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## Veterinary Medicine: Influencing Animal and Human Medicine

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# Veterinary Medicine: Influencing Animal and Human Medicine

*VMD-PhD student LaTasha Crawford shares similarities found in animal and human anxiety and what the connection may mean for therapy options*

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**W**hat started in childhood as a love for animals and an intrigue with biology has become a launching pad for a budding career in translational research.

Veterinary medicine has become an invaluable foundation of comparative pathology and comparative medicine that enriched the research I performed while enrolled in the VMD-PhD program at Penn Vet, influencing everything from the types of questions I asked to the interpretations of the answers as they applied to medical syndromes.

I pursued a PhD in neuroscience working with the Stress Neurobiology Group at the Children's Hospital of Philadelphia Research Institute among researchers who explore neural pathways underlying stress and stress-related disorders such as anxiety, aggression and depression. Working under the mentorship of Dr. Sheryl G. Beck, I was drawn towards animal models of anxiety because so little is known about the disease process, despite its prevalence in both human and veterinary medicine.

## TAKING ON ANXIETY

Collectively, anxiety disorders are the most prevalent psychiatric disorders in adult humans in the United States and tend to have an earlier age of onset than other mental disorders. Anxiety disorders often exist with other mental disorders and are associated with a wide range of other

medical conditions, including sleep disorders, interstitial cystitis, cardiovascular disease, chronic pain and irritable bowel syndrome.

Anxiety-related behavioral disorders are also prevalent in veterinary patients including dogs, cats and horses, among other companion animals. Frequent, repetitive actions and certain types of self-mutilation are behavioral disorders in equine patients for which social stress and social isolation can be contributing factors. Canine acral lick dermatitis resulting from over-grooming is often considered an animal model of obsessive-compulsive disorder with obvious parallels to the grooming behavior often seen in human obsessive-compulsive disorder.

Interestingly, these stress-related disorders in veterinary medicine are responsive to the same types of drugs that are useful in human anxiety disorders, suggesting a common biology and a mechanism rooted in conserved neural systems.

## FOCUSING ON SEROTONIN IN THE BRAIN

To begin to tease out the mechanisms underlying anxiety, my thesis research centered on the serotonin system, which is conserved among mammals and has a role in steadying mood, appetite, vasomotor tone and panic behavior amongst several other behaviors and physiological functions. Serotonin is known to play a role in anxiety and is altered by anxiety-relieving drugs.

However, we now understand that there are several types of serotonin cells that interact with distinct brain regions,

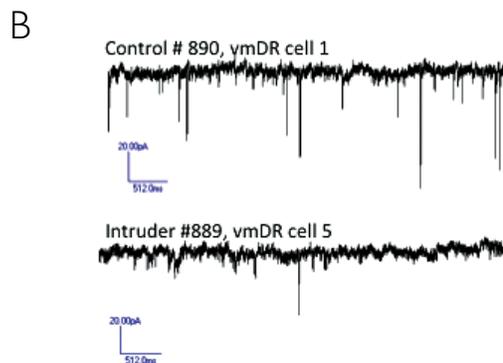
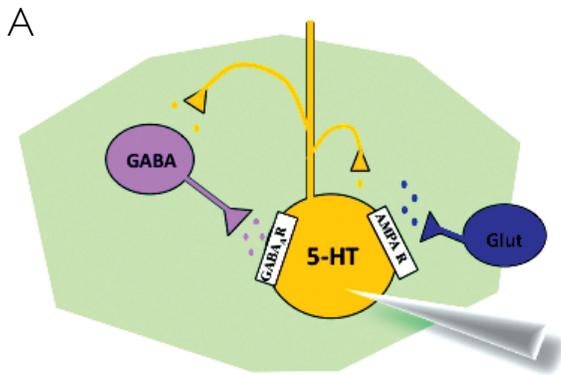


Diagram A shows how serotonin cells receive “messages” released by nearby GABA cells and Glutamate (Glut) cells. When these messages reach the serotonin cell, they cause changes in the flow of ions in and out of the serotonin cell and alter its electrical activity. We can measure the flow of ions to characterize the types of messages that serotonin cells receive and to determine if those messages come more frequently or less frequently in the anxious brain.

Diagram B shows examples of traces obtained from recordings of GABA input. The top trace is from a control mouse; the bottom trace is from a mouse that was anxious after 5 days of social defeat.

function in different ways and may play different roles in anxiety. My project explored serotonin cells within the dorsal raphe nucleus of the midbrain (DR), which is made up of several subpopulations of serotonin cells.

The dense collection of serotonin cells in the ventromedial subregion (vmDR cells) are well characterized and known for their connections to the higher-level processing regions of the brain like the prefrontal cortex, a fear-processing region known as the amygdala and a learning and memory-processing region known as the hippocampus.

I characterized the physiology of a less dense, poorly understood subpopulation of serotonin neurons in the lateral wing subregion of the DR (lwDR). The lwDR subpopulation sends connections to many subcortical brain regions such as the periaqueductal gray, where release of serotonin minimizes panic behaviors, and the rostral ventro-lateral medulla, where release of serotonin dampens the sympathetic tone within the vascular system.

## THE PROCESS

In addition to immunohistochemistry and confocal microscopy imaging, I became adept in *in vitro* electrophysiology, a technique that uses rapidly dissected brain slices to visualize, stimulate and record the electrical activity of individual living neurons. Because the local neural circuits remain intact in the slice, electrophysiology can also be used to characterize the signals from neurons that connect or synapse onto serotonin cells, including inhibitory signals from local GABA neurons. This led to several findings that differentiated lwDR cells from vmDR cells in the healthy brain of untreated mice: lwDR cells have a larger, more complex morphology and are more easily excited by electrical activity. However, the most intriguing difference between these two subtypes of serotonin cells was clarified using a model of anxiety.

## Dr. LaTasha Crawford

**HOMETOWN:** Washington, DC area

**UNDERGRADUATE SCHOOL:** Yale University

**UNDERGRADUATE DEGREE AND MAJOR:** Bachelor of Science, Molecular Biophysics and Biochemistry

**Why Penn Vet’s VMD-PhD program?** “There were many reasons why Penn’s VMD-PhD program was a good match for me,” said Dr. Crawford. “In short, the Vet School is really unique in its focus on cutting-edge medicine and its location within a strong interdisciplinary biomedical research community. In addition, Penn has a stellar neuroscience graduate program with a wide range of research labs at Penn and at neighboring institutions like the Children’s Hospital of Philadelphia.”

**What are her plans?** “I will obtain veterinary anatomical pathology residency training and more independent research experience in the comparative pathology post-doctoral program at Johns Hopkins University. Eventually, I’d like to use a background in comparative neuropathology to investigate animal models of neurological diseases and the sequelae in peripheral organ systems that are associated with those diseases. I’m particularly interested in a research career in academia, since there is a part of me that loves to teach in the lab setting and in the form of seminars and talks.”

Dr. Crawford pictured here with Bailey, a Ryan-VHUP patient, whom she examined as part of her cardiology rounds.



I sought a mouse model that used social stress as an etiological agent with hopes of stimulating pathways that are consistent with naturally occurring disease states, for which social stress plays an important part.

Chronic social defeat is a type of model used in male rodents where repeated exposure to intimidation, bullying and threat of attack produces behaviors that are typical of anxiety and depression and that can be alleviated by anxiolytic and antidepressant drugs. Using behavioral tests, I characterized a five-day social stress paradigm that induced anxiety-like behaviors in stressed mice, such as increased stress-induced grooming and increased avoidance behavior where individuals avoid vulnerable areas of an apparatus and seek more enclosed, protected areas.

Though it was beyond the focus of my lab, I was also able to perform partial necropsies following the stress paradigm and identified several changes in peripheral organs of stressed mice, including increased bladder weight, potentially due to urine retention and bladder wall thickening, and increased heart weight, potentially due to increased blood pressure or direct sympathetic stimulation and consequent ventricular hypertrophy. The hypertrophy of the bladder and heart are likely sequelae (the negative aftereffects) of increased sympathetic tone, which is a common feature of human anxiety. The changes in behavior and peripheral organ systems underscored the utility of social defeat as a model to study anxiety and to examine the potential role that anxiety plays in the often comorbid clinical syndromes.

## THE FINDINGS

I used the chronic social defeat model to investigate changes in serotonin cell function, and found that social stress caused distinct changes in the two subtypes of serotonin cells examined. Using electrophysiology in brain slices obtained from stressed mice and controls, I found that serotonin cells were capable of functioning normally; however the inhibitory input from nearby GABA cells was drastically altered by social stress.

Following social stress, vmDR serotonin cells received less inhibition from GABA inputs due to inactivity or death of GABA neurons and due to fewer GABA receptors on vmDR neurons. The loss of inhibition of vmDR serotonin cells could increase serotonin release in the cortex, amygdala and hippocampus in the anxious brain, as has been suggested in the literature. This would result in the enhanced fear processing and increased salience of fearful memories that are typical of anxiety disorders. A maladaptive reduction in inhibitory input to vmDR cells in anxiety could also explain the clinical efficacy of benzodiazepines, which act by increasing the potency of GABA signals and could theoretically counteract the pathological changes that disinhibit vmDR serotonin cells.

On the other hand, lwDR serotonin cells were affected in a distinct way following social stress: lwDR cells received more

potent inhibitory signals due to slower kinetics of the GABA receptor. This implies that in the anxious brain, fewer messages are traveling from the lwDR to the brainstem to prevent panic responses. This could then correspond to the exaggerated panic behaviors that typify anxiety disorders. In addition, because lwDR cells usually help to dampen sympathetic vasomotor tone, anxiety-induced inhibition of lwDR cells could lead to increasing blood pressure and consequent changes in heart musculature.

It is likely that stress-induced inhibition of lwDR serotonin projections to other brainstem regions could likewise contribute to increased sympathetic tone, urine retention and bladder wall hypertrophy, though this has not been shown definitively.

Collectively, these findings have contributed to a better understanding of the heterogeneity of serotonin cells, the way they are regulated by local inputs and the way they are dysregulated in anxiety.

## WHAT THIS RESEARCH MAY MEAN

Experiments of this type lend clarity to the mechanisms of known anxiolytic drugs, and may one day aid in the development of novel therapies as well. Being better able to target pharmacotherapy to specific subsets of serotonin cells may also help us to one day target particular components of anxiety-related syndromes or to avoid undesirable side effects of drugs that modulate serotonin neurons.

For me, these findings have also piqued an interest in neuropathology and the interaction between the brain, the autonomic nervous system and visceral organ dysfunction. I have been exhilarated by the opportunity to incorporate my clinical background into my research and look forward to the unique perspective additional anatomical pathology training will grant me as I continue towards a career in research. With a better understanding of mechanisms of stress susceptibility and stress-related disorders, we can build on correlations observed on the clinical side of human and veterinary medicine to improve the diagnosis, treatment – and even prevention – of comorbid diseases. It is my hope that my pursuit of mental disease research with an eye for the sequelae seen in veterinary and human patients will be a step in that direction. ■

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