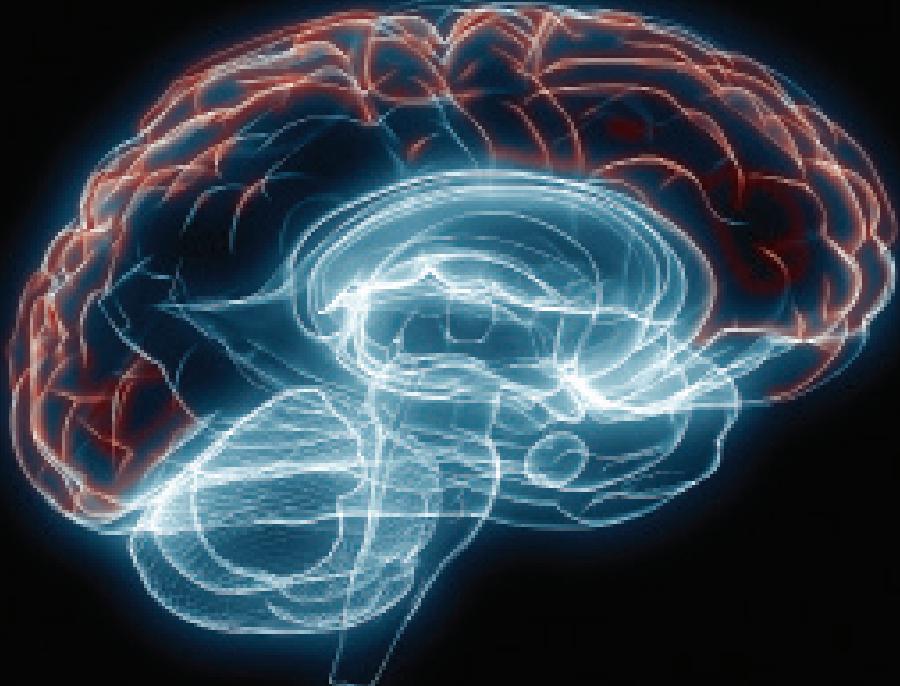


# RACING TO DETECT BRAIN TRAUMA



Scientists search for **Biomarkers and Imaging Tools** to diagnose concussion-related brain disease while a person is still alive

LAUREN K. WOLF, C&EN WASHINGTON

**T**O FINISH OFF AN OPPONENT, Chris (The Canadian Crippler) Benoit sometimes used a move called the diving headbutt. This involved the professional wrestler scaling the ropes at the edge of the ring and launching himself headfirst toward a downed competitor below. Once Benoit's head slammed into the other wrestler someplace—a shoulder perhaps—the match was over, the Crippler victorious.

In 2007, after 22 years in the ring, Benoit murdered his wife and seven-year-old son and then hanged himself. Some in the wrestling community speculated that steroid use was to blame. Others pointed to a tumultuous relationship between the two-time world champion and his wife. A few conspiracy-minded fans even proposed that Benoit, 40 when he died, had been framed.

But Julian E. Bailes Jr., then the chair of neurosurgery at West Virginia University,

had other ideas. He wondered about the effects of all those headbutts. All the concussions. All the chairs to the back of the head.

After the murder-suicide in June 2007, Bailes and colleagues examined slices of Benoit's brain. The tissue was riddled with aggregates of a protein called tau. Just as Bailes had suspected, the pattern of these tangled fiberlike deposits was consistent with a progressive, brain-damaging condition called chronic traumatic encephalopathy (CTE).

SHUTTERSTOCK

Benoit is just one in a long line of troubled athletes to be diagnosed with CTE during autopsy in the past decade. Signs of the disease, thought to be caused by repetitive brain injury, have been spotted in ice hockey players, boxers, and National Football League (NFL) retirees.

Before their deaths, many of these athletes struggled with symptoms such as memory loss, depression, and violent mood swings. Although their diagnoses helped explain the athletes' conditions, the reports came too late to save them. So researchers are now trying to identify people with CTE while they're still alive. The scientists are developing imaging techniques that pinpoint accumulated tau and detect nerve cell damage in the living brain. They're also searching for biomarkers in bodily fluid that might betray the disease's presence years before symptoms appear.

"We have to find a way of detecting CTE while someone is still alive," says Bailes, now codirector of the Chicago-area North-Shore Neurological Institute. A diagnostic test would not only give researchers a better understanding of the relatively new brain disease but may also eventually lead to treatments, Bailes says. In the meantime, he adds, "intervention with psychiatry or family counseling could be done if you make a diagnosis before it's too late."

## FIGHTING FOR RECOGNITION

The idea that repetitive blows to the head cause neurodegenerative disease has been around since the 1920s. Back then, Harrison S. Martland noted that boxers—especially the ones who got knocked down a lot—suffered from tremors, difficulty walking, and mental sluggishness. So the Newark, N.J.-based forensic pathologist took a closer look. He conducted surveys of former fighters, concluding that the athletes had "some special injury due to their occupation," a condition he called being "punch drunk" (*JAMA* 1928, DOI: 10.1001/jama.1928.02700150029009).

But boxing wouldn't be boxing without fighters taking repeated punches to the head and jaw. That these men became impaired wasn't much of a surprise to the medical community. So even though the connection between head trauma and brain disease got some attention, it wasn't until a higher-profile sport entered the picture that the link caused a public uproar.

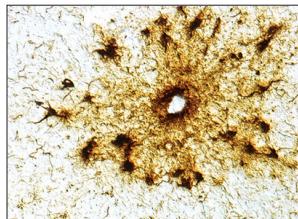
## CTE At A Glance

**Full Name:** Chronic traumatic encephalopathy

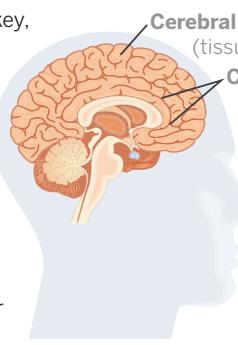
**Cause:** Repetitive mild traumatic brain injury

**Who's affected:** Athletes in contact sports such as boxing, football, ice hockey, soccer, and wrestling; military veterans; victims of domestic abuse; headbangers

**Symptoms:** Memory loss, depression, suicidal thoughts, explosive or aggressive behavior, and in some cases, trouble walking or speaking



10 µm



**Pathology:** Unlike in Alzheimer's disease, in CTE, tau protein tangles

first accumulate in the brain's cortex. What also sets CTE apart from other brain disorders is that tau collects around blood vessels (above, left) and deep in the cortical sulci (above, right) of the brain.

**Treatment:** None

## Stages Of Disease



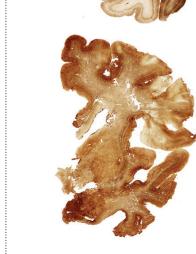
**Stage I:** Hot spots of tangled tau pop up in isolated areas of the cortex (black circle).



**Stage II:** Multiple hot spots of tangled tau appear in the cortical sulci, and tau begins to migrate.



**Stage III:** Tau hot spots begin to blend with one another. Tangles appear more diffusely throughout the ridges of the brain. Tau begins to collect in the hippocampus (involved in learning and memory) and amygdala (involved in decision making and emotions).



**Stage IV:** Dense tau tangles cover the brain's cortex and appear in most other regions, including the spinal cord.

**NOTE:** Stages proposed by Ann C. McKee, Boston University, still need to be validated by other research groups. Based on *Brain* 2013, DOI: 10.1093/brain/aws307.

In 2002, neuropathologist Bennett Omalu discovered extensive brain damage in a deceased NFL player. Before his death, Mike Webster, a Hall of Famer who once was an offensive lineman with the Pittsburgh Steelers, had fallen on hard times. He was homeless. His behavior had become erratic. And he couldn't remember significant details about his life, such as whether he'd ever been married.

When Omalu, then working at the Allegheny County coroner's office, in Pittsburgh, dissected Webster's brain, he spotted an unusual distribution of tau aggregates in the tissue. He reasoned that the condition, which looked similar to punch-drunk syndrome, might be a result of the thousands of hits Webster took during his career. Omalu revived an old term, calling the disorder chronic traumatic encephalopathy (*Neurosurgery* 2005, DOI: 10.1227/01.neu.0000163407.92769.ed).

Initially, the NFL and its doctors tried to discredit Omalu and force him to retract the report he'd published. But the pathologist wasn't the only one who had identified CTE in a former athlete's brain.

In 2003, neuropathologist Ann C. McKee received the brain of a former boxer who had been diagnosed with Alzheimer's disease while he was still alive. When the Boston University medical researcher opened up his brain, though, "it was clear he had anything but Alzheimer's," she recalls. Large amounts of tau peppered the tissue in a distinctive, florid pattern. "He had extraordinary tauopathy, the likes of which I'd never seen before," she adds.

After that first case, McKee spotted the same pathology in the brains of other professional athletes for whom hits to the head had been all in a day's work. Working with West Virginia University's Bailes, Omalu continued to rack up CTE diagnoses too. Benoit's was among them.

Beginning on Oct. 28, 2009, the pathologists presented their findings on Capitol Hill during House Judiciary Committee hearings about head injury in football. These hearings turned the tide for CTE, bringing greater public awareness to the condition and changes to the NFL's rules about concussions.

## CHARGING AHEAD

Having finally established CTE as a disease that can no longer be ignored, Omalu, McKee, and others are now trying to learn

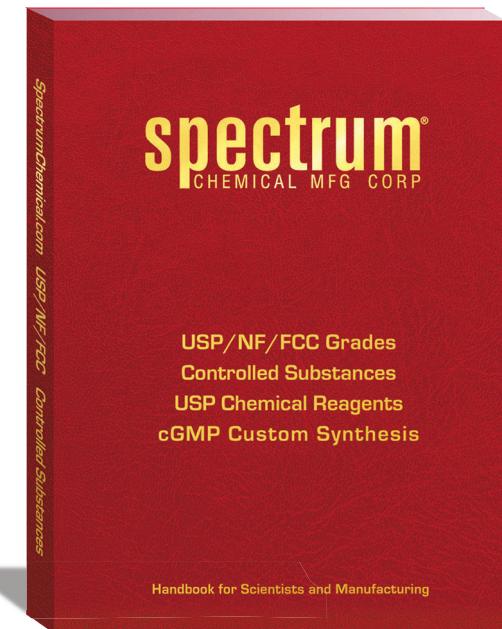
more about why repeated hits to the head trigger it. With financial support from the likes of the NFL and the National Institutes of Health, research teams have begun to launch large-scale studies of the condition.

The longest running of these is the Professional Fighters Brain Health Study. Launched in 2011 and run by the Cleveland Clinic's Lou Ruvo Center for Brain Health

in Las Vegas, the program has so far recruited more than 400 boxers and mixed martial arts fighters.

Neurologist Charles Bernick, who's leading the study, says he relied on decades' worth of Alzheimer's research to help plan the project. "We learned from Alzheimer's that this type of neurodegenerative disease may start many, many years

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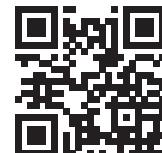
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before you show symptoms," Bernick says. For instance, scientists now know that amyloid- $\beta$ , a peptide in Alzheimer's that misfolds, clumps, and damages nerve cells, begins accumulating in a person's brain 15 to 25 years before memory loss starts.

So in addition to examining retired fighters who already have symptoms suggestive of CTE, Bernick and his team are studying a number of healthy, active fighters. By following these athletes, the researchers hope to track the early stages of CTE and figure out why it progresses in the brains of some individuals.

One question they'd like to answer is how much brain injury a person can handle before CTE sets in. With support from the Nevada Athletic Commission and local fight promoters, the group is gathering data by periodically testing its fighters and comparing them with a control group of age- and education-matched people who have never had head trauma. When the test subjects visit the Lou Ruvo Center, they update their fight records, take cognitive tests, and lie down inside a magnetic resonance imaging machine.

"We're looking at a variety of MRI modalities," Bernick explains. He isn't yet sure which combination of MRI scan types will be most useful for detecting CTE-related brain damage and tracking it over time, so his group is running a full battery of them.

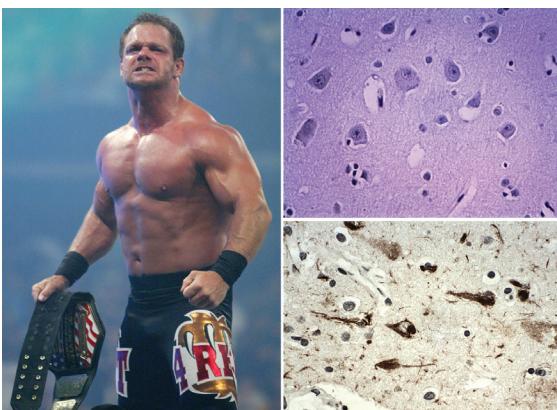
A few have shown promise so far. Volumetric MRI, which constructs a three-dimensional view of the brain, has indicated that subjects who fought more bouts during the study's first year had greater tissue loss in regions of the brain called the corpus callosum and putamen. The corpus callosum is a bundle of nerve fibers that connects the right and left hemispheres of the brain, and the putamen is a structure deeper within the brain that helps regulate movement and learning.

The results of diffusion tensor imaging, another type of MRI, also suggested that some of the study's fighters have a thinning corpus callosum. This type of imaging maps the 3-D movement of water throughout the brain. Water typically flows parallel to nerve fibers, so when that flow pattern changes in a particular brain region, scientists take it as a sign of neuronal damage in that spot.

According to Bernick, more long-term MRI and cognitive data are needed to say for sure, but "it's possible these are brain



**DIAGNOSED AT DEATH** Pelly, an 18-year-old rugby player (above), had hot spots of aggregated tau (circles) in his brain when he died. Benoit, a 40-year-old professional wrestler (below), had advanced-stage CTE at death. His brain (bottom image, 600x magnification) displayed large amounts of tau tangles (brown spots) compared with a normal brain (purple-stained image).



structures that can be used to monitor ongoing damage in those that have had repetitive head trauma." He hopes to eventually have a set of diagnostics that can help physicians decide when it's time for a fighter to leave the ring.

## GETTING IN THEIR HEADS

Having seen the signs of CTE in more than 100 autopsied brains, Boston University's

McKee has thought a lot about why some athletes succumb to the disease but others don't.

"Some people have tremendous cognitive reserve," she says. For instance, 30% of normal, healthy people over the age of 80 have amyloid- $\beta$  aggregates in their brains but no symptoms of Alzheimer's. According to McKee, their brains seem to be genetically gifted with the ability to function despite these lesions.

The same may be true of CTE: Just because a person begins to accumulate tangled tau fibers in his or her brain doesn't mean he or she will ever display disease symptoms.

Still, McKee has begun piecing together a picture of how she thinks CTE progresses once tau aggregates do appear (*Brain* 2013, DOI: 10.1093/brain/aws307). To do so, she's drawn upon donations to a brain bank she helped establish in 2008 at Edith Nourse Rogers Memorial Veterans Hospital, in Bedford, Mass.

She's characterized the early stages of CTE by dissecting the brains of young people who were involved in contact sports prior to death. For example, McKee found what she dubs stage I CTE in the brain of Eric Pelly, an 18-year-old rugby player for North Allegheny High School, near Pittsburgh, who died after a severe concussion caused his brain to swell.

Although Pelly had no outward symptoms of CTE before his death, his brain tissue revealed the disease's characteristic tangled tau: A few hot spots of the fibrils dotted his cortex, which is the outer, wrinkled part of the brain. These aggregates clustered around his blood vessels and collected in his brain's crevices.

"We're not sure if this first stage always progresses to CTE or whether we should even call it a stage," McKee says. But she is sure that these same tau hot spots also show up in advanced CTE cases in the same places.

And she thinks she understands why.

"Imagine that the brain is Jell-O and that blood vessels are spaghetti noodles encased within," McKee says. "When you shake the whole thing up, there's more damage to the area around the spaghetti than any other area." The reason for this, she says, is that the applied shaking force causes a large amount of stress at the interface between the two materials.

Similarly, the greatest concentration of stress in brain tissue is deep within the

highly folded wrinkles, known as the cortical sulci. So knocks to the head initiate damage here, too.

Why tau protein forms aggregates in those who suffer repeated head trauma, though, is still a mystery. Tau normally helps stabilize a nerve cell's scaffolding system, called the cytoskeleton. For some reason, McKee says, head injury jump-starts a process in which the protein becomes abnormally phosphorylated, breaks off from the cytoskeleton, and begins to aggregate inside neurons.

Whatever the reason for this accumulation, McKee says, more hot spots of tau crop up in the brain's cortex and begin to spread in what she's labeled stage II CTE. In stage III, the tau tangles occupy large swaths of the cortex and even appear in parts of the inner brain. For instance, aggregates show up in the hippocampus, which regulates memory and learning, and the amygdala, responsible for decision making and emotional reactions. By stage IV, "in the worst cases, you can see tau everywhere in the brain," McKee says. Often, it's crept beyond the brain, to the brain stem and spinal cord.

Benoit's brain showed signs of advanced-stage CTE (*J. Forensic Nurs.* 2010, DOI: 10.1111/j.1939-3938.2010.01078.x).

## SCOUTING OUT TAU

Seeing the characteristic pattern of tau in a person's brain during autopsy has helped researchers define CTE. But it's not useful to researchers who want to link the disease with the onset of symptoms. And it's certainly not useful to athletes such as Benoit, who might have benefited from counseling and treatment.

In recent years, scientists have developed a technique that pinpoints molecules such as amyloid- $\beta$  and tau in the living brain. Called positron emission tomography (PET), this imaging method maps features in the brain with radiolabeled compounds. Called radiotracers, these compounds are designed to latch onto specific targets in the brain—tau aggregates, for instance—and emit positrons that are registered by a PET scanner.

Four years ago, when Robert A. Stern was writing grants for a large-scale CTE study, he says it was far-fetched that scientists would be able to see phosphorylated tau in the living brain anytime soon. "Much to my delight, there are a couple groups

who have now done it," says Stern, a neuropsychologist at Boston University who collaborates with McKee.

Stern is working with one of the groups, now at Eli Lilly & Co. subsidiary Avid Radiopharmaceuticals, to test a tau radiotracer in retired NFL players. The trial has been tacked onto Stern's larger study, called DETECT (Diagnosing & Evaluating

Traumatic Encephalopathy using Clinical Tests), which is comparing participants with a control group of age-matched athletes, such as baseball players, who never played contact sports.

In addition to cognitive evaluations, blood tests, and MRI scans, subjects will undergo PET imaging with the experimental tau tracer, T807. Mark A. Mintun, chief

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medical officer at Avid, says T807 binds tightly and selectively to neurofibrillary tangles of tau, not only in the Alzheimer's brain but also in the CTE brain, something Avid confirmed with brain slices donated by McKee.

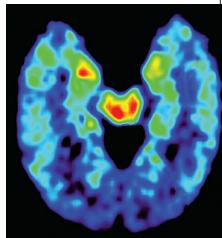
The DETECT study, which recently began conducting PET scans on subjects at Boston University's CTE Center, will help determine whether T807 sticks to tau in a living person's brain and tracks cognitive decline, a vital step in validating the tracer as a CTE diagnostic, Mintun says.

"When it comes to technology and chemistry, in my mind, the most exciting thing going on in CTE diagnostics is this PET imaging," Stern says.

Another group of scientists thinks so too. Led by Jorge R. Barrio, a molecular pharmacologist at the University of California, Los Angeles, this team is testing another radiotracer, FDDNP. Developed a number of years ago, this compound sticks to both tau tangles and amyloid plaques in the brain. Barrio's team is using it to image the brains of former NFL players with memory impairments. In a study last year, the researchers saw FDDNP binding intensity increase with the number of concussions athletes experienced during their careers (*Am. J. Geriatr. Psychiatry* 2013, DOI: 10.1016/j.jagp.2012.11.019).

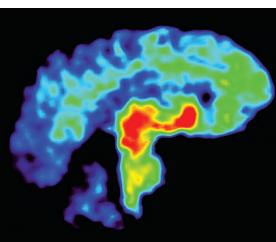
Because FDDNP isn't selective for a single target, some in the CTE community call it a "dirty tracer." Barrio argues that it doesn't matter that FDDNP binds to amyloid because amyloid plaques aren't usually present in the CTE-afflicted brain. McKee's pathology results, however, suggest that amyloid appears in 40 to 45% of CTE cases, albeit as diffuse plaques.

NorthShore's Bailes, who collaborates with Barrio, also supports the use of FDDNP. "If you're looking for Diet Pepsi and you open the refrigerator and there's also Sprite in there, it's okay," Bailes says. "You still found what you're looking for." He adds that FDDNP PET images of a CTE patient and an Alzheimer's patient look completely different because of the radiotracer's intensity and location in the brain.



## CTE Diagnostic Hopefuls

Researchers are testing a battery of techniques to find the ultimate detection tool-kit for CTE.

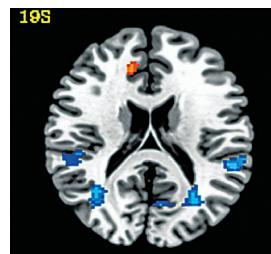


### PET Imaging:

Radiolabeled compounds are injected into a person's body, where they target features in the brain such as aggregated tau protein. Once bound, these radiotracers emit a signal that can be used to map their location. In the image shown, the tracer FDDNP lights up the brain of a person with suspected CTE.

### Diffusion Tensor Imaging:

Water molecules in the brain tend to flow parallel to nerve fibers. This type of MRI measures this flow, which gets disrupted in regions of neuronal damage. In the DTI image shown, blue areas in a boxer's brain indicate decreased neuronal activity, and the orange area indicates increased activity.



### Cerebrospinal Fluid Sampling:

The liquid that bathes the brain and spinal cord may contain molecular markers of CTE. Scientists extract the fluid via lumbar puncture and then analyze its contents with antibody assay kits.



PET = positron emission tomography. MRI = magnetic resonance imaging.

## FLUID MOVEMENT

Taking another page from Alzheimer's research, CTE-focused scientists have also been searching for indicators of the disease in athletes' cerebrospinal fluid. In the

early stages of Alzheimer's, amyloid- $\beta$  decreases and tau increases in the fluid that bathes the brain and spinal cord.

Henrik Zetterberg, a neuroscientist at the University of Gothenburg, in Sweden, has pinpointed cerebrospinal fluid markers for acute head injury. He and his colleagues collected lumbar puncture samples from a group of Swedish Olympic boxers shortly after they'd had a bout. Compared

with a control group, the fluid contained increased levels of tau and neurofilament light protein, typically found in the coatings of long nerve fibers in the brain (*PLoS One* 2012, DOI: 10.1371/journal.pone.0033606).

"If you get a blow to the head that rotates your brain, you get tearing of these fibers," Zetterberg explains. The fibers then shed

components such as neurofilament light.

Zetterberg hasn't yet sought cerebrospinal fluid markers for chronic head injury in retired boxers, so he isn't sure whether his findings will apply to CTE. Boston University's Stern, on the other hand, has already begun to take lumbar punctures as part of his DETECT study. "I was expecting elevated levels of phosphorylated tau in the fluid, and indeed, that's what we're finding," Stern divulges.

## END GAME

It will be a while before neuroscientists can officially label the various methods they've been testing as CTE diagnostics. "The science is still in its infancy," Stern says.

But once they isolate and validate a set of techniques that detect CTE while people are alive, new doors will open. "We'll be able to start doing epidemiological studies to figure out how common CTE is," Stern says. And instead of officials making "knee-jerk responses to media hype," he adds, "we can have scientifically sound data to guide policy and rules for athletes." ■

