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14. ABSTRACT

Military traumatic brain injury (TBI) is complex, often involving both diffuse and focal components. The contribution of each of these types of injury to epileptogenic brain activity and ultimately post traumatic epilepsy (PTE) is unclear, as are the mechanisms underlying this transition. Using a large animal model (pig) with adequate white matter pathways and a gyrencephalic brain, we are comparing these injury phenotypes and their potential contribution to PTE. After injury, we chronically implant high density electrodes in the hippocampus, above the cortex near the site of the focal contusion, and ECoG in the contralateral hemisphere. Pigs are monitored via video and electrophysiology up to nine months post injury, and blood biomarkers are being analyzed throughout in order to evaluate them as potential prognostic measures for the development of PTE. A full post-mortem neuropathological examination of axonal and neuronal injury will be performed to have circuitry changes, number and frequency of seizures and inter-ictal events correlated with the neuropathological outcomes to determine the mechanistic underpinnings of PTE.

15. SUBJECT TERMS
Traumatic Brain Injury, Post Traumatic Epilepsy, Contusion, Inertial Injury, Diffuse Brain Injury, Temporal Lobe Epilepsy

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Introduction

Military traumatic brain injury (TBI) is a heterogeneous injury, often involving both focal and diffuse components, and sometimes an accumulation of mild repetitive injuries such as concussion. The high incidence of post-traumatic epilepsy (PTE) is well-established in the military and civilian population. However, the degree to which each of these components of TBI leads to alterations in brain activity that ultimately results in PTE is unknown, as is the mechanism of this transition. In order to address these problems, a pre-clinical model of PTE must accurately reproduce the diffuse as well as the focal components of injury. Since it is known that injury to the axonal connections is a key component of diffuse brain injury, a large animal model (pig) is being utilized to investigate the contributions of each component of traumatic injury to the development of epilepsy. We are therefore developing a large animal model that will determine to what extent each component of the TBI, as well as which combinations, contribute to epileptogenesis. We therefore subject pigs to diffuse and focal injury, as well as combining these injuries. We are also elucidating what abnormal changes in the contused cortex, as well as the circuitry within the hippocampus after injury lead to epileptiform patterns of activity and whether they result in seizures. We are therefore chronically implanting electrodes within the hippocampus and on the surrounding cortex of injured pigs and analyzing changes in the electrophysiology, as well as correlating these findings with post-mortem neuropathology. Finally, we are examining blood biomarkers in this model thought to be indicators of TBI severity to determine if they predict the development of PTE. We are therefore developing a pre-clinical model of PTE that will investigate the mechanisms by which different components of TBI such as focal vs. diffuse injury lead to epileptogenesis. After validation, this new model will serve as a platform for future treatment targets and therapy development.

Keywords

Post Traumatic Epilepsy (PTE)
Traumatic Brain Injury (TBI)
Hippocampus
Epileptogenesis
Electrophysiology
Diffuse brain injury
Focal brain injury
Axonal pathology
Epilepsy monitoring unit
Chronic Implantation
Wireless telemetry
Contusion
Concussion

Accomplishments:

Major Goals

The major goals for this project are captured in the following three Specific Aims in the proposal:

1) Determine the relative contributions of diffuse, repetitive diffuse and focal brain injury to the development of PTE. Epileptogenesis in a large animal model of purely focal and purely diffuse brain injury will be compared with mild repetitive diffuse brain injury and focal injury superimposed on a diffuse injury.
2) Elucidate the circuitry alterations underlying hyperexcitability and epileptogenesis in the above forms of TBI using high density chronic electrophysiology of the hippocampus and cortex, as well as neuropathology.
3) Determine the utility of established blood biomarkers associated with axonal injury in identifying the progressive white matter degeneration leading to epileptogenesis via deafferentation.

Task Summary:

Specific tasks for the third year of these Aims were to continue to injure and implant animals from each injury group, begin characterization/quantification of the chronic electrophysiology of the contusion site and hippocampus as well as video monitoring, and begin neuropathological characterization of the contusion and rotational injury circuitry changes based on axonal injury. In addition, our goal was identify biomarkers that may correlate with PTE in this timeframe. Our first year schedule for the SOW was very aggressive and did not include some needed development steps, but we have met many of the above goals in this timeframe, as well as exceeding some specifications for our recording setup. We developed appropriate electrodes for the study and implanted them in pigs for most of the groups proposed (see below), and are currently monitoring them with both EEG and video. In year 2, we also decided that our initial contusion injury was not approximating an injury that would reproducibly induce localized epilepsy, and developed a new more dynamic contusion injury. In addition, we have characterized the neuropathology of the contusion site, as well as examined historic rotational injuries to begin to assess axonal pathology for the rotation only animals in the temporal lobe circuitry. For the biomarker studies, we have collected bloods from all animals at the times described, and have successfully identified NF-l, GFAP, and UCHL-1 as potential biomarkers of TBI that may prove useful. We are in the process of analyzing the electrophysiological data from these animals, and have identified seizures in 3 of the 4 animals implanted following the new CCI injury in year 3.

Specific Objectives, Major Activities, Results and Conclusions:

A) New contusion injury validation and neuropathology
B) Grid electrode development and testing
C) Wireless Large Animal Custom Enclosure System (LACES) development and deployment
D) Chronic implantation of Sham and Injured animals, Enrollment
E) Pig Epilepsy Monitoring Unit development, Chronic Monitoring, and Electrophysiology
F) Collection and testing of blood biomarkers for TBI in the pig model
G) Neuropathological examination of axonal injury in rotational injury archival tissue

A) New contusion injury validation and neuropathology:

The injury device that was initially utilized for these injuries was predominantly developed for juvenile pigs. The injury appeared to induce little damage at the time of contusion, with only minor surface hemorrhage where the impact occurred. We survived the animals until the 48 hour time point in order to characterize the extent of the pathology, and to examine the brain at the time of implantation in the chronic study animals. The initial assessment at the time of injury was misleading, as the brain had swollen extensively in response to the injury, and extensive effects of the injury to the targeted gyrus and the adjacent gyri were grossly apparent. The neuropathological assessment was performed with H&E, APP, and SNTF as proposed. Red blood cells from the contusion are apparent deep in the gyri, and axonal pathology is present in the depths of the gyri as evidenced by SNTF and APP staining. Although electrophysiological alterations are present in the adjacent tissue as well (see below), these were not impressive enough for us to continue with the injury model. We believe the differences between the pathology generated from the initial injury device, and the implanted animals, had to do with the repair of the skull defect during implantation. When this was repaired, the ischemia that was responsible for much of the pathology did not occur due to the reduced brain swelling and extrusion that occurred.

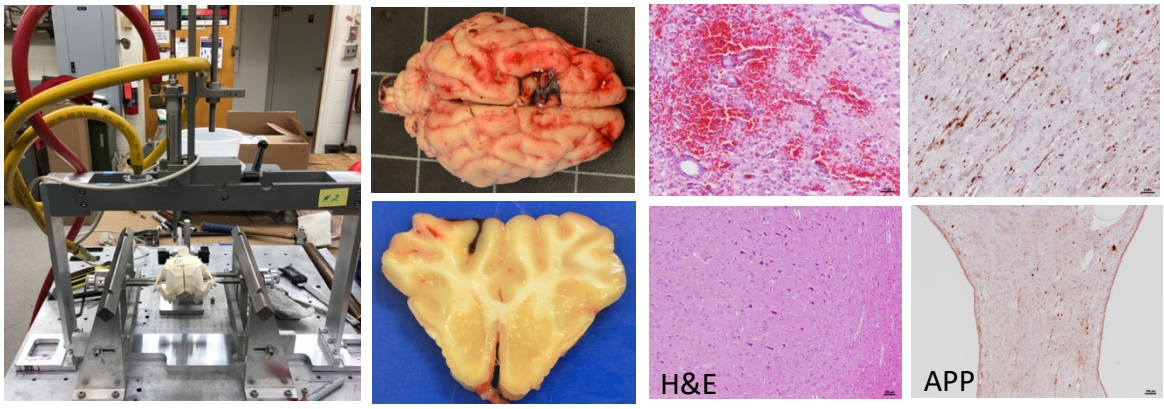


Fig.1 New CCI injury at 72 hours post injury. More appropriate biomechanics of the contusion injury lead to subdural, subarachnoid, and intraparenchymal hemorrhage. Compression of the cortical gyrus is visible, as well as a partial midline shift at this level. H&E demonstrates intraparenchymal hemorrhage as well as pyknotic neurons in adjacent gyri. Axonal pathology extends beyond the level of the blood brain barrier breakdown, suggesting primary biomechanical injury of the white matter. This extends into the collosum and fimbria fornix, suggesting that this injury may be primarily focal but with a diffuse component, replicating important aspects of human injury/contusion. An animal with this injury has now been implanted with both a surface grid and a hippocampal depth.

We therefore implemented a new, dynamic CCI model (**Fig.1**) using dimensions and biomechanics taken from the literature. This modified CCI device mounts on the stereotax, and as such can be targeted appropriately to the same location that the other device was impacting. We believe this contusion (12mm diameter, 9mm depth, 150ms dwell time, 3.5 M/s) produces an injury that will be better representative of the type that develop epileptogenic cortical foci in human PTE. We have tested the device now on age appropriate Yucatan miniature swine and replicated the injury at various early survival points, as well as implanting 2 animals for chronic ephys. We characterized the injury at 8, 9 and 10mm depth, and chose a depth of 9mm for these animals based on the depth of pathology and the 72-hour survival data. The animals had minor neurological deficits for the first 24-48 hours post injury. Gross pathology revealed subdural hemorrhage at the injury site, subarachnoid hemorrhage at the injury site and remote to the injury, as well as intraparenchymal bleeds at the site of contusion. There was a slight midline shift at the level of the contusion, as well as compression of the cortical gyri due to the sub and epidural blood.

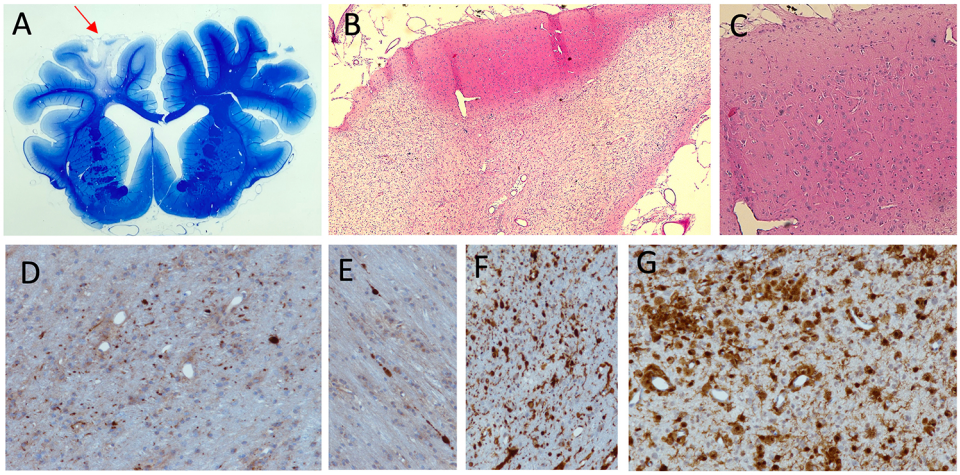


Fig.2 Pig CCI Neuropathology 8 months post injury. A) Luxol Fast Blue staining reveals loss of white matter integrity in the contused gyrus (arrow). B) A small strip of cortex remains above this, with intact laminar structure in C). D and E) APP labeling reveals ongoing axonal pathology, surprisingly active at this late time point. F and G) IBA1 labeling reveals active microglia/macrophages in the white matter tract suggesting ongoing inflammatory processes.

Histopathological examination revealed a breakdown in the blood-brain barrier, with disrupted cytoarchitecture in the three gyri biomechanically affected by the injury. In addition, pyknotic neurons were present in nearby gyri, suggesting that other cortical architecture was affected by the injury. The deep white matter was also profoundly affected, particularly at the base of the gyri and even into the corpus collosum and fimbria fornix. See below for a description of the epileptogenic activity from this new contusion.

To date, four animals have been implanted with continuous electrophysiological and video monitoring post-CCI. Notably, electrophysiological analyses have demonstrated significant electrophysiological abnormalities, including periods of electrographic seizure in both cortex and the hippocampus by 3 and 4 months post-CCI. Analyses currently indicate ongoing electrographic seizure out to the 8 month timepoint across all 3 metrics of assessment (cortical grid, screws and hippocampal depth probe). Analyses to quantify the nature and frequency of electrographic seizure are underway, as well as dynamics and origin of the epileptogenesis (see below). At 8 months, the animal underwent transcardial perfusion and processing for neuropathological analysis. We have since completed gross neuropathological assessment followed by preliminary histological examination. Specifically, sections at the level of contusion and hippocampus were subjected to standard H&E staining as well as immunohistochemistry specific for APP, GFAP, IBA-1 and fibrinogen.

Gross neuropathological findings indicate atrophic change in the brain, with widening of the cortical sulci, particularly notable in the fronto-parietal region ipsilateral to the impact site. On gross dissection, the contusion site was visible with brown discoloration and thinning of the cortex at the site of impact, but with no overt cavitation. Moreover, evidence of dilation of the lateral ventricles was observed, again particularly notable in the ipsilateral hemisphere. Immunohistochemical analyses revealed a striking degree of ongoing axonal pathology, as evidenced by APP+ swellings, indicative of active ongoing degeneration secondary to axonal transport interruption. This was observed, not just at the immediate impact site but extended deep into the white matter (**Fig.2**). Importantly, this finding appears to recapitulate with what has been reported in a subset of human severe TBI cases at post-mortem in the months and years following TBI. In addition to the white matter degeneration, marked inflammation was observed both

surrounding the contusion, as well as more remotely, particularly in the white matter. Indeed, amoeboid macrophage-like cells (IBA-1+) were abundant and again consistent with what has been observed chronically in some cases of human TBI. Finally, evidence of ongoing BBB permeability was also observed, with evidence of marked fibrinogen extravasation, particularly in the white matter underlying the contusion, but also observed more widespread in the brain, including contralaterally. This constellation of preliminary findings indicate that there is a surprising degree of active and ongoing, often-widespread pathologies persistent to 8 months post-TBI in association with electrographic evidence of seizure.

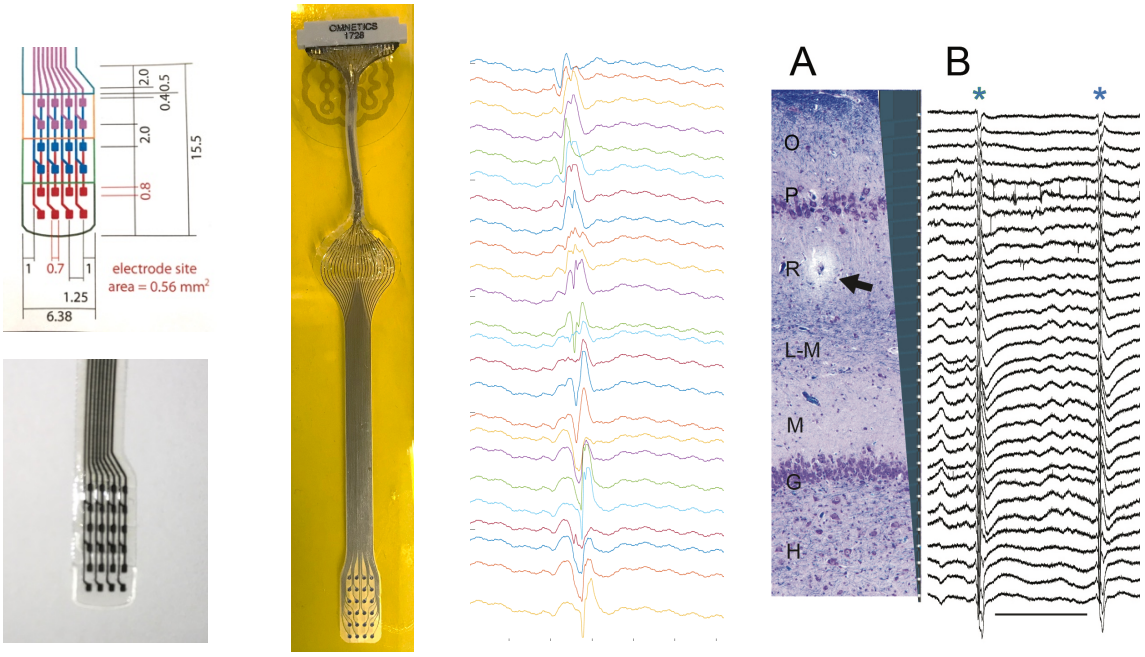


Fig.3. Custom grid electrode design, example grid electrode from Ripple, and the new one from CorTec. 200ms of recordings from the cortical surface of a pig during implantation. Note the differential activity that occurs during the cortical burst, demonstrating the localization of the 300μM electrode contacts. These will be utilized to track the progression of epileptogenic activity from the site of the cortical lesion. On the right is the 6.1mm long depth probe with 32 electrodes that is implanted in the hippocampus.

B) Grid electrode development and testing with depth electrode:

The electrodes first needed to be optimized that are implanted in each of the pigs that is recorded post-injury. These are the hippocampal depth probe, and the cortical micro-ECoG arrays that are being utilized to record activity in the laminar structure of the hippocampus as well as on the cortical surface near the lesion site in the contused animals. We therefore developed a custom depth electrode with 32 channels (6.1mm in length) to capture changes in the hippocampal circuitry post injury. This electrode has been performing extremely well, giving both units and fields post injury up to 6 months post implantation so far. The grid electrode, which provided the greatest challenge last year, was stable and appears to be resolved at this point by using the CorTec electrodes (**Fig.3**).

C) Wireless Large Animal Custom Enclosure System (LACES) development and deployment, Epilepsy Monitoring Unit

We have worked with Neuralynx to redesign our wireless headstages and enclosures for implantation so that higher fidelity recordings can be made from the pigs over even longer periods of time (up to 14 hours), termed the LACES enclosure (Large Animal Custom Enclosure System). The initial iteration of the Cube had a battery life that could be adapted up to 12 hours, but had to transmit data continuously to the Cheetah recording system. The new version of the device designed for this project has the ability to record for 14 hours before requiring a change, and importantly can save data directly to a microSD card built in to the unit. In addition, the device has been separated into an analog front end (AFE) which contains the headstage, and the wireless transmitter. Importantly, the AFE headstage is small and inexpensive enough to be permanently mounted on the pigs head, and the transmitter with the SD card and battery can then be swapped out for data downloading and battery changes. This new design has doubled the amount of time that we will be able to record from the pigs during the study, as well as potentially giving us the possibility of recording from all 4 implanted pigs simultaneously should the cost of the transmitter come down (and sufficient funds available through other savings or new funding). A smaller design this year has made the implants less bulky, a compromise between recording time and longevity time, however we are designing a compromise version to ensure longevity for these experiments (**Fig. 4**). One challenge that arose out of this was the stability of the implants over time. Bone begins to erode underneath the implants, leading to a softening of the anchoring points, as well as an increase in skin thickness that presses against the bottom of the enclosure towards the end of the experiments. In consultation with primate electrophysiologists who

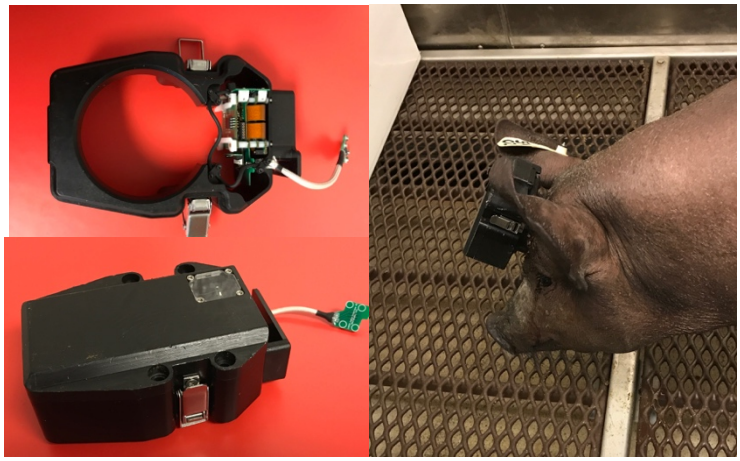


Fig. 4. LACES (Large Animal Custom Enclosure System) for the wireless CUBE2 transmitter. Made of aluminum and delrin, this system allows for the easy change of batteries at the 24hr time point, as well as removal of the SD card (256Gb) for data retrieval. While somewhat bulky due to the 24 hour batteries required, the pig tolerates the implant well even weeks post surgery. The system is designed so that most parts are replaceable over the duration of the 6-9 months of recordings.

Fig. 5. Pig Epilepsy Monitoring Unit (EMU). Images above demonstrate the video acquisition using IR for 24 hour recordings/monitoring of the pigs (2 shown). Also depicted is the acquisition system for the wireless data, as well as the home cage with the IR illuminators, cameras, and wireless hubs.

have experience with longer term implants, a revised procedure has been implemented for the remaining study animals. This includes use of a Palacos bone cement directy to the skull, longer screws in the rear wall of the skull, and Geristore as the skin/implant material for adherence. So far this has resulted in longer implants, and will be continued for the remainder of the study. However, there is some variability still in this process, and improvements will still be made for the next sham animal to be implanted. The weakness in the implant appears to shift, and animals needed to be sacrificed at 4 and 5 months respectively for this cohort.

For the Epilepsy Monitoring Unit, a dedicated room with 5 cages has been provided for the use of this project. We have acquired the appropriate GigE cameras with infrared lighting and filters so that we can monitor the pigs 24/7, and software and a server that is capable of recording from the required number of animals simultaneously with this acquisition equipment. As demonstrated (**Fig.5**), the pigs are readily visible in IR at night and during the day so that appropriate monitoring and seizure/behavioral analysis can be performed without concern for the lighting in the room. We have tested this recording setup over 7 days, and with the above wireless system is consistent enough to be utilized as a daily Epilepsy Monitoring Unit.

D) Chronic Implantation of Sham and Injured animals, Study Enrollment and Electrophysiology

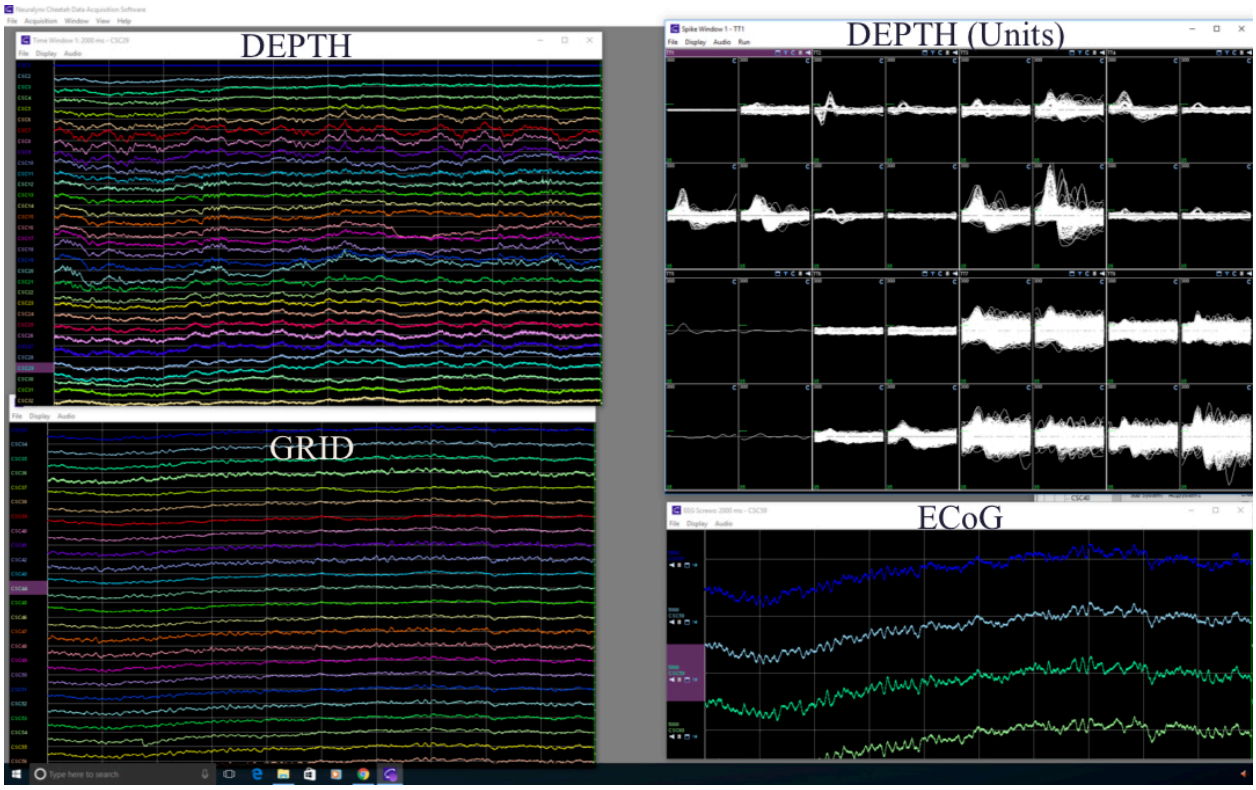


Fig. 6. Awake Wireless Recordings from Chronically Implanted Pig from 3 Types of Electrodes. In a Sham animal, the hippocampal depth is represented in the top left portion, where laminar structure is present on the probe throughout the hippocampus. The grid is underneath, demonstrating changes in the local higher frequency activity between contacts. On the depth there were a number of units present in the days after implantation as well, which may allow for a unique opportunity to examine how individual neurons respond to network level changes following during PTE development. The ECoG panel demonstrates the 3 ECoG electrodes in the contralateral hemisphere, as well as one ipsilateral to the injury site. These will be the equivalent of the traditional monitoring electrodes in PTE experiments. This data was collected with the LACES wireless enclosure and the 64-channel wireless CUBE2 at 30kHz in the animals home cage.

We have currently implanted depths with grids in both sham and contusion animals, as well as depth only in the rotational animal as proposed. Our rotational plus contusion pig is currently scheduled for implantation on Nov.2nd with both depths and grids (new from CorTec). As discussed below, the repetitive rotational pig enrollment was abandoned as the new contusion model took precedence (with programmatic approval). In order to make up for the injury and electrode development time and complete the groups in the current proposal time frame, animals from the last proposed implantation group will be added to each of the next groups, or as animals are removed from the study for epilepsy development, so that the final numbers remain as proposed.

The initial results from the contusion, rotation and sham animals were compelling. Combined grid and depth recordings from an awake animal are depicted above, as well as the ECoG screws located ispi-and contralaterally to the injury site (**Fig.6**). Note the low noise levels present due to the new LACES enclosure and the wireless system, although the animal is ambulating in its home cage during the recordings. The full spectrum of hippocampal circuitry is present, while the grid depicts the higher frequency activity from the cortex, and EEG like activity on the ECoG electrodes. There were prominent changes on the depths, as well as baseline shifts and spikes on the ECoG following rotation plus the earlier contusion injury (**Fig.7**).

Upon implantation of a pig following the new contusion injury, it became clear that not only was the cortex more severely affected, but that there was hippocampal involvement in the injury and response as well. At the two week time point, the animal was brought into the behavioral space, where focal epileptiform discharges were noted on the grid prior to and after agitated vocalization. Interestingly, a high level of ripple-like events followed this event in the hippocampus, which were

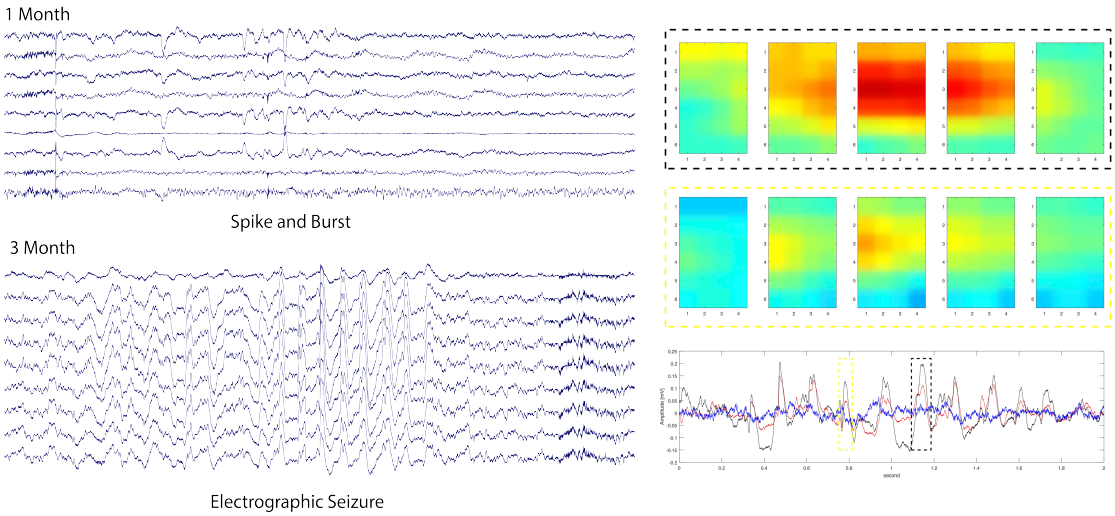


Fig.8 Epileptogenesis Post Contusion. Spikes and bursts of epileptiform discharges were present on the grid near the contusion at the 1 month time point, and had progressed to electrographic seizures by the 3 month time point (only 5 sec. displayed). Analysis of the spread of activity in a brief ictal rhythmic discharge on the grid demonstrates two patterns. From left to right, 5 frames demonstrate invasion of the hyperexcitability from the right side near the contusion. Another pattern demonstrated activation from the left side during the same burst, but this subtlety was lost at the 3-month time point.

appreciated also on the cortical electrode. Single units could be seen entraining to fast gamma oscillations during this period as well in the radiatum layer. At the one month time point, the epileptologists on the project noted 5 events in a 20 minute period concerning for seizures in the awake state (**Fig.8**). The onset was characterized by sharply contoured delta/theta activity which evolved mainly in frequency, with EEG offset consisting of diffuse slowing. At the two month time point, in a 20 minute period there were 3 events with identified as brief ictal rhythmic discharges (BIRDs), as well as frequent generalized discharges during the sleep state. At the 3 month time point, electrographic seizures and shorter bursts of epileptiform activity not long enough meet seizure criteria were noted as well as BIRDs in 2 randomly chosen 20 minute increments for assessment. While it is too early to confirm, a recently implanted animal with the same injury appears to have early hyperexcitability on both the depth and the grid, suggesting that this may be a reproducible phenomenon

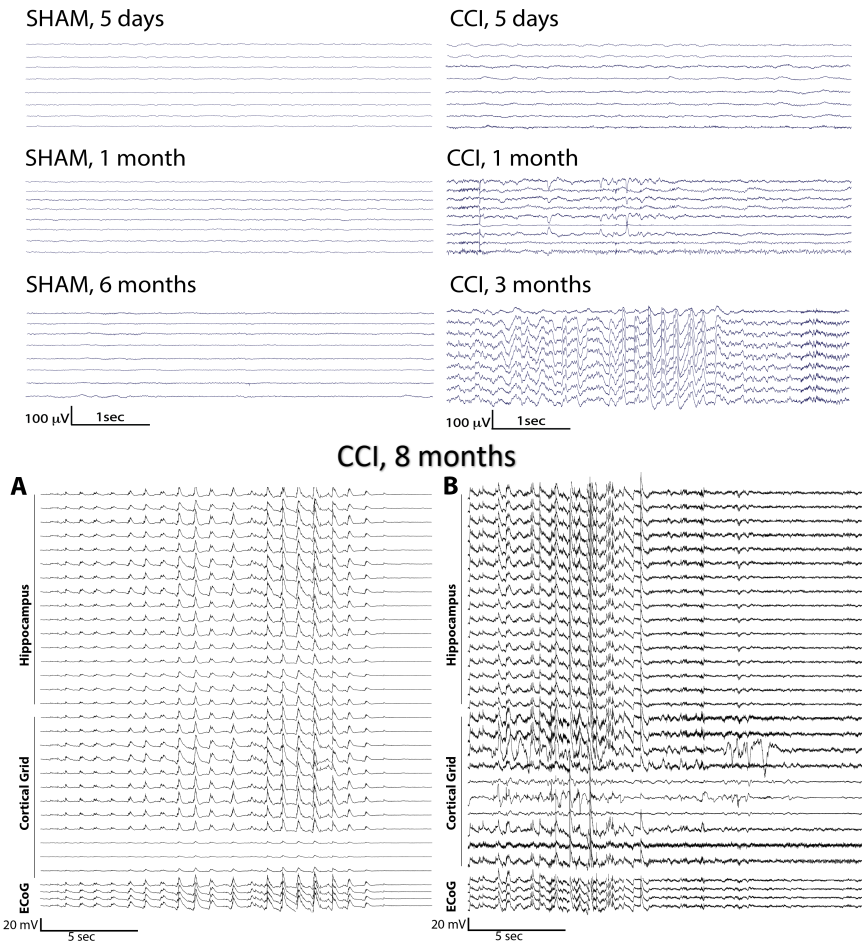


Fig.9 Epileptogenesis Post Contusion. Comparison of Sham and CCI recordings revealed spikes and bursts of epileptiform discharges present on the grid near the contusion at the 1 month time point, and had progressed to electrographic seizures by the 3 month time point, while the Sham presented normally out to 6 months. Two different seizures are presented from a 20 minute recording that was composed of 4 seizures, BIRDs, and runs of epileptiform activity that did not meet seizure criteria. Another recording period revealed only diffuse slowing with no definitive epileptiform discharges. Analysis of the spread of activity in a brief ictal rhythmic discharge on the grid demonstrates a left to right pattern - 5 frames demonstrate invasion of the hyperexcitability from the right side near the contusion. Another pattern demonstrated activation from the left side during the same burst, but this subtlety was lost at the 3-month time point.

Table 1. Summary of Animals in DoD_PTE Study. * CCI – Controlled Cortical Impact brain injury.

Injury	**Sham**	**Rotation**	**Contusion**	**Rotation + Contusion**	**CCI***	**DoD_PTE Study**
Animals (path)			2		6	**8**
Animals (ephys)	3	1	1	1	3	**8**
Total in each group	3	1	3	1	9	**16**

E) Collection and testing of candidate blood biomarkers for TBI in the pig model

One of our aims is to test the hypothesis that the brain injury to axonal circuitry is responsible for the development of PTE, and may be identified by a blood test for biomarkers for brain damage. A leading candidate biomarker for the prognosis of PTE is SNTF, a proteolytic fragment (residues 1-1176) of the abundant axonal protein alpha-II-spectrin. SNTF accumulates within damaged axons after human TBI, its blood levels are elevated acutely post-injury and are prognostic for white matter structural abnormalities and persisting brain dysfunction following concussion in humans. However, when detection assays for this biomarker were tested using serum from sham, rotation, and contusion injured pigs, detection levels in the blood suggested no difference between sham and injured animals. Biomarkers associated with both axonal, neuronal and glial pathology were therefore examined using the new Quadplex SIMOA assay (**Fig.10**). While tau and GFAP were uninformative so far, UCHL-1 and NF-l demonstrated interesting post-injury time courses that may correlate well with PTE development. We found it particular interesting that at the one month time course, the new contusion injury was demonstrating more NF-l than the rotation injury, which appears to peak earlier. This suggests either ongoing neurodegeneration, or a persistent blood-brain barrier breakdown.

F) Neuropathological examination of axonal injury in rotational injury archival tissue

We have quantitatively assessed the axonal pathology in the temporal lobe region of the archival rotational injury animals in order to assess areas of interest for animals being enrolled in the electrophysiology portion of the study. While not unexpected, there was a surprising level of axonal pathology when quantitatively assessed in both the entorhinal region, the fimbria-fornix, and the alveus of the hippocampus, as well as the axonal pathways along the ventricle in this region (**Fig.9**, Table 1).

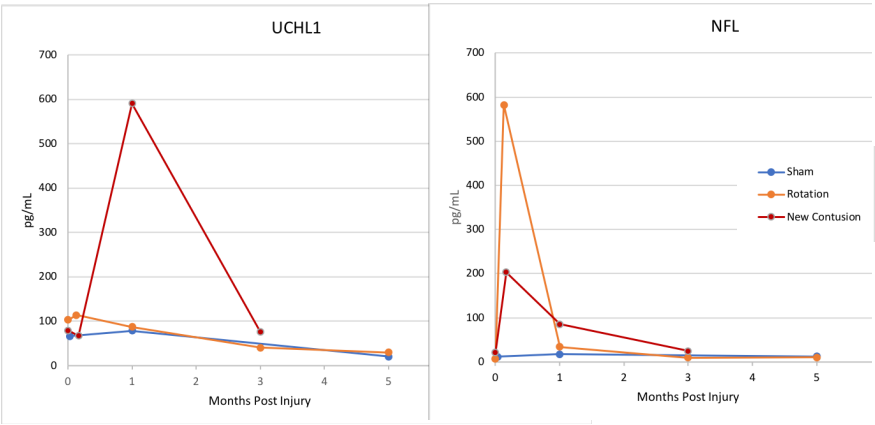


Fig.10 Blood Biomarkers Post TBI. Using a SIMOA assay, we assessed quantities of TBI related proteins in the blood following injury in one animal from sham, rotation, and a new contusion injury. UCHL1 peaked at 1 month in the contusion animals, potentially due to ongoing neurodegeneration. NF-l peaked at 3 days in inertial injury, potentially due to the greater overall burden of axonal pathology, and was greater than in the contusion animal. However, this reversed at the one-month time point, where the contusion injury showed greater NF-l levels than the rotation. This suggests either the BBB is still open at this time point in the contusion, or there is greater neuronal and/or axonal pathology ongoing in the contusion compared to the rotation at one month.

Table 2: Number of Axonal Profiles at Coronal Hippocampal Level

2016-5	2016-12	2016-13	2017-1	2017-2
Injured	Sham	Injured	Injured	Injured
2202	0	1027	3772	2498

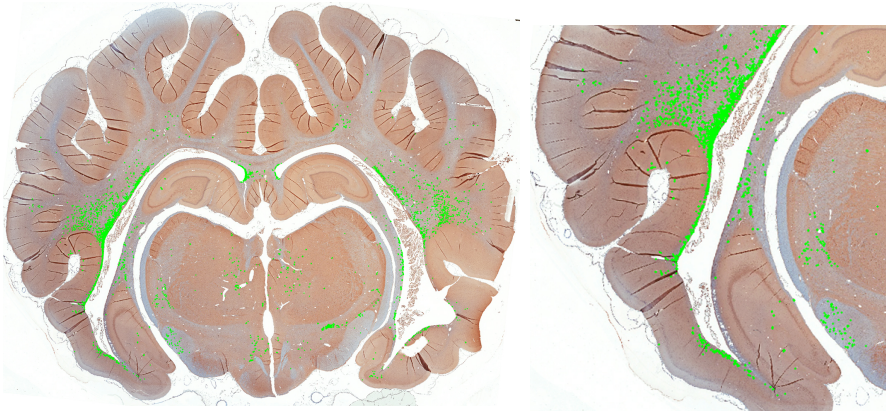


Fig.8. Axonal Pathology from Archive Inertial Injury Animals. APP labeling at 72 hrs post injury allows for quantification of axonal profiles (bulbs and varicosities, marked here in green). Note the diffuse nature of the axonal injury in the white matter, as well as the prominence of axonal pathology in the fimbria-fornix, entorhinal cortex, and pathways leading into the temporal lobe. Assessment of these pathways in our chronically implanted animals may help determine whether deafferentation of the temporal lobe is linked to an increase in TLE post trauma.

Summary of Accomplishments

- Successful design and implementation of depth and grid electrodes for evaluation of pigs following both contusion and rotational injury
- Successful development and deployment of chronically implanted, swappable wireless battery powered system for up to 24 hours of continuous recordings (>12 hrs mentioned in initial proposal)
- Development of an Epilepsy Monitoring Unit for Pigs (PEMU), and successful combination of monitoring of video and these channels post-hoc
- Quantification of axonal pathology in the regions implicated in PTE (deafferentation.)
- Identification of potential biomarkers for PTE in the pig TBI model, with promising results with NF-l and UCHL-1
- Validated the first large animal model of PTE in 3 of 4 animals, waiting for analysis on the 4th (sacrificed at 6 weeks due to an implant issue.)

Stated Goals not Met:

As discussed above and with our program managers, the repetitive injury group was removed from this proposal as the number of orthopedic injuries from the second rotation in the coronal plane was not mitigated. Future proposals will utilize the sagittal plane, which is more resilient, but is not directly comparable to the coronal plane in this proposal and needs to be characterized.

What opportunities for training and professional development has the project provided?

This project was not intended for training and professional development, however has provided excellent opportunities for both the Investigators and the post-doctoral fellows to get more involved in the PTE community, engage with other researchers in the field, and have a significant mentorship component in this area from experts in other areas.

How were the results disseminated to communities of interest?

The current results were disseminated by one talk to a national Functional Neurosurgery conference (H.I Chen, M.D.) and by poster presentations at AES 2019 (see below.) In addition, this project was presented as part of the CURE Epilepsy 2017 consortium on PTE, where additional funding for the pathological portion of the program was obtained for comparison with human cohorts. Multiple papers describing the new CCI model, the development of PTE in these animals, and the biomarker data are in preparation.

What do you plan to do during the next reporting period to accomplish the goals?

During the next quarter we will finish injury and implantation of the final group of sham animals, and continue acquiring data from them including the full Video-EEG configuration that allows for every other day assessment of the animals. In addition, we will continue quantifying both biomarker data and epileptogenic data, as well as collecting serum from the animals pre and post injury at the desired time points. Data structures for storage and analysis of video and EEG have been developed for ease of analysis, and analysis is ongoing.

IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

This project has had an impact on the development of chronic monitoring systems for large animals in PTE, simply by demonstrating that this is possible. The innovative wireless solution to this problem should be translational both up to humans and down to rodents for epilepsy monitoring. This is also the first project to our knowledge that is utilizing both rotational and contusion models and their combination to examine the underlying causes of PTE, and has therefore raised awareness in presenting it that these may be contributions to PTE. To the best of our knowledge, it is the only current program examining PTE in a large animal model as well. The results of the project so far are developmental in nature for the model, but will soon contribute better understanding of the etiology of PTE as we examine the circuitry involved in inertial and contusion models leading to epileptogenesis. In addition, axonal pathology in the temporal lobe in this model suggests an underlying mechanism for the previous reports that diffuse brain injury alone can lead to PTE.

What was the impact on other disciplines?

The chronic implant, laminar electrode technology, and wireless enclosure have significant interest and applications outside of PTE. There are many free roaming large animal experiments that are not being performed currently since this technology is not available. While the goal of this program is the development of a PTE large animal model, we are glad that our contribution to the development of these systems may be translational to other arenas that require freely roaming behavioral, such as social behavior interactions.

What was the impact on technology transfer?

Nothing to report, although the new LACES enclosure and demonstration of a wireless EMU may have an impact on the standard for both animal and human work.

What was the impact on society beyond science and technology?

Nothing to report, although we believe this project will eventually raise public awareness of PTE and potential treatment decision making.

CHANGES/PROBLEMS:

Changes in approach and reasons for change

The initial contusion methodology was discovered to be not severe enough, which required adoption of a more well controlled injury model (Controlled Cortical Impact (**Fig.2**)).

Actual or anticipated problems or delays and actions or plans to resolve them

The adoption of the new contusion model delayed the start of the full group of animals, as did the grid electrode development. We will be adding additional animals to the cohorts to account for this time delay.

Changes that had a significant impact on expenditures

The development of the custom electrodes for the grid rather than an "off the shelf" solution led to two "development" charges from both electrode suppliers that were unexpected expenditures. The animal charges for the development of the contusion model were not included in the initial budget.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

PRODUCTS:

Publications, conference papers, and presentations

Ulyanova, A. V., Koch, P. F., Cottone, C., Grovola, M. R., Adam, C. D., Browne, K. D., Weber, M. T., Russo, R. J., Gagnon, K. G., Smith, D. H., Isaac Chen, H., Johnson, V. E., Kacy Cullen, D., and Wolf, J. A. (2018) Electrophysiological Signature Reveals Laminar Structure of the Porcine Hippocampus. *eNeuro* DOI:http://dx.doi.org/10.1523/ENEURO.0102-18.2018

Platform Presentation: "Contribution of diffuse versus focal injury to the development of epileptiform activity in swine models of TBI." Annual Meeting of the Functional Neurosurgery Society. H. Isaac Chen, M.D.

Abstract: "Acute and chronic in-vivo electrophysiological changes following diffuse and focal traumatic brain injury in a large animal model of post traumatic epileptogenesis."
A. ULYANOVA**,** P. F. KOCH, C. D. ADAM, M. T. WEBER, D. K. CULLEN, B. LITT, D. H. SMITH, V. E. JOHNSON, H. I. CHEN, J. A. WOLF.
Society for Neuroscience, 2017.

Abstract: "Identifying the contributions of contusion and/or inertial injury to epileptogenesis in a large animal TBI model using a wireless epilepsy monitoring unit."
H. I. CHEN**,** A. ULYANOVA, P. F. KOCH, C. D. ADAM, M. T. WEBER, D. K. CULLEN, B. LITT, D. H. SMITH, V. E. JOHNSON, J. A. WOLF.
American Epilepsy Society, 2017

Ulyanova AV, Adam CD, Cottone C, Litt B, Smith DH, Cullen DK, Chen HI, Johnson VE, and JA Wolf. Identifying the contributions of contusion and/or inertial injury to epileptogenesis in a large animal TBI model using a wireless monitoring unit. American Epilepsy Society Annual Meeting 2018.

Ulyanova AV, Cottone C, Litt B, Chen HI, Johnson VE, and Wolf JA. Chronic electrophysiological and histopathological changes in a translational, large animal model of post-traumatic epileptogenesis. American Epilepsy Society Annual Meeting 2019.

Website(s) or other Internet site(s)

http://www.med.upenn.edu/wolflab/

This site will be the location for the dissemination of the publications and links to the electrophysiology and neuropathological databases that are being generated.

Technologies or techniques

The LACES enclosure was developed with Neuralynx, and will be available through them in addition to the CUBE2 and the system we have developed for chronic implantations.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

We are generating a database of ECoG, grid, and depth electrophysiology with these animals that will be made available to other investigators to analyze. In addition, we are generating a neuropathological archive with these animals that will be the first large animal PTE neuropathology comparing these two models (inertial and contusion) and we hope will have an impact on the mechanistic understanding of PTE.

In addition, the wireless methodology and the LACES system will have a significant impact in others replicating this work. Our custom grid and depth electrodes will also be made available to the community via Neuronexus (depth) and CorTec (grid).

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name: John A. Wolf, Ph.D.
Project Role: Principal Investigator
Researcher Identifier (e.g. ORCID ID): ORCID 0000-0002-6950-2303
Nearest person month worked: 1.8
Contribution to Project: Dr. Wolf is the PI/PD of this project.

Name: Victoria E. Johnson, MBChB, Ph.D.
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.2
Contribution to Project: Dr. Johnson is leading the efforts in neuropathology on this project.

Name: H.Isaac Chen, M.D.
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.6
Contribution to Project: Dr. Chen is the neurosurgeon on this project, and is contributing his surgical and epilepsy expertise to the design of the implants and to clinical care of the animals.

Name: Brian Litt, M.D.
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): 0000-0003-2732-6927
Nearest person month worked: 0.6
Contribution to Project: Dr. Litt is heading up the efforts to store and analyze the data from this project using his expertise in seizure detection and cloud storage.

Name: Robert Siman, Ph.D.
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.6
Contribution to Project: Dr. Siman is developing the SNTF and GFAP biomarker platforms for the detection of post-injury markers that may be prognostic for PTE.

Name: Alexandra Ulyanova, Ph.D.

Project Role: Post-Doctoral Fellow
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 10
Contribution to Project: Dr. Ulyanova has decided she will be the post-doc for this project, which we are grateful for as she has experience with PTE and large animal models. She has helped design the Video/EEG paradigms for the chronically implanted animals.

Name: Carlo Cottone, Ph.D.
Project Role: Post-Doctoral Fellow
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 3
Contribution to Project: Dr. Cottone is an expert in the analysis of oscillatory electrophysiology data. He is paid off of an R01, but contributes his effort here as well.

Name: Christopher Adam
Project Role: Research Specialist
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 2
Contribution to Project: Mr. Adam is an expert technician with large animal electrophysiology. He is paid off of another grant, but donates time to this project as well.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

SPECIAL REPORTING REQUIREMENTS

Updated Quad Chart Attached.

Diffuse and Focal Brain Injury in a Large Animal Model of PTE: Mechanisms Underlying Epileptogenesis

EP150058
W81XWH-16-1-0675

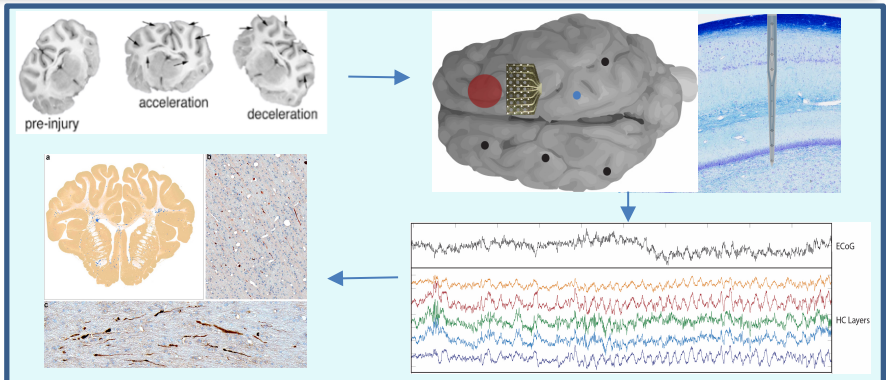
PI: John A. Wolf, Ph.D. **Org:** University of Pennsylvania **Award Amount:** $799,887

Study/Product Aim(s)

- Aim 1: Determine the relative contributions of diffuse, repetitive diffuse and focal brain injury to the development of PTE.
- Aim 2: Elucidate the circuitry alterations underlying hyperexcitability and epileptogenesis in the above forms of TBI using high density chronic electrophysiology of the hippocampus and cortex and neuropathology.
- Aim 3: Determine the utility of established blood biomarkers associated with axonal injury in identifying the progressive white matter degeneration leading to epileptogenesis via deafferentation.

Approach

Epileptogenesis in a large animal model of purely focal and purely diffuse brain injury will be compared with mild repetitive diffuse brain injury and focal injury superimposed on a diffuse injury. We will assess the contribution of each type of injury to the development of PTE, creating the first large animal model of PTE as well as the role of each type of injury. In addition, potential biomarkers for PTE will be assessed.



Pigs will be injured either with an inertial injury, a focal injury (contusion), repetitive inertial, or a combination of inertial and focal injury. These animals will then be implanted with depth and cortical electrodes to monitor epileptogenesis. Biomarkers and neuropathological outcomes will be compared to electrophysiological outcomes.

Accomplishment: We have developed a new CCI model and characterized it, as well as demonstrating a electrographic seizures in this large animal model.

Timeline and Cost

Activities CY	16	17	18	19
Approvals/Design of Monitoring				
Implant Group (all injuries)				
Video EEG Monitoring/Analysis				
Assess Biomarkers and Pathology, Compare to EEG				
Estimated Budget ($K)	**$67K**	**$267K**	**$267K**	**$200K**

Updated: 10/15/2019

Goals/Milestones

CY16 Goal – ACURO/IACUC Approval
☑ Electrode Design and Testing
CY17 Goal – Injury and Implantation of Group 1 EEG Monitoring
☑Complete EMU Setup and LACES development
☑All injury types represented and implanted
☑Complete Biomarker Development
CY18 Goals – Monitoring and Group 2, 3 Implanted
☑ Monitor with VideoEEG, Neuropath. Correlation to VideoEEG
☐ Comparison of Biomarkers with EEG outcome measures
CY19 Goal – Group 4 implanted, Completion and Analysis
☐ Compare PTE outcomes with Neuropath and Biomarker Predictions

Comments/Challenges/Issues/Concerns

- Revised implants to address bone loss
- Seizures in 3 animals according to expert interpretation

Budget Expenditure to Date

Projected Expenditure: $ 799,887
Actual Expenditure: $794,000