Breaking into the Blood-Brain Barrier

INTERVIEW WITH: PROFESSOR DANIELA KAUFER

BY: Sania Choudhary, Miriam Goodwin, Jordan Shellow, and Andrew Delaney

Daniela Kaufer, PhD, *is a professor within the Department of Integrative Biology and the Helen Wills Neuroscience Institute. She is also the Acting Associate Dean of Biological Sciences at UC Berkeley. Dr. Kaufer earned her PhD from the Hebrew University of Jerusalem and completed a postdoctoral fellowship at Stanford University before joining the faculty at UC Berkeley. Her research focuses on the effects of chronic stress on the blood-brain barrier and how blood-brain barrier dysfunction impacts aging. Specifically, Dr. Kaufer investigates astrocyte dysfunction and TGF-β inhibition in order to better understand the degradation of the blood-brain barrier. In this interview, we had the pleasure of discussing the history of blood-brain barrier research as well as Dr. Kaufer's own journey within the field and her vision for the future of aging research.*

BSJ: What exactly is the blood-brain barrier? What functions does it serve within the brain, and how does it impact human health?

DK: Let me start by telling you what the blood-brain barrier is not: it is not some kind of impenetrable wall built inside the brain that nothing enters or exits. In reality, it is a very intricate filtration system on the walls of all the blood vessels in the brain. Cerebral vasculature refers to this rich network of blood vessels in the brain. There are pictures that show how almost every cell in the brain is supplied with oxygen, glucose, and blood by the cerebral vasculature. Unlike other blood vessels in our body, there are extra filtration systems within the cerebral vasculature, so things do not just spill out from the blood vessel into the tissue in the same way that they do in the lungs, gut, or elsewhere in our body.

The best way to think about the blood-brain barrier is as a filtration system built from layers within and around every blood vessel. Tight junctions between endothelial cells, which comprise blood vessel walls, inhibit the passage of molecules between cells. These cells also express an array of proteins that inhibit the movement of molecules across the barrier; specific transporters allow only certain molecules, like glucose, to enter. Outside of the blood vessel walls are layers of other cells, like pericytes and astrocytes, that make up each succeeding layer.

BSJ: What first sparked your interest in the blood-brain barrier and cognitive deterioration?

DK: My interest began many years ago as a graduate student at the Hebrew University in Jerusalem just after Gulf War syndrome came to light. There were initial reports, of mostly American soldiers, that came back from the war with unexplainable neurological symptoms. During this time, I wrote extensively on exposure to chemical warfare and Gulf War syndrome. The prophylactic they received for chemical warfare, pyridostigmine bromide, was something that should not have passed the blood-brain barrier. However, the mystery was that their symptoms were exactly the same symptoms that one would expect to see if the drug was in the brain, as if it had access to the central nervous system. So, along with Alon Friedman—a friend, collaborator, and, at the time, member of the Israeli army involved with chemical warfare—I thought the blood-brain barrier was more dynamic than previously thought. In fact, my whole career afterward became very focused on changes in the blood-brain barrier and how they relate to stress. Alon Friedman left the army and opened his own lab while I went to do my post-doctorate degree at Stanford University before opening my lab at Berkeley. But, we continue to collaborate with regard to the ongoing issue of the blood-brain barrier.

At some point, we shifted our interest to what is happening once the blood-brain barrier opens. We began asking ourselves not why the blood-brain barrier opens but rather what happens downstream of this change. This became a way to explain individual variability in outcomes of brain diseases. A lot of people suffer a traumatic brain injury, stroke, or some sort of neural infection. However, only a subset of such patients ends up developing seizures or seizure-like events. Now, we have observed that this subset of patients have a dysfunction in the blood-brain barrier. Through follow-ups, we have noticed that protein from the blood enters the brain and binds to a receptor, where an astrocyte then activates them. What happens to the astrocytes, what happens to the cells around them, and how they affect excitation or inhibition in the brain are how one might develop epilepsy.

When we learned this about the blood-brain barrier and seizures, it became clear to us that this mechanism could help explain cognitive decline in aging. We hypothesized that the blood-brain barrier may be dysfunctional in aging since a lot of neurological disorders actually involve some level of dysfunction to the blood-brain barrier. We could not find much research concerning normal aging and the blood-brain barrier. So, we decided that this was a topic well worth investigating.

Now, with many traumatic brain injuries, you see epilepsy, but in a lot of them, you see cognitive decline. A lot of times in my talks, I show a picture of Muhammad Ali, who was one of the first individuals recognized as developing cognitive decline from traumatic brain injury. However, you may have also heard about chronic traumatic encephalopathies that are seen in football players. So, there was more and more evidence that we might think of the blood-brain barrier as something that drives brain aging. That is how my lab arrived at this question a few years ago, and the answer turned out to be, "Yes, traumatic brain injuries damage the blood-brain barrier and lead to hastened cognitive decline."

BSJ: Since physicians hypothesized that pyridostigmine caused ailments in soldiers, there has been a lot of research into the blood-brain barrier in humans. How has our understanding of and your research on the blood-brain barrier evolved since you began your career?

DK: Our understanding has changed very dramatically. We were very naive at the beginning. We thought the blood-brain barrier was a lot less dynamic; we believed that once an organism completed some stage of development, there was a barrier around the brain that kept things from entering and exiting. We now know that it is a lot more dynamic. The barrier actually opens and closes throughout the day. We have learned there are activity-dependent changes in a lot of situations, and our research is showing us a more dynamic range of dysfunction in the blood-brain barrier. This is definitely one way that our understanding has evolved.

The other way that our understanding has evolved is that we initially thought of the blood-brain barrier as either open or closed. We know now that it is more complicated and a lot of different mechanisms govern the blood-brain barrier, especially something called tight junctions. Tight junctions are the small gaps between epithelial cells, and these tight junctions are what makeup barriers throughout the body. But it turns out that this is not the only way through which things can enter the brain. There is also transcellular transport, where molecules do not go between the cells but rather through the cells. There are transporters that pump specific molecules into or out of the brain. So, when we say blood-brain barrier dysfunction, it could mean a lot of different things; usually, it is a complex assortment of different mechanisms that are being affected.

Different neurological ailments also affect the brain differently; you might see different dysfunction in a stroke than you would in a traumatic brain injury or in aging. For example, what we mainly observe in aging is a decrease in transcellular transport but not in movement through tight junctions. Now, we understand a lot more

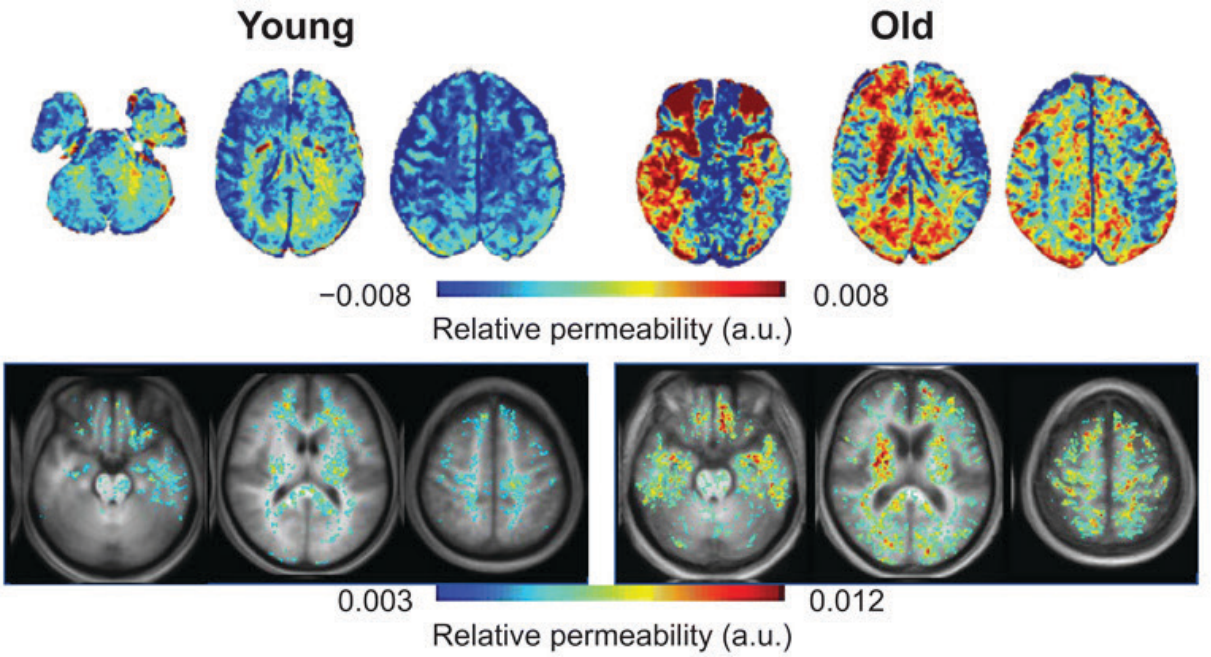

Figure 1: Permeability of the Brain Across Age Ranges. These DCE-MRI scans show the relative permeability of the brain of a young subject (30 years old) versus an old subject (70 years old). The intensity of the signal is reflective of the relative permeability of the blood-brain barrier.

about nuances in the system of the brain, and we know more about the cells that play a role in that system. For instance, within the field, there is a popular neurocentric view of the brain. Throughout the years, people were, and still are, focused on neurons and how they talk to one another. This is because, at the end of the day, the way that neurons communicate directly contributes to cognitive function. But now, with a lot more knowledge, we can say that astrocytes, endothelial cells, and other cell types are also important in creating an environment where neurons function properly. Now, my research focuses less on neurons as the drivers of disease but rather on blood-brain barrier dysfunction.

BSJ: Could you elaborate on astrocytes' function and their role in your research?

DK: I did not start my research looking specifically at glia or astrocytes—a specific type of glial cell. Glial cells, which are non-neuronal (nerve cells that do not communicate via electricity), can be divided into three types of cells: astrocytes, oligodendrocytes, and microglia. The astrocytes can serve as the glue between cells. The thought is that they are there to keep the brain and neurons in place, allowing the brain to properly function. Then oligodendrocytes are the cells that produce myelin, which wraps around neurons. This myelin acts as insulation—like how plastic around an electrical wire helps the electrical signal travel faster and more effectively. Then, there are microglia, which are little immune cells. The blood-brain barrier ensures that foreign molecules do not get into the brain. Because of this feature of the blood-brain barrier, we depend on microglia to serve as the brain's immune responses.

When I started my lab 20 years ago, there was very little interest in glial cells, and I also shared this position. Yet, my research brought me to investigate both astrocytes and oligodendrocytes. My interest in glial cells began when I realized that there was a blood protein, albumin, entering the brain. I tagged it fluorescently to see how it reached the brain. I performed a histochemical stain, using antibodies to deduce if the protein was entering through excitatory or inhibitory neurons. However, it was not passing through any type of neuron. So, I called Alon Friedman and said, "None of the cell types we analyzed have this protein. Where is it?" After this conversation, a realization hit me that maybe I need to stain for other cell types. Then, in a separate experiment where we looked at stress. I was investigating neural stem cells, which were not making new neurons in the experimental context. I checked the astrocytes and oligodendrocytes, and it turned out that the problem resulted in dysfunction with the latter. So, it was merely following the data that made me realize that glial cells were important.

When new discoveries are made in any research field, you see waves in the community. All of a sudden we saw a lot more papers coming out saying astrocytes are really important in neurodegenerative disorders. Yet, there is still a lot more to prove about the importance of astrocytes in electrical activity in the brain. For example, we have to work to look at the mechanisms in order to say why changes in astrocytes actually change surrounding neurons and glutamate and potassium levels.

A few years after my research on astrocytes, I was invited to present in a session where the whole topic was astrocytes' role in epilepsy. It felt like I was in some ways "preaching to the choir" since everybody understood that astrocytes drive disease—at least in epilepsy. Since my initial work with astrocytes, it has become clear that microglia are a big player in disease, especially in Alzheimer's disease. I think we are in the midst of finding out how glial cells play a very big role in pathogenesis—not just as bystanders, but as drivers of the disease process.

BSJ: How does astrocyte and microglia degradation relate to cognitive decline?

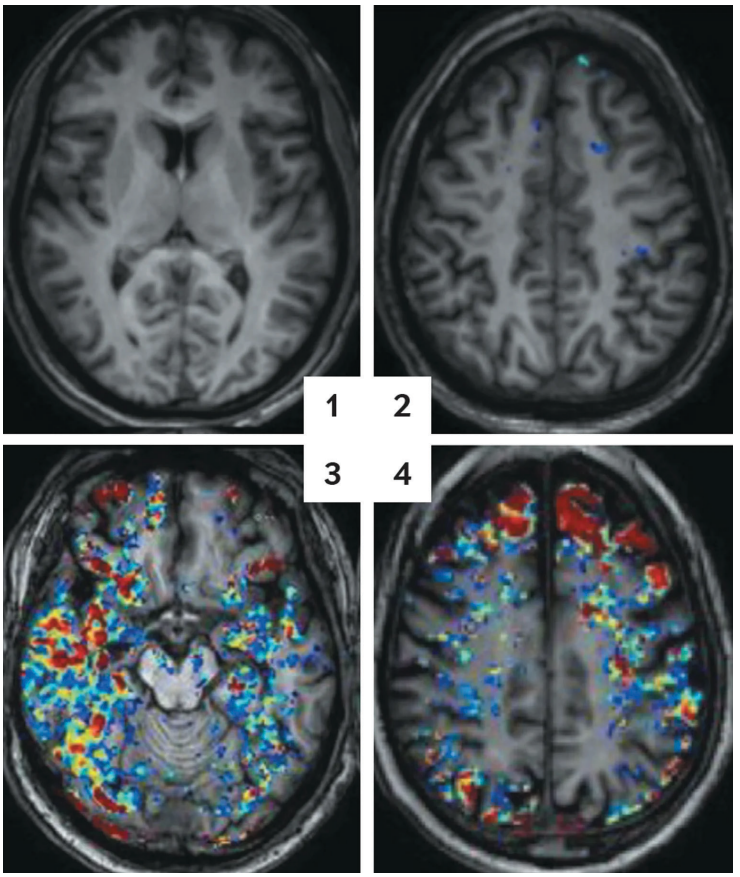

Figure 2: Leakages in the Blood Brain Barrier. These brain scans show increased leaks in the blood-brain barrier as people age (illustrated by a colored tracer molecule in the blood). The scan of the 30-year-old (1) looks clear, while blue spots show small leaks in the 42-year-old brain (2). By 65 years old (3), yellow and red spots indicate increased flows, a pattern that appears to progress as one ages, as seen in the 76-year-old brain (4).

DK: What we are understanding now is that blood-brain barrier dysfunction, as well as the accompanying activation of astrocytes, is what really leads to disease progression. Before this was established, my lab wanted to do a causative study to see if astrocyte activation and blood-brain barrier dysfunction cause aging. To do this causative study, we needed to do two things. First, we injected that blood protein, albumin, into the hippocampus of young mice since the hippocampus is the part of the brain that is most affected by aging. We wanted to study whether we could get them to look like aged brains within one or two weeks. Moreover, do they start to show processes of aging and cognitive decline? We measured this by testing if the experimental subjects could learn to navigate a maze as well as they would before. After experimentation, we found that, yes, we could quicken the aging process.

Then, we attempted the opposite. We took mice that were aged and blocked astrocyte degradation in two ways. One method stems from a very strong neuron inflammatory response in the astrocyte that starts when albumin binds to a receptor called the TGF-β receptor. Our plan was to give our older mice a drug that blocks TGF-β receptor activation in order to prevent albumin-induced signaling. The second way was even more elegant: we created a mouse strain in which we knocked out the TGF-β receptor only in astrocytes, and it turned out that these mice aged better. We showed that they have no neural inflammation and did not exhibit changes in electroencephalography (EEG) neural activity. They learned better, and several biochemical and molecular markers that are common physiological indicators of brain activity were improved compared to the control-aged mice.

With this result in mind, we then took mice that have already experienced cognitive decline and blocked TGF-β signaling to see if aging-related effects could be reversed. We went in with the hypothesis that whatever damage was done has been done; in other words, you cannot effectively turn back the clock. However, to our surprise, both methods of blocking TGF-β signaling in these aged mice resulted in improved brain function. The best way to explain how this works is to picture the activation of astrocytes as some kind of inflammatory fog that clouds the brain and significantly slows down brain function. Conversely, if you then inhibit astrocyte activation, the inflammatory fog goes away within days. Physiological markers of brain activity return to levels that are similar—in fact, identical—to those in young mice; these changes are also concurrent with improvements in learning. This is not to say that you can turn the clock back, but it is to say that the damage we are seeing in older mice seems to be from this astrocytic activation. Now that, of course, is not 100% of the time. For example, if damage to neurons or neuronal death has taken place, I do not think you can recover cognitive capability that easily. However, in our experiments, we found that cognitive decline related to typical aging could be reversed in mice.

BSJ: Much of your research focuses on the analysis of different cognitive pathways, especially the TGF-β pathway. What are the benefits of inhibiting TGF-β signaling early? Could this lead to the prevention of neurological diseases?

DK: As I mentioned earlier, inhibition of the TGF-β pathway works incredibly well in mice; however, it is probably not suitable for clinical use. This is because there is TGF-β signaling throughout the body, and it is not clear that TGF-β inhibition is a safe route to go. So, I think about this research as more of a proof-of-concept.

Mechanistically, it turns out that TGF-β signaling inhibition actually reverses blood-brain barrier dysfunction. When TGF-β signaling is not inhibited in astrocytes, they create a lot more TGF-β, so albumin activates a positive feedback loop that creates more TGF-β, which keeps the blood-brain barrier continuously open.

Thus, if you block TGF-β signaling, you restore the blood-brain barrier, and in doing so, you restore an environment in which the brain can "fix" itself. I believe this is a proof of concept of how we can heal cognitive decline. However, TGF-β blockers are not a feasible therapeutic to restore the blood-brain barrier. In fact, we had a start-up and tried to do this, but there was so much disillusionment in the pharmaceutical field with TGF-β receptor antagonists. Companies tried to use these drugs to treat cancer for many years, but it turned out that their safety profile was not ideal. Nonetheless, our recent research offers a new way of thinking about drugs that combat cognitive decline as drugs that restore the blood-brain barrier. From our initial studies, there are other drugs besides TGF-β blockers that can do that. We are currently looking at these much safer drugs and their respective targets to see if and how they can restore the blood-brain barrier.

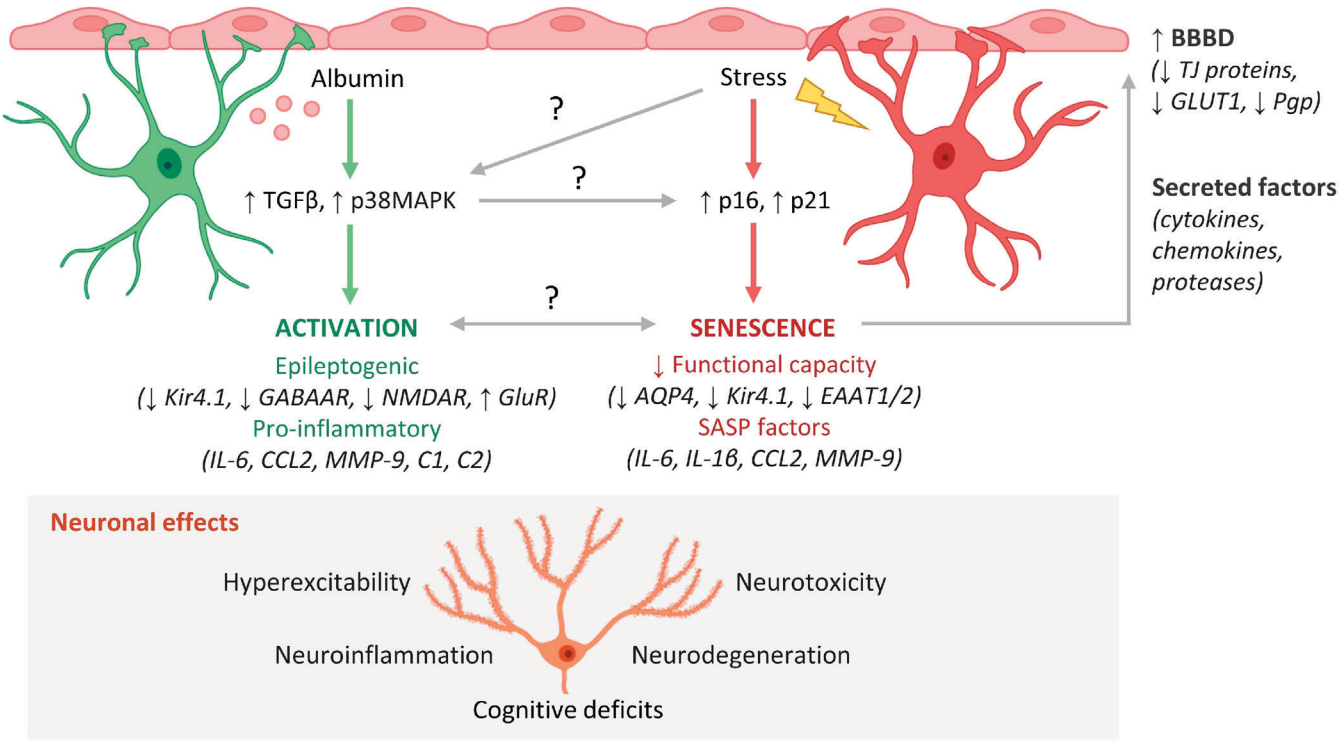

Figure 3: Mechanisms of astrocyte-mediated neuropathology in the aging brain. Blood-brain barrier dysfunction can lead to two different astrocyte phenotypes; astrocyte activation (left) or astrocyte senescence (right). Astrocyte activation leads to TGF-β and p38MAPK signaling, while senescence is characterized by the expression of tumor suppressors p16 and p21.

BSJ: What kinds of therapies do you see arising from your research?

DK: I think that the best therapies would be ones that restore blood-brain barrier function safely. I cannot tell you for certain that direct TGF-β inhibition would not work, but in practice, it just seems that this is a molecule that comes with an extensive negative history. It has been used in terminal cancer patients with no other alternative, and these patients tend to not live for that long after. So, to prescribe this drug to normal, fairly healthy individuals seems entirely irresponsible. But, there are other drugs that are much safer than the ones we are testing now.

The real excitement that my lab had, with how translatable this whole story is, comes from two ideas. One is understanding the mechanism of the blood-brain barrier because, really, this is not a mechanism that people know a lot about. This is completely novel, so it offers a target that was never looked at before. Secondly, we actually can now choose the right patients for these blood-brain barrier-centric treatments because there is a biomarker, the presence of albumin in the brain, which we can use to map barrier dysfunction pretty easily with FDA-approved means.

BSJ: Is it possible for the blood-brain barrier to show signs of dysfunction at young ages?

DK: Absolutely. When we looked at the general population across individuals older than twenty, we saw small amounts of people that show early-onset blood-brain barrier dysfunction. We looked at healthy individuals that do not have a neurological condition. However, if somebody has epilepsy, we know that epileptic seizures open up the blood-brain barrier. Furthermore, if somebody has a brain infection, like encephalitis or meningitis, they probably will show a blood-brain barrier dysfunction. When we looked at football players that were 20 years old, a lot of them had blood-brain barrier dysfunction. Even when a kid falls off a bike they might get a concussion that impacts the blood-brain barrier. So, I think there are many ways where young people can show blood-brain barrier dysfunction, but it normally heals by itself.

BSJ: How do you see the results of your research helping to create therapeutics for treating Alzheimer's disease?

DK: I am currently working with Professor William Jagust, who has been studying pathologies relating to amyloid and tau phosphorylation in Alzheimer's disease. Professor Jagust is very good at understanding and mapping the brain as well as doing PET scans to see where these markers of Alzheimer's disease develop. What we are trying to figure out now is how these amyloid plaques and tau tangles interact with the blood-brain barrier. This is very hard to do in humans, so he is going to do some work on trying to build statistical modeling of the human brain to look at its cognitive function. Then, my lab is going to do the same thing in animal models, where we can then manipulate different variables.

We are doing this because we can do many things in animal models that we cannot do in humans. We can open and close the blood-brain barrier and look at amyloid and tau separately, two things you cannot

do in humans. From these models, we will hopefully gain a lot of insight into what affects blood-brain barrier dysfunction. The next step will be to use this insight to develop therapies because the best therapies today target mechanisms late in Alzheimer's disease. So there is a big thirst in the clinical world for a therapeutic that targets something completely outside of the box from amyloid.

BSJ: For our last question, could you provide some insight into the future trajectory of your research?

DK: My research really turned into asking questions about individual variability in outcomes from different neurological disorders. Even when we work with mice—which are thought to be genetically identical—we see a huge variance in response to traumatic stress. In my lab, we had a very interesting finding when using transcriptomics on mice categorized as non-responders—mice whose phenotype did not change as a result of neurological stress. It turns out that the non-responders had the most changes in gene expression in order to keep them at the same place. So, I would like to continue research to help figure out what causes some people to have catastrophic responses to neurological stress and others to not.

One portion of my lab is very interested in psychedelics, and understanding how psychedelics affect the glial cells that we analyze. Again, most other labs are looking at how psychedelics affect neurons, but it turns out that receptors for these psychedelics are present in non-neuronal cells and that is what my lab is interested in. There is another sector of my lab that analyzes how stress affects aging. With this, we have had a lot of success in figuring out neural substrates of empathy. Lastly, I am very excited to make my work translational and hopefully affect meaningful change in how we treat neurological disorders.

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