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ADVANCES IN NEUROLOGY & NEUROSURGERY

JULY 2010

A Promising New Imaging Technique for Intra-operative Brain Tumor Diagnosis

Pramod Butte, PhD

Gliomas represent 40 percent of all primary brain tumors and treatment of them poses a challenge because of their tendency to infiltrate the surrounding normal brain. The current 18-month survival rate of glioblastoma patients treated with surgery (including biopsy only, partial resection and complete resection) ranges from 15 to 34 percent, putting gliomas among the most lethal tumors.

There are multiple options available for treatment of gliomas. The most common is surgery followed by chemotherapy and radiation therapy, with the extent of tumor resection the single most important factor for longer survival. Several techniques are currently employed by surgeons to ensure near-complete removal of the tumor, including stereotactic image-guided surgery based on pre-operative MRIs, intra-operative magnetic resonance imaging (iMRI) and intra-operative ultrasound. Time-resolved laser-induced fluorescence spectroscopy (TR-LIFS) represents a promising new technique that may similarly assist resection if it can be shown to reliably and safely distinguish between normal tissue and tumors.

Spectroscopy probe placed on the brain tissue to acquire the time-resolved laser-induced fluorescence spectra.

Intra-operative diagnosis

While actual pathological diagnosis of gliomas can only be provided by biopsy and frozen section, TR-LIFS offers the possibility of a useful adjunctive technique for *in-vivo* tissue diagnosis.

A TR-LIFS pilot study* led by Adam Mamelak, MD was conducted at Cedars-Sinai in a limited number of patients undergoing surgical resection of gliomas. The objective of our study was to determine the ability of TR-LIFS to intra-operatively discriminate glioma tumors, both high- and low-grade, from the surrounding normal brain tissue.

Using ultraviolet laser pulses to excite brain tissue during surgery, we recorded the resulting fluorescent light emission from the tissue at 200 picoseconds resolution. Measurements were initially conducted from the core of the tumor followed by the margins. For later histopathologic validation, intra-operative biopsy was conducted at each point of the spectroscopic investigation, except at the areas not considered suitable for physical biopsy due to risks posed to the patient.

Using information gained in this way, we devised a classification algorithm to characterize gliomas based on their fluorescence characteristics. The initial findings are encouraging since we achieved sensitivity and specificity up to 90 percent with TR-LIFS compared to the subsequent pathological diagnosis.

Interestingly, we also noted that the fluorescence characteristics (such as average lifetime) in oligodendral tumors seem to be distinct from those of astral tumors, with oligodendral tumors demonstrating shorted average lifetime at 390 nm of wavelength compared to astral tumors. We believe this may be related to IDH-1 & IDH-2 mutations, which disrupt normal metabolism in tumors and lead to variations in the level of glutamate in

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Theta-Frequency Phase-Locking Predicts Strength of Memory Formation

Adam Mamelak, MD

While it has long been hypothesized that there are preferential modes of brain activity that facilitate the learning of new information, it has been difficult to demonstrate this concept in people. A new research study conducted jointly by researchers at Cedars-Sinai, the California Institute of Technology and Huntington Memorial Hospital and recently published in the journal *Nature*[†], pinpoints a mechanism by which the presence of a specific kind of brain wave allows neurons to work together to improve memory retention.

Background

Theta rhythms are a pattern of synchronized brain activity that occurs in the range of three to eight hertz (Hz). In animals such as rats or mice, the presence of the theta rhythm recorded from the hippocampus has been linked to new memory formation and the ability of an animal to learn its way around a new environment. For example, animals exhibiting high amplitude and frequent hippocampal theta activity learn to navigate a maze quicker than those in which theta is diminished or abolished.

In humans, theta waves are less well understood, but are often observed in transitions between states of waking and sleep, as well as during periods of learning and memory formation.

The medial temporal lobe (predominately made up of the hippocampus and amygdala) is well-known to be a critical structure for learning from novel experiences and maintaining long-term memory. Patients with damage to these structures often have difficulty learning and remembering new information, but can recall information from the distant past. Patients with damage to these structures on both sides of the brain have essentially no capacity to learn new information. In the medial temporal and elsewhere in the brain, the modification of synapses and neuronal circuits—a process termed "plasticity"—is thought to underlie memory formation. In animal models and cell culture experiments, the induction of synaptic plasticity is enhanced by coordinated action potential timing across populations of neurons. Such coordinated activity of neuronal populations can give rise to oscillations of different frequencies, recorded in local field potentials.

Oscillations in the theta range are associated with enhancement of synaptic plasticity as well as behavioral memory in animals, but this result had previously never been observed in humans.

Methodology

Most research on theta waves has been conducted in rats, with only a few studies in humans—in part because EEG electrodes need to be placed directly on the brain's surface for highly precise measurements. This study was conducted with eight volunteers who suffer from epilepsy and were undergoing EEG clinical studies with intracranial electrodes placed to identify the exact site of seizure onset. Several factors were considered to ensure that the patients' underlying medical condition did not affect the study outcome, and electrodes were implanted for clinical reasons only.

The experimental task consisted of a simple learning and recall test (Figure 1), followed immediately by a distracting reaction-time test to prevent subjects from actively rehearsing what they had seen. Fifteen to 30 minutes later, a recognition trial was administered to assess memory retention. While these activities were in progress, we recorded the activity of single neurons, 296 in all, and the local field potentials (EEGs) around these neurons in the medial temporal lobe using microwires implanted bilaterally in the amygdala and hippocampus (Figure 2).

Based on the results of the participants' performances, we used several measures, including spike-field coherence, spike-triggered average and strike-triggered power, to test our hypothesis that successful memory formation is more likely when neurons fire synchronously with their neighbors.

Results

Our data indicate that the timing of individual spikes during learning trials relative to an ongoing theta frequency oscillation can predict the subsequent chance an image will be correctly remembered during the recall phase. We refer to this predictability as memory strength. Memory strength was not influenced by other related factors, such as the neuron firing rate or the amplitude of the theta oscillations. Simply stated, when the individual neurons fired in a set phase synchronized with the surrounding background theta, the subject was far more likely to correctly remember the object than if the neuron firing was not synchronized to a set phase of the theta.

In addition, during the learning process, the phase-locking of spikes to theta waves was significant not only following stimulus presentation but also just prior to onset. This underscores the importance of neural synchrony immediately preceding and during learning. In other words, learning is improved when the medial temporal lobe is in a receptive, synchronous state.

Discussion

While previous studies have identified correlations between memory strength and the activity of neurons in the medial temporal lobe, the relationships between these

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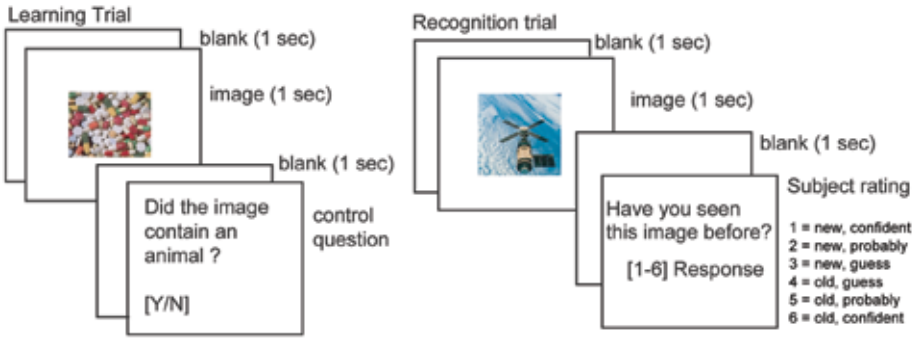

Figure 1: *Subjects were presented with 100 photographs each viewed for one second. Fifteen to 30 minutes later, in a recognition trial, they were shown another set of 100 photos, 50 that were new and 50 that had been in the first set. They were asked to recall which photographs they had seen before and to estimate how confident they were in their answers. Originally published in* Nature *(1).*

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events have not been well understood. Our research shows that when memory-related neurons are well-coordinated to theta waves during the learning process, memories are stronger. We have yet to discover all factors that influence theta oscillations and the coordination of spike timing, but this study establishes a direct relationship between events at the circuit level of the brain—individual neuron spike timing relative to the local brain wave environment—and their effects on human behavior.

Further exploration of these events could have implications for the development of new therapies to treat learning disabilities and some types of dementia.

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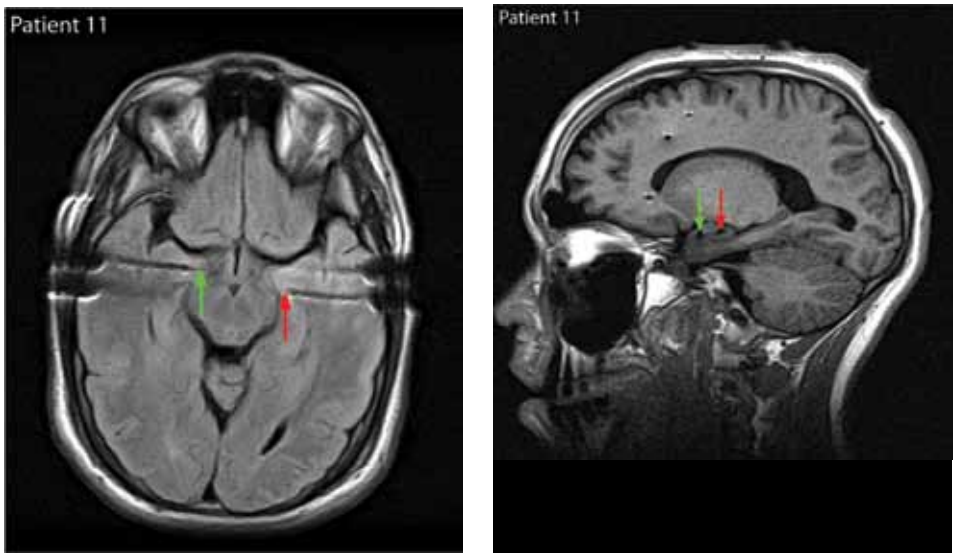


Figure 2: *MRI scans of the brain demonstrating the position of the electrodes used to record neuronal activity. The green arrow indicates an electrode placed in the amygdala and the red arrow placement in the hippocampus. Originally published in* Nature *(1).*

† *IRB #13369.* Rutishauser U, Ross I, Mamelak A, Schuman E. *Nature* 464, 903-907 (8 April 2010).

High-frequency Oscillations in Intracranial EEGs May Pinpoint Seizure's Area of Onset

Jeffrey M. Chung, MD

For the clinician seeking the most effective surgical treatments and the best outcomes for patients suffering from intractable epilepsy, current explorations of high-frequency oscillations (HFOs) may lead to some of the most significant advances in the science of electroencephalography.

German psychiatrist/neurologist Hans Berger recorded the first human electroencephalogram just over 80 years ago, confirming the notion that the brain's electrical activity could be detected, recorded and evaluated. The early ink-on-paper analog systems began the transition to the digital age in the 1980s and 1990s, and over time, technological improvements have increased the available number of sampling channels. This in turn has advanced our abilities in seizure-onset localization.

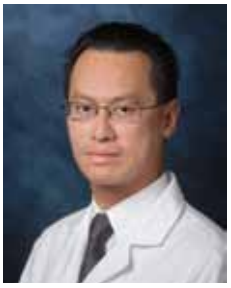
Through most of the history of EEGs, clinical systems have rarely offered a frequency range exceeding 100 Hz. Today, however, the trend is toward sampling at higher rates to record HFOs of 100 Hz, 250 Hz and even 500 Hz and higher.

In animal and human studies conducted in the past decade, such HFOs have been found in both normal and abnormal brain. It is generally believed that some of these oscillations play a role in normal brain function, such as memory encoding. Certain HFOs, however, have been found to occur in the interictal period, as well as at the onset of seizures in epilepsy patients. This observation suggests that detecting and isolating high-frequency oscillations may help in localizing the precise point of seizure onset. Tracked over time, HFOs may also provide hints of the development of epilepsy in an individual without a previous history of seizures.

While new technology allows us to visualize these high-frequency oscillations, the further refinement of devices and methods that will enable epileptologists to effectively apply this information requires the collaboration of clinicians in the field and neurophysiology engineers in the laboratory. The preliminary data from our research collaboration with partners at California Institute of Technology is encouraging and shows promise in identifying the seizure onset area.‡

For patients with intractable epilepsy, surgical resection of seizure-generating tissue offers the greatest opportunity for relief. Identifying the area of onset, evaluating the function of the tissue, and predicting benefits versus risks is a long, complex process. Our hope is that a more complete understanding of HFOs will result in more precise interventions and improved patient outcomes.

‡ *IRB #13369*



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Cerebral Microdialysis Provides Early Warning for Patients with Secondary Brain Injury

Chad Miller, MD

Observation of the natural course of neurological disease has revealed that further neuronal injury is common after a primary neurological insult. Traditionally, this injury was appreciated only when it manifested itself in gross deterioration of motor or cognitive function. In reality, evidence of neurobiological and neurochemical distress proceeds this outward deterioration by hours. Today, the numerous brain monitors employed in specialized neuro-critical care units can detect tell-tale chemical changes in the brain and allow adjustment of oxygenation, perfusion and other clinical parameters to minimize permanent brain injury. Among the most insightful of these tools is cerebral microdialysis.

Cerebral microdialysis is a monitoring technique that allows a real-time assay of various metabolic constituents within the brain. The technique employs a thin flexible catheter with a semi-permeable membrane located at its tip. The catheter is implanted into an area of the brain of particular interest through a small burr hole. Saline is then delivered to the tip through small tubing connected to a micro pump and then circulated back to a collection vial. The fluid collected within the vial has equilibrated with interstitial fluid within the brain through the catheter tip. True analyte concentrations from the brain's interstitial fluid can then be extrapolated from the dialysate after consideration of the properties of analyte recovery.

Dialysate is collected on an hourly basis and analyzed for glucose, pyruvate, lactate, glutamate and glycerol concentrations. These values provide insight into the brain's requirements and use of fuel substrates, neuronal integrity and the presence of cellular distress. Knowledge of the biochemical health of the brain allows for clinical decision-making that takes into account individual patient characteristics, such as level of sedation, cerebral metabolic need, and resilience and impact of intracranial pathology. Without these monitors, patient care relies on generalizations regarding the brain's needs that may or may not be accurate. For example, a cerebral perfusion pressure of 60 mm Hg may be adequate to perfuse the brain of a 23-year-old motor vehicle accident victim suffering from a subdural hemorrhage. However, this same perfusion pressure may prove inadequate in a 73-year-old male with severe carotid stenosis and fever who is suffering from a similar lesion.

Cerebral microdialysis has provided insights into the frequency and the type of secondary injury that occurs after a brain insult. It allows the physician to be proactive in assessing for individual cerebral requirements and enables tailored therapy to be provided at an early stage—when intervention may be more effective in limiting permanent cerebral injury. Cerebral microdialysis technology has been implemented in the care of patients with traumatic brain injury, ischemic and hemorrhagic stroke, and subarachnoid hemorrhage. Its value as a cerebral early warning system is helping intensivists learn more about secondary brain injury and provide improved care to today's brain-injured patient.

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tumor cells. Glutamate regulates the levels of glutamate decarboxylase, an enzyme known to fluoresce at 390 nm. We are in the process of designing a new study to confirm this hypothesis. The ability to detect IDH-1 mutation in tumors is important since the presence of these mutations is known to correlate with a better diagnosis.

At our present stage of research, TR-LIFS does not compare with the existing standard of diagnosis using histopathology. However, our results suggest that this technique may have the potential to reliably delineate brain tumors from normal cortex and help achieve improved tumor excision. The main goal of developing this technique is not to replace the existing gold standard of histopathology, but to provide a additional tool for the neurosurgeon to guide tumor resection.

References:

1. van den Bent M, *et al. New England Journal of Medicine* 360:765-773 (8), February 2009.
2. Yan *et al. New England Journal of Medicine* 360:2248-2249 (21), March 2009.

**IRB #3444. Cedars-Sinai has a relationship with Microsurgeon, Inc., the company sponsoring this study. Cedars-Sinai owns the patent rights on the technology being tested and has licensed this product to Microsurgeon, Inc. In addition, Keith Black, MD, one of the study co-investigators, serves as a Board Member for the sponsor and has a significant financial interest in the sponsor. These relationships have been disclosed to and considered by the Cedars-Sinai IRB in accordance with institutional policy.*

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