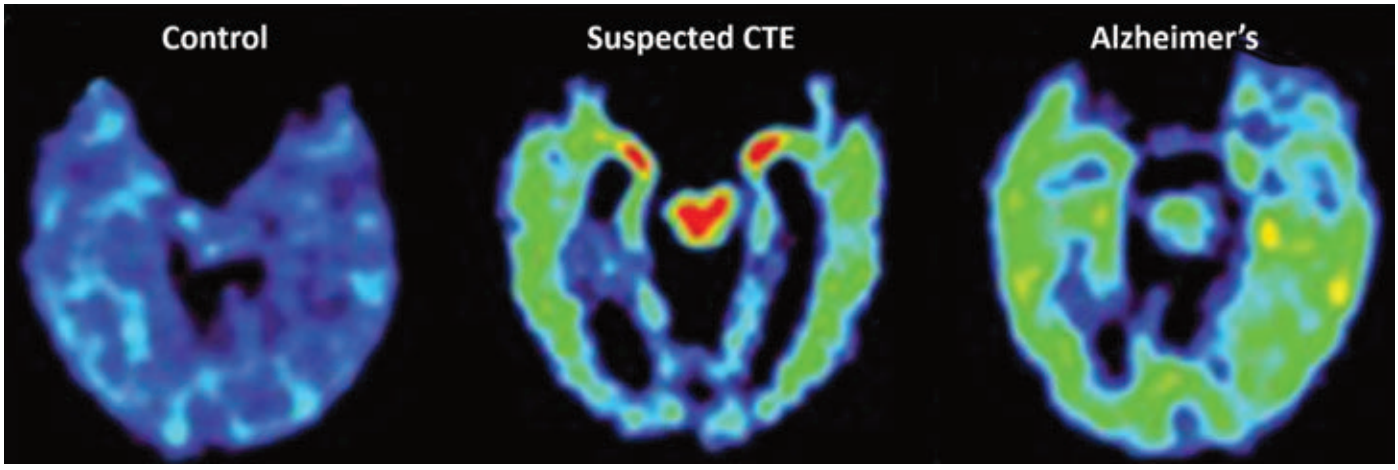
The Real Science Behind Concussions

New imaging techniques are much better equipped to see inside the living brain.

by Michelle Taylor, Editor-in-Chief



Normal PET brain scan (left), brain with suspected CTE (center) and brain with Alzheimer's (right). More red and yellow indicates more tau and amyloid, abnormal brain proteins. The image on the cover is a replication of the center image. Photo: David Geffen School of Medicine at UCLA

On Christmas Day, Sony Pictures released *Concussion*, a biographical sports medical drama film based on research by Dr. Bennet Omalu identifying chronic traumatic encephalopathy (CTE) in retired NFL players. While the movie may be classified as entertainment, the story is real, and the scientific research behind it is very real.

CTE is a type of traumatic brain injury associated with repeated blows to the head. The progressive degenerative disease can be spurred on by symptomatic concussions as well as sub-concussive hits to the head that do not cause immediate symptoms. Thanks to Omalu's work, CTE has most commonly been found in the brains of professional athletes participating in football, but is also associated with other contact sports, such as ice hockey, wrestling and boxing and cheerleading. The disease is characterized by degradation of brain tissue and the accumulation of tau protein, causing symptoms such as memory loss, aggression, confusion and deep depression that generally appear years after initial brain trauma.

Omalu first encountered CTE in 2002 during the autopsy of former Pittsburg Steelers center Mike Webster when he was a forensic pathologist with the Allegheny County, Penn. coroner's office. With the help of former Steelers team doctor Julian Bailes, Omalu published a paper on his findings, which was initially dismissed by the NFL. *Concussion* follows the "David v. Goliath" story of Omalu trying to reveal the truth with the NFL being less than cooperative. Eventually, Omalu is vindicated and amid growing scrutiny from retired players and Congress, the NFL is forced to take the concussion issue more seriously.

Although the circumstances have changed mightily since 2002, the same problem exists today for CTE research—it can only be diagnosed posthumously. However, thanks to continued research by Omalu and fellow researchers at UCLA, as well as significant research out of Boston University, we are getting closer and closer to the goal line of more accurate diagnosis.

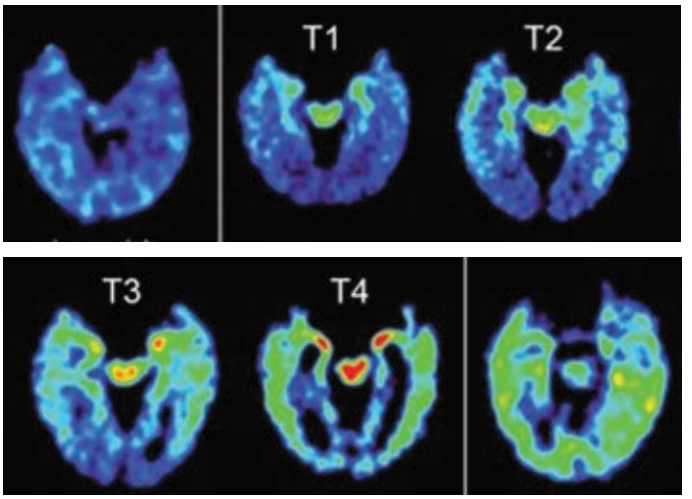
UCLA's PET imaging technique

CTE is characterized by the accumulation of the protein tau in regions of the brain that control mood, cognition and motor function. Tau is also one of the abnormal protein deposits found in the brains of people with Alzheimer's, sometimes making it difficult to image the difference between CTE and Alzheimer's.

In 2013, Gary Small, Director of the Geriatric Psychiatry Memory and Aging Research Center at UCLA, conducted a study that became the first to identify the abnormal tau proteins associated with CTE in five retired NFL players who were still living.

The study—which included Omalu and Bailes as authors—relied on a chemical marker Small and collaborator Jorge Barrio previously created for assessing neurological changes associated with Alzheimer's disease. The marker, FDDNP, binds to deposits of amyloid beta plaques and tau tangles, which are the hallmarks of Alzheimer's.

After the players received



PET scan brain images show four distribution patterns (T1-T4) of the FDDNP signals that reflect different levels of abnormal brain proteins. According to researchers, the patterns may signify early (T1) to advanced (T4) stages of CTE as reflected by increased levels of the abnormal proteins. For comparison, the first PET scan (far left) shows a normal brain and the last PET scan (far right) reflects an Alzheimer's patient's brain. Photo: David Geffen School of Medicine at UCLA

intravenous injections of FDDNP, the researchers used positron emission tomography (PET) imaging to perform brain scans on the living players. Compared to healthy men, the NFL players had elevated levels of FDDNP in the amygdala and subcortical regions of the brain. According to the study, the FDDNP binding patterns in the players' scans were consistent with tau deposit patterns that have been observed at autopsy in CTE cases.

"Early detection of tau proteins may help us understand what is happening sooner in the brains of these injured athletes," Small told *Laboratory Equipment*.

Small and his colleagues further elaborated on this work in an April 2015 paper published in PNAS. The new, larger study included 14 retired NFL players (including the five previously used), in addition to 12 men and 12 women with Alzheimer's disease and 19 men and nine women with healthy brains as controls.

The researchers identified four distinctive patterns of brain FDDNP PET signal in the 14 retired NFL players, each of whom has suffered at least one concussion. The four stages of deposits could signify early to advanced levels of CTE.

According to the paper, the observed deposit patterns are defined as follows:

i) Pattern T1 is predominantly subcortical in brainstem (midbrain) with localized involvement of the limbic medial temporal lobe structures (limited to amygdala).

ii) Pattern T2 shows FDDNP PET signal in all subcortical areas analyzed in this study, in all limbic medial temporal lobe areas [amygdala and medial temporal lobe (MTL); hippocampus, entorhinal cortex, parahippocampalgyrus)], and in parts of the frontal cortex, including anterior cingulate gyrus (ACG).

iii) Pattern T3 shows further increases in signal intensity and pattern complexity: all affected areas in the T2 pattern plus additional cortical areas [posterior cingulate gyrus (PCG), lateral temporal lobe (LTL), and

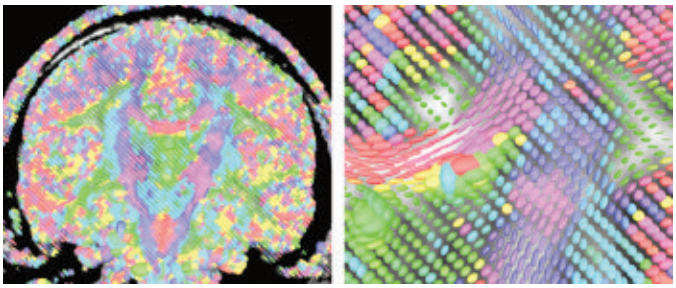
parietal lobe]; this pattern is not associated with severe ventricular enlargement and prominent cortical atrophy commonly observed in aged retired boxers with dementia pugilistica.

iv) Pattern T4 shows high FDDNP PET signal throughout the cortical, subcortical and limbic medial temporal lobe structures, as well as in the white matter areas; this pattern was associated with significant brain atrophy (MRI or CT); possible comorbidity of CTE with other neurodegenerative diseases may be suspected, such as Alzheimer's or end-stage CTE progressing to and simulating Alzheimer's disease.

Verifying—and extending—the results of the 2013 study, the PET scans revealed the former athletes had higher levels of FDDNP in the amygdala and subcortical regions of the brain, which are areas that control learning, memory, behavior, emotions and other mental and physical functions. These are also the types of symptoms experienced by some of the former players in the study, as well as former players diagnosed with CTE postmortem. In contrast, those subjects with Alzheimer's disease had higher levels of FDDNP in areas of the cerebral cortex that control memory, thinking, attention and other cognitive abilities.

"The distribution pattern of the abnormal brain proteins, primarily tau, observed in these PET scans presents a 'fingerprint' characteristic of CTE," said Barrio.

"One of the advantages of FDDNP is it shows both amyloid and tau proteins, which are important in many forms of neurodegeneration," explained Small. "It's not the specific protein that is useful at this point, but it's the pattern of deposition that seems to be informative."



Images generated by diffusion tensor imaging, which is based on the random motion of water molecules in the brain. It is an excellent technique to image microstructural changes in the living brain. Photo: Martha Shenton, BWH, Harvard Medical School

The present work, according to researchers, suggests the use of neuropathology deposition as a brain tissue target for PET molecular imaging probes. But, since tau deposits are not specific to CTE, probes can only provide significant *in vivo* information when combined with the regional sensitivity of PET. The resulting information must then also be in agreement with CTE autopsy results and known mood/cognitive symptoms.

"We want to understand how these imaging tools [like FDDNP PET] predict future cognitive decline," said Small. "For years we've worked with Alzheimer's to try to use these tools to identify problems early so we can test interventions to help people. The article is exciting [since] it shows there are patterns that appear to differentiate these two subject groups."

Multi-modal, multi-center technique

Barrio and Small note in their paper that FDDNP PET scan is just one approach to *in vivo* CTE detection—with alternative techniques such as blood-based biomarkers, functional MRI and diffusion tensor imaging being explored by other researchers.

In fact, on Dec. 22, 2015, researchers from Boston University, the Cleveland Clinic, Banner Alzheimer's Institute and Brigham and Women's Hospital were awarded a seven-year $16 million grant from the National Institutes of Health/National Institute of Neurological Disorders and Stroke (NIH/NINDS). The multi-center grant will be used to create methods for detecting and diagnosing CTE during life, including examining risk factors for CTE through the use of multiple advanced imaging modalities.

The study is headed by Robert Stern, Clinical Core director of the Boston University Alzheimer's Disease and CTE Center. Other PI's include Cleveland Clinic's Jeffrey Cummings; Eric Reiman from the Banner Alzheimer's Institute; and Brigham and Women's Hospital's (BWH) imaging expert Martha Shenton.

"Years ago, people stopped using imaging to detect mild-TBI because, for the most part, it was not informative," said Shenton, Director of the Psychiatry Neuroimaging Laboratory at BWH and professor of psychiatry and radiology at Harvard Medical School. "What this translates to, and what this means, is that the right technology was not developed yet, nor were [the researchers] using the right tools. But we are now."

Among the "right" tools Shenton will be using throughout the duration of the grant is a technique known as diffusion tensor imaging (DTI). General diffusion imaging is a type of MRI developed in the early 1990s for use in humans that is based on the random motion of water molecules. According to a 2012 paper by Shenton, this motion in the brain is affected by the speed of water displacement depending on the tissue properties and type, such as gray or white matter.

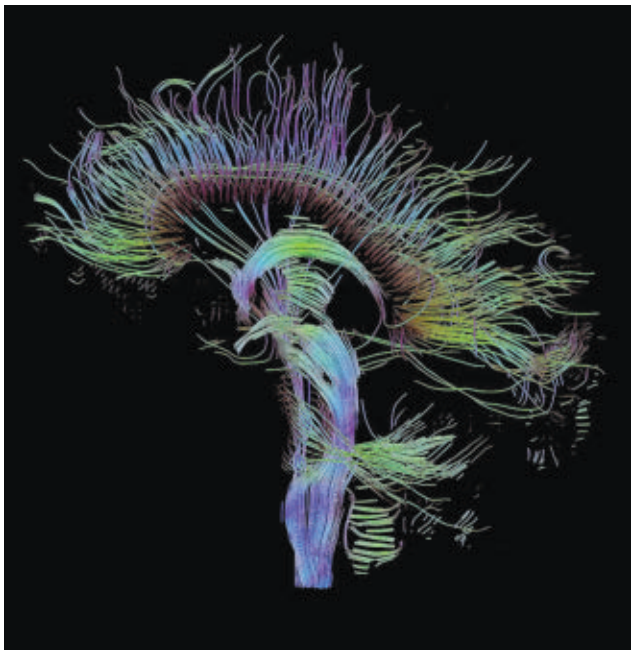
What makes DTI especially suited for *in vivo* CTE brain imaging is its ability to provide information about white matter that is not possible using other imaging techniques. DTI differs from CT and conventional MRI in that it is sensitive to microstructural changes. Thus, subtle changes using DTI can reveal microstructural axonal injuries, which is the most common injury in concussions.

Diffusion tensor imaging data can be used to perform tractography—a 3-D modeling technique used to visually represent neural tracts—within white matter. Tractography is a useful tool for measuring deficits in white matter. Its estimation of fiber orientation and strength is increasingly accurate, making DTI the most sensitive way to view elements of the brain indicative of possible CTE.

The researchers involved in the grant will image retired NFL players, former college football players and a control group of individuals without any history of contact sport or brain injury.

"We're going to be looking at structural imaging for volume or area changes," Shenton told *Laboratory Equipment*. "We will be focusing on neural inflammation since we're going to be examining college athletes as well to see if we can pick up early signs of problems. And we're also going to be using PET with florbetapir to measure the buildup of amyloid beta plaques in the brain, as well as T-807, a tau ligand, to measure the buildup of tau proteins in the brain."

The latter is important as



Tractographic reconstruction of neural connections in the brain via DTI measurement. Depicted are reconstructed fiber tracts that run through the mid-sagittal plane. Photo: Thomas Schultz

tau pathology seems to have a different signature in the brain in postmortem findings where CTE is diagnosed than is observed in, for example, Alzheimer's disease.

The additional use of magnetic resonance spectroscopy will allow the researchers to see any metabolic changes affecting the brain. They intend to use a method to look at many different biochemical peaks in the MRS spectrum, not just the typical five or six peaks researchers use most of the time.

"If you combine a lot of these imaging techniques in the same patients, you're looking at multiple images with different information from each of the imaging modalities," explained Shenton. "It will make a big difference in terms of getting a full picture for us to characterize what is really going on in the brain. You'll have the neural chemistry, the structure and what is going on in terms of changes in the ligands. We think this kind of multi-modal approach is going to be very important to understanding what is going on in the brain so that we can try to pinpoint what some of the consequences of repetitive head impacts are, including CTE. Eventually, we may be able to intercede early to try to prevent the cascade of changes that occur before they become neurodegenerative. Once you are able to diagnose it, you may be able to predict better prognosis. Is there anything you can do that is neuro-protective that will help? You can't even try treatment until you are able to detect and diagnose. That is the first step."

In addition to state-of-the-art imaging methods, participants in the study will undergo extensive clinical examinations, experimental blood tests, genetic testing and head impact exposure tests to refine and validate specific criteria for the diagnosis of CTE.

Not everyone who plays football ends up with a neurodegenerative disease like CTE. That's why it's important to use methods that identify a wider range—not just symptomatic, but asymptomatic as well, said Shenton. This will give researchers a better idea of what the contributing factors of the disease are, what predicts who will get it and who won't, and what makes some brains more prone to CTE than others.

The project involves a group of approximately 50 investigators representing 17 research institutions, with all patient examinations taking place at four centers across the country. This allows the study to combine the efforts of the best researchers with differing/complimentary expertise across the best sites. And—perhaps more importantly—more sites means more subjects.

"This is true in a number of studies," said Shenton. "You are always trying to acquire more and more data. Everything is moving toward multi-site because it allows you to do that. The more you sample anything, the more representative your findings are." LE