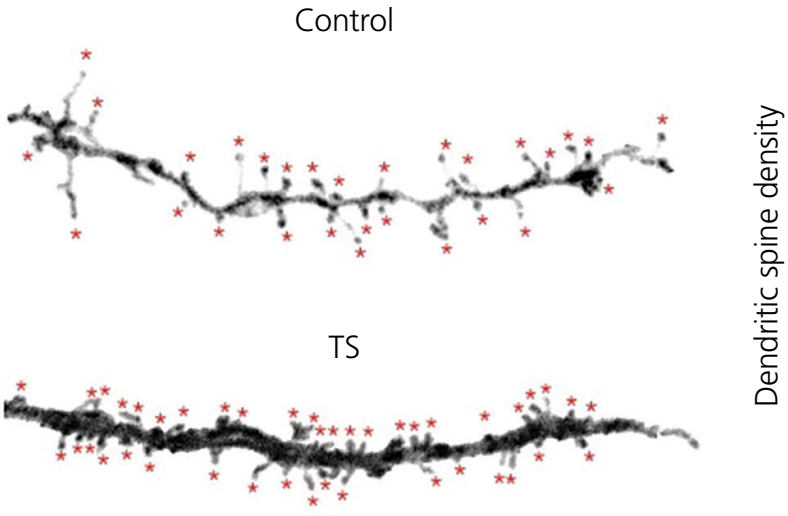
FACTORING IN THE IMMUNE SYSTEM

Among the questions that define any set of experiments with brain organoids is "what types of cells do you want to have in the organoid?" This observation, by **Simon T. Schäfer, Ph.D.**, one of Dr. Gage's mentees at Salk who has established his own lab at the Technical University in Munich, Germany, establishes a context for another important milestone in organoid-based experiments.

In a recent paper appearing in *Cell*, Dr. Schäfer, a 2021 and 2018 BBRF Young Investigator, along with Dr. Gage and other colleagues, reported success in engrafting into the rodent brain an organoid consisting primarily of human cortical neurons, but importantly, also including an important cell type called microglia. Microglia are the only cells of the body's innate immune system that live and function in the human brain. In the healthy brain, these cells are constantly surveilling the environment, looking for toxins and responding



The power of the organoid transplantation strategy to explore emerging pathology in developmental disorders is seen in these images. ABOVE: Eight months after being transplanted into the rodent brain, cortical neurons in organoids based on samples from a healthy person (left) are much larger and have different structure compared with those based on cells sampled from patients with Timothy Syndrome (right), which shares many features with autism spectrum disorders.



ABOVE: The density of connection points called dendritic spines (red stars) is much higher in dendrites of neurons in the transplanted organoids based on Timothy Syndrome patients (immediately above) than in those based on neurotypical donors (top).

to damage. No one had previously succeeded in growing a human brain organoid with microglia that completed the journey to functional maturity.

Microglia are generated from a fundamentally different stem cell precursor type than other cells of the brain, such as neurons, helper cells like glia, or fatty cells called oligodendrocytes which protectively insulate nerve pathways. Those and other cells of the human nervous system are products of stem cells from one of the three layers of the early embryo called the ectoderm. Microglia derive from stem cells in the original embryo's mesoderm, which is also the source of all blood cells.

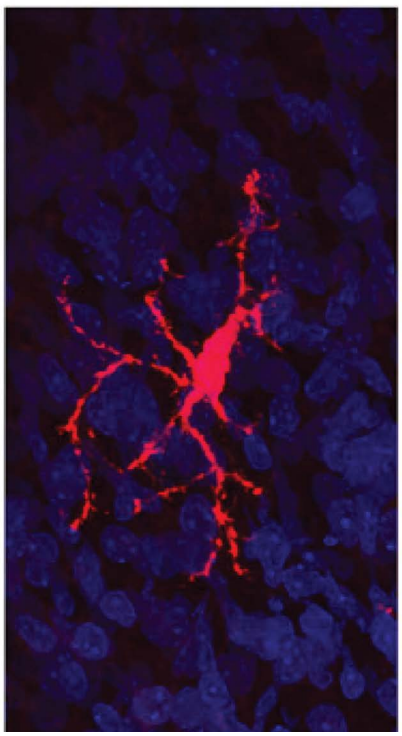
Microglia can be generated in the lab and added to organoids composed of neurons. But they fail to thrive. Drs. Schäfer, Gage and colleagues wanted to see what would happen if microglia were incorporated in the lab into a human-derived cortical organoid and then immediately transplanted into the living rodent brain.

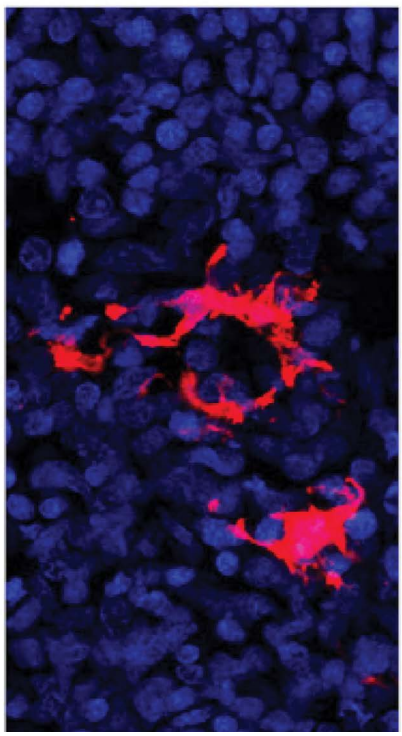
Their hypothesis was that signals sent and received only in the environment of a living brain might enable the microglia to mature and start to perform their immune surveillance function. This is precisely what happened. Once a protective layer called the blood-brain barrier forms, microglia become "trapped" in the engrafted organoid, just as they do in the developing human brain.

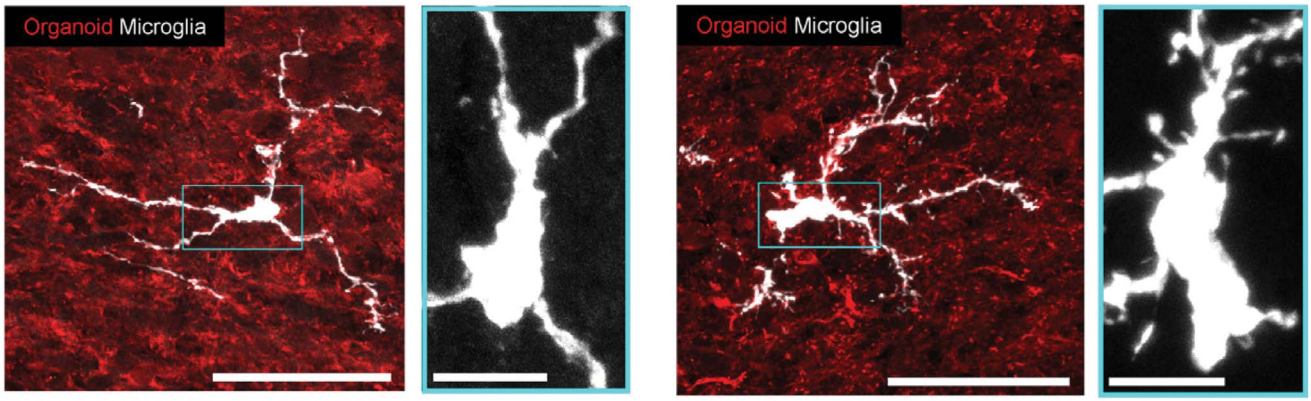
The microglia then began to respond and function when they sensed factors emanating from the human cells within then engrafted organoid. Now it was time to perform a parallel experiment. Just as Dr. Pasca's team had implanted into the rodent brain cortical organoids derived from patients with Timothy's Syndrome, Dr. Schäfer and colleagues now grew cortical organoids "colonized" with primitive microglia derived from patients diagnosed with autism and a co-occurring condition called macrocephaly (enlarged head size, which in severe cases has serious neurological and developmental consequences).

Two remarkable observations followed. One was that inside these patient-derived organoids functioning within the rodent brain, the microglia became very active. The team thinks this recapitulates something that happens in the brains of children with the combined condition.

By transplanting cortical organoids into the living rodent brain the team could observe microglia become fully mature and perform their immune surveillance function, thanks to signals they received from the living brain environment. Image on right shows microglia that failed to mature when grown in the lab environment.







Microglia in organoids based on samples from patients with autism spectrum disorder + macrocephaly (right) show structural differences compared with those based on samples from neurotypical individuals (left). Equally important, their much higher level of activity may be linked causally with inflammation seen in ASD + macrocephaly patients.

The other observation was that intense microglial activity resulted in inflammation within the engrafted organoid. And this is important because brain inflammation is often seen not only in autism, but a host of other neuropsychiatric conditions including schizophrenia and depression. Itself a source of pathology, inflammation has been hard to study in patients, but the organoid transplantation strategy provides one way to do just this.

The team's observations led to a crucial question: why did microglia become so active in these models of patient brain tissue "living" within the functioning rodent brain? Was there something about the microglia themselves that accounted for their unusual activity? Or was their activity dependent upon something in the organoid environment in which the microglia were functioning? Sophisticated experiments were performed which led the team to conclude that it was not the microglia, but signals from their immediate environment—that unique environment of a living, functioning brain—that prompted their high level of activity, leading to the inflammation that likely has a role in the pathology of the combined condition of ASD + macrocephaly.

"We knew from autopsy and biopsy tissue that patients with autism often show inflammation," Dr. Schäfer explains. "Well, we wanted to know where this inflammation comes from. The experiments suggested it is the developing brain environment that changes the activity of the microglia, which may result in inflammation."

This is something that begins early in development, it appears, and, says Dr. Schäfer, "it probably has very long-lasting consequences" for patients. "I think this also may prove relevant in other disorders, where you see contributions [to pathology] that are immune cell-driven." A new question the team now studies is whether microglia, in the very early stages of brain development, are, in effect, "trained incorrectly" due to environmental signals, such that they become overactive, opening the way to inflammation. The experimental approach is to perform experiments with transplanted organoids that enable the team to observe these processes as they occur, ideally when they first occur.

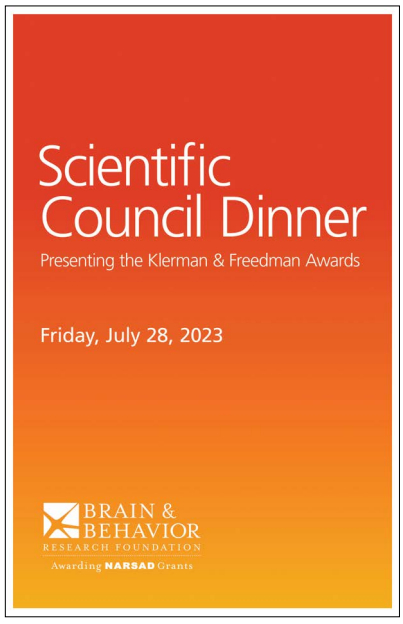
From such insights, it is hoped, may come new concepts for therapeutics. "We're just now building a center for organoid systems," Dr. Schäfer says. "We're bringing together people with different kinds of expertise to build systems that we can translate, not only to disease models, but maybe also to treatments.

"This is why I'm interested in organoids. We're used to doing science in a certain way: we make deductions based on our observations of complex systems [in which pathology is already present]. With organoids we have the chance to observe things from the very beginning of the process."

❖ **PETER TARR**

BBRF President and CEO Dr. Jeffrey Borenstein (far left) and BBRF Board Chairman Geoffrey A. Simon (far right) with prizewinners Drs. Neir Eshel, Linden Parkes, Madeline Andrews, Ritchie Chen and Danella M. Hafeman. (Photo Credit: Chad David Kraus)

The 2023 BBRF Klerman and Freedman Prize Winners



On Friday, July 28th, the Brain & Behavior Research Foundation presented the 2023 Klerman and Freedman Prizes to five outstanding young researchers at our annual Scientific Council Dinner in New York City.

The Klerman & Freedman Prizes recognize exceptional clinical and basic research conducted by BBRF Young Investigator grantees. BBRF's Young Investigator grant program supports early-career scientists as they gather pilot data and "proof of concept" for their innovative clinical and basic research.

The prizewinners are selected by committees of the Foundation's Scientific Council, led by its founding President, Dr. Herbert Pardes.

ANNUAL KLERMAN PRIZE FOR EXCEPTIONAL CLINICAL RESEARCH

Danella M. Hafeman, M.D., Ph.D.

University of Pittsburgh School of Medicine

Dr. Hafeman's research focuses on youth diagnosed with or at risk for bipolar disorder. She is interested in understanding clinical and neural mechanisms of risk and resilience in these youth, with the goal of preventing progression of mood disorders in this vulnerable population. Much of her recent work has focused on predictors of bipolar disorder in youth at familial risk, working with BBRF Outstanding Achievement Prizewinner Dr. Boris Birmaher. Dr. Hafeman hopes to use these data to construct a risk calculator for the development of bipolar disorder in at-risk youth.

HONORABLE MENTION

Linden Parkes, Ph.D.

Rutgers University & University of Pennsylvania

Dr. Parkes is a computational neuroscientist who seeks to uncover pathways that track the emergence of psychopathology. He approaches this goal from a neurobiological perspective by studying how complex neural systems shape behavior and cognition, and how dysfunction in these systems predicts psychopathology. His goal is to develop a set of robust, reliable, and predictive biomarkers that can be used in clinical trials to assess treatment stratification and response

ANNUAL FREEDMAN PRIZE FOR EXCEPTIONAL BASIC RESEARCH

Ritchie Chen, Ph.D.

University of California, San Francisco

Visceral sensations such as heart palpitations, hunger pangs, and pain profoundly shape our mental state and behavior. Dr. Chen is inventing technologies for modulating affective and social behaviors, opening new possibilities for treating mental health disorders. He has developed a cutting-edge technology that can non-invasively control cells throughout the mammalian body which could revolutionize our understanding and treatment of neurological and psychiatric conditions.

HONORABLE MENTIONS

Madeline Andrews, Ph.D.

Arizona State University

Dr. Andrews is a developmental neuroscientist who uses human cell cultures to explore the gene programs and cell signals that are essential for how brain cells grow, change shape, and become organized.

Neir Eshel, M.D., Ph.D..

Stanford University

Dr. Eshel's research focuses on the two neuromodulators that form the basis for most existing psychiatric treatments: dopamine and serotonin. By uncovering the functional diversity within these systems, he hopes his findings will lead to novel, more targeted treatments for symptoms such as irritability and aggression.

MONTHLY GIVING HELPS BBRF AND YOU!

What's the most effective and efficient way to impact brain science research at BBRF? By becoming a Monthly Donor.

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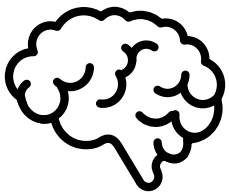

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100% OF EVERY DOLLAR DONATED FOR RESEARCH GOES TO RESEARCH

BRAIN & BEHAVIOR RESEARCH FOUNDATION

Awarding **NARSAD** Grants

A Giorgio Armani Benefit for BBRF

On Tuesday, April 11th the iconic global fashion brand Giorgio Armani presented a private VIP shopping experience to benefit the Brain & Behavior Research Foundation.

The event was hosted by BBRF Board Member Carole Mallement and her friend Michelle Reisman and included cocktails and hors d'oeuvres in addition to a beautiful display of the Armani Spring/Summer 2023 Men's and Women's Ready to Wear Collections + Accessories.

BBRF President & CEO, Dr. Jeffrey Borenstein, introduced Dr. Dolores Malaspina, a 2007 BBRF Distinguished Investigator Grantee, a 2001 BBRF Independent Investigator Grantee, and a 1995 and 1993 BBRF Young Investigator Grantee, who spoke about the enormous personal family and societal costs of depression and new breakthrough therapies for treatment-resistant depression. These include medications and various types of neurostimulation interventions.

Proceeds raised over $55,000 from both donations and shopping and will support the BBRF Young Investigator Grant Program.

Due to the success of the event, a second event in Florida and a New York City event are in discussion for 2024.

CLOCKWISE FROM TOP LEFT:

1. Dr. Jeffrey Borenstein, Michelle Reisman, Dr. Dolores Malaspina, Carole Mallement
2. Nancy Weber, Dr. Jeffrey Borenstein, and Lisa Eisner
3. Brenda Netkin and Michelle Reisman
4. Dr. Jeffrey Borenstein
5. Judy Wertheim, Harvey Wertheim, Hillary Kupferberg, Harvey Mallement, and Cara Kupferberg
6. Beth Guttman, Hazel Shanken, Carole Mallement, Janet Levy, and Debra Feinstein
7. Dr. Malaspina addressing the crowd

PHOTO CREDIT - HEATHER HOLT PHOTO

Warning Signs & What to Look For: Anxiety and Depression in Childhood

Q&A for teachers and parents by Dr. Jeffrey Borenstein, BBRF President & CEO, with Dr. Joan Luby

Adapted from a special BBRF webinar aimed at educators and parents

Jeffrey Borenstein, M.D.
President and CEO, BBRF

Joan Luby, M.D.

Samuel and Mae S. Ludwig Professor of Child Psychiatry

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2020 BBRF Ruane Prize for Outstanding Achievement in Child & Adolescent Psychiatric Research

2004 BBRF Klerman Prize for Exceptional Clinical Research

2008, 2004 BBRF Independent Investigator
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IN BRIEF

Symptoms of depression in children as young as age 3 are often missed by parents and teachers. One might see withdrawal, sadness, expressions of guilt. Teachers should look for persistent signs of a child's inability in school to enjoy joyful activities, a lack of motivation to engage in social relationships, and negative self-perception; these signs should be shared with parents. In adolescence, one may see passive or active suicidality and self-harming behavior, which should occasion referral to a mental health professional.

Dr. Luby, how early can symptoms of anxiety and/or depression start to appear?

That is a really important question that we've been interested in. We don't really know how early symptoms can begin, but available empirical data show that we can identify depression as early as age 3. You may be able to identify some anxiety disorders even before that. But the age of three is when clinicians can start looking for it and we can provide guidance on the signs and symptoms.

What does depression look like at such a young age?

For a very long time, there was a resistance in the field to accept the idea that children could be depressed. It really wasn't until the 1980s when empirical studies came out showing that children could be depressed, and that depressed children had the same fundamental symptoms as depressed adults. Previously, people said either children were developmentally too immature to experience the core symptoms of depression, or they said they would experience other symptoms, like stomach aches or aggressive behavior.

But then the research started to show that no, this was not the case, that children were a lot more emotionally sophisticated than we had previously understood. One landmark paper provided data showing that in children, depression looks just like it does in adults. In other words, children have anhedonia, which is decreased ability to enjoy activities and play. They have sustained sad mood; the inability to sustain joyful moods; and disturbances in sleep and appetite.

We essentially capitalized on that work, which had studied children as young as 6, and then asked the question: what this would look like in even younger children? We discovered we could find depression in children as young as 3. We found symptoms like anhedonia and decline in joyful behavior. Children are inherently so joyful that these markers are very important to pay attention to.

And what do symptoms look like in a slightly older child, a child of elementary school age who may be experiencing depression or anxiety?

That's where you're going to see some social withdrawal. Certainly, with anxiety, you will generally see a lot of social withdrawal. You see children showing more sadness, decreased motivation to engage in joyful and social activities. You might see changes in appetite. You might see psycho-motor slowing and fatigue. But the reason why these symptoms are so often missed—and they are often missed—is because caregivers and teachers tend to pay more attention to disruptive behaviors, and depressed kids fade into the background. Perhaps parents may be more sensitive to the symptoms.

What types of things should a teacher look for? And what should a teacher do if they see these signs?

With regard to depression, a teacher would be looking for a change in behavior, unless they're meeting a child when they're already depressed. Sometimes you see children who have more of what we call a chronic dysthymic condition—chronic low mood and other depressed symptoms—and in such children you wouldn't necessarily see a change.

Depression in children looks a lot like it does in older people. Depressed children have anhedonia, decreased ability to enjoy activities and play. They have sustained sad mood; the inability to sustain joyful moods; and disturbances in sleep and appetite.

But, in cases of new depression, often you will see more withdrawal, more sadness. Another very important sign is increased guilt. Young children who are depressed experience high rates of guilt. They feel guilty for things that aren't their fault. When they commit a transgression, it's much harder to reassure them or for them to shake it. Teachers should also look for the inability to enjoy joyful activities, lack of motivation to engage in social relationships, and also negative self-talk or self-perception.

When a child is depressed, these symptoms should be sustained over a period of a couple of weeks. Obviously, any child can be sad or irritable for one day (irritability is another sign, but it's a very non-specific sign, which is why I didn't mention it). But a child with clinical depression has these symptoms in a persistent way. They will brighten at times, so they don't have to be vegetatively depressed [this refers to symptoms affecting basic bodily processes, for instance, sleep habits, appetite, or the digestive system]. But because children are just inherently happier, depressed children will have sadness for large parts of the day more than usual, or for more days than not in a week, or for a couple of weeks. And when that's observed, then it would be time for referral to a mental health clinician.

How common is this? Do we have a sense of what percentage of kids experience these symptoms?

Prior to adolescence, there's about a 2% prevalence rate. Adolescence is when the prevalence of psychiatric disorders goes up sharply (particularly for girls, according to some data) to around 8% to 10%. We do see depression as something that runs in families. So when there's a family history of depression, we would be more suspicious of it, and those children would be at a somewhat higher risk.

What are the symptoms for adolescents as they go through the teenage years, middle school, high school?

There's a lot of continuity of the symptoms of depression across the lifespan—increased sadness, increased guilt, anhedonia, changes in sleep and appetite. When you get into adolescence, that's when you might also see more passive suicidality, and maybe active suicidality. Suicidality

can, of course, occur outside of a mood disorder, outside of depression, but as we know, there's an increasing prevalence of suicidality currently, and that is an important marker that often becomes clear in adolescence. Self-harming behavior might be another sign, although that can be a non-specific sign as well.

But obviously, a very important one to take action on. If a child is having thoughts of hurting themselves, is acting on that, these are issues that need to be addressed right away.

Absolutely. Another thing we're starting to understand is that suicidality is being observed and occurring much earlier in childhood than we previously understood. We've seen suicidality at surprisingly high rates in depressed preschoolers as early as 4 and 5. We've done studies looking at whether these children understand the permanence of death and found that those who have suicidal ideation understand the permanence of death even more than other kids who don't. So expressions of suicidal ideation, either passive or active, can arise early in childhood and should be taken seriously. That doesn't mean we should panic or take kids to the emergency room, but it does mean we should take it seriously and address it.

Tell us about the importance of early identification of these symptoms, why that makes a difference for the child.

Early identification is so important, and that's because, generally speaking, when you look at cognitive, social, and emotional skills across development, that's where we see impairments in depressed children. These affected skills and processes are much more changeable earlier in development, when the brain is much more "plastic," i.e., it will change more in response to environmental and psychosocial experiences. So that's one of the reasons we think early identification of psychiatric disorders and particularly mood and anxiety disorders is so important. We believe there's a window of opportunity earlier in development to more effectively treat.

It's important to remind our readers that brains are still developing at that younger age, and even among teenagers and adolescents into their early 20s. So the good news is that treatment can have an even greater impact on those developing brains.

Exactly. And many people believe that adolescence is another period of very high neuroplasticity, which is another reason why that's a real focus of attention.

One of the concerns in adolescents is the issue of substance misuse. I'm curious about the interplay between anxiety and depression and the risk of then experimenting with and misusing drugs and alcohol.

I think that's a huge risk. Because of the stigma associated with mental disorders, children and families don't necessarily identify, focus, and seek treatment. Therefore, it leaves these conditions untreated, although still very distressing and impairing. And

'Any child can be sad or irritable for one day… But a child with clinical depression has these symptoms in a persistent way.'

that's why, in some cases, adolescents turn to substances as a way of managing symptoms. But of course, it is a very maladaptive way of managing the symptoms that will ultimately exacerbate the symptoms.

If a child was walking with a limp, it's more straightforward for the teacher to say to the parent, "Your child's walking with a limp. Have that checked out." How can teachers approach the sensitive topic of depression or possible substance misuse with a parent?

You're right. That is very tricky. I think that's a huge risk. One of the problems that we have with the stigma associated with mental disorders is that children and their families don't necessarily identify, label, focus, and go for treatment, and therefore, it leaves these conditions untreated. I do think that it's important for teachers to let parents know when they see concerning signs—across the board.

Now, they may run into parents who are not very receptive, who might be defensive, who might want to write it off. I still think it's important for teachers to let parents know. Sometimes a parent might not initially or immediately embrace your concerns or take them seriously, but you may be planting a seed. It may take some time for the parent to accept that this is something they need to grapple with.

And if the teacher from last year told them, and now, the current teacher tells them, that reinforces it. So I would encourage teachers not to hesitate to notify parents. The work of really educating parents on the existence of mental disorders, their validity, their causes and treatments, is beyond the scope of what a teacher can do. But this is where school systems might come in and educate about mental health.

Sometimes parents know their kids better than anybody. They may have noticed signs on their own, and having an educator say something could be helpful. One idea would be to even say to the parent, "You may want to discuss this with the child's pediatrician and get some feedback and see what, if anything, needs to be done by getting further professional help."

Exactly. I agree. I've had so many patients come to my clinic where the parent may say, "The teacher thinks there's something wrong. I don't see it, or I don't really agree, but the teacher thinks so." I think parents do take what teachers say quite seriously, and then they do go seek professional help, and sometimes that leads to a much greater understanding on the part of the parent, ultimately.

Underlying issues of depression or anxiety in a young child are sometimes telegraphed via changes in eating or sleeping habits.

Now let's talk about treatment. What does treatment look like? What happens when somebody goes for an evaluation?

For kids older than 6, there are several forms of psychotherapy, for instance, cognitive behavioral therapy, that are proven to be effective. There are some age-adapted forms of interpersonal psychotherapy (IPT) that have been tested in pre-adolescent kids as well. Of course, there are an array of medications that have been tested for children as young as age 6, which are also proven to be effective. So when you talk about kids 6 and older, there's a number of treatment options and many of these options are quite effective, even though, of course, we are still searching for more effective treatments.

However, when you look at kids under 6, that's where you get into the zone where the intervention literature and the interventions are much sparser. First of all, the use of medications for depression in children under age 6 is not recommended because there is no data looking at the safety or efficacy of those medications for these children. There is at least one form of psychotherapy that we have worked on developing and testing at Washington University called Parent-Child Interaction Therapy—Emotion Development (PCIT-ED), which is a manualized form of therapy [i.e., performed according to specific guidelines for administration, maximizing the probability of the therapy being conducted consistently across settings, therapists, and clients]. It was tested in a large-scale, randomized controlled trial. It targets the parent-child relationship,

and it targets the child's emotional competence, and that's proven to be very effective. The problem is it's really not widely available right now, even though the manual with instructions on how to do it can easily be downloaded, and there are a number of therapists who know these approaches. But it's one of those types of therapies that needs to become much more readily available across the country. And that's where we run into a roadblock.

In PCIT-ED, how long does it take to start seeing results?

For kids under 6, we used an 18-week psychotherapy treatment delivered by a master's-level clinician, and we saw very positive results after this 18-week period. We saw kids starting to get better even after the first few weeks of treatment, which is part of the reason why I think parents remained so engaged. Now, again, that's early

Dr. Luby stresses that "it's important for teachers to let parents know when they are seeing concerning signs." This is all the more important because of the undeniable pressure of stigma: parents may tend to resist signs of depression or stress in their children and leave the matter unresolved. Sometimes a teacher's expression of concern may plant a seed that comes to fruition later on.

'The brain is highly plastic in early development. This is why we believe there's a window of opportunity early in development for more effective treatment.'

childhood, where we have the plasticity working for us.

When you get into older kids, sometimes the process might be slower. As for medications, it can take two to three weeks to begin to see effects. So you do have to be patient. It's a process, not a quick fix.

Another thing to be aware of is that a child who is depressed is vulnerable to another episode, even if they are effectively treated. So it's something you need to be attentive to over the course of development. And you might need therapy course-corrections, you might need boosters. It's something to keep your eye on in a lifelong way.

A key point is that with treatment, children get better. That for these fully treatable conditions, therapies may need tweaking at some points, but the children get better. They can function at a high, full level with appropriate treatment.

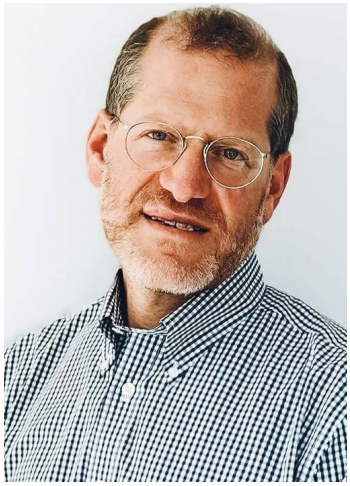
Absolutely. And the other reason it's so important to treat it in childhood is because during childhood, children are traversing a steep developmental curve. They have a lot of things to do, developmentally. They face high levels of social challenge, social-emotional development, cognitive challenges, motor development, and if a child has an anxiety disorder or depressive disorder, it doesn't just impair them in their daily life and increase their distress (which is a problem in and of itself), but it also drags down their development, which can then become a vicious cycle with long-term effects.

You're speaking to a good number of teachers, and to parents. What do you say to them? What's your guidance to them?

I would stress that children are very vibrant emotionally, even early in development. They're much more aware; they have much deeper feelings than we used to think. They have a much broader range of emotions. They're very capable of complex emotions. They feel intense guilt. These are things that are very important to pay attention to. Just as much as you're paying attention to their motor skills, to their language skills, children are really burgeoning in this domain and it's really, really important for us as educators, as caregivers, to foster and facilitate this. And this is the reason why we have to think about emotional functioning, and try to identify disorders as soon as they possibly arise and work to treat them. ❖ **EDITED BY FATIMA BHOJANI**

Helping Children & Adolescents with Emotional Problems

A Q&A with Daniel S. Pine, M.D.



Daniel S. Pine, M.D.

Chief, Emotion and Development Branch

Chief, Child and Adolescent Research in the Mood and Anxiety Disorders Program

National Institute of Mental Health (NIMH)

Thursday, November 30, 2023
7:00pm EDT, 4:00pm PDT via Zoom

Teachers, school counselors and other educational professionals are on the front line of dealing with kids with mental health issues and can often be among some of the first people to see that a child is struggling. Enhancing the potential for early intervention is important and because educational professionals have relationships with students and their families, they are often the people who guide students and their families to resources. As educational professionals learn more about mental health issues, their ability to make appropriate referrals for evaluation will improve for students and their families.

This conversation will share with parents and educators the key symptoms and attributes associated with pediatric mood and anxiety disorders. BBRF President & CEO Dr. Jeffrey Borenstein and Dr. Daniel Pine will discuss novel insights for improving treatment and offer tools to help families and educators address how best to help children and teens with emotional issues. The webinar will also highlight particularly pressing questions in research on pediatric mood and anxiety disorders while outlining an agenda for future research.

Registration will be required for this FREE event.

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Pregnancy-Related Brain Changes May Help New Mothers Prepare for and Bond With Their Children

Pregnancy is a time of profound changes in the female body, including the endocrine system—the system that secretes and regulates hormones. Changes in female sex hormones such as estrogen orchestrate numerous adaptations throughout the body, including the brain. Yet there is notably little data reflecting the impact of the reproductive process on the female human brain.

To address this, 2017 BBRF Young Investigator **Elseline Hoekzema, Ph.D.**, of the University Medical Center of Amsterdam and the University of Leiden, the Netherlands, led a team that recruited 80 women of childbearing age, half of whom became pregnant over the period of the study, and a matching group who were not pregnant during that time.

Dr. Hoekzema and colleagues used four technologies to examine brain structure and function in these women, at four time-points. Tests were performed on all of the women at baseline, which was prior to conception for those women who became pregnant; and then at times corresponding with late pregnancy, post-childbirth, and the late postpartum period among those participants who became pregnant.

These tests enabled the team to assess brain structure and function across the four time periods, and supported a series of important findings about how pregnancy affects resting-state brain activity, the structure of the brain's white and grey matter, and levels of neural metabolites—molecules generated by or as a result of metabolic processes occurring in brain cells.

Broadly speaking, the team, reporting in *Nature Communications*, said their data revealed "pronounced and selective structural and functional" changes in brain plasticity, "which may confer adaptive advantages" affecting the mother's behavior in the forging of bonds with a new child. Plasticity refers to changes in the strength of connections between neurons and is a key factor in how well the brain functions, for example, in learning, memory, and the ability to respond and adapt to changing bodily or environmental conditions. Deficiencies in neuroplasticity have also been linked with depression and other psychiatric illnesses.

The tests conducted at baseline showed that there were no pre-existing differences in the volume of the brain's grey matter among women in the two groups. Grey matter corresponds, roughly, with portions of the brain composed of neuronal cell bodies; white matter refers to the structures such as axons that connect neurons into complex networks.

Based on data from structural MRI scans that each woman received over the course of the study, the team was able to confirm its own previous finding indicating that women experiencing pregnancy and the postpartum period have *reductions* in the volume of their grey matter described as "highly significant" with "very large effects." The new findings confirmed not only the magnitude of the previously observed reductions but also the locations in the brain where they appear to be most prominent.

Resting-state fMRI scans showed that despite reductions in grey matter among women who were pregnant or in the postpartum phase (compared with the controls who were not pregnant at these time points) there was a notable "*increase* in functional connectivity" that was evident in the brain's

default mode network (DMN). The DMN regulates brain activity at moments when an individual is not focusing on the external world. Specifically, the team found that reproductive processes enhanced the DMN's "temporal coherence."

A different brain-scanning technology called diffusion tensor imaging revealed that there was no significant change in white matter structure among the pregnant/postpartum women when compared with those in the control group. Similarly, measurements of neural metabolite concentrations revealed no strong changes.

Perhaps the study's most important finding was that pregnancy-related neural changes were likely associated with the stimulation of behavioral and bodily adaptations that new mothers normally make to prepare for motherhood. For instance, pregnancy-related neural changes were associated with changes in the mothers' physiological responses to infants, to nesting behaviors, and to bonding with newborns in the postpartum period.

While the observed changes in brain structure were maintained, the increases in DMN coherence gradually reverted back to pre-pregnancy levels during the postpartum period. The total duration of breastfeeding positively correlated with the gains in DMN coherence, suggesting to the team that "prolonged breastfeeding may stimulate a prolonged maintenance of pregnancy-related neural changes."

During the perinatal period, changes in the DMN, which plays a key role in self-perception, may even "underlie transformations in the neural representation of the self when becoming a mother," the researchers speculated. Sex hormones and especially estrogen appear to contribute to these adaptive brain changes. Broadly speaking, they said, "our findings suggest pregnancy-related neuroplasticity plays a role in psychological and physiological gestational maternal processes that help a woman to prepare for the arrival of her baby" and to "the establishment of the mother-infant dyad." ❖

Researchers Identify Habit-Related Neural Circuitry That is Likely Perturbed in Binge-Eating Disorders

A team of investigators led by a BBRF grantee has gained new insight into circuit-based mechanisms in the human brain that underlie habitual behaviors, specifically those involved in two eating disorders, binge-eating disorder (BED) and bulimia nervosa (BN).

In BN, there are recurring episodes of eating large amounts of food at one sitting with a sense of loss of control, usually coupled with efforts to purge. BED, in contrast, involves similar binge-eating episodes that are not paired with purging.

One of the mysteries posed by eating disorders as well as other disorders including substance-use disorders is why an individual will persist in behaviors that they know will hurt them. The answer is thought to involve, among other things, problems in circuitry underlying the formation of habits. Better understanding such circuitry provides a potential path toward new treatments.

Habits are formed through repeated associations between a rewarding behavior—say, the satisfaction of taking in nourishment—and contextual stimuli experienced or perceived at the time of the behavior—say, a feeling of hunger, or, quite different, the mere sight or thought of food. Eventually the "contextual stimuli" themselves become sufficient to drive the behavior, independent of the reward actually obtained. For this reason, behaviors that are habitual are resistant to changes in

what happens as a result of engaging in the behavior. People will continue to smoke or take addictive drugs long after they learn of the likely damage being done to their health. Similarly, people with disorders involving binge eating and/or purging habitually engage in the behavior despite knowing their health is being adversely affected.

Casey H. Halpern, M.D., a 2016 BBRF Young Investigator at the University of Pennsylvania and the VA Medical Center, Philadelphia, and colleagues including **Cara Bohon, Ph.D.**, a 2012 BBRF Young Investigator, began with the hypothesis that people with recurrent binge eating will have altered habit circuitry, compared with healthy controls. They used a combination of functional and structural neuroimaging methods to examine specific parts of the brain in a cohort of eating disorder patients, 21 with BED and 13 with BN. All were female.

The team, reporting in *Science Translational Medicine*, was inspired by an extensive literature based on experiments with animals—mainly rodents—that point to the brain's striatum and its complex connectivity with different parts of the cortex as playing a key role in driving and regulating habitual behaviors. The striatum has many functions, prominent among which are processing rewards and bodily movements related to reward-seeking. Much of what happens in the striatum is regulated by dopamine signaling—which has been implicated in the acquisition of habits and the carrying out of habitual behaviors.

Maladaptive behaviors—harmful to the individual—have been central in studies of addiction, and as Drs. Halpern, Bohon and colleagues note, recent evidence has suggested that habitual behavior may also be important in eating disorders. Specifically, "as eating becomes habit-driven, it may be influenced more by external food cues than by the individual's feeling of satiety [fullness] or actual bodily needs."

Part of the new study was designed to identify subregions of the human striatum that are most likely to be involved in habitual behavior. This yielded two regions, called the sensorimotor putamen and the associative caudate. Study of the imaging data from eating disorder patients revealed to the team that connectivity of the sensorimotor putamen was altered, both in BED and BN patients, and that the degree of alteration correlated with the severity of the disorder in individual cases.

The observed striatal connectivity alterations were also found to correlate with grey matter microstructure in the affected subregion and with dopamine signaling with the ventral portion of the striatum. The associative caudate subregion of the striatum, though involved in habitual behavior, was not found to exhibit alterations in connectivity in the same patients. "This suggests the sensorimotor putamen may be the key node for promoting habitual behaviors," not just in BN and BED patients, but generally in human beings, the team said.

Binge eating in response to external cures, such as the sight of food, and "emotional eating," which is a term for eating that is prompted by depressed mood or other emotional states, have previously been regarded as involving separate processes. The new study, however, provides evidence that both "may be conceptualized as habit-driven behaviors, i.e., related to habit circuitry, and that it is these which may drive the frequency of binge eating episodes."

The evidence in this study did not indicate that habit circuitry involved in binge eating behaviors was also involved in "restrictive eating" behaviors, for example the restrictive type of anorexia nervosa that involves consistent self-restriction of food intake.

The team suggested that "future treatments involving modulation of circuitry-based mechanisms may potentially provide a means to treat habitual behaviors that underlie the treatment-resistant nature of many human psychiatric disorders, not limited to eating disorders." ❖

Four Subtypes of Autism Spectrum Disorder Are Distinguished, Helping to Explain Individual Differences in Symptoms

Researchers led by a BBRF grantee have used a large set of neuroimaging data to identify distinct sets of alterations in functional connectivity that may help explain differences among individuals with autism spectrum disorder (ASD). The finding could have implications for the development of new treatments.

The autism "spectrum" refers to wide variations in the types of symptoms that affect those diagnosed, as well as the degree to which symptoms impact individual function. Social communication and interaction skills are usually affected, although to varying degrees. As noted by the U.S. Centers for Disease Control, people with ASD also may have restricted or

repetitive behaviors or interests. In addition, some patients may have delays in acquiring language skills, movement skills, or cognitive and learning skills. Some may exhibit hyperactive, impulsive, or inattentive behavior; or have unusual eating or sleeping habits, gastrointestinal issues, or issues with mood, anxiety or fear.

"Our limited understanding of the neural mechanisms underlying ASD variability has impeded the development of therapeutic interventions," notes a research team led by 2013 BBRF Young Investigator **Conor Liston, M.D., Ph.D.**, of Weill Cornell Medicine, reporting in *Nature Neuroscience*. Dr. Liston's team sought to discover consistently identifiable subtypes of ASD as a way of generating testable theories "about how different biochemical genetic and cellular processes may shape" the wide range of ASD's clinical manifestations.

There was good reason to use neuroimaging data to try to discern ASD subgroups. Past functional magnetic resonance imaging (fMRI) studies have found that impaired social cognition and language processing in ASD are associated with atypical activity in the thalamus and visual areas of the brain, as well as in the salience network, composed of several brain regions that work together to determine which stimuli should command attention. Repetitive and ritualistic behaviors also have been linked in imaging studies with specific brain circuitry.

Dr. Liston and colleagues sought to discover how atypical connectivity contributes to individual differences in ASD symptoms and behaviors. They drew upon two large-scale fMRI datasets curated by the Autism Brain Imaging Data Exchange. The data analyzed was derived from 299 individuals with ASD and 907 neurotypical controls. The analysis enabled the team to relate functional connectivity patterns to three "dimensions" of ASD symptoms—those affecting verbal ability, social affect, and repetitive behaviors and restricted interests.

When study subjects with ASD were assessed according to this schema, the team found that they "clustered" in four subgroups, each with distinct patterns of functional connectivity in ASD-related neural networks. The same four subgroups emerged when the team applied the same functional-connectivity analysis to an independent sample of ASD patients.

The next step was to consider the connectivity data for the four ASD subgroups in the light of data on gene expression patterns in the brain. Of the approximately 21,000 human genes, many, but not all, are activated in brain cells, and at different moments and in different brain regions. Activation patterns vary depending on what tasks the brain is performing. The team hypothesized that distinct genetic pathways may

be important in subsets of ASD patients, and may confer risk for specific symptoms by impacting functional connectivity in ASD-related brain networks.

That is what the analysis revealed. Each of the four ASD subtypes was associated with distinct gene expression patterns and the biological processes they affect. This led to a number of interesting observations. Individuals in two of the subgroups, for example, were alike in being "highly impaired" by core ASD symptoms, the team noted, but differed notably in verbal ability, and had dissimilar patterns of atypical connectivity and gene expression. The other two subgroups "had average verbal ability" but differed in the degree to which they were impaired by two of the core ASD symptoms, social affect and repetitive and restricted behaviors.

The four ASD subgroups identified by Dr. Liston and colleagues provide insight into the biological mechanisms "that may regulate changes in brain function that lead to ASD behaviors," the team said. The analysis also makes it possible, they said, to form "multiple testable hypotheses that could be explored in future studies."

In ASD subgroup 4 for example, which is characterized by strong repetitive and restrictive behaviors and notably diminished "social affect," i.e., signals to others about how one is feeling, atypical connectivity was linked with decreased expression of a gene called *HTR1A*. That gene encodes a cellular receptor for the neurotransmitter serotonin that has been associated in past research with severe repetitive behaviors and restricted interests. Expression of *HTR1A* is known to be reduced in people with ASD, which in turn is associated with stress and anxiety. Problems with serotonin signaling have also been implicated in altered reward processing in the brain, as well as impairments of the sensorimotor system during development—which contribute to repetitive and restrictive behaviors. These linkages suggest that drugs targeting the serotonin system could potentially be beneficial for reducing these behaviors in some people with ASD, the researchers note.

More broadly, the researchers say their results can generate testable ideas that can be explored in animal models of ASD and in future clinical studies. "They suggest distinct alterations in brain function that could be targeted using circuit-based neuromodulation" such as TMS or other brain-stimulation technologies. They also "predict distinct biological pathways that could help inform studies of drug targets specific to each ASD subtype," the team said. ❖

Therapy Update

Recent news on treatments for psychiatric conditions

AFTER 'PRIMING' WITH KETAMINE, PATIENTS RECEIVING SELF-ESTEEM TRAINING HAD EXTENDED ANTIDEPRESSANT BENEFITS

Sanjay J. Mathew, M.D.

The experimental drug ketamine has been shown to reduce symptoms of major depression, even in treatment-resistant patients, within hours of a single intravenous administration. A medicine based on ketamine—a chemical derivative called esketamine—was approved by the FDA for use in treatment-resistant depressed patients in 2019.

Yet the search continues for better versions of ketamine. This includes drugs that might act as rapidly to reduce major depression symptoms but have fewer risks of adverse side effects, as well as agents with a longer-lasting antidepressant effect. In most patients, ketamine's therapeutic effects fade within a week, at which point the underlying depression reasserts itself. Some patients have received repeated ketamine infusions over a period of months to maintain the antidepressant effect. The safety profile of this strategy remains uncertain, however.

A team of researchers led by Rebecca B. Price, Ph.D., of the University of Pittsburgh School of Medicine, now report in *The American Journal of Psychiatry* that they have tested a way of extending ketamine's antidepressant effect. It involves pairing a single intravenous administration of ketamine in treatment-resistant patients with a computer-administered training procedure given in the days following the treatment. Results were encouraging.

A randomized, double-blinded trial conducted by Dr. Price and colleagues who included **Robert H. Howland, M.D.**, a 1991 BBRF Young Investigator, and **Sanjay J. Mathew, M.D.**, a 2009 BBRF Independent Investigator and 2006 and 2001 Young Investigator, was performed with a study cohort of 154 adults with treatment-resistant depression, most in the their 30s and 40s.

The participants were divided randomly into three groups. One group (53 participants) received a single treatment of ketamine plus a series of computer-administered training sessions called ASAT that are designed to increase self-esteem. A second group (50) received a placebo injection instead of ketamine plus the ASAT training. A third group (50) received ketamine and a placebo version of ASAT training.

ASAT stands for Automated Self-Association Training. Dr. Price and her team wanted to test it in connection with a hypothesis they hold about how ketamine exerts its therapeutic effects. The team posits that one central reason ketamine works is because it causes a rapid and pronounced increase in the plasticity of neurons and brain circuits. Plasticity refers to the ability of brain cells to change the strength of their connections. It is widely thought that increases in plasticity are at least part of, and may be central to, the antidepressant effect, of ketamine and possibly other existing depression treatments.

"We have hypothesized that these [plasticity] effects [rapidly induced by ketamine] may produce a corresponding neurocognitive shift," the team noted in its paper. It was their hope that "we can extend rapid mood relief during a window of opportunity, using behavioral learning-based approaches."

The approach they used, ASAT, is designed "to reinforce adaptive patterns of cognition through automated [i.e., computer-administered] training." By adaptive, the team means patterns of behavior that help the individual adapt to or cope with challenges. This is relevant because depression is typically associated with low self-esteem and chronic, repetitive patterns of negative thinking.

The idea of using the ASAT training program following a single ketamine treatment was that the drug would boost plasticity and thus mood, opening a "window of opportunity" in which the ASAT training might "consolidate beneficial processing patterns and prolong ketamine's rapid [positive] mood effects."

Nearly all of the participants in the trial—148 of the initial 154—received full courses of either ASAT training or a placebo version of it, following a single ketamine treatment or a placebo version of one. The training was given in the 4 days following ketamine or placebo injection being administered. Two training sessions of about 20 minutes were given each of those days.

In those who received both "active" ketamine and ASAT, the team found that ketamine's initial antidepressant effect was extended for at least 30 days. (In-person trial monitoring ended at that point, although a questionnaire-based follow-up will continue for a year, and results will be forthcoming.) Trial participants who received a placebo injection but an "active" version of ASAT training received relatively little benefit over the 30-day period. Those who received active ketamine and a placebo version of ASAT responded over the month as do most patients who take only ketamine; their depression symptoms steadily returned.

The team offered this conclusion: "After priming the brain with ketamine, training positive self-associations could provide an efficient, low-cost, portable, noninvasive, and highly dissemination-ready strategy for leveraging and extending ketamine's rapid antidepressant effects."

Larger trials involving more diverse patient populations must follow this initial study, the team said, before ASAT or similar strategies might be paired with rapid-acting antidepressants including ketamine to extend their therapeutic effects. ❖

TALK THERAPY + BRAIN STIMULATION REDUCED SUICIDAL IDEATION IN PATIENTS WITH BORDERLINE PERSONALITY DISORDER AND TREATMENT-RESISTANT DEPRESSION

Anthony C. Ruocco, Ph.D., C.Psych.

About 1.4% of U.S. adults (over 3 million people) experience Borderline Personality Disorder (BPD) in a typical year. BPD is difficult to treat. Evidence for the effectiveness of medications alone to treat BPD symptoms is limited. A form of talk therapy called dialectical behavior therapy (DBT) has often been effective in treating people with BPD. But BPD often co-occurs with other psychiatric illnesses including major depression, and it is associated with an exceptionally high risk of suicidality, especially among patients with comorbid depression.

DBT can help many people to reduce their self-harming behaviors, but additional treatment approaches are urgently sought to address the risk of suicidal behavior associated with BPD.

Now, a team of researchers led by 2014 BBRF Young Investigator **Anthony C. Ruocco, Ph.D.**, of the University of Toronto and Centre for Addiction and Mental Health, reports encouraging results of a small feasibility trial of a new treatment combination for severely suicidal patients with BPD and co-occurring treatment-resistant depression. The team treated nine such patients with DBT and "conjoint" magnetic seizure therapy (MST) for 5 weeks, and compared impacts on their symptoms—especially suicidal ideation and depression, as well as cognitive performance—with 10 similar patients who received only DBT for 5 weeks.

Conjoint therapies are those administered in concert. In this case, participants in both groups received 1 hour weekly of individual DBT and 1 hour of weekly DBT skills training focused on distress tolerance. Participants in the "conjoint therapy" group received these DBT treatments plus up to

15 MST treatments (up to 3 per week) over the 5 weeks of the trial. MST is a form of non-invasive brain stimulation that has been associated in some trials with significant reduction of suicidal ideation in patients with treatment-resistant depression. It had not been tested previously in BPD. MST uses magnetic pulses to induce a brief seizure in the brain that is intended to have therapeutic effects.

Although the trial led by Dr. Ruocco and colleagues was small, it did generate hopeful results. Combined DBT and MST treatments led to a "rapid, significant, and clinically meaningful reduction in suicidal ideation" at the end of the 5-week study period, the team reported in the inaugural issue of *Nature Mental Health*. This reduction in suicidality was sustained at a 4-month follow-up assessment.

Conjoint DBT + MST was also associated with "significant reductions in depression and BPD interpersonal symptom severity," the team reported, "but neither effect was sustained at the 4-month follow-up. Importantly, there were no observed impacts of MST therapy on cognition, and there were no treatment-related serious adverse effects.

These initial results lead the team to suggest that the DBT + MST combined therapy is "feasible" to offer, and "warrants further exploration" in a larger placebo-controlled clinical trial. They also suggest that rTMS, a commonly used form of brain stimulation, might be tested in combination with DBT in suicidal patients with BPD. For now, they said, their results "represent a step toward addressing the long-standing problem of suicidality in BPD."

The researchers note that their results do not suggest that DBT alone is ineffective, rather that the combination of DBT and MST was associated with a more rapid reduction in suicidality compared with 5 weeks of DBT alone. ❖

The research team also included senior member **Zafiris J. Daskalakis, M.D., Ph.D.**, BBRF Scientific Council, 2008 BBRF Independent Investigator, 2006 and 2004 Young Investigator; co-first author **Jenna M. Traynor Ph.D.**, 2022 BBRF Young Investigator; and **Daniel M. Blumberger, M.D.**, 2010 BBRF Young Investigator.

RAPID-ACTING BRAIN STIMULATION METHOD MAY REDUCE MAJOR DEPRESSION BY REVERSING INFORMATION FLOW BETWEEN BRAIN REGIONS

Nolan R. Williams, M.D.

In their efforts to understand the beneficial effects of SAINT, a new fast-acting brain stimulation treatment for refractory major depression, researchers now think they understand why it works, and for whom. Remarkably, they propose, the treatment works because it helps to reverse the direction of signaling within an important brain network that is likely out of synch in major depression. The finding has potentially significant implications for fitting patients to the treatment and for understanding depression more generally.

In September 2022, the U.S. Food and Drug Administration approved commercialization of SAINT for people with severe major depressive disorder who have not responded to multiple conventional antidepressant therapies.

Developed by a team led by **Nolan R. Williams, M.D.**, of Stanford University, a 2018 and 2016 BBRF Young Investigator and 2019 winner of BBRF's Klerman Prize for exceptional clinical research, SAINT has been impressive in three clinical trials. It has provided rapid remission for about 80 percent of the several dozen severely depressed, treatment-resistant individuals involved in the trials. SAINT is an accelerated and intensified form of rTMS (repetitive transcranial magnetic stimulation), which has been widely used to treat depression since its approval in 2008. Unlike conventional rTMS, SAINT is individually targeted for each patient. Patients also receive stimulation in much shorter treatment sessions, lasting only a few minutes, compared with 37 minutes in conventional rTMS. One of the chief innovations of SAINT is to deliver 10 stimulation sessions per day over just 5 days (vs. one session for 5 days a week over 4–6 weeks in rTMS). SAINT sessions are separated by an interval of 50 minutes "to build upon one another to amplify the antidepressant effect," Dr. Williams has explained.

Now, Anish Mitra, Ph.D., a postdoctoral investigator in the Stanford laboratory of BBRF Scientific Council member and two-time grantee **Karl Deisseroth, M.D.**, Ph.D., and a team that includes his Stanford co-mentor Dr. Williams and **Marcus E. Raichle, M.D.**, 2004 BBRF Goldman-Rakic Prize winner, have used Dr. Mitra's innovative method of analyzing data from fMRI functional brain scans to probe how, why, and for whom SAINT works.

The team used rs-fMRI scans of 33 severely depressed patients who took part in SAINT clinical trials—scans made before treatment began and after it ended. The same kind of scans from 85 healthy controls were used for comparative purposes. These latter scans revealed that in the undepressed brain, an area called the anterior insula, which has the role of integrating information about bodily sensations, sends signals to the anterior cingulate cortex (ACC), one of the regions that regulates emotions.

But analysis of the SAINT patient scans showed that in about three-fourths of patients, this "normal" information flow was reversed. The ACC was sending signals to the anterior insula. The more severe an individual's depression symptoms, the greater the proportion of signals between the two areas that were flowing the wrong way.

When SAINT patients were treated, the flow of neural activity changed—now moving from the anterior insula to the ACC—as is seen in undepressed people. The team also was able to determine that those patients with the highest depression scores pre-treatment were those who were most likely to benefit from SAINT.

In a paper in *Proceedings of the National Academy of Sciences* reporting their results, the team was careful to note that the biomarker they discovered regarding the flow and timing of signaling applies specifically to signaling within the brain's "salience network." This network is involved in processing emotions and evaluating one's internal state. The team's analysis points to the dorsal portion of the ACC as a "major hub" in the salience network, whose "early signaling," i.e., prior to signaling it should be receiving from the anterior insula, appears to be directly tied to major depression symptoms.

Depression is "heterogeneous"—it affects patients in a large number of ways, with symptoms varying widely over the total population of depressed people. It is likely that there are multiple mechanisms involved in depression symptoms, involving other brain regions. SAINT may be more or less effective in a broader population of patients with varying manifestations of major depression.

Since stimulation of other brain areas has been found to relieve depression symptoms in some patients, it will be necessary to determine if other mechanisms in addition to the "ACC-salience network" mechanism are involved. In other words, have they pinpointed a unique subtype of severe, refractory major depression? Or is the finding more generally applicable in depression?

Another of the interesting questions to be explored in future research is whether noninvasive stimulation of other areas—for example, the insula—might also have the effect of reversing the "wrong-way" flow of information discovered in the current analysis. ❖

GLOSSARY

CHOLINERGIC SYSTEM (p. 6) Refers to signaling by the neurotransmitter acetylcholine, which is important for neurons throughout the body, and which, in the brain, plays an important role in learning, memory, stress response, and broadly, cognitive functioning.

ANTICHOLINERGIC BURDEN (p. 6) Some medicines, including many taken by schizophrenia patients, act to impede the operation of the cholinergic system. When the total anticholinergic burden—summing the impact of all medicines taken by a given patient—is very high, core cognitive impairments in schizophrenia may be exacerbated. Managing the total anticholinergic burden (ACB) may be a way to reduce potential adverse cognitive impacts.

MMN and P3a (p. 8) These are features of EEG (electroencephalogram) readouts of brain activity. MMN, or "mismatch negativity," tests the brain's ability to detect subtle changes in an otherwise repetitive background of sounds. P3a similarly gauges the degree to which an individual responds to sounds. Both are innate responses, and both are blunted in schizophrenia, with the degree correlated with an individual's ability to function in real-world settings. The blunting effect is significantly greater in patients with high total anticholinergic burden scores (see above).

ORGANOIDS (p. 14) Assemblies of cells derived from reprogrammed pluripotent stem cells (see below) that clump together and form functional units. Grown in culture dishes in the lab, they can be transplanted into the living rodent brain, where they forge connections, mature physically, and acquire organic functional capabilities.

PLURIPOTENT STEM CELLS (p. 15) Precursor cells that, in the embryo, are capable of developing into all of the cell types that make up the adult organism. Researchers can sample mature cells, e.g., skin cells, from children or adults and induce them to return to a pluripotent state. Such cells can then be reprogrammed to re-develop as various cell types, e.g., brain cells such as neurons or microglia. This is called **INDUCED PLURIPOTENT STEM CELL (iPS)** technology.

ASSEMBLOIDS (p. 17) Fused-together combinations of three-dimensional organoid cultures that represent different regions of the brain. This is a way of modeling inter-regional complexity in the living brain.

MICROGLIA (p. 19) The only cells of the body's innate immune system that live and function in the human brain. In the healthy brain, these cells are constantly surveilling the environment, looking for toxins and responding to damage. Recent experiments with transplanted organoids suggest that overactive microglia responding to local environmental signals in the living brain may give rise to brain inflammation that is seen in autism spectrum disorder and other psychiatric illnesses—an important clue about illness-related pathology.

ECTODERM / MESODERM (p. 20) Two layers of the early embryo from which stem cells arise. Neurons and other cells of the human nervous system are products of stem cells from the ectoderm. Microglia derive from stem cells in the embryo's mesoderm, which is also the source of all blood cells.

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