THE TRANSMITTER

The brain's quiet conductor: How hidden cells fine-tune arousal

New research published today suggests that the pericoeruleus acts as a kind of micromanager of arousal, selectively inhibiting different subgroups of locus coeruleus neurons depending on the behavioral context.

7 MAY 2025 | by MAC SHINE

This transcript has been lightly edited for clarity; it may contain errors due to the transcription process.

Mac Shine
OK, I think we're live. Hi, everyone. My name is Mac Shine. I'm a professor of systems neuroscience at the University of Sydney. I'm really interested in a lot of different things, but one of them is trying to develop an understanding and a multiscale appreciation of the brain—to really think about explanations of neuroscience that incorporate microscale detail of circuits and cells but also stretch all the way up to understand how processes might work at the whole-brain level.

To me, this is a really exciting time to be in this space. And one of the reasons it's exciting is that I get to have really fun conversations with people like Michael Bruchas, who is a professor of anesthesiology and pharmacology at the Center for Neurobiology of Addiction, Pain and Emotion at the University of Washington in Seattle. Michael works on a wide range of fascinating areas, and what I really admire is his multidisciplinary approach. He draws on details from a lot of different fields. He's interested in cellular-level details and the tools you actually need to steer those kinds of systems. But he also has a nuanced understanding of biochemistry and cares deeply about behavior. This interesting melting pot of different technologies and techniques is exactly what's required to come up with the kinds of sophisticated answers we need for modern interdisciplinary neuroscience.

I'm really excited today to talk to Michael about a new paper he has coming out. Welcome, Michael—it's great to have you on!

Michael R. Bruchas
It's good to be here. Thanks.

Mac Shine
Awesome. So yeah, I came across your paper when it was a preprint—and congrats, it's now been accepted for publication! I think this paper really captures, in microcosm, a fascinating moment in our field. Thinking about the brain is a complicated exercise—that's a fairly uncontroversial statement. It's an incredibly complex system: lots of moving parts, lots of details and many different scales. One of the things I really admire about your work is how you dive into some of those details to see where we can push the boundaries. So, maybe tell us a little about who you are, what you do and what you're interested in—and then we can dive into the paper.

Michael R. Bruchas
Yeah, absolutely. My background is actually in pharmacology. That training started me on the path to getting excited about these receptors we're going to talk about here—receptors that are one part of a broader network. We have all these different sensors and receptors throughout the brain and body. There are many types—some work on very fast timescales, some on slower ones, and some are incredibly slow.

I got interested in these receptors during graduate school because they're the kinds that modern therapies often target. Things people take in the clinic—to regulate blood pressure or mood, for example—act on these receptors. So as a graduate student, I was interested in questions like: What do these receptors do? How do they signal? How do drugs bind to them? What characteristics of drugs affect their function?

When I moved into my postdoctoral work—which, for those who don't know, is the phase where you advance your training, deepen your research focus and gain new tools—I looked for labs that had a backbone of interest in these receptors but were also applying that interest to more physiological and behavioral settings. The idea was to understand the connections between what these receptors are doing on their own—sensing things—and how they ultimately affect behavior.

That's where I got passionate about the field my lab focuses on now: neuromodulation. We try to understand neuromodulation at the level of specific receptors, particularly G protein-coupled receptors. These are one of the major players in neuromodulation.

So, when we talk about the brain's complexity and all its moving parts, it's worth noting that the mammalian genome codes for about 850 of these receptor types. That's a massive number. My lab is trying to figure out how all these different receptors—expressed in different places, doing different things, responding to different kinds of inputare actually working. How are they functioning at a very molecular, even atomic level? And how do those molecular events scale up to change brain function, ultimately influencing behavior or our ability to sense changes in our body?

It's not just about the brain in isolation. There are constant interactions between systems that control things like feeding or blood pressure and the brain. These receptors are part of a continuous back-and-forth conversation between body and brain. So our overarching goal is to understand whether there are fundamental principles that govern how these neuromodulators work—how they affect mood, affective states and pain. We're particularly interested in both the sensory and emotional components of pain. And we're asking: Are there conserved features of these receptors or neuromodulators across different moods, brain states, and behaviors?

That's a general summary of what we do in the lab.

Mac Shine
Yeah, great. That's a really lovely overview. And it kind of reminded me of why we first started chatting together, because we both have an interest in this neuromodulatory system, but we come from very different perspectives. I have a background in human neuroimaging. I actually have a clinical background, but loved biochemistry in undergrad and kind of kept that love of biochemistry with me as I went through medical school, finished my training, and did my Ph.D. I then moved into my postdoc, which, like Michael was saying, is this really rich time when you can really double down on what it is that you find fascinating. What do you think are the big open spaces that need to be captured? You need to think, how could I get to that space? And what kinds of skills do I need in order to navigate that space? And so for me, rather than coming from the rich background of pharmacology and looking for behavioral relevance, I came from the world of clinical medicine, where there were lots of stories about how the brain looked in the context of neuroimaging, but what I wanted to be able to do was to take those and turn them into the kind of microscale explanations that you've been building. So having this conversation today to me is great because we're coming from different perspectives, but we can see connections.

I thought a useful place for us to maybe start would be to kind of unpack a little bit about what makes the neuromodulatory system interesting: What makes it a little different from the rest of the neurons in the brain? So typically, when we're in our undergraduate years, if you go and look on Wikipedia of what a neuron looks like, it's a really specialized, elaborated, eukaryotic cell. It's got a cell body. It often has these dendrites that are projecting out one direction and an axon that goes in the other direction. We think of the dendrites as receiving inputs and the axons as projecting outputs. And in order to do that, they often release a certain type of chemical which we often call a neurotransmitter. It's a label that sometimes is a little bit difficult to follow all the way through to its logical conclusion. But the idea with these chemicals is what they do is they hit a ligand-gated ion channel. They open it up on a recipient neuron, and then that opens up a channel. Different ions can flow in and out that can change the biochemistry of the cell, and it can fire an action potential. But the really fascinating thing about these G protein-coupled receptors is that they work really differently. So, Michael, do you want to maybe give a little description of how these little GPCRs, as we call them, work, and why they're different?

Michael R. Bruchas
So if you look at biologists who study evolutionary history, it's kind of an interesting thing. And there's someone else who studies neuromodulation and her model system that she uses is, she does things in lobsters and crabs. Her name's Eve Marder. She's at the Brandeis University. And one of the things that she always reminds everybody of is that these neuromodulators, actually, these molecules that act on these G protein-coupled receptors actually came earliest in evolution. They came before the fast transmitters that we think of as more electrical in nature that work very, very fast on the millisecond timescales that you know cause the electrical activity to go up or down in the brain. So these are called things like glutamate and GABA. Those are the traditional ones. And glycine is the other one.

These neuromodulators actually come evolutionarily before in terms of their role in controlling how neurons and different types of animals work the modulators. These chemical transmitters seem to appear on the evolutionary map before some in terms of their importance before. And you know it doesn't necessarily mean that they're more important or not; I think, as things evolve, things complemented each other in really exciting ways and really important ways. But I always remind people, because I think it gives you a sense of how much time and how much selective pressure from the biological world over millions of years has worked to really shape the and influence, how these things are working. Right? So it gives you a sense that you know how exquisite the complexity could be, but also how you know, sort of tuned their function probably is because of the fact. They've been around in evolution for a really long time.

Mac Shine
I love the idea, Michael, that a lot of these ligands for these protein-coupled receptors are modified amino acids which may have been around in the diet. Things like tyramine and tryptophan are common in meats and foods that animals would eat. So there's all these different chemicals floating around, and if you add a hydroxy group on here, or you delete a hydroxy on there, all of a sudden now you've made a really interesting rich chemical that's quite different from all the other chemicals that are around. Now find a match in a receptor. And all of a sudden you've got this really exquisite signaling capability that you didn't have beforehand. So I really love this kind of like natural selection, using all the bits that are just lying around to almost like MacGyver solutions to problems that exist.

Michael R. Bruchas
And so we have receptors and sensors that are in animals that respond to these molecules that work, as you mentioned, through these ion channels, right? And those will cause changes in electrical activity—spiking of neurons and coordinating activity. And then we have these G protein-coupled receptors, these neuromodulatory receptors. And the way I like to think about it, which may be an oversimplification, but I think it usually gets people all on the same page: We're at a time where you know everybody that's really into music is going back into the record store and buying records. Right? They're buying these vinyl records. And the idea is that you play vinyl music, and you have a richer sound. You know it's analog sound. And it's a little bit more interesting if you think about that versus your MP3 you have on your Spotify or your iTunes account—music that's digital in nature. And of course, now we've got uncompressed versions and compressed versions. The cool thing about these GPCRs: I like to

think of them as the analog version of some of the communication that's happening in the brain. And the reason I say that is because they work on different timescales and they tend to not be quite as binary in terms of how they operate.

They tend to operate more like of your amplifier dial, or you know, actually, I always like to show people: In the back here I have this this lamp, and this lamp has a dimmer switch on it, right? So you can kind of dim it up, or dim it down versus, like the lights that are in the background in my office are just controlled by a 1 or a 0. You know, they're ON or they're OFF, right? And so neuromodulators are sort of uniquely poised because they can dial the activity down or up of a given neuron—by activity, we mean the electrical activity of the neuron. But then, in addition to doing this they are also communicating new signals and amplifying things inside of the cell.

So they receive these ligands. They bind to specialised receptors. But then these receptors have this unique ability to take that single molecule signal and they can amplify and promote a whole bunch of different changes inside the neuron, or actually in the glial cell, which is another type of brain cell that's really kind of relevant to talk about here. And that change can lead to dramatic changes in how well the neuron is going to be connected to another neuron, how much transmitter it's going to release, how much it's going to fire going forward. Or it can say: OK, we want to really amp up this signal and communicate more to the next to the neighboring neuron or to the neuron somewhere else in the brain. So they're kind of like a gain signal that integrates a lot of information but allows the neuron to have more flexibility than just kind of a yes or no switch.

Mac Shine
I love this, and I think I really like the kind of physical notion of altering an analog signal like a light switch or the volume on a radio or something. And, as I think you know, coming to the field from the neuroimaging perspective and trying to understand the complexity of something like the cellular composition of the arousal system, and all these incredibly complicated chemical signaling elements, those kinds of analogies are deeply helpful, because what you can then do is you can say, "All right, I don't fully understand the richness of this whole system, and if I was going to, I'd have to go off and do a Ph.D. AND a postdoc AND a whole year after that. What I need is the tether that lets me have a conversation now with people in that world, and to a first approximation understand a good-enough heuristic take on what might be happening." So I find those kinds of heuristics really helpful.

One area where I think maybe the dimmer switch doesn't give us the full appreciation of the complexity here, and I think it actually points to what you were speaking about before where the arousal system itself is more high, dimensional than we give it credit for. So originally, when we're taught about the arousal system, often we think of it as a 1-dimensional dimmer switch. We can turn the light up or down. But there's actually a lot of subtlety in the system. For one, there's multiple different types of these neuromodulatory hubs. There'll be certain chemicals that release acetylcholine, and there'll be others that release serotonin, and another that we're both interested in releases a chemical called norepinephrine (or if you're in Australia, noradrenaline)—the locus coeruleus. And if you want to start thinking about that complexity, that's one thing. But then you can also go down within the cellular hubs themselves, and you actually find a lot of complexity there, too.

So one of the really interesting analogies that a grad student of mine came up with a number of years ago, Gabriel Weinstein, though he's now passed on from the lab and works as a postdoc. Gabriel's a really established violin player, and we were having a conversation about this notion of gain. We do a lot of computational modelling of gain terms and see what the impact might be on the brain. But Gabriel said, as a violin player. I have multiple different ways of changing the gain of the violin. I'm not just stuck with a single knob that I can turn up and down. I can press the bow of my violin a little bit harder. I can play a little bit more frequently back and forth. I could play pizzicato with my left hand. I can play fervently or melodically. And so we really like this idea of trying to extend the analogy of gain to a much more complex and nuanced concept like playing a musical instrument. I wanted to see if that resonated with you.

Michael R. Bruchas
Yeah, I think it's a perfect way of putting it. Because you know, when you think about these receptors, they're just, they're just molecules. They're just proteins that are called serpentine receptors because they're shaped a little bit like a snake. They kind of bypass the cellular membrane. So they're sitting on the cell membrane, and they're just kind of bypassing it. And there's portions of the of them that are on the outside, and then there's portions of them that are on the inside, and the outside ones are the part that receives the signal. There's sort of the antenna out there waiting to get the ligand to act, to sort of respond. And then the inside of them is what talks to the inside of the cell, and there's many different types of them. So there's ones that can sort of boost activity in a couple different ways: There's ones that can inhibit activity, and there are ones that are that are sort of not related to either, and they can sort of change the shape of the neuron. Or how many other contact points, you know or dendritic, you know, spines that the neuron might have.

So there, there are many different receptors that can respond differently, depending upon how much ligand is present. And then they can also shut off differently depending upon whether you have a lot of ligand versus if you have a little bit of ligand. So there's a ton of flexibility in these receptors, even though they're just very simply just a protein, each one that has its sort of matching key—we think of them kind of lock and key. They have many different possible doors that you can open with that lock. I think that's the part that that is really exciting about them. But the opportune thing here is, coming from a pharmacology background, because there are all of these potential things that they can do, it means that you might be able to take advantage of those differences for mental health and neurological disorders. You know, if you can figure out what these things are doing, and you work with chemists which we've done in the past—we have some projects going forward for some new sort of depression treatment options for people. These clues give you the ability to say, "OK, now let's talk to the chemist and let's think of ways that we can really only tap into one aspect of this receptor or block this particular aspect of this receptor, and that means you have safer drugs." You may have less side effects. You maybe have a more effective treatment for people. So understanding those fundamental differences into the richness of them, I think, is one of the reasons why I'm so excited about this. It's a great analogy.

Mac Shine
Yeah, cool. And I could also imagine ways in which this space is going to get really exciting as we start to develop better machine learning tools for kind of guessing the ultimate quaternary structure of proteins, given their amino acid sequence. You know, Alphafold made some huge inroads. There's of course lots of work to be done in that space—I don't think it's a fully solved problem yet, but you can see the kind of sketch of the solution. And I love this notion of being able to almost like make designer receptors for people. You know, you come into the clinic, and you've got a particular neural signature of your receptor. We want to make a drug that'll target you and not affect all these other systems which we really want to leave alone because they're working in a complicated way we don't fully understand.

Michael R. Bruchas
Yeah, it's definitely an interesting angle. You know, the personalized medicine kind of approach, right? And you know, the Nobel Prize was won this past year for the understanding of these kinds of structures, and actually by a university colleague of mine, David Baker, from Utah, was one of the winners of the Nobel. And then some people from Google DeepMind that have been really thinking about using computational approaches to solve some of these problems. This is a super exciting time in that regard.

Mac Shine
Just to click on the on the medical angle. One of the things that really stuck with me from my medical training is that the treatment that we give is often a huge cause of this major side effects that end up causing really massive impacts in quality of life and that really comes down to the fact that we have effective treatments. But they often have really nonspecific effects on this incredibly complicated biological system built over billions of years of phylogeny to work the way that it does just good enough to solve the problems. So I think coming up with nuanced, sophisticated ways to interact with this system is going to be a really fascinating forefront for young scientists and engineers to get involved in and really dive into the details. Don't over skirt them, dive into the complexity, and really try to understand these things as they are.

That's a lovely foray into the world of neuromodulatory or chemical receptor signaling systems, as well as a heuristic overview, thinking of them as a gain control and excitability controller, something that can interface with a system that can act really quickly, but can bias and shift the system to have different kinds of operating modes? You can imagine that is a really rich computational capacity that would come from that, especially when we then start to think about the complexity of the of the brain, the differences between the cortex and the thalamus and the cerebellum and the basal ganglia. All of that complexity is now in this really interesting soup that can be steered around and controlled.

But as soon as you start to bring in control parameters, now you run into a different set of problems, which is that you need to learn how to control the controllers. And so one of the reasons that I really love this paper that you recently published is that you're really starting to think carefully about what kinds of systems could come in and shape and change those controllers. So can you tell us a little bit about the pericoeruleus and things like it, and how you got interested in that space?

Michael R. Bruchas
Yeah. So this paper that that we're now going to talk about more carefully is related to the arousal system, which we've been thinking about for a while. My lab is about is a little under 15 years old, and we've thought a lot about how the brain occupies these different states across experience within environment. And I'm keeping it very biological for the moment, just to kind of highlight something which is as we sort of started to thinking about: Namely, how does stress impact the brain? How does an animal waking up from sleep impact what's happening in the brain? We started to realize that there's a lot of different types of states that the brain can occupy across an arousal spectrum. You can think of stress as a hyper arousal state, right? There's all kinds of stuff coming at you. It's hard to sort through all the information. You're not able to filter the information as well. You're more stumbly. You can think you think of different things that happen. You know, it feels like when something stressful happens (at least personally for me), usually there's a series of dominoes, and people say I had a bad day, you know, like it was not my day. Well, sometimes it like feeds into itself, right? Because your cognitive abilities have shifted when you're in this hyper-aroused state and you lose the ability to process information.

In contrast, if you're in a really focused state, you might be able to process certain types of information, but not other types of information. And the arousal system sort of shifts you between these states. Given experiences that people have in their day-to-day interactions, there's one system that we know that controls this. There's many that do, but one that is really important, that we got interested in, because we were really interested in trying to understand how stress is changing, how different types of stress impact the brain: the noradrenergic system (which uses norepinephrine). And it is very important for controlling your what we call the "fight or flight" response. That's the classic idea: You know, the lions coming for you, your pupils dilate to let more light in, your heart rate increases to get more blood out there, There are other things that are happening, too, which we can talk about later. But you're basically like: "OK, how am I going to respond into this situation?" And you're in this hyper-aroused state. Well, in your brain you have cells that release norepinephrine and help regulate your heart rate and blood pressure, but then in your brain, you also have nuclei that make norepinephrine. And what's cool about these cells is their tiny, tiny nucleus called the locus coeruleus. And it's called the locus coerulus because "cerulean" is blue, and it turned out when they first discovered this structure, it had a blue-like haze to it. And so it became the locus coeruleus. It's a tiny set of cells in the mouse. It's like 2,000 neurons. So it's very tiny amount of neurons that make this norepinephrine.

And what's interesting about this tiny nucleus is that, while it's super tiny, it sends axonal projections that go all over the brain. So basically, the brain has this nucleus that sends norepinephrine all over the brain: from the front of your brain to deep into the amygdala where you're going to your fear center, and it even goes back down into the spinal cord, too. So there is some direct connectivity to your spinal cord, and when we think about that, we thought, OK, this is really interesting. And people have been studying the structure for a while. And one of the leading beliefs of this of the field was: When there's a stressful event, these neurons basically all increase their activity and norepinephrine is released across the brain

and when that happens that gives you the ability to sort of deal with the stressful situation sort of the classic fight or flight response that I that I mentioned.

And in the last, probably not quite a decade, there have been some papers where people started to say, "OK, we know that there's this stress sensitivity where the neurons basically just all elevate their activity. But then we also know that these neurons respond to a clap. Or hearing a bird outside my window—right now, there's a bunch of cool stellar jays that live out here, and they come around, and I and you're, oh, I'm paying attention to them now, right? Or you know there's a crow flying over here. So all these other stimuli are coming at you. And when that happens, people start studying these neurons, and they realize that these neurons fire these very tight little burst events, and they release norepinephrine in places like the front of your brain, in the cortical regions, or they might release norepinephrine deep in your brain, and some of the sort of subcortical regions like the amygdala. And they're more discrete norepinephrine release events. And so norepinephrine is not just: "Hey, I'm scared. I need to fight the stressor." It's also communicating some other information that's relevant to salient stimuli that are coming in at you at the same time. And so people have thought about the population as having two ways that it can respond. And the question was: "Do all the neurons do that? Or are there sort of subsets of them that are doing different things?" And for many, many years we didn't have the ability to test this, which comes back to your earlier point about tools and technology really kind of helping pave the way forward. We didn't have a lot of ways that we could actually understand whether there are subsets of these neurons that respond to different kinds of stimuli, things that are maybe more rewarding, or things that are maybe a little bit more aversive. And how does that relate to sort of that coming back to that arousal spectrum? You know, how does that work?

And about the last 10 years or so, people started to realize actually, there might be some subsets of these neurons that are maybe going to different places and specifically going to different places in the brain, some that are going only to the spinal cord versus those that are going to sort of the cortex, for example, in your brain. And or there might be different responses that these neurons have to things like queues, things that are paired with sort of aversive experiences, or if an animal or an individual trying to recall a prior event. And these papers came out, and we were sort of like, well, this is cool. We sort of always had a hunch that there's probably more diversity than what we originally thought in terms of this population. But at the end it became OK.

Well, how would that be? How would the neurons have this complete, you know, if there is complexity and it looked like evidence was coming out from a few labs. Actually, a really beautiful paper from Josh Johansen's lab at the Riken Institute in Japan. In *Nature Neuroscience*. I think it was a 2017 paper that found that there were different locus coeruleus neurons that respond that are projecting to the cortex versus those that are projecting to the amygdala and showing that those have different types of responses in fearful situations, started to really push this.

Mac Shine
The other paper that I really love is the one from Vincent Breton-Provenchar in which they were tracking the axons of the locus coeruleus during a reinforcement learning task, and they show that during some elements of the task you get this kind of almost like popcorn-type effect in the locus reels, where some of the neurons are active. But then, if you get a really big prediction error, the whole thing lights up like a Christmas tree, and it I think it really comes back just to kind of reinforce the analogy. You could imagine playing a violin and just like slamming your bow across the strings that would be like hitting the whole thing. But you could also imagine playing a really delicate melody and having that ability to kind of shift between these different modes, that have a complexity axis, confers such a huge adaptive benefit to an animal, because now it's not just lights on lights off, it's lights subtly on and letting this little system take the lead for a little while, and then it's also now, OK, quick! Action stations! Don't think about it. Run away from the tiger!

Michael R. Bruchas
So that's a great, and that's a that's a really good example. There's also this newer paper that that has only been out for a little while, which I also like, which is where they took people from all different species that have recorded from these neurons, and sort of compiled it all into one dataset. And what you see is a lot of consistency across the species, which is also always, as you know, as a biologist who works mostly in rodent models, being able to see that consistency up into non-human primates and something even the human work that people have been doing is a satisfying thing. Because sometimes, you know, the critique is: "Oh, you're doing all your studies in mice. Does it actually mean anything for human health or for how human brains work?" Well, in the case of the locus coeruleus, there's a lot of conservation. And I'm going to come back to that later, as we talk more about the paper.

But so we have this data. And at the same time there was a technique that kind of came about that was starting to evolve and in neuroscience. And it had been used in in other fields, but it hadn't really come to neuroscience. Until more recently, which is this approach called single-cell RNA sequencing. And basically what it does is it allows you to take populations of cells and identify in each cell what genes are there in a given cell type.

And you know there was evidence from some old papers from one of the leaders in this field for many years, and got me excited about the locus coeruleus. It's from Professor Gary Aston-Jones—He's now at Rutgers—and Rita Valentino. And there was a couple papers in rats where they'd shown that, in addition to these norepinephrine neurons, right next to them, there's a population of cells that seem to be possibly GABAergic cells. So these cells in the brain—GABAergic cells tend to be inhibitory cells—and they tend to have an inhibitory function. In other words, they're all over your brain, and they can release this chemical transmitter, GABA, that can work both on G protein-coupled receptors or ion channels, and it can act to sort of suppress the activity of the receiving neuron.

Well, there were these neurons there, and but there wasn't a ton of anatomy that was done. And there was another paper that also came out from another lab maybe a few years after that paper. And it was sort of right in the middle of our study, but it also showed that there's some GABA cells there, and they might be changing when you know, in sort of different types of arousal type of states. And it was kind of tantalizing. And we were

like: “Oh, these cells are interesting.” So Andrew, who was the graduate student in the lab at the time, said: “Let's target these cells.” But first of all, as I told you already, this nuclei is only 2,000 neurons—it's super small in a mouse. The mouse brain is about the size of an almond, and this structure is 2,000 neurons that you know, a neuron can be, you know, 50 microns across. So you're talking about this tiny, tiny space you have to hit.

Mac Shine
It’s also sitting right where a whole bunch of other structures are sitting that are really important for the mouse to stay alive. To keep the blood pressure in check, and other important functions like that.

Michael R. Bruchas
Exactly right. So Andrew comes in, and I'm telling him about these terms and saying, “You know, this is, this would be really exciting project to try to figure out more about what these cells are. They're there, but we don't really know anything about them, and I don't know that anybody else is kind of working on.” This is always the thing that we do as scientists is like: “OK, who? Who am I competing with? Am I going to have some time to figure this out before I get scooped, or before, you know?” So we always kind of have those conversations. And so I said, “Let's try to hit them.” So we took mice that are special transgenic mice that allow us to target GABAergic neurons essentially through a genetic trick where we can take a virus that expresses something like a fluorescent reporter, a GFP, for example, and we can inject it, and then it will only express in GABA cells.

So I had Andrew, and it took him about 6 months to target these things and be convinced that we could really just target them. Because, of course, as I told you earlier, there's GABA cells all over, right. So if we don't hit just these and they're starting to bleed into other structures, then we can't be sure that we're actually studying these neurons and so forth. So he was able to do that very carefully. And now other people in the lab have learned how to do it, and what he did that was kind of interesting is that he asked what these neurons respond to. In prior studies, the animals had been head-fixed, and they were not really looking at all the possible things that these neurons might respond to. It was a very focused set of things that might change their pupil diameter or things like this. So we thought: Let’s put something in them that, let's put a genetically encoded calcium sensor. And basically what this is gives us the ability to say, “OK, we're going to target these neurons. And we're going to express a sensor that whenever the neurons fire, there's going to be an increase in fluorescence. And we can measure this with a fiber optic.” We basically put a little fiber optic in the brain. And we can see whenever the neurons are active and see what happens. Well, we did that experiment, and we screened across a whole bunch of different types of behaviors that we would think of as aversive behaviors, appetitive behaviors, neutral odors, salient stimuli. A big, big spread. So we can kind of cover that arousal spectrum, if you will. And of course, these neurons are just right next to these norepinephrine cells. So you think, well, maybe they talk to each other. But just because you have two populations of cells next to each other doesn't necessarily mean they're actually talking to each other.

So we collaborated with a colleague, a friend of mine, Chris Ford, at University of Denver, and he did some simple electrophysiology experiments which basically just confirmed that these neurons actually are directly connected to the norepinephrine cells. And a couple other people had shown it. But you know, as good scientists, we like to reproduce work and make sure that that's actually the case. And then we saw that these neurons have all these different responses to these different stimuli. And we thought, “Well, these are kind of interesting. Maybe these are sort of acting as some kind of filter integrator for information that is going to influence the norepinephrine system or the noradrenergic system.”

And so we thought, “OK, well, we need to find out more. We know they're GABAergic cells.” But just because the neuron's GABAergic doesn't mean it's not also something else. People have started to appreciate more recently that these neurons can release different things. They can co-package in vesicles or release two things at once, or one thing under one firing condition, another thing under another. So if a neuron's firing like this fast, it might release one thing, but if it's firing this fast it, might release multiple things, or one thing might come out first, and another thing might come out later. So there's both temporal and spatial type of constraints.

And so we did the single-cell RNA sequencing and asked, “What are the molecular identity of these cells?” And to do that, we basically take a punch of the tissue both sides of this structure, which is on both sides of your brain, sort of towards the back of your brain, and then the hind brain. And we did a sequencing experiment. It took us a long time. There's a lot of data to analyze. And because one thing that's hard about this and it hadn't been done before, for the locus coeruleus, in part because we didn't have a lot of cells.

If you do RNA sequencing with a big nucleus, you have lots of cells. And so you get lots of reads. So we're trying to get a certain amount of confidence in our dataset. And so we had to do lots of combinations and iterations to get enough cells where we're really confident that we had norepinephrine cells in our sample, these GABA cells in our sample and there's another population of excitatory cells kind of in the middle. There's like a donut hole of glutamate or excitatory cells right in the middle of the of the GABAcells. And that sequencing data told us, “Wow, there's actually an incredible amount of diversity here.” Heterogeneity here that is, you know, in the GABA population as well as in the in the locus coeruelus population. And that was an exciting moment. I remember sitting outside my office when we started getting the first data coming in and doing the first round of analysis. And we got the first data. “Did we get the noradrenergic cells? Yes, we did.” So that meant we were confident in our sample. We had a kind of a positive control. But now we've got all these other markers. And we started collapsing these and looking for overlapping genes that were specifically in the GABA cells versus in the noradrenergic cells.

And what we found was that the noradrenergic cells themselves had different types, different types of GPCRs on them. Some would have one flavor of GPCR, one enrichment of one type, some types of GPCRs, another population group would have different types of GPCRs, and then the GABA cells themselves that are right next door, and that we showed were connected to them, make the chemical or the transmitters for those particular receptors. So it gave us the idea that maybe these neurons are actually, helping to influence the individual activity of these noradrenergic cells and provide a finer-tuned control of how this locus coeruleus is actually functioning.

Mac Shine
Just to throw in an analogy to kind of highlight the process. Let's imagine we were going to a high school. We'd be quite comfortable, saying, "We're going to go look at a bunch of high school students." But if you went down and interviewed and interacted with each one of them, they'd all be completely different from one another. They'd have their interests and music tastes, and like this sport, that sport. So at one resolution, you can say, "Oh, yeah, these are all GABAergic cells, or all these are all locus coeruleus cells." But down at the level of the individual detail, there's so much diversity that the biology can take advantage of in really interesting ways. And I think this actually kind of really puts its finger on why, it's so damn hard to think about a system like the brain in a sensible way. Because at one resolution, you can say something that, at another resolution, gets completely betrayed at its fundamental core. I think it's what makes it so difficult to be working as a neuroscientists nowadays.

Michael R. Bruchas
It is. It's a great analogy. And you know, I always try to tell people why the development of new tools and new approaches are so important. Because you if don't have the more powerful telescope, you don't know what you don't know. There's another galaxy over there, or there's another. You can't see what you can't see. And so the tools enable us to see in here. The tool is this single cell RNA sequencing.

The other thing that we did that complemented this. I had a colleague here, Liangcai Gu, who had been developing this approach to not only because when we did this we just took punches. We put the cells in this kind of sorting mechanism, and then we do analysis and look at what the genes are. But then the question also is, "OK, where are all these different types of cells? Where are they actually located in space?" Very new techniques have been coming about where you can actually do this single-cell type of approach, but you can actually do it on the tissue itself. So everything is sitting there intact. You've cut the tissue, and you can see, OK, now, here's where these genes are expressed. Here's where they are. Now, this is going to be. This technique is going to be huge for pathology, for health, for lots of different things. But we used it. We collaborated with Liangcai up in biochemistry, just upstairs. And he had just been developing this technique in a neighboring structure. And I thought, "Wow, this is really beautiful." We should try to do it for the locus coeruleus. And so we did it also in this paper, and we were able to take that data and kind of combine our more kind of crude approach with this spatial approach, and really get an idea that actually, what we're seeing is consistent that there's particular actually sub-locations where these GABA cells that might be expressing one transmitter versus another are located versus maybe another type of cell, and that it was also exactly like they have these little neighborhoods essentially, right? And so that that got us excited too, because then we thought, well, this means that maybe you know, there might be different ways that those particular neighborhoods are talking to other parts of the brain, and then, it sort of opened this door that, hey, these GABA cells could be this really critical integrator of information because they're not just GABA cells. They make other transmitters.

And on the locus coeruleus, you have different types of molecules that are represented in the receptor populations that are there. Right now, I will say as a caveat. And this is something that you've probably thought about, too is obviously there's these receptors, and we're focusing on them today because it tends to be our mutual interests as well as like the interest of neuromodulation as a whole. But you know, in the paper we have, we could sort these cells by many different criteria. We could look at their ion channel compositions. We could look at, you know, other signaling molecules. And there's a lot in that there's a lot of data in there that frankly, like we kind of put our blinders on because we're like at some point you have to write up the paper. But there's a lot more that we hope that other people will take this data set right and find new things that we didn't find, because it's that's what's nice about. This is a very high-throughput approach that gives you lots of data. And we focused on one particular way of thinking about how these neurons might work. But I personally think there's probably an exceptional amount of other rich data in this dataset that can be that can be mined for other clues to how this is all working.

Mac Shine
Yeah, I really admire you saying that, Michael. And coming from the human imaging community, we really went through a phase, maybe in the last 10 to 15 years, where people really realized the strength of this approach. Essentially, we were sort of the public face of a big replication crisis that that kind of swept through a lot of psychology and neuroscience. And one of the ways to fight against the replication crisis is to make your data and your code available. And that's I mean, it's scary. And it's also a huge cost to the people that spend the months and years, as you've just outlined, on one experiment collecting this exquisitely detailed data. But if you're willing to overcome that frustration. The benefit to you, I think, is manifold. Number one: You get to have other people look at your code and your approaches, and that can be really helpful for developing robustness. And number two: sometimes people will come up with crazy new ideas that you could never have thought of that will then come and help you become a better scientist, going forward. And I think, as a field, we really benefit, because now, as a student, you can come in, and you can learn from the really detailed data what's going on.

Michael R. Bruchas
Actually, it's funny enough that you mentioned the human example with this data. So we put it out there. And then we got an email from a lab at Johns Hopkins, Keri Martinowich's lab. And also the Allan Brain Institute, which is just down the street from me. And they're like, "Hey, you guys did this, and they'll see this is super exciting. We've been trying to get it to work. Can you share the data with us so we can compare it to our data?" So, boom, of course we did. And then Keri at Hopkins had been doing this with human tissue. And that was really exciting. And her paper, just also, I think, came online, like recently in paper. And it was great. We gave her the data, and we had her sort of take a look at what we found in the mouse, and what we see in the human and some of the things that we focus in on the paper she found similar, you know, enrichment of the genes. And you know, there's differences, right? There's going to be differences. Humans and mice aren't the same. But there was actually some very interesting similarities. And that was that was really actually reassuring. Because again, we work with these little mice. And you know we're trying to get insight that will help human health eventually. And so, being able to see that the human dataset had some similar features was really exciting. And it's again suggesting that this conserved function of this structure, this peri-LC structure across the mammalian species, which is exciting, I think.

Mac Shine
I mean that, that's how science works. It should be a process that we all apply to our curiosity and questions that leverages the specific skills and detailed backgrounds of each of the different individuals but together comes with a consensus that is sort of independent of all of us. I think that's one of the things I love the most about science is that it will give you an answer whether or not you wanted it or not. So you, by exposing yourself to this complexity, it's almost always more interesting than you think it would have been in the first place as well, which is a really fun.

Michael R. Bruchas
I like that. I mean, I think that's a really good way to put it. And I always feel like you know you can't, you know, you can't always anticipate the impact of those kinds of conversations or those kinds of interactions. Now, because we had that interaction, we're writing a review article about this topic and trying to pull together some ideas that between both of our perspectives that might kind of fit together, and some other people that are working in this space. It just adds more. And I do think it's a hard thing to communicate that science has an autocorrect mechanism, and the way it does that is by us sharing information with each other and with the public, and so forth. That's the correction mechanism. And it's not like it's immediate. It takes time for it to incubate and marinate, if you will, and then and then it corrects itself over time. And it doesn't do that if there isn't this this kind of interaction.

Mac Shine
Yeah, I firmly agree. And I think it also really highlights the challenge that I think we face as a community to figure out how we support and breed, for lack of a better term, the new generation of scientists.

You know, the scientists that I really admire, and I'm happy to say that you're one of them, Michael, doing what I would describe as brave work, which is work where we don't really know what the answer is going to be. We don't even know what it will look like, but we think that there's going to be something in this space, so we should look here. Here's where stuff will be. Let's not just go and confirm that we're right, and say, "Let's give each other a pat on the back. How clever were we?" Instead, let's go see how nuanced and complex and strange the answer is. And you know, as you've seen just in our brief conversation today—I'm speaking to the listener here—how hard it is in order to have a little idea and follow that all the way through with all the different specialists that have to come on board to really make sure that you're right, and with the young minds that you have to have there, trusting the process that at the end, with a much more interesting question. I really think that this is a difficult thing for us to grapple with as a community, and we have to think very hard about protecting what we believe in is this scientific ideal of doing slow science, of taking our time to get the right answer. And it's not simple. So I wanted to know if you had any thoughts on how we could achieve this goal.

Michael R. Bruchas
Yeah, I mean, I'll just say this started with Andrew, who moved to the University of Washington with me. I did my postdoc here, and then I started my lab at Washington, St. Louis. Fantastic place, really supportive of my work, great colleagues there. And we started there. We started this, these injections where we're just trying to hit the structure. Try to figure out some simple things there and then, you know, we moved here and Andrew came with me, and then Li joined the lab as a resident fellow, as a postdoc kind of resident fellow, as a pediatric anesthesiologist, but he joined as kind of a postdoc at the same time. I don't know if Li sleeps at night or not, like his arousal system is like ... But the two of them spent a good five years doing all this stuff. We send the manuscript out. We get revisions. It took a long time. And it's kind of funny, because by the time you're done with it you're like, "Oh, wait! There's a newer version of this technique." It's hard as a scientist, you're like, "Gosh! I wish I could just do it all."

And of course, we're now adapting and doing more with this structure. But the two people that led the study: one was a graduate student, and one was sort of a postdoc. And then there's a whole bunch of other people in the study, too, that really came in and helped out as well at all different levels of their training. They're right at the beginning of their careers. And now they've got this, this big discovery that they can use to sort of open up a whole bunch of new possibilities and new questions for their own research programs that are sort of separate from my own. Their tenacity; this is the thing with this kind of project, this tiny nucleus. There was a couple of the second time we did the experiment where we did the single-cell RNA seq we got to spend spent a lot of time on it. We got really poor dataset. And I said, "OK, well, looks like we have to do another round." And I guess, you can feel the sort of collective gasp and sigh, but they did it, and they kept with it, and because they did it, that's why we're here. That's why we're at the point where we have this whole new dataset that we've shared with lots of other groups. Now, I think we've had several groups across the world. You know, email us, and we've given them access to the data. That, you know they are really the story here in terms of, you know the effort that was put in.

Mac Shine
I'm so glad they worked on it.

Michael R. Bruchas
Yeah, I'm glad that they worked on it too. And now I have people that are joining my lab or in the middle of the experience. They're following up various aspects of this study and trying to figure out: "What does this subset of neurons do? How can we target these neurons? Specifically these subsets of them? Can we use some new genetic tricks? Or what do these subsets do?" Because in the paper at the end of the day we do the sequencing. And what we decided to do at the end was sort of focus in on a few of the populations. So there's many different types of these subsets of these GABA populations, these peri-LC neurons. But what we what Andrew and Li kind of decided at the end of the day, and we thought about it and talked about: "How are we going to make this? How do we make this connection?" Because I'm just telling you we have this map right of their identity, you know who, what they express and so forth, and where they live in the in the structure. But we don't really know what their function is, and that was missing.

So we chose a few of the subsets of the populations where we knew we could get access to them, genetically, and we looked at their activity across the same suite of arousing stimuli in exploratory behaviors, in head-fixed situations. And what we found is that the different subsets of these neurons responded differently to different stimuli. And that was also really exciting. Because that suggested, OK, this idea that maybe these neurons and these subgroups of neurons have the ability to control the norepinephrine system in a way that gives it some specificity in different parts of the brain was pretty exciting. That was unique. Now the question is: "OK. Well, what if you just turn up or turn down these populations? Do you see differences in behavior? Or do you see differences in activity in the locus coeruleus?" And we did that experiment, too, and with a subset of animals again. And we found that, depending upon how you turn on different version different subsets of these neurons, you get different responses in the animal different behaviors, different types of exploratory behavior, different types of arousal type of behaviors. And so that's kind of where we ended the story. Basically like, these are the possibilities, you know. We've concluded that there's a lot of diversity within this GABA population and heterogeneity that responds to different types of arousal along that spectrum, and that in all likelihood that difference in these GABA populations and subsets of them influences the arousal system across the brain in interesting ways. Now, you know, there's a lot to really unpack from the data side.

Mac Shine
So many new questions. Yeah.

Michael R. Bruchas
So many new questions to go after, and it's great to have these people come into your lab and to mentor them, because even though I have a large group, it is nowhere near equipped to answer all of those possibilities, and so having people to do it and continue the work, sort of pass on that, you know, those insights to the next generation, is exciting.

Mac Shine
Really takes a village ... That was absolutely fantastic, Michael. I feel like I could talk to you for a couple more hours about all of this.

Michael R. Bruchas
Yes.

Mac Shine
I think it's a really fascinating space. Thank you for all your hard work, and thanks for all the chat today.

Michael R. Bruchas
Awesome, thanks for having me; appreciate it. It was a delight.

Mac Shine
Cheers, mate.