Getting sick from worry

November 18 2010, Joshua E. Brown

Three years ago, Victor May began to wonder about an obscure protein. From nerve cells he was testing in his laboratory, "it looked as if it could be active in stress/anxiety pathways in the brain," he says, "but we didn't know where."

So May, a neurobiologist in the school of medicine, called Jom Hammack, his young colleague in the psychology department. He asked Hammack if he had time to put some experimental rats through a standardized stress routine -- and then prepare them for dissection.

Soon, May and his students were sleuthing through cells from twelve different parts of each rat's brain that Hammack thought might play a role in regulating anxiety. They were looking for the protein that May had been studying: a sometimes-neurotransmitter called pituitary adenylate cyclase-activating peptide, or PACAP.

They didn't find anything out of the ordinary, until they began to look at a little-studied region of the brain: the bed nucleus of the stria terminalis, sometimes called the BNST or just "bed nucleus" in neuroscientist shoptalk.

What May's team found in the bed nucleus of the stressed-out rats amazed him: ten times the level of PACAP found in unstressed rats. "A fifty percent change is big," says May, "but tenfold is, is...wow!" Had they stumbled onto a biochemical key to anxiety?

May picked up the phone again. In short order, Hammack and his

colleagues had a group of unstressed experimental rats -- alive -- with tubes surgically implanted directly into their bed nuclei. "We sent the PACAP over and they injected it into the rat brains," says May. "And wouldn't you believe it that the rats all became extremely anxious!"

Since then, the scientists have been able to block anxiety behaviors in rats -- by blocking PACAP's action in the bed nucleus. And they're working in a three-department team with chemist Matthias Brewer '96 to make and test a series of small organic molecules that look promising as PACAP-blocking drugs.

Their goal: find the first truly targeted medications for chronic anxiety diseases from anorexia to post-traumatic stress disorder.

Anxieties in the bed

But to better understand all this PACAP business requires a little exploration of the mammalian brain under stress. So, take a sharp pencil and poke through your ear into your brain a few inches until you hit an almond-shaped region called the amygdala. Sounds horrible and scary, doesn't it?

Of course, you're not really going to do that -- talk about a bad end to the day! -- but just thinking about something so dangerous probably activates your amygdala. A major job of this little structure is to be on the lookout for danger. When the snarling pit bull approaches, your amygdala lights up like fireworks on a PET-scan and helps launch stress responses that leave you wide-eyed and sweating.

"The amygdala has gotten a lot of attention," says Hammack.

But close to the amygdala is its less-well-known cousin, the bed nucleus of the stria terminalis. It, too, is implicated in fear reactions. But in

recent years, neuroscientists have started to tease out how its role differs from the amygdala's.

"The amygdala is important for learning to be scared of something right in front of you," says Hammack, "but the bed nucleus seems to be more connected to vigilance and vulnerability." Heading into a dark alley? Blame the dreadful feeling on your bed nucleus. In particular, it appears to control responses to long-term threats and is a major player in sustained anxiety.

And sustained anxiety can be bad news. Sure, public speaking or a first date could make anyone anxious -- for a short while. That's normal. But more than 40 million American adults suffer with long-term anxiety problems: panic disorders, social phobias, post-traumatic stress disorder, obsessive-compulsive behaviors, and others.

It's a huge public health problem, and a priority for the National Institutes of Health, yet "there is no known cure for chronic anxiety behaviors," says May.

Doctors often prescribe anti-depressant drugs, which can help, but no one knows why, nor are they highly effective, especially in profound cases.

While genetics are important, many of these anxiety problems are triggered by stress, often by extreme stress like rape, combat, abuse, or extended isolation. But the mechanisms by which stressful experiences alter the central circuits in the brain that control anxiety behaviors are still unknown.

Into overdrive

Which brings us back to the rats. When they were given a single shot of

PACAP straight into their BNST's they became anxious within twenty minutes, as indicated in a standard acoustic startle test -- "a series of tones and then measures of how high they jump," explains May.

But what was more impressive to the scientists -- as they describe in a journal whose title, Psychoneuroendocrinology, would have driven both Strunk and White to panicked flight -- is that seven days later the rats were still anxious.

"The PACAP peptide is neurotrophic," says Hammack, "it enhances the survivability of brain cells and promotes their growth." In other words, chronic stress makes the BNST swell and become more active, and the Miracle-Gro is PACAP.

"And we think it may make permanent changes," says Hammack. "Chronic stress sends the bed nucleus into overdrive and its stays there," pumping out an unneeded abundance of signals to be wary, vigilant, anxious. Signals that may contribute to the behaviors of pathological anxiety: don't eat, hit the ground when the firecracker goes off, stay inside all the time, wash your hands seventeen times in a row.

Small molecules are beautiful

But what if PACAP is cut off? In one experiment, May and his colleagues were able to block the peptide by fifty percent using another peptide, a so-called antagonist, that gummed up the PACAP receptors in the rats' bed nuclei. As they describe in a review article in the Journal of Molecular Neuroscience, this put a major damper on the rats' anxiety behaviors during a seven-day stress test.

Problem is, this PACAP antagonist had to be mainlined into their brains. "Peptides are not small molecules," says May, "as a general rule they don't cross the blood-brain barrier very well and are quickly broken

down in the bloodstream." (The blood-brain barrier is the body's nifty trick of separating circulating blood from cerebrospinal fluid in the central nervous system. It helps protect the brain from infection and toxins, but it can be a devil for drug development.)

So May began to wonder if something else might block PACAP.

"Then Victor came across a paper where a company described a small molecule antagonist that blocked PACAP," says Hammack, "A small molecule that won't break down orally and will get across the blood-brain barrier." The company was working in another area entirely, looking for ways to stimulate PACAP receptors, but May saw potential in their failure.

So he picked up the phone again, this time to chemistry professor Matthias Brewer. "He approached me to see if I could make that kind of molecule," says Brewer, "which I could." Indeed Brewer and his students were able to make some dozen variants of this molecule to test. He then delivered the compounds to May to make sure they exhibited the same PACAP knockout that the other paper had described -- and "to see which one would do the best job binding to the receptor," says Brewer.

The molecular testing proved positive in May's lab -- so now it's back to the rats in the psychology department. "Can we mimic the things I see in my culture dish in a real animal?" says May. "If yes," he says, and if "everything falls into place, we might be in touch with a pharmaceutical company."

And, yes, working with UVM's Office of Technology Transfer, May and his colleagues have taken out two provisional patents on their anti-anxiety work -- just in case you were worried.

More information: Read more about the contributions of students,

including Spencer Scholz '11, to this project in the original article, which appears in the fall 2010 issue of *Vermont Quarterly* magazine. The full issue may be viewed online at alumni.uvm.edu/vq

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