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Cholinergic Neurotransmission In Partial Limbic Seizures

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Cholinergic Neurotransmission in Partial Limbic Seizures

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Table of Contents

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Abstract

Cholinergic Neurotransmission in Partial Limbic Seizures. Geoffrey Liu, Joshua E. Motelow, Wei Li, Qiong Zhan, Asht M. Mishra, Robert N. S. Sachdev, Abhijeet Gummadavelli, Zaina Zayyad, Hyun Seung Lee, Victoria Chu, John P. Andrews, Dario J. Englot, Peter Herman, Basavaraju G. Sanganahalli, Fahmeed Hyder, Hal Blumenfeld.

Partial limbic seizures impair consciousness, but the mechanism of impairment is not known. Most views hold that structures necessary for consciousness are disrupted by overexcitation from spread of seizure activity. Against this view, we hypothesize that partial limbic seizures cause pathological long-range inhibition of cortical activity. Using a rat model for partial limbic seizures, we demonstrate BOLD fMRI signal increases in the hippocampal seizure focus, but decreases in arousal promoting regions such as the thalamus and midbrain tegmentum. Second, direct single unit recordings from cholinergic neurons in two arousal nuclei, the basal forebrain and the pedunculopontine tegmental nuclei, demonstrate suppressed firing during seizures. Finally, using enzyme-based amperometry, we probe levels of the arousal neurotransmitter acetylcholine in the cortex and thalamus and observe decreased cholinergic neurotransmission during seizures. These findings demonstrate that an arousal center is suppressed during partial limbic seizures and suggest that decreased arousal may lead to impaired consciousness.

Introduction

The mechanism by which partial seizures originating from the temporal lobe impair consciousness is unknown

Epilepsy is a serious medical condition. Defined as the tendency for recurrent, unprovoked seizures, epilepsy is common (2 million in the United States are affected (Chang and Lowenstein 2003)), debilitating (affecting driving, work performance and leading to social stigmatization (Vickrey, Berg et al. 2000, Drazkowski 2007)), and even lethal (mortality rate 2-3x above the general population (Sperling 2004)).

The most common form of epilepsy — temporal lobe epilepsy (TLE) — is a condition in which seizures originate in the temporal lobe, typically the hippocampus (Engel 1996). Temporal lobe seizures are either confined to the temporal lobe, termed 'partial seizures' or secondarily generalize from the seizure focus in the temporal lobe to the rest of the cortex. Here, we focus on partial seizures originating in the temporal lobe, which can be further subdivided based on whether consciousness is lost or preserved during seizures. 'Simple' partial temporal lobe seizures are seizures in which consciousness is preserved, and typically are characterized by epigastric sensations, emotional changes, and sometimes olfactory hallucinations (Engel 1996). 'Complex' partial seizures, by contrast, are seizures in which consciousness is impaired. Complex partial seizures have a more stereotyped phenomenology: averaging 2 minutes in duration during which patients are unresponsive, with accompanying automatisms such as lip smacking and grunting (Sharbrough 1987).

Despite being the most common form of epilepsy, TLE is arguably the poorest understood. Basic features about the pathophysiology of TLE remain unclear. In particular, one question which has eluded neuroscience research is why partial temporal lobe seizures impair consciousness. It is of interest to neuroscientists because it is not clear why a seizure confined to the temporal lobe would cause impairment of consciousness. The temporal lobe is associated with memory functions, along with audition and olfaction. But the temporal lobe is not typically thought of as a seat of consciousness. The patient HM famously underwent bilateral temporal lobectomy and became unable to encode new memories but was nevertheless conscious (Scoville and Milner 1957). Furthermore, this is a question of deep relevance to patients with TLE, since a considerable portion of patients are treatment refractory (Picot, Baldy-Moulinier et al. 2008). Uncovering the etiology of impaired consciousness in TLE may provide new avenues for preventing loss of consciousness in this treatment-refractory population.

The prevailing theory has limitations

A number of theories have been put forward to explain why complex partial seizures in the temporal lobe cause impaired consciousness (Englot and Blumenfeld 2009, Yu and Blumenfeld 2009). One popular theory posits that onset laterality of the partial seizure is an important determinant for whether or not consciousness will be preserved during partial seizures (Inoue and Mihara 1998, Lux, Kurthen et al. 2002, Hoffmann, Elger et al. 2008). Evidence for this theory comes from the observation that patients with left or bilateral temporal involvement more frequently exhibited impaired consciousness than patients with an isolated right temporal lobe seizure. This led the authors (Lux, Kur-

then et al. 2002) to suppose that the left temporal lobe is necessary for the maintenance of consciousness.

There are several limitations to this theory. 1) Impairment of consciousness in these studies was assessed by verbal responsiveness, thereby confounding consciousness with language and motor domains (i.e. patients are possibly unresponsive not because of impaired consciousness but because of inability to respond due to aphasia or lack of motor control). 2) Some isolated right temporal lobe seizures *did* cause impairment of consciousness, meaning the role of the right temporal lobe in maintenance of consciousness can not be discounted entirely. 3) The patient HM, who underwent bilateral mesial temporal lobectomy had no anterograde memory but was nevertheless conscious (Scoville and Milner 1957), arguing against the necessity of either temporal lobe for maintenance of the conscious state. While the observation that laterality of seizures correlates with impairment of consciousness may have utility as a clinical predictive rule, there are several limitations which make it less attractive as a foundation for a theory of impaired consciousness.

Human EEG demonstrate neocortical slow activity, not seizure activity, during hippocampal seizures

One potential clue to the pathophysiology of impaired consciousness in complex partial seizures comes from clinical studies in humans examining the effects of temporal lobe seizures on areas outside the temporal lobe. In an intracranial electroencephalogram (EEG) study of complex partial seizures (Blumenfeld, Rivera et al. 2004), patients demonstrated, as expected, fast polyspiking activity in the temporal lobe at seizure onset, consistent with seizure activity. Surprisingly however, EEG traces from the frontal

and ipsilateral parietal cortex demonstrated neither baseline activity nor polyspiking activity seen in the temporal lobe but a distinct rhythm altogether: high amplitude, low frequency delta-range slow activity, a pattern reminiscent of slow wave sleep, deep anesthesia and coma (Figure 1) (Steriade, Amzica et al. 1993, Haider, Duque et al. 2006). A follow-up study (Englot, Yang et al. 2010) demonstrated that this relationship between slow activity and impaired consciousness is quantitative: patients with impaired consciousness exhibited considerable delta-range activity in the cortex as assessed by spectral power analysis, whereas patients with preserved consciousness exhibited only modest changes.

These findings replicate prior intracranial EEG studies of complex partial seizures (Lieb, Dasheiff et al. 1991, Franaszczuk, Bergey et al. 1994, Mayanagi, Watanabe et al. 1996), though these authors interpreted slow wave activity as a seizure propagation pattern. This interpretation was based on the observation that slow waves are sometimes seen when recording over a seizure focus using scalp EEG (French, Williamson et al. 1993, Pacia and Ebersole 1997).

If it were the case that neocortical slowing represented seizure propagation, then one would expect to see accompanying increases in cerebral perfusion. Several studies have examined cerebral perfusion by single-photon emission computed tomography (SPECT) in partial seizures in humans (Rabinowicz, Salas et al. 1997, Chang, Zubal et al. 2002, Van Paesschen, Dupont et al. 2003, Blumenfeld, McNally et al. 2004). As expected, hyperperfusion is seen within the temporal lobe in complex partial seizures; however, there is marked *hypoperfusion* in the frontal and parietal association cortices, which is not observed during simple partial seizures. The observation that the frontal

and parietal cortices are hypoperfused during complex partial seizures argues against the interpretation that extratemporal slow activity represents a propagation pattern of seizures, but rather, represents a distinctive depressed state of cortex, perhaps mimicking slow wave sleep.

In summary, human intracranial EEG and functional neuroimaging during complex partial seizures demonstrate distinctive extratemporal manifestations of temporal lobe seizures: 1) a distinctive slow rhythm in the neocortex and 2) hypoperfusion in corresponding cortical regions. Taken together, these data argue that the cortical state during seizures represents a globally depressed state reminiscent of sleep.

A rat seizure model finds slow oscillations during partial temporal lobe seizures; modulated by subcortical inhibitory structures

To study the effects of these partial temporal lobe seizures more closely, our lab recently developed a rat model for partial temporal lobe seizures (Englot, Mishra et al. 2008). The seizure paradigm consisted of an electrical stimulus delivered to the hippocampus. This stimulus generated focal limbic seizure recapitulates several features of human temporal lobe seizures. First, rats experiencing stimulus-induced seizures mimic human symptomatology, including behavioral arrest, staring and facial automatisms. Second, these seizures recapitulate the electrographic and perfusion findings in humans: EEG demonstrated extratemporal slow activity and blood oxygen dependent level functional magnetic resonance (BOLD fMRI) demonstrated decreased activity in corresponding cortical areas (Englot, Mishra et al. 2008, Englot, Modi et al. 2009).

This model has led to several important insights into the mechanism of impaired consciousness in TLE. Recordings of multiple neurons (multi-unit activity) revealed a prominent slow-oscillatory component to slow activity as evidenced by characteristic up and down states (Steriade, McCormick et al. 1993, Crunelli and Hughes 2010). Further studies support the notion that the lateral septum plays an important role in generating slow waves. In particular, lesioning the fornix, which connects the hippocampus and lateral septum abolishes cortical slowing during seizures in this rodent model and stimulating the lateral septum is sufficient to drive slow wave activity in non-seizing animals (Englot, Modi et al. 2009).

In conclusion, rat models of temporal lobe seizures have face validity for human seizures and have led to new insights into the mechanism of impaired consciousness in TLE, including the notion that neocortical slowing is comprised of slow oscillations.

Slow oscillations during physiologic sleep are modulated by subcortical arousal systems

Why would slow oscillations appear in the neocortex during temporal lobe seizures? One possibility is that focal seizures disrupt pathways involved in maintaining arousal, instigating a transition from waking to sleep when slow oscillations appear physiologically. Here, we review what is known about slow oscillations in the context of physiologic sleep. In particular, we focus on their control by subcortical arousal systems such as the acetylcholinergic arousal system.

From a neurobiological standpoint, sleep and waking are brain states defined by their EEG and electromyographic (EMG) properties (Steriade, McCarley et al. 2005, Brown, Basheer et al. 2012). Waking is characterized by low voltage fast activity (LVFA)

and high muscle tone whereas sleep is subdivided into two categories. The first, non-rapid eye movement (NREM) sleep, is defined by high amplitude low frequency activity and decreased muscle tone. The second, REM sleep, is defined by LVFA on EEG, but muscle atonia on EMG.

The slow oscillation is one of the component EEG rhythms of NREM sleep (Steriade, Dossi et al. 1991). It appears at the onset of NREM sleep and disappear during transition to waking or REM. A low frequency (0-1Hz) high amplitude rhythm, the slow oscillation consists of two states. The downgoing (depth-negative) portion of the wave corresponds at the network level to the synchronous depolarization of neurons to threshold potential followed by a series of action potentials, called an 'up state' (Compte, Reig et al. 2008). The upgoing (depth-positive) portion of the wave corresponds to the hyperpolarization of neurons and an arrest of firing, called a 'down state'.

The slow oscillation is primarily a cortically-generated rhythm, and is thought to represent a 'default' rhythm when outside inputs to the cortex are withdrawn. Support for this assertion comes from studies demonstrating that: 1) the slow oscillation is abolished in the thalamus after decortication in cats (Timofeev and Steriade 1996) 2) the slow oscillation survives in the cortex of cats with extensive thalamic lesions (Steriade, Nuñez et al. 1993) and 3) *in vitro* slices of cortex in ferrets are sufficient to generate the rhythm (Sanchez-Vives and McCormick 2000). The function of the slow oscillation as it relates to sleep is not definitively known, but some evidence suggests that the slow oscillation represents an organizing rhythm upon which other NREM sleep rhythms are superimposed. (Steriade, McCormick et al. 1993, Steriade, Nuñez et al. 1993).

Slow oscillations are generated by the cortex when disconnected from subcortical input (Timofeev and Steriade 1996, Sanchez-Vives and McCormick 2000). How does cortical disfacilitation occur in a physiological context to instigate a transition from waking to sleep? Early studies support an important role for the brainstem and basal forebrain. Brainstem transection at the level between the inferior and superior colliculus produced comatose rats (Bremer 1935). Furthermore, brainstem reticular formation stimulation produced fast rhythms associated with waking in anesthetized cats (Moruzzi and Magoun 1949). Since that time, a number of brainstem and basal forebrain arousal systems have been identified, classified by their mode of neurotransmission, including acetylcholine (ACh), norepinephrine, serotonin, dopamine and orexin (Steriade, McCarley et al. 2005, Brown, Basheer et al. 2012).

Of these arousal systems, the acetylcholinergic contributions to the sleep-wake transition are arguably the best studied. The ACh arousal system contains two nuclei: 1) the pedunculopontine tegmental nucleus (PPT) in the brainstem, which sends projections to the thalamus (De Lima and Singer 1987, Hallanger and Levey 1987) and 2) the basal forebrain (BF) which projects diffusely to cortex (MESULAM and MUFSON 1984, Gritti and Mainville 1997). The acetylcholinergic system is thought to promote fast EEG activity (such as gamma, beta and theta rhythms) associated with REM and waking. Conversely, withdrawal of cholinergic stimulation of thalamus and cortex is thought to be important for ushering in slow activity of NREM sleep such as slow oscillations. Evidence for this relationship comes from 4 lines of evidence:

1) BF cholinergic neurons fire more frequently during REM and waking and are less active during NREM (Duque and Balatoni 2000, Szymusiak, Alam et al. 2000,

Manns, Alonso et al. 2003); increased firing of PPT cholinergic neurons anticipate sleep-wake transitions (Steriade, Datta et al. 1990, Boucetta and Jones 2009)

2) ACh concentrations are highest in the cortex and thalamus (targets of basal forebrain and PPT cholinergic neurons) during REM sleep and waking (Celesia and Jasper 1966, Williams and Comisarow 1994)

3) *in vitro* application of cholinergic agonists to cortical (datBuhl, Tamás et al. 1998, Blatow, Rozov et al. 2003) and thalamic (Lörincz, Crunelli et al. 2008) slabs promotes EEG activity associated with waking (alpha, beta, gamma rhythms)

4) stimulation of the PPT cholinergic areas enhances waking rhythms such as gamma and beta (Steriade and Dossi 1991)

While stimulation of the cholinergic system promotes waking, lesion studies of the cholinergic systems have been mixed. Some studies have shown that large lesions to the cholinergic system is sufficient to produce some sleep-"like" changes on EEG, while others show no significant changes. The failure of lesion studies to produce EEG changes is likely from physiological redundancy between multiple arousal systems (Berntson, Shafi et al. 2002, Kaur, Junek et al. 2008).

To summarize, cholinergic nuclei in the BF and PPT fire during waking and REM and shut off during NREM sleep, and cholinergic stimulation is sufficient to generate waking activity on EEG. Thus, the cholinergic arousal system likely plays an important role in the generation of slow oscillations during the wake-sleep transition.

Hypothesis and Specific Aims

Why do focal temporal lobe seizures cause impairment of consciousness? We have presented evidence to support the notion that there is diffuse cortical suppression dur-

ing partial seizures (see 'Human EEG demonstrate…' and 'A rat seizure model'). Furthermore, the appearance of a slow neocortical rhythm on EEG is reminiscent of slow oscillations seen during sleep, coma and anesthesia. It is plausible that focal temporal lobe seizures cause cortical depression by disrupting physiologic pathways important for maintaining arousal, such as those in the brainstem and basal forebrain.

To this end, we propose a network inhibition hypothesis to explain impaired consciousness in focal temporal lobe seizures (Figure 2) (Blumenfeld and Taylor 2003, Blumenfeld 2012). This hypothesis states that focal temporal lobe seizures impair consciousness by seizure spread to subcortical 'inhibitory structures', such as the lateral septum and anterior hypothalamus. These structures, rich in GABAergic connections to brainstem and basal forebrain arousal centers, suppress their firing during seizures (MESULAM and MUFSON 1984, Semba and Fibiger 1992, Varoqueaux and Poulain 1999). The loss of tonic excitation from arousal centers leads to a phenomenon analogous to a rapid wake-sleep transition in the cortex, producing neocortical slow activity on EEG and impairment of consciousness.

This hypothesis makes several testable predictions:

1) limbic and subcortical inhibitory structures such as the lateral septum and anterior hypothalamus should increase their firing during focal temporal lobe seizures; whereas the cortex and subcortical arousal areas should decrease their firing
2) neurons located in arousal nuclei such as the cholinergic basal forebrain and PPT should have depressed firing rates during seizures
3) given suppression of arousal nuclei, arousal neurotransmitter levels should show decreases during seizures

In this thesis, we present three experiments using a rat model (Englot, Mishra et al. 2008) to test these predictions. Previous experiments (Englot, Modi et al. 2009) had imaged rats using blood oxygen-level dependent functional magnetic resonance imaging (BOLD fMRI) during focal temporal seizures, but imaging had been limited to dorsal structures due to technological constraints. In particular, subcortical structures such as the upper brainstem were omitted. Here, we use an improved magnetic fMRI coil to expand our analysis of network activity to include more ventral structures to test prediction 1. Second, using single unit recordings, we assess activity of single cholinergic neurons in the PPT and basal forebrain to test prediction 2. Finally, using enzyme-based amperometry, we evaluate changes in choline, as a proxy for acetylcholine in the cortex and thalamus, where the basal forebrain and PPT project to test prediction 3.

Methods

This section is adapted from (Motelow, Li et al. 2015).

Animal Preparation

All experiments were performed in compliance with institutional animal use policies. One hundred and thirty-eight adult Sprague-Dawley female rats (Charles River laboratories) were used (10 for BOLD fMRI, 108 for juxtacellular recordings, 20 for choline recordings), weighing between 202-365 grams. All experiments shared a common protocol for 1) placement of the HC stimulating electrode 2) stimulation and 3) sacrifice, fixation of tissue and immunohistochemistry.

Placement of hippocampal stimulating electrode

All animals were anesthetized using ketamine (90 mg/kg) and xylazine (15mg/kg) and assessed by toe pinch every 15 minutes to ensure adequate depth of anesthesia. A bore hole was drilled and a biphasic electrode (E363/2-2TW, PlasticsOne for juxtacellular and choline recordings; ~0.1 MΩ, MicroProbes, WE(35)ST30.1A10 for fMRI experiments) was placed at [anteroposterior (AP),-3.8; mediolateral (ML), 2.5; superior–inferior (SI)], 2.6 relative to bregma. The stimulating electrode was cemented to the skull. In BOLD fMRI experiments, surgical placement of the hippocampal stimulating electrode occurred 6 days prior to imaging and at a 50 degree angle to allow greater proximity between the fMRI coil and the skull. In juxtacellular recordings and choline recordings, an-

imals were permitted to return to a light anesthesia phase for same-day seizure induction and experimentation, and a 70-90 degree angle of entry was used.

Seizure induction paradigm

A 2 second long 60Hz biphasic pulse with 1 millisecond phases was delivered through the implanted hippocampal electrode (procedure described above), with current titrated to seizure threshold (between 200uA - 800uA in 200uA increments).

Animal sacrifice and histology

After experimentation, animals were sacrificed using Euthasol (Virbac). Brains were harvested and analyzed histologically to confirm location of electrodes. For juxtacellular recording experiments, prior to brain harvesting rats were perfused with heparinized saline and 4% paraformaldehyde followed by immunohistochemistry (see Methods, juxtacellular recordings).

BOLD fMRI

Six days prior to experimentation, an MRI compatible hippocampal stimulating electrode was placed (described above) and fixed to the skull using 2-4 nylon screws (MN-0265-015P-C, Small Parts, Inc.). On the day of experimentation, animals were anesthetized with a cocktail of ketamine and xylazine (90/15 mg/kg, i.m. q1 hour). Animals were paralyzed with d-tubocurarine (0.5 mg/kg initial dose, 0.25 mg/kg q2 hour maintenance, i.v; Sigma-Aldrich) to decrease motion artifact during imaging, tracheotomized and artificially ventilated (70% air and 30% O2). The femoral artery was

cannulated (Intramedic PE50 tubing; Becton Dickinson) to facilitate arterial-blood gas and pressure monitoring. Carbon fiver electrodes (EL254RT, BIOPAC Systems) were placed between the scalp and the skull in order to obtain scalp EEG.

fMRI imaging was acquired by a modified 9.4T system with Varian spectrometer (Agilent Technologies, Santa Clara, CA) using a custom 2 x 1 1H surface coil in two phases: 1) anatomic imaging and 2) BOLD imaging. Anatomic images were obtained using either gradient echo or fast spin-echo in the coronal plain in 1mm slices. The resolution for acquired images was 0.1mm (superior-inferior) x 0.1mm (medial-lateral) x 1mm (anterior-posterior). BOLD data was acquired in the same plane using spin-echo contrast. BOLD images were acquired in 1 second with a 2 second delay between images. Therefore for a 600 second experiment, there were 200 BOLD acquisitions. Seizures (see induction paradigm above) initiated 1 minute after first acquisition.

t-Map analysis was performed using in-house software developed in Matlab (Mathworks Inc.). Data were masked to remove non-brain pixels. A ten point average BOLD signal was used as a baseline. A mean ictal map for each animal was computed and compared to baseline as a percent change. Statistics were calculated using a 1 sample t-test, taking a p value < 0.05 as a threshold for comparison.

Timecourse data were acquired for all ROIs bilaterally except for the intralaminar thalamus and hippocampus which were imaged on the right side only due to concern for interference from the L hippocampal stimulating wire. ROI were mapped on a high resolution brain atlas. BOLD signal at each acquisition was computed as a percent change for each ROI. For group data, each run was aligned to seizure onset and the first thirty seconds were used to create an averaged curve (3 seconds per acquisition, 10 acquisi-

tions total). From this thirty-second run, mean ictal change was calculated and compared to baseline using a 1-sample t-test to test for significance, correcting with Holm-Bonferonni for multiple comparisons.

Juxtacellular Recordings

For juxtacellular recordings, animals were prepared with a hippocampal stimulating electrode (as above, Animal Preparation, Placement of Hippocampal stimulating electrode). Additionally, in order to measure intracranial EEG from cortex, rats were implanted with a high impedance monopolar electrode in the lateral orbitofrontal cortex at a 20 degree angle, coordinates: (AP, +4.2; ML, 2.2; SI, 2.4) relative to Bregma. Juxtacellular recordings were conducted as described previously (Pinault 1996). Briefly, glass electrodes (World Precision Instruments, #1B150F-4) were pulled using a P-1000 horizontal puller (Sutter Instruments). Electrodes were bumped under a microscope to produce a resistance between 15-30MΩ. Electrodes were filled with neurobiotin (4%; Vector Laboratories, SP-1120), in saline (0.9% NaCl). The pedunculopontine tegmental nucleus (PPT) and basal forebrain were targeted and cells recorded during partial seizures induced using an electrical stimulus as described above (see Animal Preparation, Seizure induction paradigm). Single unit activity was recorded on an Axoclamp-2B amplifier (Molecular Devices). After recording activity during seizures, cells were labelled by injecting neurobiotin during current pulses (5-200nA, pulse duration 150ms, 3Hz) as described (Pinault 1996).

After experimentation, animals were sacrificed and perfused with paraformaldehyde as above (see Animal Preparation, Animal sacrifice and histology). Brainstem was cut at 60 μM and approximately thirty slices were prepared using a vibratome (Leica,

VT1000S). Slices were incubated with cyanine-3 conjugated to streptavidin (1:1000, Jackson ImmunoResearch, #016-160-084) diluted in 5% donkey serum (D9663, Sigma-Aldrich) in PBS-triton (PBS-T; 0.3% triton-X). Slices were washed x3 for 10 minutes in PBS. Slices were incubated with goat anti-choline acetyltransferase antibody (Millipore; 1:500, #AB1449). Slices were washed x3 for 10 minutes in PBS. Slices were incubated with secondary antibody donkey anti-goat (Alexa Fluor 647). Slices were washed in PBS-T x3 for 10 minutes, then one PBS wash for ten minutes and mounted on glass slides for viewing.

Juxtacellular activity was recorded in Spike2 software and analyzed using in-house software programmed in Matlab (Mathworks Inc.). Analyses were grouped into three groups based on their cellular identity per immunhistochemistry: cholinergic, non-cholinergic and unrecovered. Single unit activity during seizures was compared to thirty seconds of baseline activity using a paired t-test, corrected with Holm-Bonferroni for multiple comparisons.. Results are reported as mean change in firing rates ± standard error.

Enzyme-based amperometry

For choline recording experiments, all recordings were performed on amperometric biosensor probes (Quanteon) using a FAST system (FAST16-mkI, Quanteon). Sampling frequency is 2Hz. Three days prior to experimentation choline probes were prepared. Their preparation is described elsewhere in greater detail (Parikh, Pomerleau et al. 2004). Choline probes are ceramic electrodes with four platinum pads (15x333 um; S2, Quanteon). Two pads are coated with choline oxidase (Sigma-Aldrich, C5896-100UN), which converts choline to hydrogen peroxide, an electron donor, which generates a cur-

rent which is captured by the platinum pads. The remaining two pads are not coated in choline oxidase, and capture background electrical noise. The subtraction of the two signals therefore represents the current attributable to choline only. To coat two pads in choline oxidase, choline oxidase is suspended in a mixture of bovine serum albumin (Sigma-Aldrich, C5896-100UN), glutaraldehyde (Sigma-Aldrich, G5882 -10x1ML) and applied under a light microscope to the bottom two electrode pads only. Electrodes are 'cured' in the fridge for 48-72hrs to allow the enzyme to form a solid matrix.

On the day of experimentation, electrodes are plated in methyl-phenyldiamine (m-PD; BRAND) via electropolymerization. m-PD is a size exclusion lattice which prevents large interferents such as dopamine and ascorbic acid from donating electrons. Electrodes were calibrated by fixed voltage amperometry (supplementary Figure 2). A -0.7V voltage was applied between the electrode and a silver/silver chloride reference wire. Using sequential addition of the following substrates into a 40mL solution of PBS (final concentrations), current was recorded: ascorbic acid (250μM), choline (20, 40, 60μM), DA (2μM) and H_2O_2 (8.8μM). Only electrodes that met the following calibration criteria were used for experimentation: > 5 pA/μM sensitivity for detecting choline on the coated electrodes, limit of detection (LOD) < 350 nM choline, ratio of selectivity for choline and AA, >180:1, linearity for detection of increasing analyte concentrations (20-60 μM) on coated electrodes, $R^2 > 0.99$.

Animals were prepared with a hippocampal stimulating electrode as above (see Animal Preparation, Placement of Hippocampal stimulating electrode). Additionally, in order to measure intracranial EEG from cortex, rats were implanted with a high impedance monopolar electrode in the lateral orbitofrontal cortex at a 20 degree angle,

coordinates: (AP, +4.2; ML, 2.2; SI, 2.4) relative to Bregma. Choline recording electrodes were lowered into LOF until “pockets” of choline signal were identified (by sudden rises in current). Peaks were allowed to normalize to baseline before seizures were initiated, using the above stimulation paradigm (see Animal Preparation, Seizure Induction Paradigm). After seizures, animals were given further anesthesia and seizures and toe pinches were administered: 1 minute toe clamp, 2 minutes release, 1 minute toe clamp. After experimentation, animals were sacrificed as above.

Results were smoothed using a 10 point moving average. Signal “drift” (very low frequency oscillations between 0.01 to 0.001Hz) was removed by subtracting a 400 point average. A large artifact was produced in the first 5s of seizure by 2s hippocampal stimulation. This artifact was removed for statistical purposes so as not to skew the drift correction. A thirty second baseline was compared to mean ictal choline signal after discarding the first 12.5s to allow for achievement of steady state. Baseline was also compared to toe pinch after the first 25s to achieve steady state. Data were compared using paired t-tests, correcting for multiple comparisons using the Holm-Bonferroni correction.

Student Contribution

Enzyme-based amperometry had been used in other *in vivo* settings, but prior to this study had never been used to probe choline levels during seizures (Parikh, Kozak et al. 2007, Parikh, Ji et al. 2010). This author optimized and modified the original protocol (Parikh, Pomerleau et al. 2004) for use in an *in vivo* animal seizure model, which entailed maintaining a library of more than fifty biosensor probes and maintaining and teaching the electrode preparation protocol to others in the laboratory. This author per-

formed *in vivo* choline recordings in twelve animals, which generated 42% of data reported in the Choline Recordings section of this thesis (n=4 for choline recordings during partial seizures, n=4 for choline recordings during generalized seizures n=4 for choline recordings during toe pinch). This writer also assisted in preparation of choline recording figures, and prepared figure 2.

Results

fMRI shows decreased BOLD activity in arousal centers

Previous work in our lab demonstrated subcortical BOLD increases and cortical BOLD decreases during partial seizures in rats (Englot, Modi et al. 2009). However, due to technical limitations, ventral structures such as the anterior hypothalamus and midbrain tegmentum were poorly visualized. In order to clarify activities in ventral regions during partial seizures, we performed fMRI using an improved fMRI coil in rats during partial seizures (n=10 animals, 34 seizures, mean seizure duration: 70.72±4.01 seconds) induced by an electrical stimulus delivered to the hippocampus as described previously (Englot, Mishra et al. 2008, Englot, Modi et al. 2009).

Our t-map BOLD results indicate decreases in the lateral orbitofrontal cortex (LO) and increases in the hippocampus, which is consistent with prior investigations (Englot, Mishra et al. 2008, Englot, Modi et al. 2009). However, our t-map data also demonstrate BOLD decreases in the intralaminar thalamus, midbrain tegmentum and anterior hypothalamus, areas which have been traditionally implicated in arousal (figure 3) (Steriade, McCormick et al. 1993, Saper, Scammell et al. 2005).

In order to clarify the time course of these changes, we repeated the analysis while quantifying BOLD changes in these regions of interest (ROI) as a function of time: the lateral orbitofrontal cortex, the hippocampus, the intralaminar thalamus, the anterior hypothalamus, and the midbrain tegmentum (figure 3). We also selected the lateral septum as an ROI given previous work implicating this area in the generation of cortical

slowing (Englot, Modi et al. 2009). We observed ictal BOLD increases in three regions: the hippocampus, lateral septum and anterior hypothalamus (figure 4), followed by decline post-ictally and eventual return to baseline (figure 4). We observed ictal BOLD decreases in three regions: the lateral orbitofrontal cortex, the midbrain tegmenjtum and the intralaminar thalamus, followed by continued post-ictal decrease and eventual return to baseline (figure 4).

Cholinergic neurons in a brainstem arousal center demonstrate suppression of firing by juxtacellular recordings during seizures

Since fMRI demonstrated BOLD decreases in midbrain tegmentum, we speculated that a nucleus located in this region, the pedunculopontine tegmental nucleus (PPT), may show depressed activity during partial seizures. The PPT is a heterogeneous nucleus harboring both cholinergic and non-cholinergic populations (Hallanger and Levey 1987, Semba and Fibiger 1992). Cholinergic neurons in the PPT have been implicated in the sleep-wake transitions (Steriade, Datta et al. 1990, Steriade and Dossi 1991). We reasoned that the PPT may be involved in the generation of cortical slow waves during partial seizures. In order to determine whether there was depression of activity in the PPT during partial seizures, we performed single-cell recordings of PPT neurons.

A representative recording for a cholinergic PPT neuron is shown (Figure 5). Neurobiotin is injected via the single-cell recording electrode, marking the cell during histology. This neuron costains with choline acetyltransferase, which is a marker for cholinergic neurons (Figure 5b). At baseline, the cell is tonically firing (Figure 5a). However, during a seizure, there are slow oscillations in the cortex (as described previously (Blumenfeld,

McNally et al. 2004, Englot and Blumenfeld 2009)), and a decrease in firing in this cell. Post-ictally, cell firing normalizes after a period of recovery.

Other cholinergic cells (n=8, 8 animals) in the PPT displayed a similar pattern of ictal suppression followed by postictal recovery and eventual normalization (figure 7a-b; mean ictal change in firing rate -2.31 ± 0.71 Hz, paired t-test corrected by Holm-Bonferroni method, $P < 0.05$). By contrast, non-cholinergic cells (n=21, 19 animals) in the PPT, thought to consist primarily of glutamatergic and GABAergic cell populations, in aggregate showed no significant change from baseline firing (fig 7c-d; mean change +1.04 ± 3.45 Hz, paired t-test corrected by Holm-Bonferroni method, $P > 0.05$).

Given suppression of activity in cholinergic neurons in the PPT during partial seizures, we speculated that basal forebrain (BF) cholinergic neurons may also alter their firing patterns during seizures. The basal forebrain and the PPT are the two major outputs for acetylcholine in the brain (Brown, Basheer et al. 2012). Like the PPT, basal forebrain cholinergic neurons have also been shown to be important for sleep-wake transitions (Duque and Balatoni 2000, Szymusiak, Alam et al. 2000, Lee, Hassani et al. 2005). To probe activity of BF neurons during seizures, we performed single-cell recordings on these cells.

Like PPT neurons, we observed decreases in firing during seizures in BF cholinergic neurons (figure 7e-f; mean change in firing rate: -4.36 Hz ± 1.01 Hz; Holm-Bonferonni corrected, paired t-test, $P < 0.05$; 7 neurons from 6 animals; mean seizure duration: 77.21 seconds ± 16.17 seconds.). An example recording is depicted (figure 6).

In-non cholinergic BF neurons, some cells increased in firing, decreased, or remained the same. In the aggregate, cells showed no significant change from baseline

firing was observed (figure 7g-h; mean change in firing rate -1.97 Hz ± 1.71 Hz; paired t-test, uncorrected P = 0.4; 18 neurons from 12 animals, mean seizure duration: 86.61 seconds ± 10.16 seconds)

Choline recordings from the thalamus and cortex demonstrate decreases during partial seizures

The primary components of the cholinergic arousal system are the PPT and the basal forebrain (Steriade, McCarley et al. 2005, Brown, Basheer et al. 2012). The cholinergic basal forebrain projects primarily to cortex, while the cholinergic PPT projects to the thalamus (Hallanger and Levey 1987, Gritti and Mainville 1997). Having demonstrated suppression of cholinergic PPT neurons by single cell recordings (Figures 5-7), we sought to determine whether this suppression leads to detectable changes in levels of acetylcholine in thalamus and cortex.

To probe acetylcholine levels in the cortex and thalamus, we employ an enzyme-based amperometry assay. Traditional techniques such as microdialysis have a temporal resolution on the order of minutes (Marrosu, Portas et al. 1995). Since seizures produced in our model averaged approximately one minute in length, we reasoned that the temporal resolution of microdialysis was insufficient to capture what were likely rapid changes in acetylcholine levels on the order of seconds. Enzyme-based amperometry samples choline, as a proxy for acetylcholine at 2Hz, allowing for more rapid detection of changes in acetylcholine.

An example choline trace, recording from the lateral orbitofrontal cortex is depicted in figure 9. Partial seizures induced by hippocampal stimulation induce hippocampal

polyspiking activity and cortical slow oscillations (Figure 9b). After a brief stimulus artifact, in which signal rises in all four leads, the reference electrodes normalize to baseline whereas the choline-oxidase coated electrodes demonstrate decreases with eventual normalization several minutes post-ictally (figure 9a). The subtracted choline signal (blue line), representing the subtraction of the choline-oxidase-coated electrodes from the reference electrodes demonstrate choline decreases ictally, with eventual normalization after approximately five minutes.

In the aggregate (n=6 animals), choline signal decreased significantly during seizures when recording from the lateral orbitofrontal cortex (Figure 10a; -0.086 ± 0.032 μM, paired t-test corrected by Holm-Bonferroni method, $P < 0.05$). Choline recordings from the intralaminar CL nucleus thalamus also demonstrated significant choline decreases (Figure 10c; n=5 animals, -0.031 ± 0.009 μM, paired t-test corrected by Holm-Bonferroni method, $P < 0.05$).

While we had validated the fidelity of our assay *in vitro* (figure 8) to detect changes in choline concentration, we sought to validate the fidelity of our assay *in vivo*. Having shown that the assay is sensitive to choline decreases, we sought to measure choline *in vivo* under conditions in which we would expect choline to increase: 1) secondarily generalized seizures and 2) toe pinch. We reasoned that secondarily generalized seizures, involving excitatory activity in many brain regions, may spread to cholinergic neurons and cause choline increases in the thalamus in cortex. As a physiologic *in vivo* control, we also employed toe pinch, which is known to increase firing in cholinergic PPT neurons and increase cortical acetylcholine (Boucetta and Jones 2009).

Our choline recordings from generalized seizures show significant increases when recording from both the LO cortex and intralaminar CL nucleus in the thalamus (figure 10b,d,e; LO cortex: 5 animals, +0.076 ± 0.011 μM, paired t-test corrected by Holm-Bonferroni method, $P < 0.05$; thalamus: 7 animals, +0.030 ± 0.009 μM, paired t-test corrected by Holm-Bonferroni method, $P < 0.05$). Our choline recordings during toe pinch show significant increases in the cortex and thalamus as well (Fig. 10e; cortex: 9 animals, +0.122 ± 0.032 μM, paired t-test corrected by Holm-Bonferroni method, $P < 0.05$; thalamus: 8 animals, +0.040 ± 0.014 μM, paired t-test corrected by Holm-Bonferroni method, $P < 0.05$).

Discussion

Why do complex partial seizures cause impaired consciousness? Here, we present findings that support a network inhibition hypothesis (Blumenfeld and Taylor 2003, Blumenfeld 2012): focal seizures originating in the hippocampus cause inhibition of arousal systems leading to long-range deactivation of the cortex. First, we find BOLD fMRI signal increases in the hippocampus and lateral septum, alongside BOLD decreases in the cortex which replicates prior studies in rodents (Englot, Mishra et al. 2008, Englot, Modi et al. 2009). We also find BOLD decreases in the midbrain tegmentum, which has not been observed previously and suggests that the reticular activating systems may be suppressed during seizures. Given the possibility of suppressed activity in reticular activating systems during seizures, we probed the cholinergic neurons in PPT and basal forebrain, given the importance of cholinergic nuclei in sleep-wake transitions (Steriade, Datta et al. 1990, Steriade and Dossi 1991, Steriade, Amzica et al. 1993). There, we find marked depressed activity in cholinergic PPT and basal forebrain neurons during seizures. By contrast, we failed to observe an aggregate change in the firing patterns of non-cholinergic. Finally, to correlate decreased firing in these nuclei with neurotransmission, we assessed choline, as a proxy for acetylcholine, in the cortex and thalamus. We find transient choline decreases during seizures. Taken together, these findings provide strong evidence that suppression of arousal is an important mechanism for impaired consciousness during focal temporal lobe seizures.

Our theory coheres with current clinical findings in patients with partial seizures. A recent study examined responsiveness across multiple response domains in patients experiencing partial seizures (e.g. ability to recall name, identify a watch etc.) (Cunningham, Chen et al. 2014). Interestingly, the authors found that responsiveness across domains was either more or less preserved or entirely impaired. The bimodal distribution of partial seizures is consistent with the notion that complex partial seizures are disorders of arousal, affecting all domains of brain activity globally, rather than selective impairment of individual domains.

Our theory is discrepant with a recent imaging study of patients during sleep. In a study of 14 human subjects, the authors of this study report activity BOLD signal *increases* within frontal areas during slow oscillations in NREM sleep (Dang-Vu 2008). This finding contrasts with our interpretation of BOLD decreases in cortex of rodents as evidence of a transition to a sleep-like state. However, one explanation for the discrepancy is our different comparison groups. In their study, they compare BOLD signal during slow oscillations to non-slow oscillation rhythm, though both signals are recorded during NREM sleep. This finding is nonetheless compatible with an overall signal decrease from waking to NREM sleep. Indeed, multiple studies by PET and fMRI validate this position (Braun, Balkin et al. 1997, Maquet 2000, Nofzinger, Buysse et al. 2002, Kaufmann, Wehrle et al. 2006). Thus, this study does not contradict our interpretation of BOLD signal decreases during partial seizures as evidence of a sleep-like state.

A limitation of our series of experiments was that we did not demonstrate a causal relationship between suppressed arousal and impaired consciousness. However, complementary studies do support a causal relationship. Optogenetic stimulation of PPT

cholinergic neurons decreases slow activity during seizures in rats (Furman, Motelow et al. 2013). Furthermore, thalamic stimulation of the intralaminar CL nucleus, a target of PPT cholinergic neurons, reduces slowing and prevents behavioral arrest during seizures (Gummadavelli, Motelow et al. 2014). It is unlikely that suppression of the cholinergic system alone is sufficient to generate cortical slowing and impaired consciousness since lesioning the basal forebrain or the PPT produces only modest effects on the EEG and behavior (Berntson, Shafi et al. 2002, Kaur, Junek et al. 2008). The absence of a dramatic response in lesion studies is likely a result of redundancy in multiple arousal systems including orexin, norepinepherine, dopamine, serotonin (Saper, Scammell et al. 2005, Steriade, McCarley et al. 2005, Brown, Basheer et al. 2012). The response of these other arousal systems during seizures is unknown and worthy of further investigation.

Future directions

The finding that an arousal system is suppressed during seizures raises a number of additional questions. We have advanced a theory that seizure spread to nearby inhibitory structures causes deactivation of arousal systems. However, the identity of these inhibitory structures is unknown. Our BOLD data suggest that the anterior hypothalamus and the lateral septum are candidate regions, given that they demonstrate BOLD increases during seizures and are known to be rich in GABAergic neurons (MESULAM and MUFSON 1984, Semba and Fibiger 1992, Varoqueaux and Poulain 1999, McGinty and Szymusiak 2001). Furthermore, lesioning the fornix in rats, the connection between the hippocampus and lateral septum, abolishes ictal neocortical slowing and stimulation of the lateral septum alone is sufficient to produce neocortical slowing (Englot, Modi et

al. 2009). Future investigations into these and other candidate areas will be helpful in clarifying the mechanism of impaired consciousness in focal seizures and providing new targets for intervention.

Another question this finding raises is whether chronic pathological suppression of an arousal system (i.e. repeat seizure episodes) induces plastic changes. For example, patients with epilepsy have a higher incidence of comorbid dysomnias and report poorer quality of sleep (Bazil 2003, Weerd, Haas et al. 2004). This observation has typically been attributed to reduction in sleep quality from anti-epileptic medications (Gigli, Placidi et al. 1997). However, our study raises the possibility that long term synaptic changes in this hippocampal-arousal system circuit may underpin sleep disturbances in patients with TLE. One approach to begin investigating this possibility would be to probe for differences in patterns of gene expression before and after repeat induced seizures in rats. Regions of interest would be areas predicted to be excited during hippocampal seizures (lateral septum, anterior hypothalamus) and suppressed (basal forebrain, PPT). DNA microarray has emerged as a powerful tool of interrogating gene expression changes in the setting of neuronal plasticity (Tropea, Kreiman et al. 2006, Cazzin, Mion et al. 2011). Comparing DNA microarrays may provide a window into candidate genes and perhaps an opportunity to reverse or prevent potentially pathological plastic changes from repeat seizures.

Similarly, this finding also casts a familiar clinical observation seen in patients with TLE in a new light. Sleep deprivation is classically cited as a trigger for seizures in epileptic patients (Rajna and Veres 1993, Bazil 2003, Badawy, Curatolo et al. 2006, Haut, Hall et al. 2007). Is it possible that sleep deprivation could drive the hippocampal-

arousal circuit in reverse? One important investigation to shed light on this question would be to examine the behavior of the ventrolateral preoptic nucleus (VLPO) during seizures. The VLPO has a number of reciprocal connections with arousal nuclei, including the PPT, and it seems to act as a barometer of sleep deprivation: firing rate more than quadruples at the initiation of sleep after a period of sleep deprivation (Saper, Chou et al. 2001)

Finally, this finding raises important possibilities for new interventions in treatment refractory TLE. Epilepsy is a debilitating condition which impairs a patient's ability to drive, work, learn and function in a social context (Sperling 2004, Drazkowski 2007). Although epilepsy is a condition with effective pharmacologic treatment, a considerable portion of patients are treatment resistant, with estimates as high as 25% (Picot, Baldy-Moulinier et al. 2008). In this treatment-refractory population, can we prevent loss of consciousness during partial seizures? This study suggests a number of promising avenues for intervention. First, this work implies that complex partial seizures are disorders of arousal. An arousal-promoting medication such as modafinil may improve alertness during seizures (Ballon and Feifel 2006). A second possibility is deep brain stimulation of arousal-promoting regions during seizures to prevent loss of consciousness. Indeed, a proof-of-concept study demonstrates that stimulation of the CL nucleus of the thalamus in rats during partial seizures maintains arousal (Gummadavelli, Motelow et al. 2014).

Conclusion

What is consciousness and how does it arise from the human brain? For neuroscience, this problem has fascinated researchers for decades. Importantly, for patients,

this problem can have severe impact on quality of life. Here, in this series of experiments, we scratch the surface of this question. We demonstrate, for the first time, the suppression of an arousal center during a focal temporal lobe seizure in rats. Based on this finding, we propose a new paradigm for how we understand partial seizures: some partial seizures, it would seem, put patients transiently to sleep.

Appendix 1: a primer on enzyme-based amperometry

In this thesis, we present three experiments to investigate the cause of impaired consciousness in temporal lobe epilepsy. Two of these experiments rely on established experimental methods: BOLD fMRI and juxtacellular recordings. However, one experiment employs enzyme based amperometry, a relatively novel technique, which is worthy of further discussion. Here, we review the rationale for enzyme-based amperometry in the context of this particular scientific problem, the mechanism of choline detection, the extent of its validation with controls and finally its limitations. We conclude that amperometry is the most appropriate assay to study this scientific problem particularly given the limitations of its alternatives such as microdialysis.

We have proposed a network inhibition hypothesis for impaired consciousness during partial temporal lobe seizures (figure 2). One prediction of this hypothesis is that suppression of arousal centers during partial seizures leads to decreased levels of arousal neurotransmitters in the cortex, such as acetylcholine. Therefore, we sought a method to capture changes in acetylcholine levels during seizures.

Microdialysis is a technique pioneered in 1985 and relies on the principle that the concentration of a substrate of interest (e.g. acetylcholine) should equilibrate between two fluid compartments separated by a semi-permeable membrane (Osborne, O'Connor et al. 1991, Pepeu and Giovannini 2004, Nirogi, Mudigonda et al. 2010). Exploiting this principle, the investigator inserts a probe consisting of a dialysis membrane at its tip into a brain region of interest, and draws off serial vials of dialysate. Acetylcholine concen-

tration is then probed by analytic chemistry, typically high-performance liquid chromatography, though other techniques are under development (Wang, Roman et al. 2009, Nirogi, Mudigonda et al. 2010). This technique is highly sensitive (lower limit of detection of 50μM) (Wang, Roman et al. 2009) and well-validated in live animals (mOsborne, O'Connor et al. 1991, Williams and Comisarow 1994, Marrosu, Portas et al. 1995). However, one feature of this assay which limits its application to our scientific problem is its temporal resolution: established microdialysis techniques can sample, at best, once every 2.4 minutes (Schultz and Kennedy 2008, Wang, Roman et al. 2009). Problematically though, induced seizures are transient events (in our model, the average seizure length was ~1 minute, see Results). We reasoned that any changes in acetylcholine, if present, would happen within this time frame, meaning that a sampling rate of once per 2.4 minutes may conceivably miss rapid changes. This limitation made microdialysis less than ideal for the study of ictal acetylcholine concentrations.

Recently, an enzyme-based microelectrode assay to detect choline, as a proxy for acetylcholine, was developed (Burmeister, Palmer et al. 2003). Electrochemical methods, broadly speaking, involve converting a non-electroactive substrate of interest (such as choline) into an electroactive reporter such as hydrogen peroxide. An electrode probe then detects current generated by the reporter, the magnitude of which can then be back-correlated to the concentration of the substance of interest. For the choline probes, Burmeister and colleagues apply a choline oxidase coating (which converts choline to hydrogen peroxide) to a micro electrode (figure 11). Then, using known concentrations of choline in vitro, investigators can generate a choline-response curve which can be extrapolated to infer concentrations of choline in vivo (a sample calibra-

tion, figure 8). Importantly, this electrochemical technique has a theoretical temporal resolution upwards of 20,000Hz, though it has been currently validated up to 2Hz (Parikh, Pomerleau et al. 2004).

One historical limitation of this technique is that electroactive interferents in the extracellular space such as ascorbic acid and dopamine typically drown out the signal. To overcome this pitfall, they make two additional modifications (Parikh, Pomerleau et al. 2004). First, a size exclusion lattice of methyl-phenyl diamine is applied to prevent penetration of interferents into the electrode space. Second, each choline-oxidase channel (a total of two per electrode) is normalized to a non-choline oxidase coated channel, further improving signal-to-noise ratio.

Electrochemical choline probes have been validated in both exogenous and endogenous control paradigms. Exogenous addition of choline and acetylcholine into the same stereotactic space as the choline probe produced spikes in current (Parikh, Pomerleau et al. 2004, Parikh, Apparsundaram et al. 2006). Potassium chloride, a depolarizing agent, also produced choline spikes, presumably due to depolarization of the presynaptic cell and acetylcholinergic vesicular fusion (Parikh, Apparsundaram et al. 2006). Finally, various biochemical manipulations produced their expected results: 1) application of the acetylcholinesterase inhibitor neostigmine decreased choline signal (due to decreased breakdown from acetylcholine to choline) 2) addition of tetrodotoxin-1, an agent which prevents vesicular fusion, decreased choline signal and 3) the addition of hemicholinum-3, a presynaptic choline reuptake inhibitor, causes increase in choline concentration (Parikh, Kozak et al. 2007). Taken together, these findings argue in favor of the robustness of this assay.

Since its initial introduction, choline biosensors have been employed in several scientific studies to yield novel scientific insights. In one study, investigators found that acetylcholine spikes in the cortex (as detected by choline biosensors) predicted cue detection in rats (Parikh, Kozak et al. 2007). Another study found that in dopamine transporter knocked-down mice, there is decreased capacity for choline reuptake, which was inferred from experiments performed using these probes (Parikh, Apparsundaram et al. 2006). Finally, investigations into glutamate neurotransmission employing electrochemical probes with the same basic design have yielded important insights into altered glutamate signaling in animal models of Parkinson's (Fan, Zhao et al. 2014). Despite the relative novelty of the technique, electrochemical sensors have been quickly assimilated into the neuroscientist toolkit.

One main criticism of enzyme-based amperometry is that choline (which is directly measured) is not necessarily a reliable proxy for acetylcholine. For instance, varying concentrations of acetylcholinesterase at the synapse could conceivably impact the rate of generation of choline. While it is true that there may be theoretical conditions under which choline and acetylcholine concentrations uncouple, this is unlikely to make an measurable difference in the physiologic context. Acetylcholinesterase has the highest rates of turnover of any enzyme with a KES of 104 s-1, which is equivalent to 25,000 molecules of acetylcholine converted per molecule of acetylcholinesterase per second (Quinn 1987). Therefore, even wide variation in concentrations of acetylcholinesterase are unlikely to limit the reaction rate and therefore the generation of choline from acetylcholine. Furthermore, in this study, we seek to answer a question with a qualitative answer: whether or not cortical and thalamic choline decreases, increases or remains the

same during hippocampal seizures. While reaction kinetics and local variation in receptor count/acetylcholinesterase concentration may impact the quantity of choline generated, it will not affect the direction of the relationship (e.g. choline will not decrease in the face of choline increases). For these reasons, we find that enzyme-based amperometry is better suited to this scientific problem than microdialysis.

Appendix 2: Figures

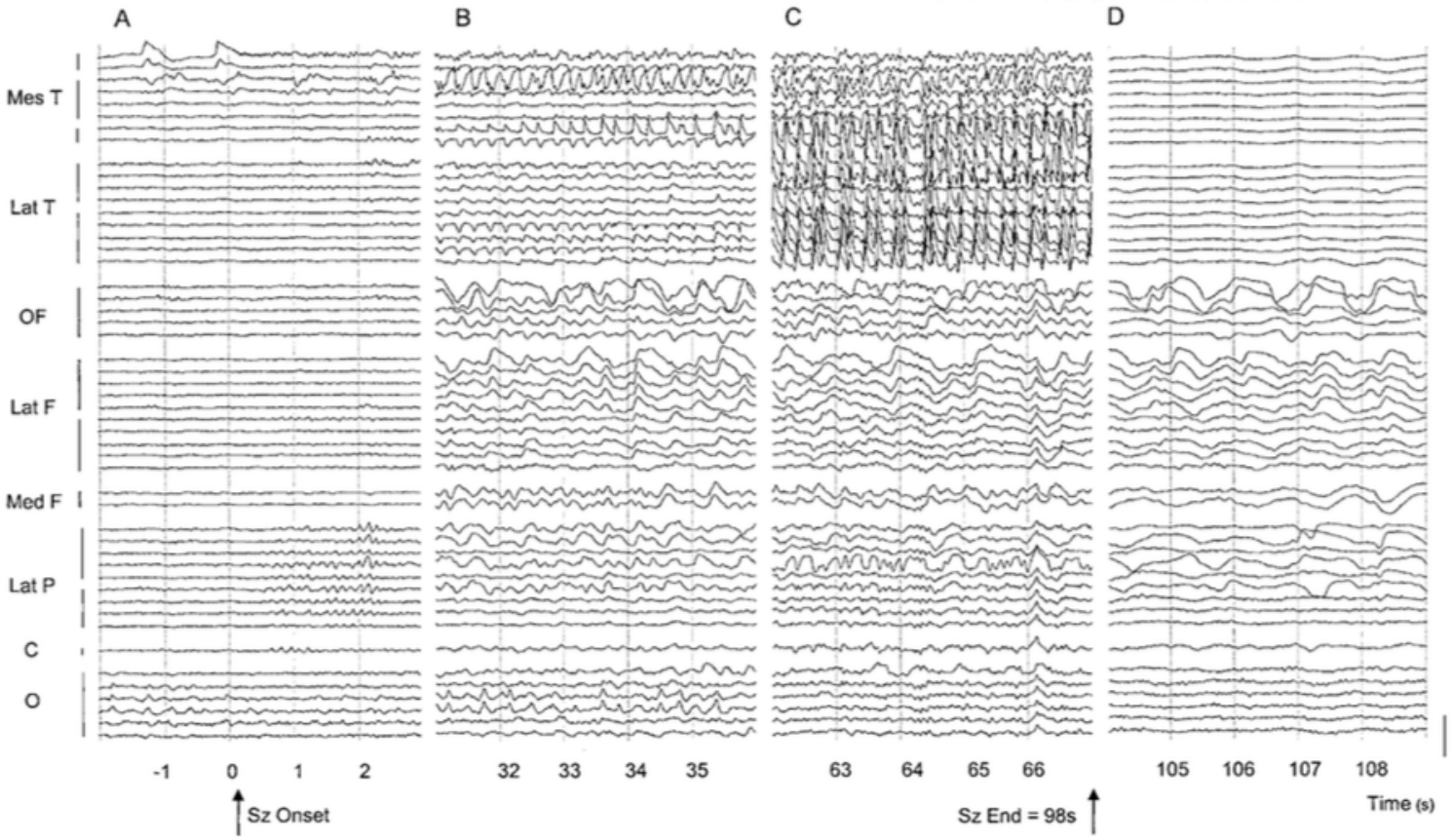

Figure 1: human intracranial EEG during focal temporal lobe seizures demonstrates neocortical slow activity. A representative intracranial EEG trace from various cortical leads at temporal lobe seizures onset (A), 30 seconds into seizure (B), 60 seconds into seizure (C) and post-ictally at 100s (D). The seizure ends at 98s. Seizure activity in the temporal lobe is accompanied by slow activity most prominently in lateral and orbitofrontal leads thirty seconds after seizure onset (B). The occipital and perirolandic areas are relatively spared. The phenomenon continues post-ictally. Mes T - mesial temporal; Lat T - lateral temporal; OF - orbitofrontal; Lat F - lateral frontal; Med F - medial frontal; Lat P - lateral parietal; C - perirolandic; O - occipital. Adapted from (Blumenfeld, Rivera et al. 2004)

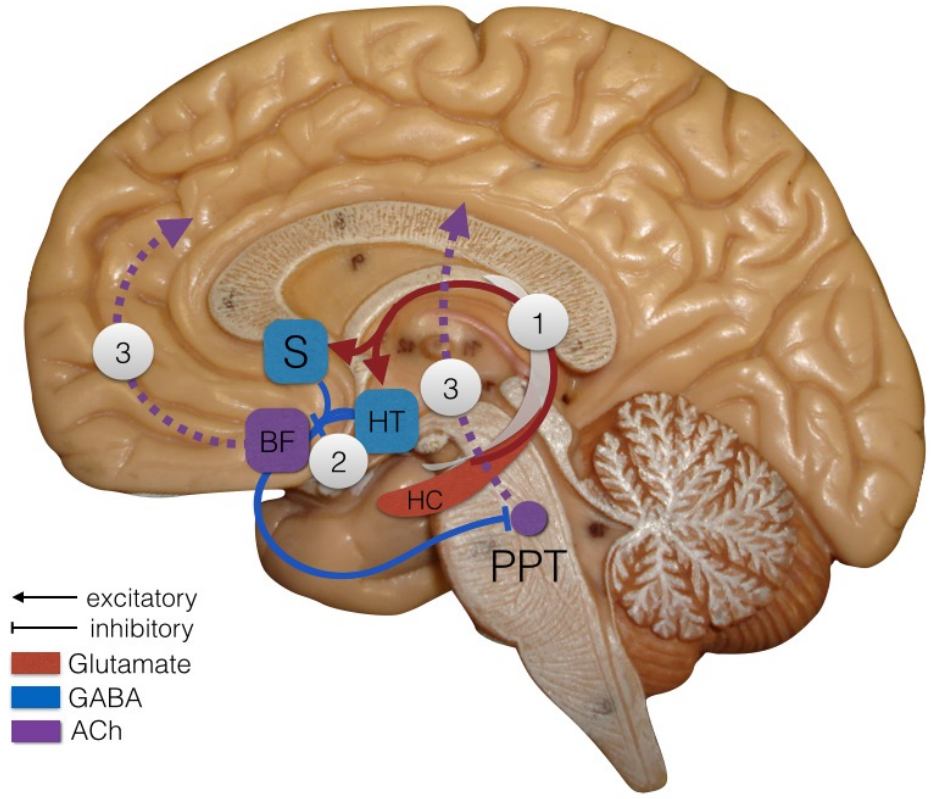

Figure 2: Network inhibition hypothesis. (1) Hippocampal seizures spread via the fornix to inhibitory structures such as the hypothalamus and lateral septum. (2) These inhibitory structures suppress subcortical arousal systems such as the pedunculopontine tegmental nucleus (PPT) and basal forebrain, causing decreased excitatory drive to the cortex (3).
This hypothesis makes three predictions
(A) increased activity in the hippocampal seizure focus and inhibitory structures such as the lateral septum and hypothalamus and decreased activity in brainstem arousal centers, the thalamus and the cortex
(B) suppression of firing of brainstem arousal nuclei such as the PPT and basal forebrain during seizures
(C) decreases in acetylcholine in the thalamus and cortex during seizures
ACh - acetylcholine; BF - basal forebrain; Glu - glutamate; HC - hippocampus; HT - hypothalamus; S - lateral septum; PPT - pedunculopontine tegmental nucleus.

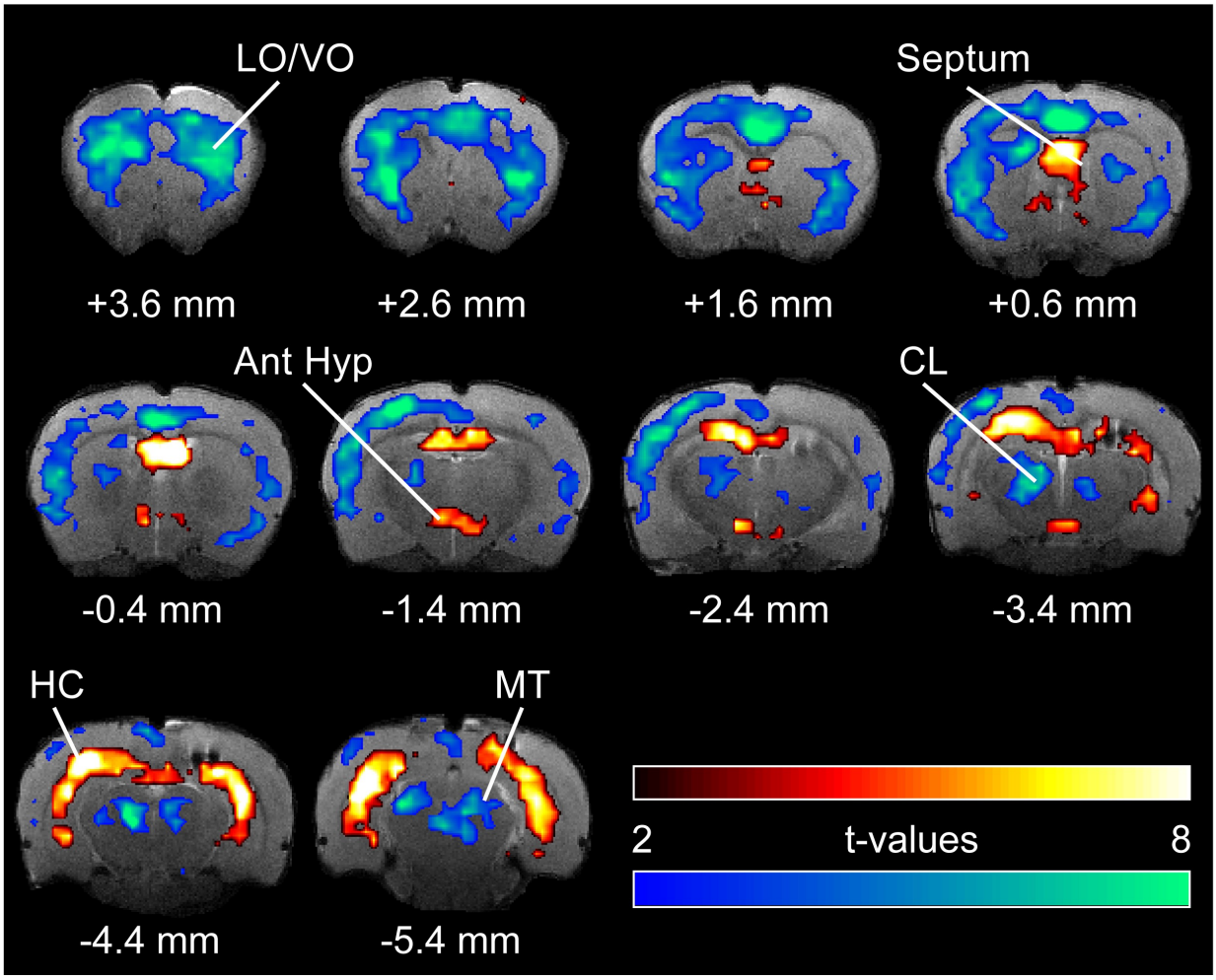

Figure 3: BOLD signal changes during focal hippocampal seizures. BOLD signal is superimposed on coronal sections, where red represents signal increases and blue represents decreases. There are signal increases in the hippocampus which is the site of the seizure stimulus, as well as increases in anterior hypothalamus and septum, which are known to contain many GABAergic neurons (MESULAM and MUFSON 1984, Varoqueaux and Poulain 1999). There are prominent decreases in frontal cortex and centrolateral thalamus, as well as midbrain tegmentum which contains among other structures, the superior portion of the brainstem reticular activating system., which contains Ant hyp - anterior hypothalamus; CL - thalamic centrolateral nucleus; HC - hippocampus; LO/VO - lateral orbitofrontal/ventral orbitofrontal cortex; MT - midbrain tegmentum. Taken from (Motelow, Li et al. 2015).

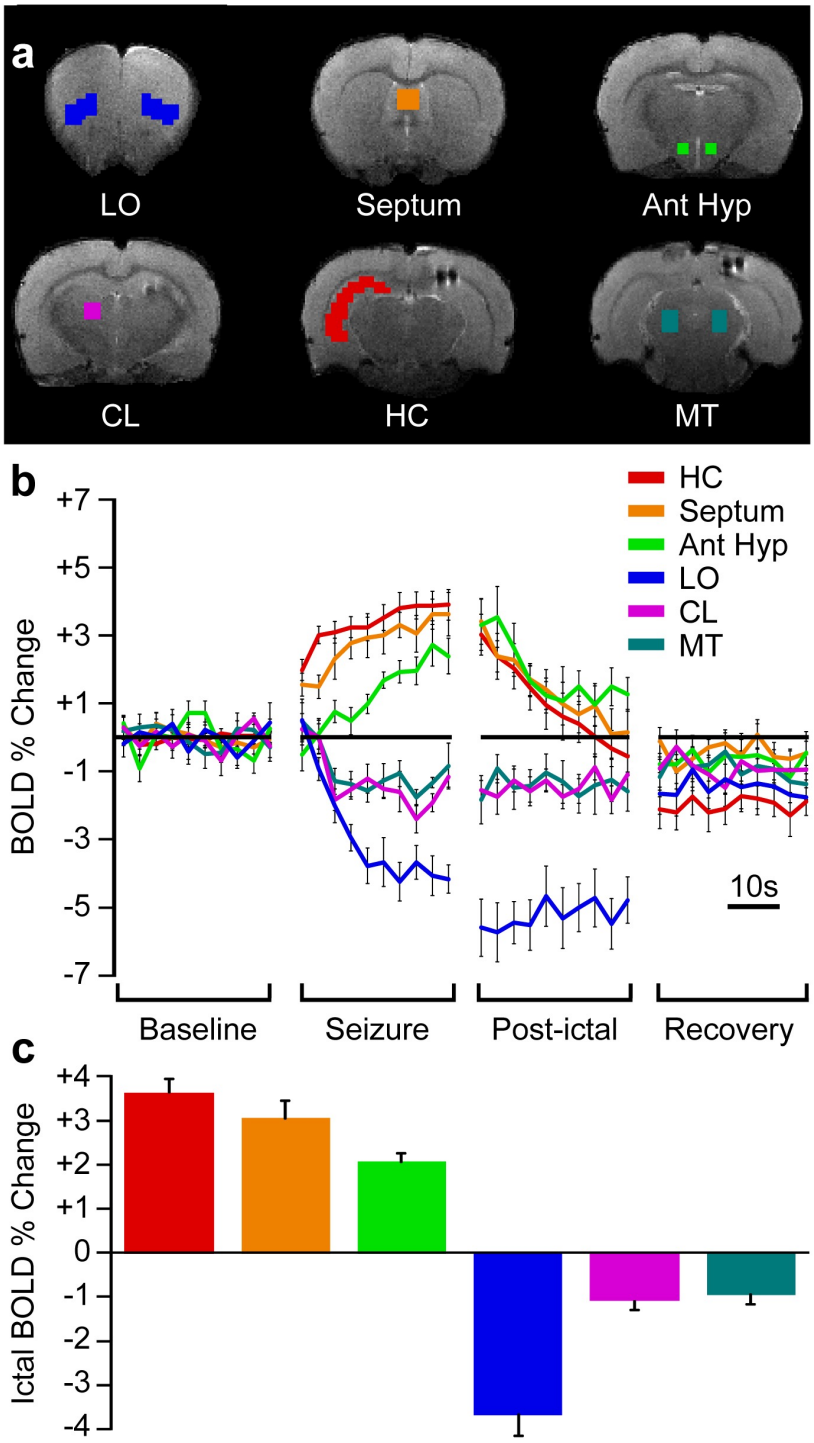

Figure 4: Region of interest (ROI) time course analysis reveals that signal increases and decreases occur ictally with eventual normalization. (A) ROI targeted for this analysis shown on representative coronal sections. (B) ROI averaged time courses (±SEM) for thirty seconds of baseline prior to seizure, during seizure, post-ictally and during recovery. Note ictal increases in hippocampus, septum and anterior hypothalamus and decreases in lateral orbitofrontal, CL nucleus and midbrain tegmentum. These changes normalize after a recovery period. (C) Mean ictal signal change (±SEM). All changes are significant as compared to baseline. Ant Hyp - anterior hypothalamus; CL - thalamic centrolateral nucleus; LO - lateral orbitofrontal cortex; MT - midbrain tegmentum. Taken from (Motelow, Li et al. 2015).

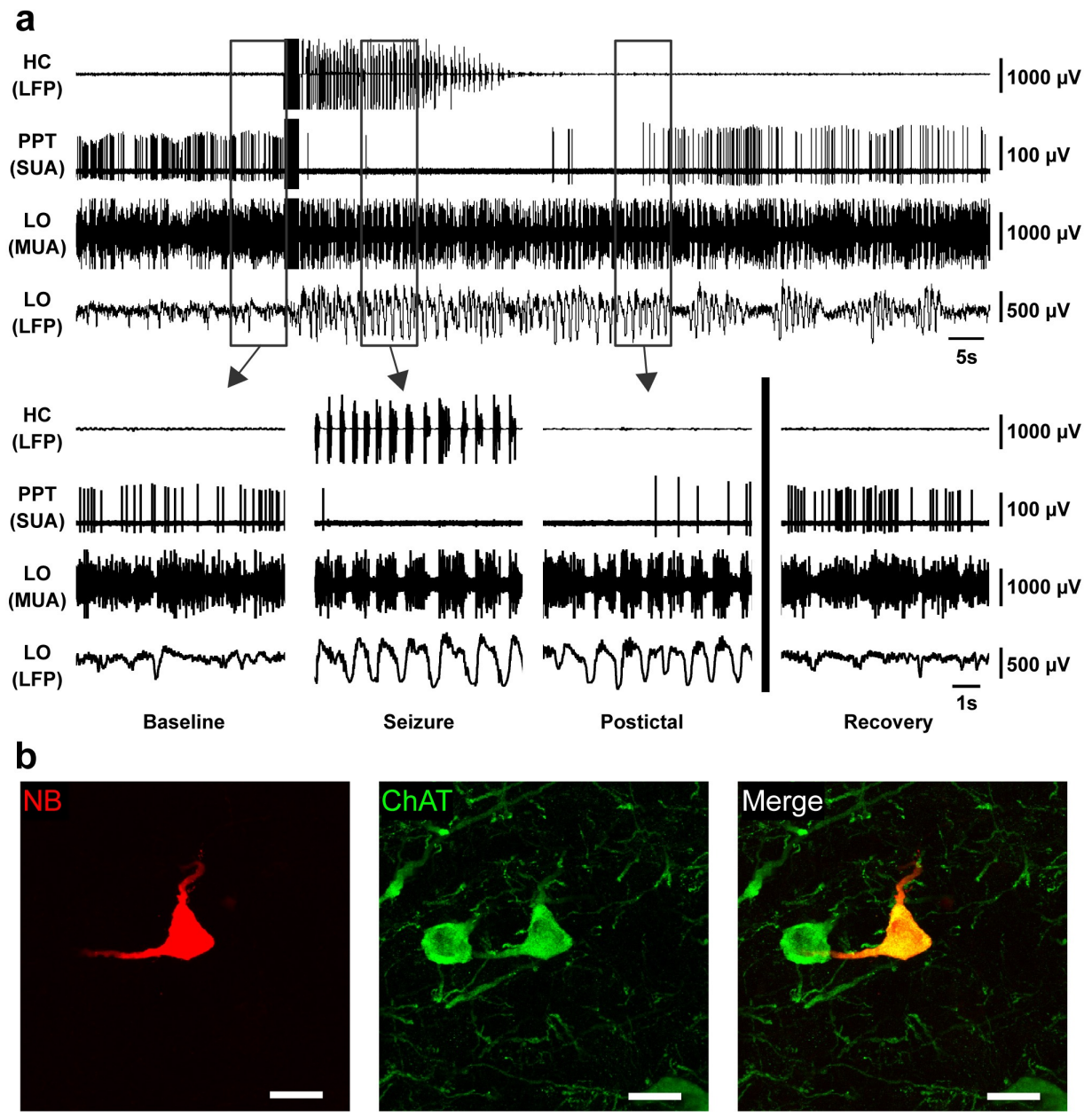

Figure 5: a representative pedunculopontine tegmental (PPT) nucleus cholinergic neuron demonstrates depressed firing during seizures. (A) simultaneous multi-unit (MUA), single-unit (SUA) and local field potential (LFP) recordings capture changes in neurophysiologic properties during seizures. Recording from the hippocampus (HC) LFP demonstrates baseline activity, followed by a stimulus (black box), followed by polyspiking activity of a seizure (inset magnified) followed by quiescence post-ictally. LFP recording from the lateral orbitofrontal cortex (LO) demonstrates baseline fast activity but slow activity following the hippocampal stimulus, continued slow activity post-ictally, with eventual normalization (recovery). MUA recordings from the LO demonstrate characteristic synchronous firing with the depth-positive and depth-negative portions of the wave recorded from the LFP trace, consistent with the up and down states of slow oscillations, respectively. Single unit recordings from one cell in the PPT demonstrate marked decreases in firing during seizures as compared with baseline, with eventual normalization post-ictally. (B) Cell stained with neurobiotin (NB; left panel) indicates recorded cell from single unit recording. Cell stained with choline acetyltransferase (ChAT; middle panel), a marker for cholinergic neurons. Images overlayed (right panel). Taken from (Motelow, Li et al. 2015).

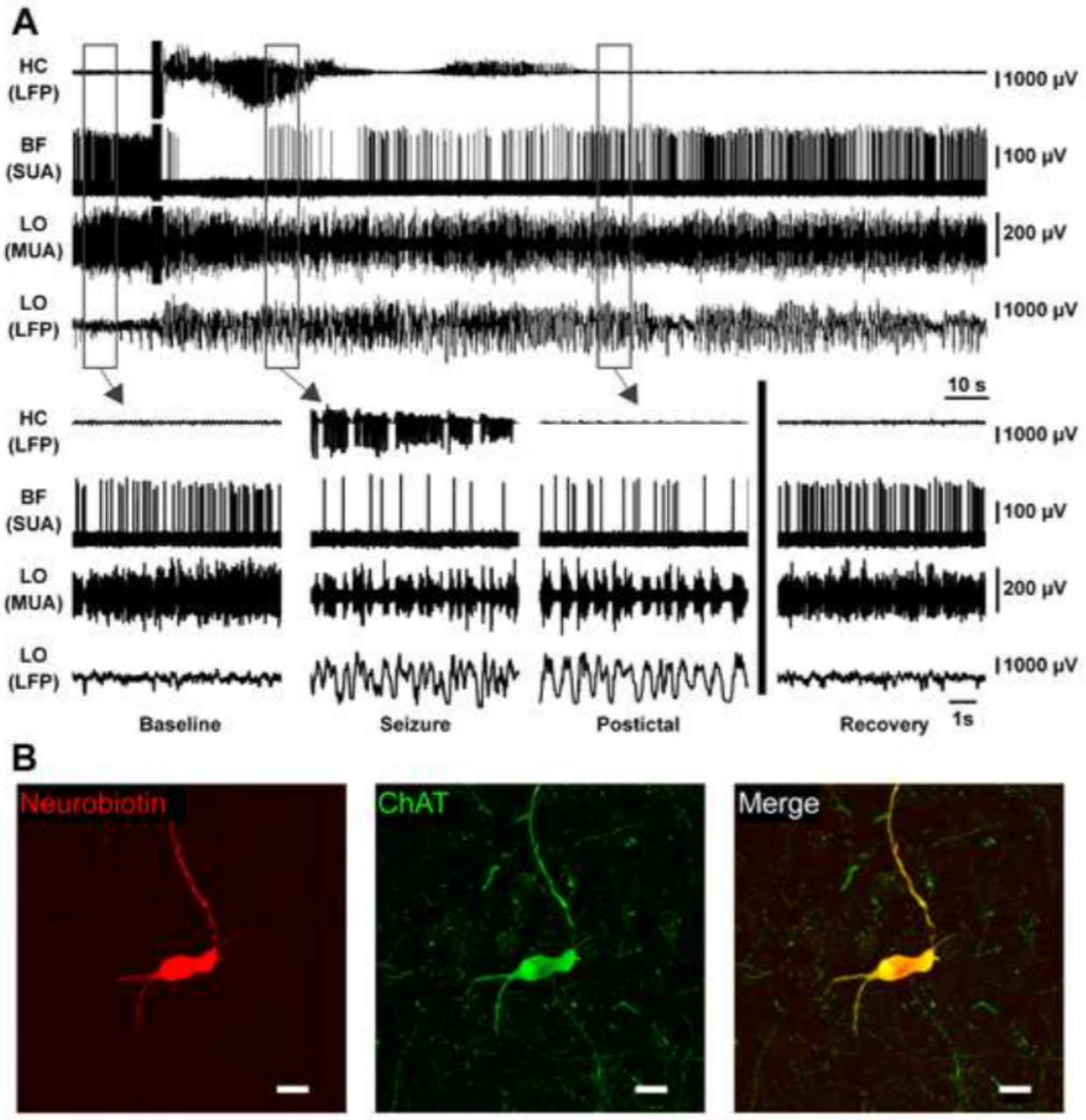

Figure 6: a representative basal forebrain (BF) cholinergic neuron demonstrates depressed firing during seizures. (A) simultaneous multi-unit (MUA), single-unit (SUA) and local field potential (LFP) recordings capture changes in neurophysiologic properties during focal limbic seizures. Single unit recordings from the BF demonstrate marked decreases in firing during seizures as compared with baseline, with eventual normalization post-ictally. (B) Cell stained with neurobiotin (NB; left panel) indicates recorded cell from single unit recording. Cell stained with choline acetyltransferase (ChAT; middle panel), a marker for cholinergic neurons. Images overlayed (right panel). Taken from (Motelow, Li et al. 2015).

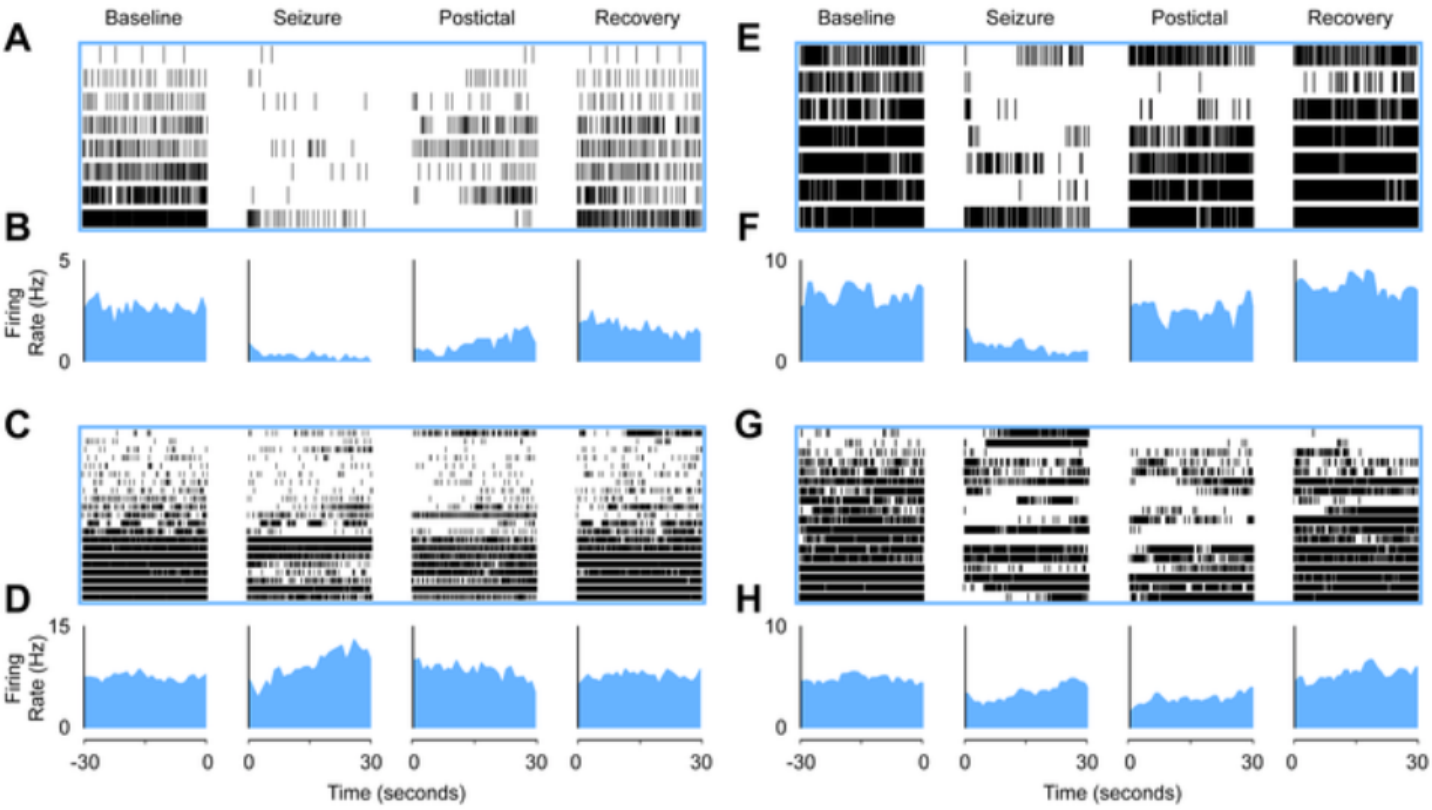

Figure 7: pedunculopontine tegmental nucleus (PPT) and basal forebrain (BF) cholinergic neurons have depressed firing rates while non-cholinergic neurons are mixed. Single unit recordings from cholinergic PPT neurons (A and B), non-cholinergic PPT neurons (C and D), cholinergic BF neurons (E and F), and non-cholinergic BD neurons (G and H) during baseline activity, seizures, post-ictally and during recovery. Firing represented as a raster plot and mean firing rates histogram. Note decreases in firing rates during seizures in PPT and BF cholinergic neurons only (A and B, E and F).
Baseline panels show thirty seconds prior to seizure, seizure panels show first thirty seconds of seizure, post-ictal panels show first thirty-seconds after the end of the seizure and recovery panels show thirty seconds prior to reapplication of anasthetic or neuronal labelling by juxtacellular method. Firing rates are binned by 1 second. Taken from (Motelow, Li et al. 2015).

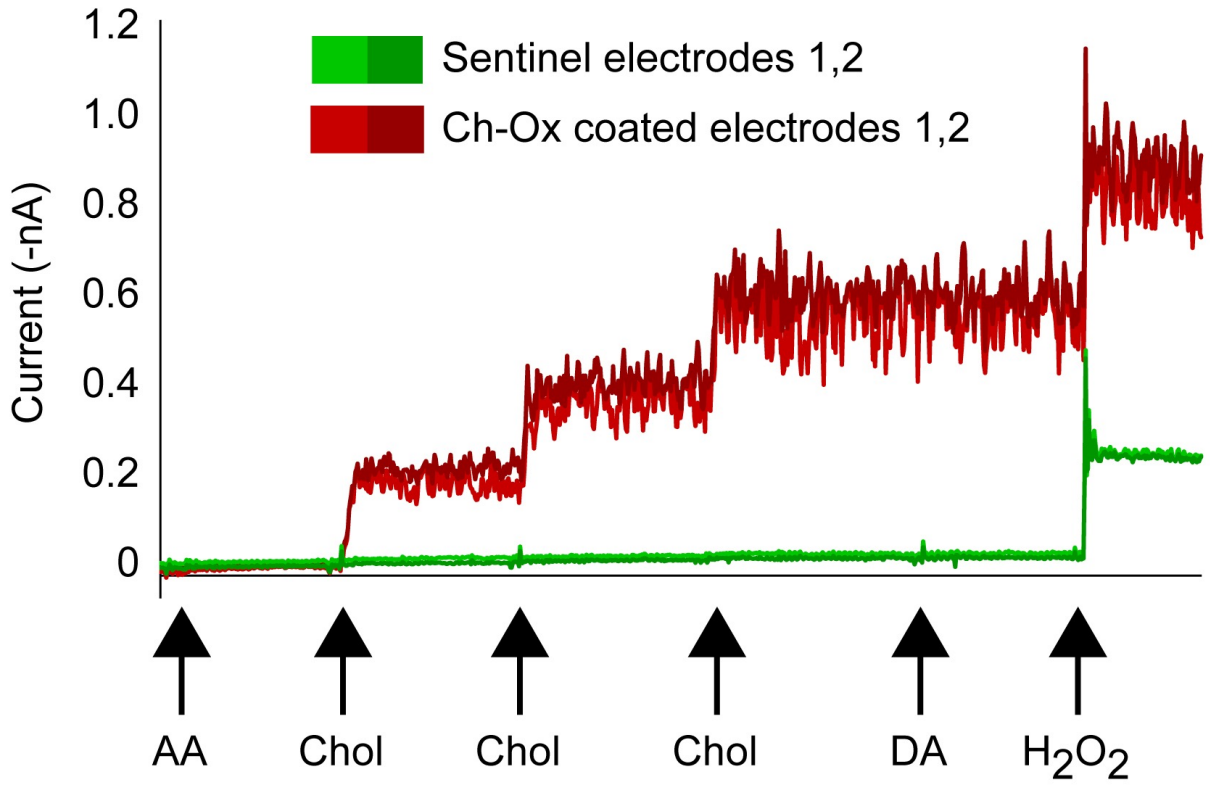

Figure 8: sample *in vitro* calibration of a choline-detecting biosensor electrode. Biosensor probes are ceramic electrodes with four platinum channels. Two channels are coated in choline oxidase, which converts choline to hydrogen peroxide which generates a current. Two channels are uncoated and act as 'sentinel' pads, capturing background noise. The subtraction between the mean signal of the two sets of channels represents the current attributable to choline alone. Here, the electrodes are suspended in a beaker containing PBS. The addition of 250μM ascorbic acid (AA) does not alter currents since a size exclusion layer of methyl-phenyldiamine (m-PD) prevents entry of AA to the platinum pads. Three additions of 20μM choline (Chol) produces step-wise signal increase in the choline-oxidase coated pads only. The addition of 2μM dopamine (DA) does not alter current since it is also excluded by the m-PD layer. Addition of 8.8μM hydrogen peroxide (H_2O_2) yields signal increase in all four pads. Taken from (Motelow, Li et al. 2015).

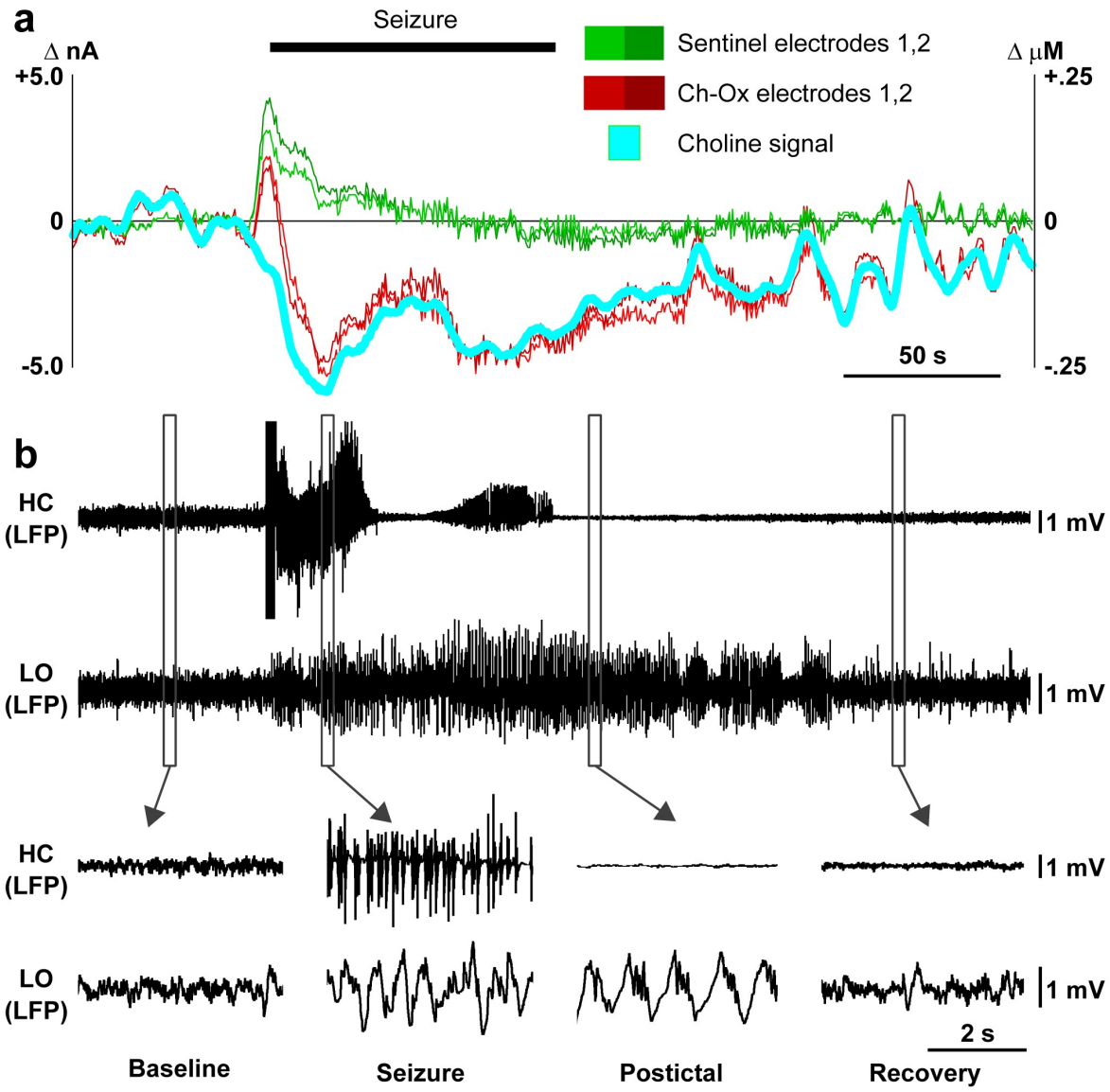

Figure 9: choline signal decreases during seizures in the lateral orbitofrontal cortex. A sample set of recording from a representative animal during an electrically induced limbic seizure. (A) after a brief stimulus artifact, choline-oxidase coated channels demonstrate ictal decreases in current (red line), with eventual return to baseline (not shown). Non choline-oxidase coated electrodes (green line) reflect the stimulus artifact but return to baseline levels during seizures. The subtracted choline signal (blue line) subtracts the stimulus artifact and demonstrates ictal decreases. (B) Simultaneous recording from the hippocampus (HC) and lateral orbitofrontal (LO) cortex using local field potential electrodes (LFP). After the electrical stimulus LFP traces from the hippocampus demonstrate polyspking activity while LFP traces from the lateral orbitofrontal cortex demonstrate slow activity. Lower insets depict magnified traces. Taken from (Motelow, Li et al. 2015).

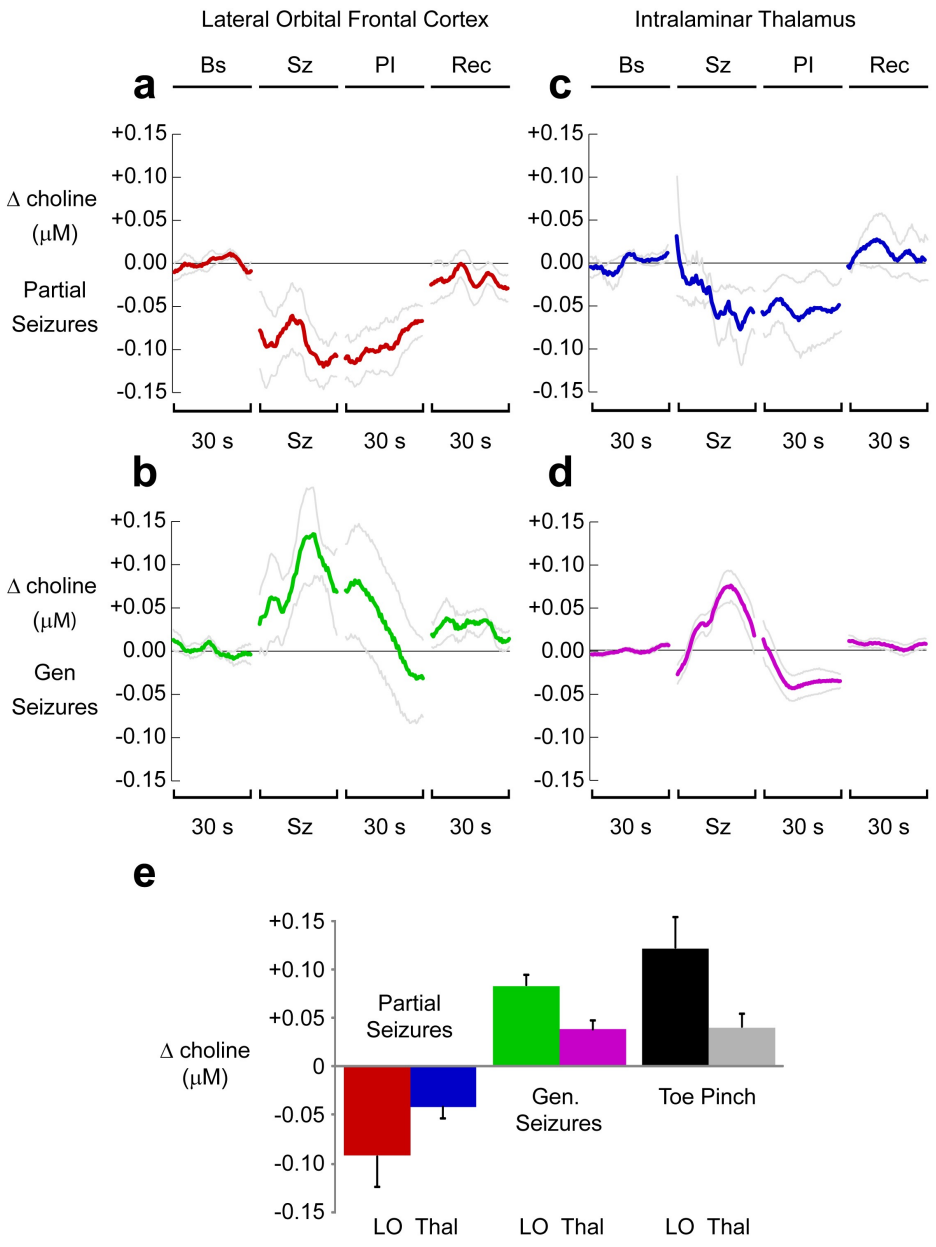

Figure 10: choline signal in the lateral orbitofrontal cortex (LO) and thalamus (Thal) decreases during partial seizures, and increases during generalized seizures and in response to toe pinch. (A and C): mean choline signal ± SEM during electrically-stimulated partial seizures decreases in the lateral orbitofrontal cortex and thalamus. Choline signal shown for thirty second periods during baseline (Bs), seizure (Sz), post-ictally (PI) and in recovery (Rec).
(B and D): mean choline signal ± SEM during electrically-stimulated secondarily generalized seizures increases in the lateral orbitofrontal cortex and thalamus. Secondarily generalized seizures can be electrically induced in the same manner as partial seizures by increasing the current delivered to the hippocampus. Choline signal increases during generalized seizures.
(E): mean ictal changes in the lateral orbit frontal cortex and thalamus during partial, generalized seizures and toe pinch. All changes are statistically significant (paired t-tests, corrected with Holm-Bonferroni, $P < 0.05$). Taken from (Motelow, Li et al. 2015).

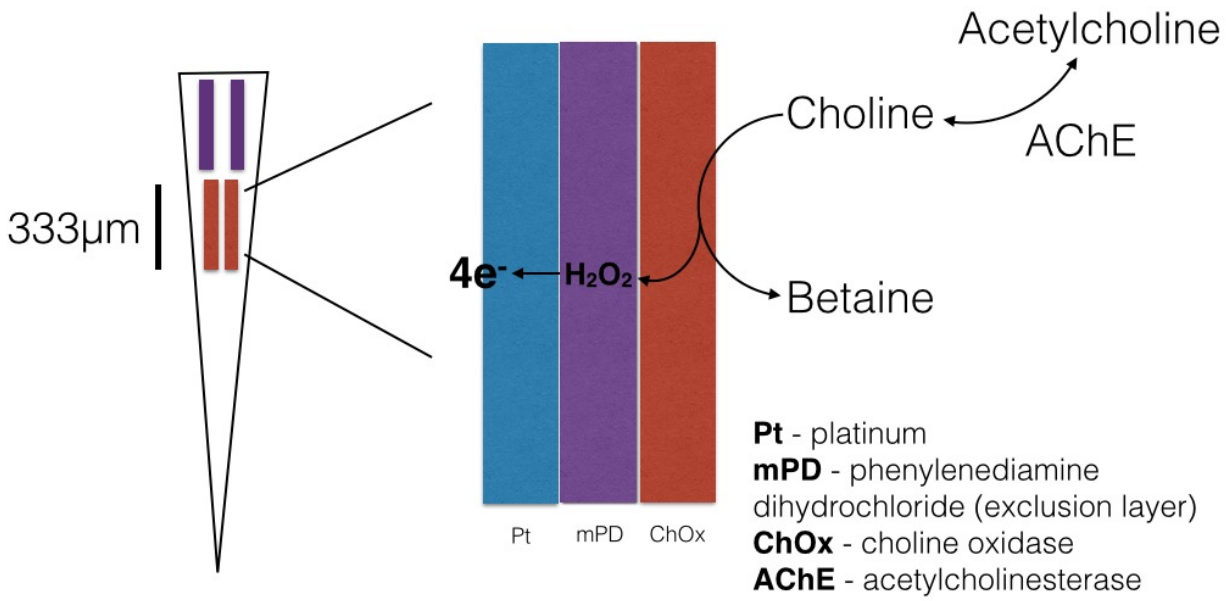

Figure 11: detection principle for enzyme-based amperometry. Choline-oxidase coated micro-electrodes convert endogenously produced choline to hydrogen peroxide, which generate an electric current, captured by platinum electrodes. methyl-phenylenediamine dyhydrochloride is a size exclusion layer which prevents the penetration of interferents like dopamine and ascorbic acid. Each electrode consists of four channels of which two channels are choline-oxidase coated. The remaining two are reference electrodes which capture background noise. The subtraction of the reference signal from signal derived from choline-coated electrodes represents the true choline signal. Modified from (Sarter and Parikh 2005).

Bibliography

Badawy, R. a. B., J. M. Curatolo, M. Newton, S. F. Berkovic and R. a. L. Macdonell (2006). "Sleep deprivation increases cortical excitability in epilepsy: syndrome-specific effects." Neurology 67: 1018-1022.

Ballon, J. and D. Feifel (2006). "A systematic review of modafinil: Potential clinical uses and mechanisms of action." Journal of clinical Psychiatry.

Bazil, C. W. (2003). "Epilepsy and sleep disturbance." Epilepsy & Behavior 4: 39-45.

Berntson, G., R. Shafi and M. Sarter (2002). "Specific contributions of the basal forebrain corticopetal cholinergic system to electroencephalographic activity and sleep/waking behaviour." European Journal of … 16: 2453-2461.

Blatow, M., A. Rozov, I. Katona, S. G. Hormuzdi, A. H. Meyer, M. a. Whittington, A. Caputi and H. Monyer (2003). "A Novel Network of Multipolar Bursting Interneurons Generates Theta Frequency Oscillations in Neocortex." Neuron 38: 805-817.

Blumenfeld, H. (2012). "Impaired consciousness in epilepsy." Lancet Neurol 11(9): 814-826.

Blumenfeld, H., K. A. McNally, S. D. Vanderhill, A. L. Paige, R. Chung, K. Davis, A. D. Norden, R. Stokking, C. Studholme, E. J. Novotny, Jr., I. G. Zubal and S. S. Spencer (2004). "Positive and negative network correlations in temporal lobe epilepsy." Cereb Cortex 14(8): 892-902.

Blumenfeld, H., M. Rivera, K. A. McNally, K. Davis, D. D. Spencer and S. S. Spencer (2004). "Ictal neocortical slowing in temporal lobe epilepsy." Neurology 63(6): 1015-1021.

Blumenfeld, H. and J. Taylor (2003). "Why do Seizures Cause Loss of Consciousness?" The Neuroscientist 9: 301-310.

Boucetta, S. and B. E. Jones (2009). "Activity profiles of cholinergic and intermingled GABAergic and putative glutamatergic neurons in the pontomesencephalic tegmentum of urethane-anesthetized rats." The Journal of neuroscience : the official journal of the Society for Neuroscience 29: 4664-4674.

Braun, A., T. Balkin and N. Wesenten (1997). "Regional cerebral blood flow throughout the sleep-wake cycle. An H2(15)O PET study." Brain: 1173-1197.

Bremer, F. (1935). "Cerveau isolé et physiologie du sommeil." CR Soc. Biol.(Paris) 118: 1235-1241.

Brown, R. E., R. Basheer, J. T. McKenna, R. E. Strecker and R. W. McCarley (2012). "Control of sleep and wakefulness." Physiological reviews 92: 1087-1187.

Buhl, E., G. Tamás and A. Fisahn (1998). "Cholinergic activation and tonic excitation induce persistent gamma oscillations in mouse somatosensory cortex in vitro." The Journal of Physiology: 117-126.

Burmeister, J. J., M. Palmer and G. a. Gerhardt (2003). "Ceramic-based multisite microelectrode array for rapid choline measures in brain tissue." Analytica Chimica Acta 481: 65-74.

Cazzin, C., S. Mion, F. Caldara, J. M. Rimland and E. Domenici (2011). "Microarray analysis of cultured rat hippocampal neurons treated with brain derived neurotrophic factor." Mol Biol Rep 38(2): 983-990.

Celesia, G. and H. Jasper (1966). "Acetylcholine released from cerebral cortex in relation to state of activation." Neurology.

Chang, B. S. and D. H. Lowenstein (2003). "Epilepsy." The New England journal of medicine 349: 1257-1266.

Chang, D., I. G. Zubal, C. Gottschalk, A. Necochea, R. Stokking, C. Studholme, M. Corsi, J. Slawski, S. Spencer and H. Blumenfeld (2002). "Comparison of Statistical Parametric Mapping and SPECT Difference Imaging in Patients with Temporal Lobe Epilepsy." Epilepsia 43: 68-74.

Compte, A., R. Reig, V. F. Descalzo, M. a. Harvey, G. D. Puccini and M. V. Sanchez-Vives (2008). "Spontaneous high-frequency (10-80 Hz) oscillations during up states in the cerebral cortex in vitro." The Journal of neuroscience : the official journal of the Society for Neuroscience 28: 13828-13844.

Crunelli, V. and S. W. Hughes (2010). "The slow (<1 Hz) rhythm of non-REM sleep: a dialogue between three cardinal oscillators." Nature neuroscience 13: 9-17.

Cunningham, C., W. C. Chen, A. Shorten, M. McClurkin, T. Choezom, C. P. Schmidt, V. Chu, A. Bozik, C. Best, M. Chapman, M. Furman, K. Detyniecki, J. T. Giacino and H. Blumenfeld (2014). "Impaired consciousness in partial seizures is bimodally distributed." Neurology 82(19): 1736-1744.

Dang-Vu, T. (2008). "Spontaneous neural activity during human slow wave sleep." Proceedings of the ….

De Lima, a. D. and W. Singer (1987). "The brainstem projection to the lateral geniculate nucleus in the cat: identification of cholinergic and monoaminergic elements." The Journal of comparative neurology 259: 92-121.

Drazkowski, J. (2007). "An overview of epilepsy and driving." Epilepsia 48 Suppl 9: 10-12.

Duque, A. and B. Balatoni (2000). "EEG Correlation of the Discharge Properties of Identified Neurons in the Basal Forebrain." Journal of …: 1627-1635.

Engel, J. (1996). "Introduction to temporal lobe epilepsy." Epilepsy research 26: 41-50.

Englot, D. J. and H. Blumenfeld (2009). "Consciousness and epilepsy: why are complex-partial seizures complex?" Prog Brain Res 177: 147-170.

Englot, D. J. and H. Blumenfeld (2009). "Consciousness and epilepsy: why are complex-partial seizures complex?" Progress in brain research 177: 147-170.

Englot, D. J., A. M. Mishra, P. K. Mansuripur, P. Herman, F. Hyder and H. Blumenfeld (2008). "Remote effects of focal hippocampal seizures on the rat neocortex." J Neurosci 28(36): 9066-9081.

Englot, D. J., A. M. Mishra, P. K. Mansuripur, P. Herman, F. Hyder and H. Blumenfeld (2008). "Remote effects of focal hippocampal seizures on the rat neocortex." The Journal of neuroscience : the official journal of the Society for Neuroscience 28: 9066-9081.

Englot, D. J., B. Modi, A. M. Mishra, M. DeSalvo, F. Hyder and H. Blumenfeld (2009). "Cortical deactivation induced by subcortical network dysfunction in limbic seizures." J Neurosci 29(41): 13006-13018.

Englot, D. J., L. Yang, H. Hamid, N. Danielson, X. Bai, A. Marfeo, L. Yu, A. Gordon, M. J. Purcaro, J. E. Motelow, R. Agarwal, D. J. Ellens, J. D. Golomb, M. C. Shamy, H. Zhang, C. Carlson, W. Doyle, O. Devinsky, K. Vives, D. D. Spencer, S. S. Spencer, C. Schevon, H. P. Zaveri and H. Blumenfeld (2010). "Impaired consciousness in temporal lobe seizures: role of cortical slow activity." Brain 133(Pt 12): 3764-3777.

Fan, X. T., F. Zhao, Y. Ai, A. Andersen, P. Hardy, F. Ling, G. A. Gerhardt, Z. Zhang and J. E. Quintero (2014). "Cortical glutamate levels decrease in a non-human primate model of dopamine deficiency." Brain Research 1552: 34-40.

French, J. a., P. D. Williamson, V. M. Thadani, T. M. Darcey, R. H. Mattson, S. S. Spencer and D. D. Spencer (1993). "Characteristics of medial temporal lobe epilepsy: I. Results of history and physical examination." Annals of neurology 34: 774-780.

Furman, M., J. Motelow, B. Lerner, W. I., K. Deisseroth, F. Hyder, J. Cardin and H. Blumenfeld (2013). Optogenetic stimulation of cholinergic mesopontine neurons for preventing cortical dysfunction during seizures. Society for Neuroscience.

Gigli, G. L., F. Placidi, M. Diomedi, M. Maschio, G. Silvestri, A. Scalise and M. G. Marciani (1997). "Nocturnal Sleep and Daytime Somnolence in Untreated Patients with Temporal Lobe Epilepsy: Changes After Treatment with Controlled-Release Carbamazepine." Epilepsia 38: 696-701.

Gritti, I. and L. Mainville (1997). "GABAergic and other noncholinergic basal forebrain neurons, together with cholinergic neurons, project to the mesocortex and isocortex in the rat." Journal of Comparative … 177: 163-177.

Gummadavelli, A., J. E. Motelow, N. Smith, Q. Zhan, N. D. Schiff and H. Blumenfeld (2014). "Thalamic stimulation to improve level of consciousness after seizures: Evaluation of electrophysiology and behavior." Epilepsia.

Haider, B., A. Duque, A. R. Hasenstaub and D. a. McCormick (2006). "Neocortical network activity in vivo is generated through a dynamic balance of excitation and inhibition." The Journal of neuroscience : the official journal of the Society for Neuroscience 26: 4535-4545.

Hallanger, A. and A. Levey (1987). "The origins of cholinergic and other subcortical afferents to the thalamus in the rat." Journal of … 124: 105-124.

Haut, S., C. Hall, J. Masur and R. Lipton (2007). "Seizure occurrence Precipitants and prediction." Neurology 02192.

Hoffmann, J. M., C. E. Elger and A. a. Kleefuss-Lie (2008). "Lateralizing value of behavioral arrest in patients with temporal lobe epilepsy." Epilepsy & behavior : E&B 13: 634-636.

Inoue, Y. and T. Mihara (1998). "Awareness and Responsiveness During Partial Seizures." Epilepsia 39: 7-10.

Kaufmann, C., R. Wehrle, T. C. Wetter, F. Holsboer, D. P. Auer, T. Pollmächer and M. Czisch (2006). "Brain activation and hypothalamic functional connectivity during human non-rapid eye movement sleep: an EEG/fMRI study." Brain : a journal of neurology 129: 655-667.

Kaur, S., A. Junek, M. a. Black and K. Semba (2008). "Effects of ibotenate and 192IgG-saporin lesions of the nucleus basalis magnocellularis/substantia innominata on spontaneous sleep and wake states and on recovery sleep after sleep deprivation in rats." The Journal of neuroscience : the official journal of the Society for Neuroscience 28: 491-504.

Lee, M. G., O. K. Hassani, A. Alonso and B. E. Jones (2005). "Cholinergic basal forebrain neurons burst with theta during waking and paradoxical sleep." The Journal of neuroscience : the official journal of the Society for Neuroscience 25: 4365-4369.

Lörincz, M. L., V. Crunelli and S. W. Hughes (2008). "Cellular dynamics of cholinergically induced alpha (8-13 Hz) rhythms in sensory thalamic nuclei in vitro." The Journal of neuroscience : the official journal of the Society for Neuroscience 28: 660-671.

Lux, S., M. Kurthen, C. Helmstaedter and W. Hartje (2002). "The localizing value of ictal consciousness and its constituent functions A video-EEG study in patients with focal epilepsy." Brain.

Manns, I. D., A. Alonso and B. E. Jones (2003). "Rhythmically discharging basal forebrain units comprise cholinergic, GABAergic, and putative glutamatergic cells." Journal of neurophysiology 89: 1057-1066.

Maquet, P. (2000). "Functional neuroimaging of normal human sleep by positron emission tomography." Journal of Sleep Research 9: 207-231.

Marrosu, F., C. Portas, M. Mascia and M. Casu (1995). "Microdialysis measurement of cortical and hippocampal acetylcholine release during sleep-wake cycle in freely moving cats." Brain research 671: 329-332.

McGinty, D. and R. Szymusiak (2001). "Brain structures and mechanisms involved in the generation of NREM sleep: focus on the preoptic hypothalamus." Sleep Medicine Reviews 5(4): 323-342.

MESULAM, M. and E. MUFSON (1984). "Neural inputs into the nucleus basalis of the substantia innominata (Ch4) in the rhesus monkey." Brain: 253-274.

Moruzzi, G. and H. Magoun (1949). "Brain stem reticular formation and activation of the EEG." Electroencephalography and clinical ….

Motelow, J., W. Li, Q. Zhan, A. M. Mishra, R. N. Sachdev, A. Gummadavelli, Z. Zayyad, H. S. Lee, V. Chu, J. P. Andrews, D. J. Englot, P. Herman, B. G. Sanganahalli, F. Hyder and H. Blumenfeld (2015). "Decreased subcortical cholinergic arousal in focal seizures." Neuron (In Press).

Nirogi, R., K. Mudigonda, V. Kandikere and R. Ponnamaneni (2010). "Quantification of acetylcholine, an essential neurotransmitter, in brain microdialysis samples by liquid chromatography mass spectrometry." Biomedical Chromatography 24: 39-48.

Nofzinger, E., D. Buysse and J. Miewald (2002). "Human regional cerebral glucose metabolism during non-rapid eye movement sleep in relation to waking." Brain.

Osborne, P. G., W. T. O'Connor, J. Kehr and U. Ungerstedt (1991). "In vivo characterisation of extracellular dopamine, GABA and acetylcholine from the dorsolateral striatum of awake freely moving rats by chronic microdialysis." Journal of Neuroscience Methods 37(2): 93-102.

Pacia, S. V. and J. S. Ebersole (1997). "Intracranial EEG substrates of scalp ictal patterns from temporal lobe foci." Epilepsia 38(6): 642-654.

Parikh, V., S. Apparsundaram, R. Kozak, J. B. Richards and M. Sarter (2006). "Reduced expression and capacity of the striatal high-affinity choline transporter in hyperdopaminergic mice." Neuroscience 141: 379-389.

Parikh, V., J. Ji, M. W. Decker and M. Sarter (2010). "Prefrontal beta2 subunit-containing and alpha7 nicotinic acetylcholine receptors differentially control glutamatergic and cholinergic signaling." The Journal of neuroscience : the official journal of the Society for Neuroscience 30: 3518-3530.

Parikh, V., R. Kozak, V. Martinez and M. Sarter (2007). "Prefrontal acetylcholine release controls cue detection on multiple timescales." Neuron 56: 141-154.

Parikh, V., F. Pomerleau, P. Huettl, G. A. Gerhardt, M. Sarter and J. P. Bruno (2004). "Rapid assessment of in vivo cholinergic transmission by amperometric detection of changes in extracellular choline levels." Eur J Neurosci 20(6): 1545-1554.

Pepeu, G. and M. G. Giovannini (2004). "Changes in Acetylcholine Extracellular Levels During Cognitive Processes Changes in Acetylcholine Extracellular Levels During Cognitive Processes." 21-27.

Picot, M.-C., M. Baldy-Moulinier, J.-P. Daurès, P. Dujols and A. Crespel (2008). "The prevalence of epilepsy and pharmacoresistant epilepsy in adults: A population-based study in a Western European country." Epilepsia 49(7): 1230-1238.

Pinault, D. (1996). "A novel single-cell staining procedure performed in vivo under electrophysiological control: morpho-functional features of juxtacellularly labeled thalamic cells and other central neurons with biocytin or Neurobiotin." J Neurosci Methods 65(2): 113-136.

Quinn, D. M. (1987). "Acetylcholinesterase: enzyme structure, reaction dynamics, and virtual transition states." Chemical Reviews 87(5): 955-979.

Rabinowicz, A. L., E. Salas, F. Beserra, R. C. Leiguarda and S. E. Vazquez (1997). "Changes in Regional Cerebral Blood Flow Beyond the Temporal Lobe in Unilateral Temporal Lobe Epilepsy." Epilepsia 38: 1011-1014.

Rajna, P. and J. Veres (1993). "Correlations Between Night Sleep Duration and Seizure Frequency in Temporal Lobe Epilepsy." Epilepsia 34: 574-579.

Sanchez-Vives, M. and D. McCormick (2000). "Cellular and network mechanisms of rhythmic recurrent activity in neocortex." Nature neuroscience 3.

Saper, C. B., T. C. Chou and T. E. Scammell (2001). "The sleep switch: hypothalamic control of sleep and wakefulness." Trends in Neurosciences 24(12): 726-731.

Saper, C. B., T. E. Scammell and J. Lu (2005). "Hypothalamic regulation of sleep and circadian rhythms." Nature 437: 1257-1263.

Sarter, M. and V. Parikh (2005). "Choline transporters, cholinergic transmission and cognition." Nature reviews. Neuroscience 6: 48-56.

Schultz, K. N. and R. T. Kennedy (2008). "Time-resolved microdialysis for in vivo neurochemical measurements and other applications." Annu Rev Anal Chem (Palo Alto Calif) 1: 627-661.

Scoville, W. B. and B. Milner (1957). "Loss of recent memory after bilateral hippocampal lesions." J Neurol Neurosurg Psychiatry 20(1): 11-21.

Semba, K. and H. C. Fibiger (1992). "Afferent connections of the laterodorsal and the pedunculopontine tegmental nuclei in the rat: A retro- and antero-grade transport and immunohistochemical study." The Journal of Comparative Neurology 323(3): 387-410.

Sharbrough, F. (1987). "Complex partial seizures." Epilepsy: Electroclinical Syndromes.

Sperling, M. (2004). "The consequences of uncontrolled epilepsy." CNS spectrums.

Steriade, M., F. Amzica and A. Nunez (1993). "Cholinergic and noradrenergic modulation of the slow (0.3 Hz) oscillation in neocortical cells." J Neurophysiol 70.

Steriade, M., S. Datta and D. Pare (1990). "Neuronal activities in brain-stem cholinergic nuclei related to tonic activation processes in thalamocortical systems." The Journal of …: 2541-2559.

Steriade, M. and R. Dossi (1991). "Fast oscillations (20-40 Hz) in thalamocortical systems and their potentiation by mesopontine cholinergic nuclei in the cat." Proceedings of the … 88: 4396-4400.

Steriade, M., R. Dossi and A. Nunez (1991). "Network modulation of a slow intrinsic oscillation of cat thalamocortical neurons implicated in sleep delta waves: cortically induced synchronization and brainstem." The journal of neuroscience.

Steriade, M., R. W. McCarley and SpringerLink (Online service) (2005). Brain Control of Wakefulness and Sleep. Boston, MA, Kluwer Academic/Plenum Publishers, New York,.

Steriade, M., D. McCormick and T. Sejnowski (1993). "Thalamocortical oscillations in the sleeping and aroused brain." Science 262: 679-685.

Steriade, M., A. Nuñez and F. Amzica (1993). "Intracellular analysis of relations between the slow (< 1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram." The Journal of Neuroscience.

Szymusiak, R., N. Alam and D. McGinty (2000). "Discharge patterns of neurons in cholinergic regions of the basal forebrain during waking and sleep." Behavioural Brain Research 115: 171-182.

Timofeev, I. and M. Steriade (1996). "Low-Frequency Rhythms in the Thalamus of Intact-Cortex and Decorticated Cats." Journal of neurophysiology 76.

Tropea, D., G. Kreiman, A. Lyckman, S. Mukherjee, H. Yu, S. Horng and M. Sur (2006). "Gene expression changes and molecular pathways mediating activity-dependent plasticity in visual cortex." Nat Neurosci 9(5): 660-668.

Van Paesschen, W., P. Dupont, G. Van Driel, H. Van Billoen and a. Maes (2003). "SPECT perfusion changes during complex partial seizures in patients with hippocampal sclerosis." Brain 126: 1103-1111.

Varoqueaux, F. and P. Poulain (1999). "Projections of the mediolateral part of the lateral septum to the hypothalamus, revealed by Fos expression and axonal tracing in rats." Anatomy and Embryology 199(3): 249-263.

Vickrey, B. G., A. T. Berg, M. R. Sperling, S. Shinnar, J. T. Langfitt, C. W. Bazil, T. S. Walczak, S. Pacia, S. Kim and S. S. Spencer (2000). "Relationships Between Seizure Severity and Health-Related Quality of Life in Refractory Localization-Related Epilepsy." Epilepsia 41: 760-764.

Wang, M., G. T. Roman, K. Schultz, C. Jennings and R. T. Kennedy (2009). "Improved Temporal Resolution for in Vivo Microdialysis by Using Segmented Flow." Anal Chem 80: 8-18.

Weerd, A. D., S. D. Haas and A. Otte (2004). "Subjective Sleep Disturbance in Patients with Partial Epilepsy: A Questionnaire-based Study on Prevalence and Impact on Quality of Life." … 45: 1397-1404.

Williams, J. and J. Comisarow (1994). "State-Dependent Release of Acetylcholine in Rat Thalamus Measured by in vivo Microdialysis." The Journal of … 14.

Yu, L. and H. Blumenfeld (2009). "Theories of impaired consciousness in epilepsy." Annals of the New York Academy of Sciences 1157: 48-60.