

Zweig

From the Harry M. Zweig
Memorial Fund for Equine
Research at Cornell University
College of Veterinary Medicine



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Cornell radiologist creates new brain atlas for horses

By Olivia Hall

Dr. Philippa Johnson knows her way around brains. A clinical radiologist and diplomate of the European College of Veterinary Diagnostic Imaging, she specializes in advanced magnetic resonance imaging (MRI) techniques. Now she has mapped her findings in an equine brain atlas to help guide colleagues to better diagnostics in veterinary medicine.

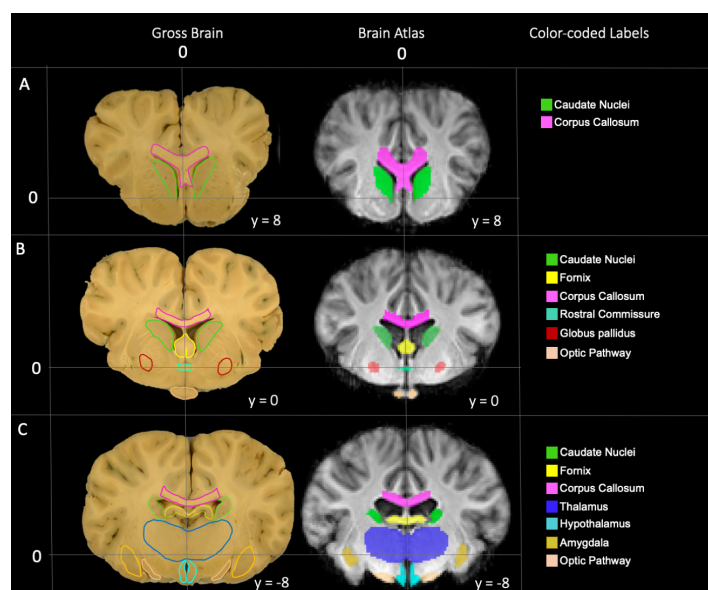
"Currently, our state of neuroimaging is to perform standard MRIs," said Johnson, who joined the College of Veterinary Medicine's Department of Clinical Sciences as an assistant professor four years ago. "But there are limitations, because readouts can be variable between radiologists, and some of the lesions — such as in epilepsy — aren't visible on standard MRIs. The diagnosis is therefore presumptive. So, I wanted to start applying the most advanced forms of neuroimaging currently being done in human clinical research in the veterinary world. The big tool that we were missing was the brain atlas."

Cutting-edge, high-resolution, so-called stereotaxic brain atlases bring data obtained from multiple individuals into a standardized virtual space. "There they can be compared to each other," Johnson said. "The atlas also enables us to identify a standardized region in the brain that we want to compare across subjects." She hopes that the newest publications — freely available for download online — will enhance the few existing tools and fill significant gaps.

The equine brain had not yet been mapped. Supported by the Harry M. Zweig Memorial Fund for Equine

Research, Johnson and her colleagues used powerful 3-Tesla magnets with twice the power of routine MRI devices to image the brains of 15 horses euthanized for reasons unrelated to the study. They averaged the measurements of 10 horses to create a generalized model and used the remaining five to test whether, when brains are registered to — or integrated into — the atlas, the data quality is maintained (a process called "validation"). The team also made sure to equip the equine brain atlas with subcortical masks, a feature that lets users separate specific parts of the brain for more detailed insights.

"Each manuscript has its own specific purpose, and they can always be improved on," she said. "Our lab really welcomes collaboration and data sharing, so we're very open to different labs reaching out to us. It's nice to be able to support the application of advanced MRI techniques in veterinary neurology, and we'd like to promote this level of research as much as possible." ■



An example of the equine brain atlas developed by Dr. Philippa Johnson, assistant professor in the Department of Clinical Sciences. Image provided.

Collaboration reveals potential new therapy for osteoarthritis

By Lauren Roberts

Osteoarthritis affects 240 million people worldwide and is one of the most common causes of disability in both humans and animals.

Currently, no therapeutics exist to prevent this disease, but recent collaborative research at Cornell reveals that the application of a proprietary peptide known as SS-31 may protect cartilage from the injury that leads to arthritis.

Michelle Delco '98, D.V.M. '02, Ph.D. '16, is senior author of "Mitoprotective Therapy Prevents Rapid, Strain Dependent Mitochondrial Dysfunction After Articular Cartilage Injury," which was published Dec. 16 in the *Journal of Orthopaedic Research*.

Delco is a board-certified large-animal surgeon and assistant research professor in the Department of Clinical Sciences, in the College of Veterinary Medicine (CVM). Her time spent in clinical practice treating equine athletes for sports injuries motivated her to search for ways to treat and prevent osteoarthritis.

"Just like human athletes," Delco

said, "horses are particularly prone to injury-related arthritis. In human athletes, the disease is often career-ending. In our patients, it can be life-threatening."

While the prevalence of osteoarthritis continues to rise, current drugs target only the symptoms, not the underlying disease itself. "Forget preventing osteoarthritis," she said, "right now we don't have a single drug that even slows down progression of the disease."

In younger individuals and athletes, arthritis typically develops following joint trauma. But how injury to the cartilage surface is translated into an ongoing degenerative process has been unclear. Delco believes mitochondria, the "battery pack" of the cell, are key mediators of this injury-to-disease cascade, but there was no direct evidence for that role.

Now, Delco and colleagues in biomedical engineering and physics have found that mitochondria are a linchpin in the body's response to injury. They've also found a drug that can interrupt the injury response.

That drug, SS-31, was developed by Hazel Szeto, M.D. '77, Ph.D. '77, former professor of pharmacology at Weill Cornell Medicine and a co-author of the paper. SS-31 is known to protect and heal mitochondria in other parts of the body.

Delco and her CVM colleagues were the first to explore its effects in cartilage, and revealed in an earlier study that SS-31 helped protect injured chondrocytes days after an injury. Delco wanted to further understand how mitochondria respond to injury, and how SS-31 might protect cells.

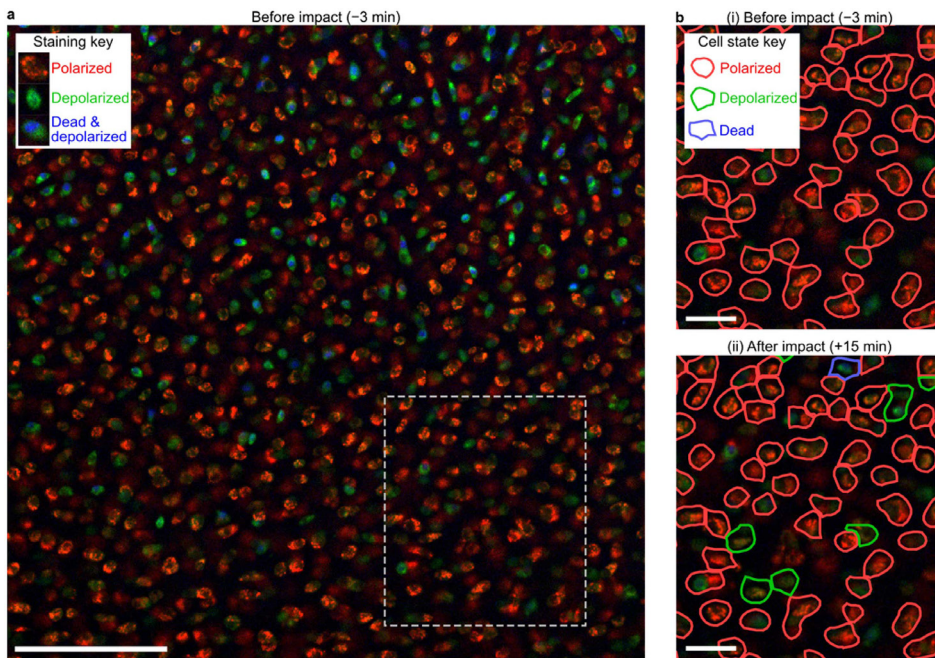
"Since osteoarthritis is caused by both biological and mechanical factors," she said, "we need to evaluate them simultaneously to understand what is happening during injury."

To do this, the team developed a novel experimental setup, one that allowed them to observe and compare huge numbers of cartilage cells and their mitochondria during and immediately after injury.

"Typically, to study mitochondria, researchers crush up the tissue and isolate the cells or individual mitochondria," Delco said. "But to study the effects of tissue injury, we needed to monitor mitochondrial function in cells within the dense cartilage matrix during a rapid impact. We also had to track the fate of thousands of individual cells over time."

Left, Delco attends a horse at the Cornell Equine Hospital. Photo by the College of Veterinary Medicine.





To do this, Delco used a custom-made, spring-loaded piston that was developed with Dr. Lawrence Bonassar, the Daljit S. and Elaine Sarkaria Professor in Biomedical Engineering, and Dr. Itai Cohen, professor of physics in the College of Arts and Sciences. The device, designed to sit on a confocal microscope stage, delivers a single, high-speed impact to cartilage samples. To visualize the physiological effect of injury on cells and their mitochondria, the team used special dyes that indicate if mitochondria are healthy or dysfunctional, and if cells are alive or dead.

Next, the team needed to observe the real-time effects of injury on cartilage — which presented an experimental challenge. “We needed to collect data at rates of roughly 1,000 points per second and with micron-scale spatial resolution,” Delco said.

Delco and her collaborators captured and analyzed these myriad digital images and videos during impact with the help of Lena Bartell, M.S. ’15, Ph.D. ’18, a former member of Cohen’s lab and first author on the paper.

“Lena’s expertise in image analysis,

statistical modeling and machine learning was instrumental in this study,” Delco said. “That’s the power of our collaboration — every contributor to this study brought a completely different skill set and perspective. Together, we got new insights into these very early events that initiate disease.”

Cohen seconded this view. “That’s the whole point of radical collaboration,” he said. “It takes the expertise that ranges across the campus and brings it together to do something that neither group could do on their own.”

Using their newly developed injury-imaging system, the experiment yielded insights into the fate of individual cells during impact.

“We discovered that in control

Above: Delco and her team imaged cartilage cells with fluorescent dye that would tell them if a cell was alive with functioning mitochondria (red), dysfunctional mitochondria (green) or dead (blue) before and after being injured with a spring-loaded piston. Image provided. Right, Delco in the lab. Photo by the College of Veterinary Medicine.

samples, mitochondrial dysfunction is immediate after injury,” Delco said. “The organelles are responding to the mechanical forces of the impact. They become depolarized — like a discharged battery, they can no longer drive energy production. They also become swollen and lose their tightly folded inner membrane structure.”

In contrast, the mitochondria in SS-31-treated cartilage maintained their normal, healthy form; dramatically fewer cells died compared with the control samples. “Treated samples looked very similar to those that hadn’t been injured at all,” Delco said.

While SS-31’s mechanism of action is not completely known, scientists do know that the peptide enables mitochondria to maintain membrane structure and function during various types of cellular injury — referred to as “mitoprotection.”

“Our finding that SS-31 has this protective effect after mechanical injury is exciting,” says Delco. “It suggests mitoprotection may be a new strategy for preventing arthritis after joint trauma.”

Lisa Fortier, Ph.D. ’99, the James Law Professor of Large Animal Surgery, also contributed to this work. ■



College clinicians garner Grayson-Jockey Club grants

From Grayson-Jockey Club online

Two Cornell veterinary researchers have received grants from the Grayson-Jockey Club to further equine health.

Soon Hon Cheong, Ph.D. '12, associate professor of reproductive medicine, will be developing a diagnostic test that can improve treatment outcomes of fungal infection in horses. Fungal infections in horses can cause major health concerns and are difficult and expensive to treat.

"Right now, there can be long lag-times for veterinarians to get results from fungal culture and antifungal susceptibility testing," says Cheong.

"This delay forces veterinarians to guess which treatment to start with while waiting for results, and even discourages veterinarians from submitting the sample at all. Making the wrong antifungal treatment can lead to worsening of the disease, antifungal resistance, high treatment costs and can erode trust between the owner and the veterinarian," he notes.

Adding to the issue is the lack of available information to help guide veterinarians in their treatment selection.

Cheong seeks to change this issue through his grant work, which will leverage a specific type of polymerase chain reaction (PCR) test that can detect and identify fungal organisms. Up until now, this type of test has not been proven to work on fresh samples or in horses.

"We propose to first test this assay on the supposedly clean uterine samples of horses that are collected using sterile technique and compare the results to the conventional fungal culture method," says Cheong. "We believe that the development of this

test will be an invaluable diagnostic tool to improve the treatment of uterine fungal infection in horses."

Cheong also plans to gain a better understanding of equine antifungal resistance through this study. He plans to gather fungal samples from horses and to culture them in biofilm, a complex mixture of secretions from the fungal organisms that can help protect them from antifungal drugs.

"We will save the fungal organisms that are resistant to antifungal drugs and determine if they have genes known to provide antifungal resistance. The hope is that these genetic markers can be used to develop rapid PCR diagnostic tests in the future. We expect biofilm from different fungal species will have different antifungal drug susceptibility and this information will be beneficial to guide treatment decisions for fungal infection in horses."

"Our long-term goals are to develop a rapid diagnostic test for fungal infection in horses," says Cheong. "This should improve antifungal susceptibility screening to help guide clinician treatment selection for fungal infections in horses."

Heidi Reesink, Ph.D. '16, the Harry M. Zweig Assistant Professor in Equine Health, will be determining the prevalence of bisphosphonate use in racehorses and whether the drugs are associated with fatal breakdowns in racing.

Fatal musculoskeletal injuries (FMI) have plagued the racehorse industry, causing tragic loss of equine life, risk to jockeys and increasingly negative public perception.

"While the cause of FMI is complex, there is widespread concern that

recent clusters of fatalities may be due, in part, to the approval of the bisphosphonates clodronate (Osphos®) and tiludronate (Tildren®) in 2014," says Reesink. "Speculation abounds about whether bisphosphonate administration in young racehorses could be a contributing factor to recent spates of FMI at prominent racetracks; however, there is a paucity of evidence to support or refute these claims."

Bisphosphonates are known to interfere with the normal remodeling process of replacing old bone with new bone and result in increased microfractures in human bone. "There is concern that these drugs might impair the normal repair processes in equine bone during race training," says Reesink. "However, this is an open question, and one that the racing industry needs to answer."

Currently, no data is available about how common bisphosphonate administration is in the young Thoroughbred population. "We are literally racing blind," says Reesink. "Often owners, trainers and racing jurisdictions are unaware of whether their horses have been treated with bisphosphonates, how frequently and how many doses they've received; and if and how bisphosphonates places horses at risk of injury."

To help answer these questions, Reesink will be estimating how common bisphosphonate administration is in the young, exercising Thoroughbred racing population in New York by gathering bone samples from 182 horses and conducting nuclear magnetic resonance spectroscopy and small molecule detection methods to detect bisphosphonates in the bone tissue. Reesink will also evaluate samples that do contain bisphosphonate to see how



Above: Cheong. Photo by the College of Veterinary Medicine. Below: Reesink. Photo by Elvina Yau, D.V.M. '20/College of Veterinary Medicine.

bone tissue of two- and three-year-old Thoroughbreds with racing and training histories have been affected by the drug.

Additionally, Reesink will study how a single doses of bisphosphonate drugs can show up in blood, urine, bone, hair and hoof tissues using nuclear magnetic resonance spectroscopy.

“Optimizing the way we measure bisphosphonates will advance the current state-of-art in racing medication drug testing efforts,” says Reesink. “Our ultimate goal is to help racehorse veterinarians, owners and trainers make informed decisions about fracture risk while improving equine welfare, mitigating negative publicity and enhancing our understanding of the impact of bisphosphonates on bone remodeling and fracture risk.” ■



2020 HARRY M. ZWEIG MEMORIAL FUND FOR EQUINE RESEARCH AWARDS



The Harry M. Zweig Memorial Fund for Equine Research honors the late Dr. Harry M. Zweig, a distinguished veterinarian, and his numerous contributions to the state’s equine racing industry.

In 1979, by amendment to the pari-mutuel revenue laws, the New York State Legislature created the fund to promote equine research at the College of Veterinary Medicine at Cornell University.

New Awards

\$78,244 to Julia Felipe, Ph.D. '02, for “Diagnostic markers in mares with placentitis”

\$76,782 to Dr. Kathleen Kelly for “Genomics of Autopsy–Negative Sudden Cardiac Death in Racing Thoroughbreds”

\$71,571 to Dr. Bettina Wagner for “Nasal immunity and its function in preventing transmission of EHV-1 in immune horses”

\$57,621 to Heidi Reesink, Ph.D. '16, for “Unraveling lubricin signaling in equine joint injury”

\$55,768 to Michelle Delco '98, D.V.M. '02, Ph.D. '16, for “The role of mitochondrial Damage Associated Molecular Patterns (mDAMPs) in equine joint injury and disease”

\$49,552 to Dr. Gerlinde Van de Walle for “Studying the replication kinetics of equine parvovirus hepatitis (EqPV-H)”

\$7,000 to Dr. Douglas Antczak '69 for “2020 Horse Genome Project Workshop at Cornell”

Continuations

\$99,643 to Jonathan Cheetham, Ph.D. '08, for “Accelerating recovery after Laryngeal Nerve Graft in Horses”

\$83,921 to Heidi Reesink, Ph.D. '16, for “Does Proximal Sesamoid Bone Mineral Loss Lead to Increased Fracture Risk?”

\$68,537 to Dr. Douglas Antczak '69 for “Functional gene annotation in the horse”

New test predicts horse's risk of contracting EHV-1 during an outbreak

By Patricia Waldron

Despite widespread vaccination, outbreaks of the potentially deadly equine herpesvirus 1 (EHV-1) occur sporadically across the United States, triggering apprehension and quarantines. Now, a new diagnostic test can tell owners whether their horse will be at risk in the event of an outbreak.

The test, developed by Dr. Bettina Wagner, chair of the Department of Population Medicine and Diagnostic Sciences at the Cornell University College of Veterinary Medicine, is called the EHV-1 Risk Evaluation Assay, and is available at the Animal Health Diagnostic Center. It measures the level of a key antibody that protects against the virus to let owners know a horse's immediate risk. Wagner is using this and similar tests to understand how the horse immune system interacts with EHV-1 and to develop better vaccines against the virus.

"We can take a serum sample and define if this horse today is at high or low risk of EHV-1 infection," said Wagner. "It also provides guidance on when the horse should next be vaccinated."

Typically, EHV-1 causes a mild respiratory infection in horses. It circulates by close nose-to-nose contact through droplets and through contaminated surfaces or clothes. Most horses are exposed to the virus before the age of two and carry a dormant form that can re-emerge during times of stress, making them perpetual carriers.

During an outbreak, however, about five to 10 percent of sick horses develop a neurological form of the disease called EHV-1 myeloencephalopathy. The virus travels via

blood vessels into the spinal cord and causes strokes, which manifest as a lack of coordination, weakness in the hind limbs, lethargy and an inability to maintain balance. About one-third of horses who develop the neurological form will die from the disease.

New York saw four outbreaks of EHV-1 in 2019 alone. Besides treating affected horses, the course of action is to quarantine the facility and its horses for up to 28 days to stop further spread of the disease, which can paralyze horse movement, shows and sporting events.

Several licensed EHV-1 vaccines protect against the respiratory infection and prevent pregnant mares from experiencing abortions, but none exist that stop horses from developing the neurological form of the disease. Also, there are numerous cases where vaccinated horses still became sick during an outbreak.

"One important thing is to note that not every horse responds evenly to the vaccination," said Wagner, who sees this phenomenon in her own horses, Peaches and Betty. While Peaches' immune system reacts strongly to the vaccine, Betty has a much weaker response, and needs more frequent vaccination to maintain protection. "For some horses, the vaccination interval of a year may be fine. For others, six months may not be enough. The test is designed to find that out," said Wagner. A horse will always react to the vaccine in the same way, so the testing only needs to be performed once, before and after vaccination, for veterinarians to optimize a horse's vaccination schedule.

When Wagner started working with her Icelandic horses in

Zweig Memorial Trot 2020

August 21, 2020

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Above, Wagner with one of her Icelandic horses. Photo by the College of Veterinary Medicine.

2012, the prevailing belief was that T cells — a type of white blood cell that targets the virus as it enters the bloodstream — would be the key to stopping the neurological form of the virus. But after monitoring how the horses respond both to vaccination and to exposure to the virus, Wagner realized that it wasn't T cells, but a specific type of antibody, called IGg4/7, in the lining of the nose that can completely protect the horse.

"It turned out that if these IGg4/7 antibodies are in the nose of the horse at the time of infection, they immediately deal with the virus at the local infection site, and thereby prevent the virus from entering the lymphatic tissues and white blood cells, so that neurologic signs could not happen," said Wagner. "We now can use those antibody values to predict if the horse can be infected or not with the EHV-1 Risk Evaluation Assay."

Another related test currently under development in the Wagner lab may help New York manage EHV-1 outbreaks more effectively. The test detects biomarkers in the nose that indicate whether a horse exposed to the virus is protected and thus can be released early from quarantine. Thanks to a new grant from the Zweig Memorial Fund, she also plans to investigate whether those horses can pass the virus to susceptible horses, to ensure that they don't pose a risk to the greater population.

Additionally, Wagner has been testing different variations of the EHV-1 vaccine to see if they stimulate a better immune response in adult horses that would protect against the neurological form. She is also working on vaccines for foals, whose immune systems are less mature compared to adult horses.

"We're going to find a vaccine at some point that helps against neurologic disease in horses," said Wagner. "But in the meantime, a test like this can help evaluate risk, especially for horses that travel a lot and are at risk of being infected." ■

2020 HARRY M. ZWEIF MEMORIAL FUND COMMITTEE

The Harry M. Zweig Committee is established for the purpose of administering the fund and is composed of individuals in specified state agencies and equine industry positions and others who represent equine breeders, owners, trainers and veterinarians.

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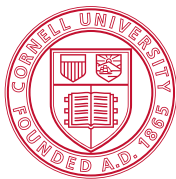
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