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TITLE: Dissecting neuronal participation to focal epileptic events in vivo

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CONTRACTING ORGANIZATION: Boston VA Research Institute, Inc.

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13 SUPPLEMENTARY NOTES

14. ABSTRACT

The high prevalence of epilepsy among veteran populations with traumatic brain injury (TBI) makes epilepsy one of the congressionally directed topic areas. In previous studies, the electroencephalography (EEG) recording at the cortical surface during TBI induced epileptic seizures revealed hyper-synchronous epileptic bursts, whereas single cell recordings found heterogeneous neuronal spikes during the hyper synchronous EEG bursts (Truccolo et al., 2011). To define the correlation between the EEG and single neuron activity and to determine how different cell types participate in seizure events, we monitor the individual activity of a large number of neurons in vivo using 2 photon microscopy. As a model of the long term effects of TBI, we inject tetanus toxin (TeT) into the visual cortex of mice to induce seizures. The activity dependent calcium indicator GCamp6, which in our case is expressed in selective neurons by gene modification, or in all neurons by virus infection, reports the activity of individual neurons. Several types of neurons from multiple layers of the visual cortex are recorded at several time points. The experimental timeline is shown in Figure 1.

15. SUBJECT TERMS

NONE LISTED

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1. INTRODUCTION

The high prevalence of epilepsy among veteran populations with traumatic brain injury (TBI) makes epilepsy one of the congressionally directed topic areas. In previous studies, the electroencephalography (EEG) recording at the cortical surface during TBI-induced epileptic seizures revealed hyper-synchronous epileptic bursts, whereas single-cell recordings found heterogeneous neuronal spikes during the hyper-synchronous EEG bursts (Truccolo et al., 2011). To define the correlation between the EEG and single-neuron activity and to determine how different cell types participate in seizure events, we monitor the individual activity of a large number of neurons in vivo using 2-photon microscopy. As a model of the long-term effects of TBI, we inject tetanus toxin (TeT) into the visual cortex of mice to induce seizures. The activity-dependent calcium indicator GCamp6, which in our case is expressed in selective neurons by gene modification, or in all neurons by virus infection, reports the activity of individual neurons. Several types of neurons from multiple layers of the visual cortex are recorded at several time points. The experimental timeline is shown in Figure 1.

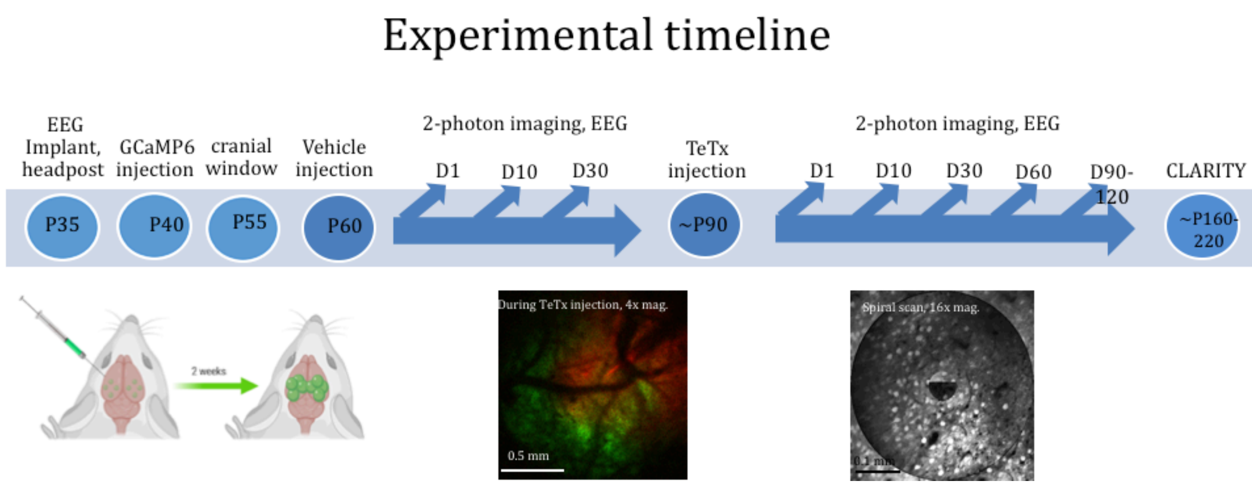


Figure 1 The experimental time line.

2. KEYWORDS:

synchronization,
excitation and inhibition balance,
systems neuroscience,
visual cortex,
traumatic brain injury,
epilepsy,
tetanus toxin,
seizure,
GCamp6
calcium indicators,
patch-Clamping

3. ACCOMPLISHMENTS

Major Goals and Objectives:
(as stated in the modifies SOW, site: Jamaica Plain VA Hospital)

START DATE OF THE AWARD (BAYLOR COLLEGE OF MEDICINE: October, 1, 2015.
TRANSFER DATE TO BVARI (JAMAICA PLAIN VA HOSPITAL): June 1, 2016.
ACURO APPROVAL FOLLOWING TRANSFER: May 31, 2017.

NEW PERIOD COVERED BY THIS REPORT: 10/31/2019 – 9/29/2020.
Please note that this report also serves as the final report for the duration of this award, ie from 10/1/2015 to 9/29/2020.

Please note that the original award was initiated at Baylor College of Medicine 10/1/2015. Subsequently, I moved to Jamaica Plain Veterans Administration Hospital, Harvard Medical School 12/27/2015. My laboratory's move was completed on 6/2016. New IACUC and ACURO approvals were necessary before restarting experiments. Final ACURO approval was received 6/1/2017. Therefore due to the administrative delays (in excess of 18 months) incurred by the transfer we requested and were granted first a 1-year no cost extension and then a further no cost extension to complete the original aims. The modified SOW to account for the requested extension is appended below.

SOW

Goals / Timeline as originally stated **Specific Aims 1, 2 will proceed in parallel**	**New Proposed Timeline**	**Site**
Study pyramidal and PV+ interneuron cohorts	Months	JP VA H.
Hire a new postdoc, train personnel, Set up the TeT injection experiments, IACUC and ACURO approval. Originally anticipated to take 4 months.	Completed. Originally this was completed at Baylor College of Medicine. It was completed again after transfer at JP VA Hospital (ACURO approval granted 6/1/2017)	BCM and JP VA H.
SA#1,2 proceed in parallel, studying the pyramidal neurons. Originally anticipated to take 16 months (from month 4 to month 20)	Experiments completed. Analysis completed.	BCM and JP VA H.
Studying PV+ interneuron cohorts. Originally planned from month 9 to 24.	Experiments completed. Initial analysis completed.	JP VA H.
Milestone(s) To Achieve:		
Write a first manuscript.	Manuscript is in progress. We expect to submit it for publication by12/2020	JP VA H.
Study SOM+ and VIP+ interneuron cohorts		
Have SA#1,2 proceed in parallel studying SOM+ interneurons. Originally planned from month 16-30.	Experiments completed. Initial analysis in progress – data are integrated to present in manuscript form.	JP VA H.

Have SA#1,2 proceed in parallel studying VIP+ interneurons. Originally planned from month 24-34.	Instead of completing this goal, we opted to focus on completing additional patch-clamp experiments as these give more important scientific information. We therefore deferred the performance of the VIP experiments. We did not have sufficient funds to pursue both goals at this time.	JP VA H.
Write 1-2 additional manuscripts. Originally planned from month 30-36.	We decided to concatenate the planned second manuscript with the first manuscript that was originally planned to be published separately. We will now publish one more comprehensive, larger paper.	JP VA H.
Milestone(s) Achieved: 1. 1-2 Manuscripts	Presentation in American Epilepsy Society Conference 2019 (See below); One comprehensive manuscript is in progress to be submitted ~12/2020	JP VA H.

<u>What Was Accomplished during the (entire) period of this award :</u>

1) We established and optimized a two-photon (optical methods) experimental approach for studying the TeT model of epilepsy. We developed algorithms for analyzing calcium imaging data from epileptic cortex in conjunction with EEG recordings. Importantly, we modified the TeT injection protocol, increasing the concentration of TeT injected. This made this seizure model more reliable in terms of exhibiting seizure events, and therefore better amenable to in-vivo 2-photon microscopy analysis. We reported this at the Boston VA Research Week Conference, May, 2018.
2) We discovered that micro-seizure foci develop in cortex over time following TeT injection and characterized their evolution over time. Overall, we found that there were stronger micro-seizure events in layer 4 compared to L2/3, more so in animals injected with a high (versus a low) TeT dose. The microseizure events peaked around day ~30-40 post injection and then subside between day 60 and day 90, following also the known trajectory of epileptic seizures in this animal model.
3) In vivo patch-clamp recordings describe the recruitment of neurons into the observed micro-seizure events. In brief, the potential of cells that engage into micro-seizures increases sharply by ~40mV at the beginning of a micro-seizure event. This sharp depolarization blocks action potential firing until the end of the depolarization event, when an additional brief burst of spike activity occurs. The potential then returns to baseline slowly, followed by a period of hyper-polarization.
4) We imaged 7 mice with genetically labeled parvalbumin (PV)-expressing interneurons (3 low-dose, 4 high-dose), and 5 with genetically labeled somatostatin (SOM)-expressing interneurons (2 low-dose, 3 high-dose). Preliminary analysis of the PV+ populations shows that participation in microseizure events was more diverse in PV+neurons than in pyramidal units (see figure 3). Analysis of SOM+ populations is ongoing at this time.

5) In 4 high-dose animals, we employed a new low-magnification imaging strategy utilizing the one-photon fluorescence of GCaMP6 and a 100Hz frame-rate sCMOS camera to capture the spread of microseizure events at the cortical surface in quasi-real-time. This allows us to determine the full spatial extent of the abnormal discharge events, and we are currently finalizing their analysis.
6) Our observations confirm that the TeT model serves as a useful paradigm for studying the effects of mild traumatic brain injury on hyper-excitability and epileptogenesis.

We present some of the basic findings in the figures below:
EEG changes: Figure 2 illustrates data from animals that received the higher dose of TeT, compared with animals injected with Bovine Serum Albumin (BSA). EEG events are classified into 1) seizures (defined as >10 sec long high-amplitude (>3 SD) events that contain at least ~2 sec of high-frequency oscillations (>10 Hz), and that are clearly visible as an episode with a clear beginning and an end. 2) Interictal spikes: Single, high-amplitude events (>5 SD)and a half-width of ~10 ms. 3) spike-wave events: 4-8 Hz oscillations with clearly visible, alternating spike and wave components, >1 sec long. 4) other abnormal EEG signatures with high amplitudes (>3 SD) that may consist of combinations of event classes 1-3, but lack consistent behavior or are not long enough.

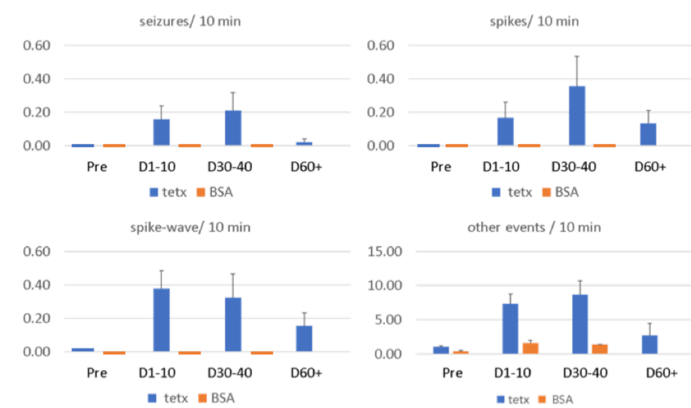
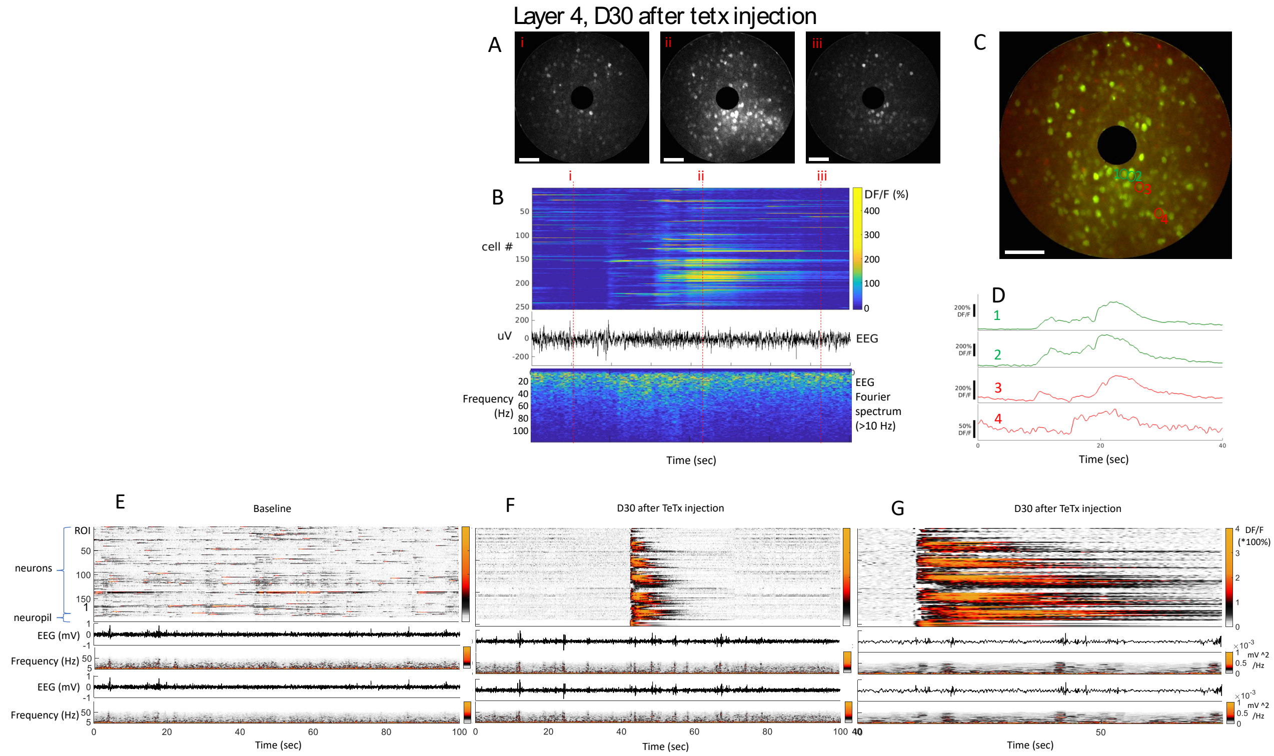


Figure 2: ***Pre:*** prior to injection. ***D1-10***: first 10 days post injection; ***D30-40:*** days 30-40 post injection; ***D60+:*** days 60-80 post injection. ***Y-axis:*** Event frequency (number of events/10min).

Development of Micro-Seizures: Figure 3 illustrates the observation that local groups of synchronously hyperactive neurons appear over time (Layer 4 > layer 2/3). **Panel 3A** shows a typical calcium event recorded from layer 4 of area V1 in an animal injected with the high dose of TeT injection on day 30 (D30) post injection (**i:** before, **ii:** during, **iii:** after the event). We observed multiple such calcium events evolving over several seconds as indicated in the figure. These events were never noted in control animals injected with BSA (**fig 3E**). Note that the EEG spectrum has increased power (but not increased amplitude) in the range of frequencies 20-60Hz (**fig. 3B lower panel**), when the calcium signal is also increased (**fig. 3B upper panel**). Note also that the focal abnormal calcium events sometimes have no obvious correlate on the EEG, even though they presumably represent “mini-seizures.” **Panels 3C,D** show how the calcium signal evolves during the event shown in **panel 3A** in 2 pyramidal (#1,2) and two PV+ (#3,4) interneurons. This animal had its PV+ interneurons labeled with Td+ so they look orange when they are double labeled with the green GCamp6s. Note that signals largely co-vary, in pyramidal neurons, but appear more diverse in PV+ cells. It is likely that a relative failure of PV+ interneurons to follow pyramidal cell activity contributes to ictal generation in this model.

Figure 3. Local groups of synchronously hyperactive neurons appear over time (Layer 4 > layer 2/3). A) Neural population labelled with GCaMP6m, scanned in spiral mode, 400 µm below the dura.



Scan was taken 30 days after TeT injection. Panel i and iii show a snapshot of baseline activity, while ii is taken during a period of hypersynchronous, elevated activity that is clearly visible in most cells. See **B)** for a raster plot of the DF/F calcium activity (top) with simultaneously acquired EEG (voltage: middle, Fourier spectrum: bottom). Note that there is no change in EEG amplitude during the elevated cellular activity, but the high-frequency spectral power is increased during the event. **C)** parvalbumin-expressing (PV+) interneurons were co-labelled with tdTomato and appear yellow, while all other neurons are green. **D)** putative pyramidal cells (green) exhibit a highly correlated activity increase, whereas some PV+ interneurons have distinct time courses. **E)** Reference activity in another animal prior to TeTx injection, and 30 days after BSA injection. ***Top:*** calcium traces (DF/F) for neurons (top) and neuropil (bottom). ***Below:*** EEG ipsilateral to imaged window, relative power spectrum. ***Bottom two rows:*** contralateral EEG and power spectrum. Note that the time point prior to TeTx injection corresponds to D30 after vehicle injection in the same area. **F)** Activity from the same group of cells 30 days later, showing a microseizure in the calcium activity plot. **G)** Same event shown in F, zoomed in to 15 seconds around the seizure event. Note that neuropil activity changes before neurons respond, EEG shows only subtle changes.

Evolution of Micro-seizures as a function of time after TeT injections: Percent of time spent in a state of micro-seizure in L4 vs L2/3 as a function of time after TeT injection for both low- and high-dose TeT injections. Note that the micro-seizures are detected in both L2/3 and L4 after high-dose injection, and are most abundant in L4 21-40 days post high-dose injection (using Kruskal-Wallis nonparametric ANOVA with multiple comparisons correction).

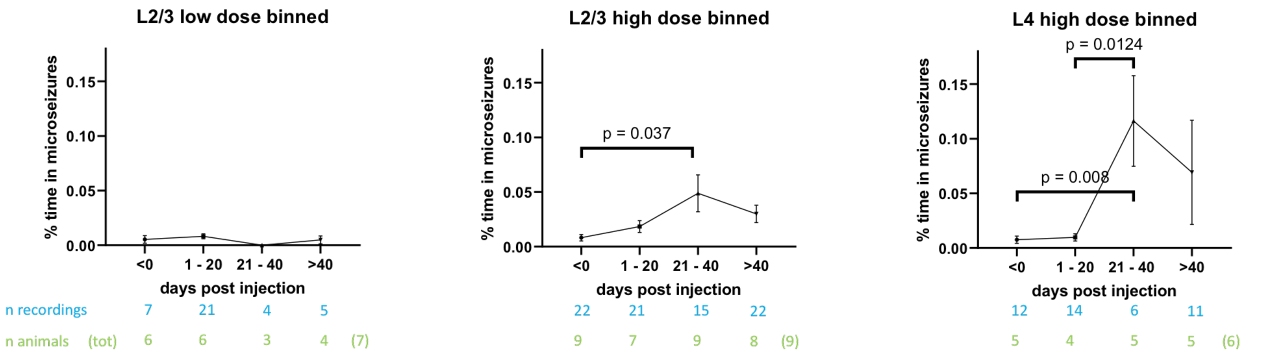
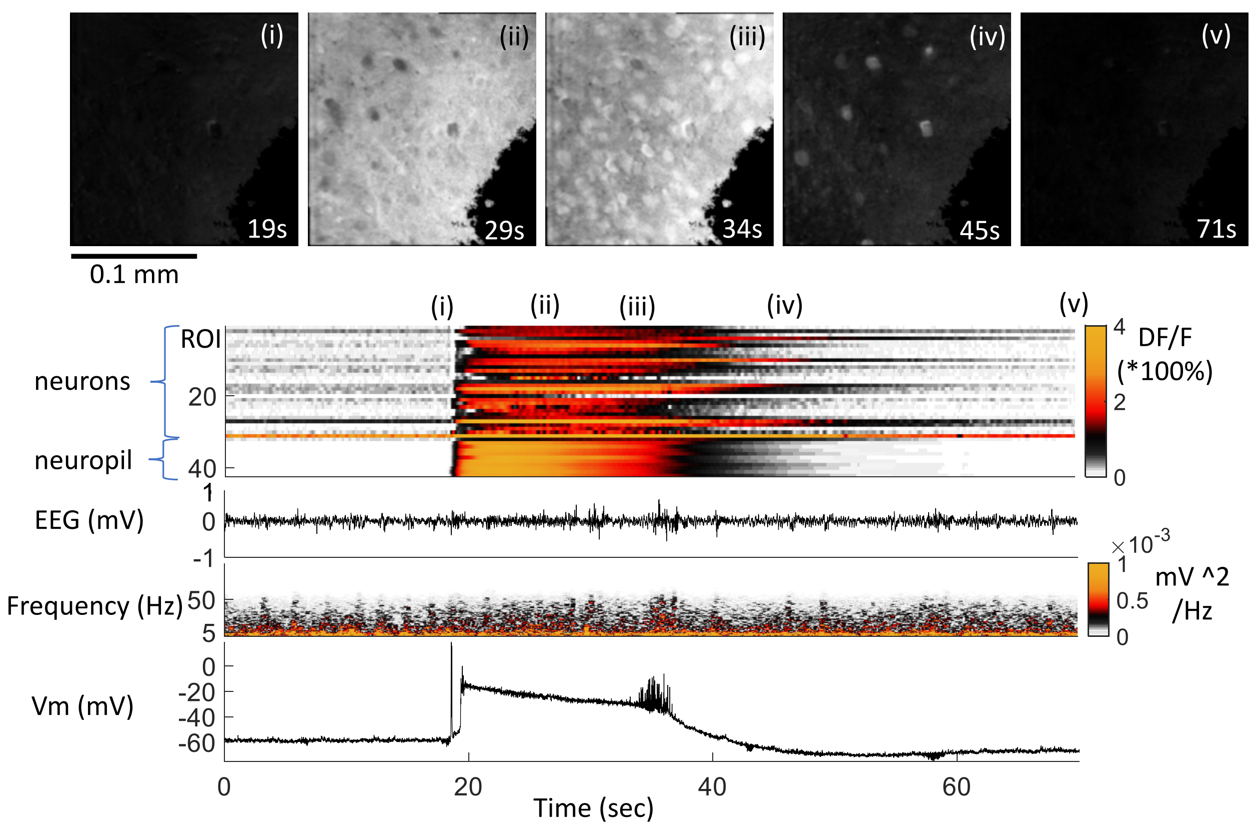


Fig. 4: Percent of time spent in a state of micro-seizure in L4 vs L2/3 as a function of time after TeT injection.

In vivo patch clamp electrophysiology corroborates calcium imaging results:

Figure 4: ***Top:*** calcium images before (i), during (ii, iii) and after (iv, v) the event recorded in vivo during spiral scanning. The raster plot shows the calcium signal (DF/F) in both neurons and neuropil patches following the convention in figure 3. The EEG trace appears below the raster plot. ***Bottom:*** Spectrogram of the whole-cell voltage trace (orange) and simultaneously recorded EEG voltage (red) during a "microseizure" event in L4 of a high-dose TeTx injected animal, D45 post injection. The plot below is the membrane voltage of a pyramidal neurons (whole-cell recording). Note the sharp rise of the potential inducing a depolarization block, followed by a burst of spikelet firing at the end of the depolarization wave and a slow return to baseline following a long period of slight hyperpolarization. These events occurred at an average rate of 1-5 per hour in high-dose animals around day D30-45 post -injection.



Summary: Tetanus toxin injection in L5/L6 of mouse V1 causes local hyperactivity to emerge over several weeks. We show that mice develop several different types of abnormal EEG discharge patterns, including bilateral spikes and less frequently seizures after unilateral injection of TeT. These patterns (seizures, single spikes, spike-wave complexes, irregular oscillations) evolve dynamically over several weeks, peak around 10-30 days post TeT injection, and gradually subside thereafter. Longitudinal calcium imaging over several weeks shows prominent, localized groups of hyperactive and hypersynchronous neurons. Although the toxin spreads to upper layers during injection, most of the hyperactivity occurred in deeper layers. The abnormal events of brief hyperactivity ("micro-seizures") are characterized by abnormally high DF/F signals calcium signals (>7 SD above mean activity) in most of the local neurons, typically last for a few seconds and are not always accompanied by overt changes in the EEG. However, many of these events are accompanied by elevated high-frequency (HF) power in the EEG. We are currently investigating under which conditions these HF oscillations preferentially coincide with micro-seizure events, and to what degree they represent a signature of local hyperactivity. Single-cell patch clamp recordings reveal that neurons inside the "microseizure" focus are strongly depolarized throughout these events. Furthermore, we are currently analyzing whether baseline activity (i.e. activity outside of micro-seizure events) several weeks after injection differs from pre-injection activity, by computing pair-wise correlation coefficients and functionally clustering neurons to determine whether/how overall network dynamics have been impacted by TeT injections. Taken together, this indicates that the TeT 2-photon imaging model of epilepsy can serve as a useful paradigm for studying the effects of mild traumatic brain injury on hyper-excitability and epileptogenesis.

We are concluding the analysis of how groups of neurons evolve synchronous firing patterns over time, including how interneurons contribute to chronic seizure generation, and are in the process of writing a comprehensive manuscript outlining our observations. We expect it will be ready for submission ~12/2020.

Opportunities for training and professional development:

Dr Meyer, Lombardo and Palagina worked on these experiments and developed and honed their skills for 2-photon imaging in general, and as it applies to the study of epilepsy in particular. Specifically they learned to perform calcium imaging and simultaneous EEG recordings in TeT injected animals, how to perform EEG analysis, and how to perform and analyze patch-clamp data (J Meyer) in conjunction with EEG monitoring.

Results Disseminated to communities of interest:

We reported our results in the American Epilepsy Society Meeting 12/2019: AES presentation 12/2019: **J. F. Meyer, Z. Hao, S. Smirnakis,** "Local disinhibition via tetanus toxin injection reshapes neural activity and EEG patterns in mouse neocortex", abstract #3.007, American Epilepsy Society Annual meeting, Baltimore, 2019, as well as in the Boston VA Research Week Conference, May 2018: **Z Hao, JF Meyer, J Lombardo, A Palagina, S Smirnakis**. "Network-level Interrogation of Cortical Seizures in a Mouse Model of Focal Epilepsy."

Final dissemination of our results will be through our current manuscript which is in preparation, expected to be submitted ~12/2020.

Plans for the next reporting period:

The award has now closed. We expect a final manuscript to be submitted for publication ~12/2020.

4. IMPACT:

Preliminary results from this work were presented in the American Epilepsy Society Conference, 12/2019, and the Boston VA Research Week Conference, 5/2020 and were well received. We are currently preparing a comprehensive manuscript, to be submitted ~12/2020.

The impact of our project on:
1) the development of the principle discipline**: i)** We have established the 2-photon paradigm for analyzing the TeT model of Epilepsy**. ii)** We improved our understanding of how seizures get generated in the TeT model of epilepsy and discovered that neurons, especially in cortical layer 4, form small hyper-synchornized groups that fire exuberantly together entraining the circuit. These "microseizure" events have at times no obvious EEG correlate and demonstrate the importance of using optical imaging methods for studying epileptogenesis. We studied the role that interneurons play in the generation of aberrant patterns of activity.
2) other disciplines: nothing to report
3) technology transfer: we will make available all software methods developed for EEG and Calcium analysis upon completion of the analysis and publishing of the manuscript.
4) society beyond science and technology: nothing to report

5. CHANGES / PROBLEMS

We faced several challenges during the execution of this award. However these did not prevent us from achieving our planned goals. We summarize these challenges and their resolution below: As reported previously, there was a delay associated with the Award transfer from Baylor College of Medicine to BVARI at JP VA Hospital (Boston VA System). The new ACURO approval was obtained on 5/31/2017. Subsequent to this, there was also a delay incurred from 11/2017 to 2/2018 due to procedural issues involving our IACUC approval that we reported previously, which were resolved. Given these delays we have obtained a 1-year no cost extension and submitted an amended SOW that was approved on 9/2018. In august 2019 we extended this. Finally the covid-19 pandemic resulted in suspension of all experiments starting 3/2020. Experiments are only restarting now with special petition (9/2020). Despite these challenges, we were successful in completing the large majority of the proposed aims (See SOW). The goals were entirely completed, except for the original proposal to study of the VIP+ interneurons. The reason this was deferred was that we felt that it would be more valuable scientifically to obtain patch-clamp recordings from the neurons that participated in the microseizures, to validate and understand the mechanism of the observed calcium events. Unfortunately dedicating personnel time to this new aim, meant that there was not enough funding left to study in parallel VIP+ cells. One reason this was not possible is that there had been already a large delay to transfer the grant from Baylor College of Medicine to the JP VA, and re-obtain ACURO and IACUC approval after the transfer to the new institution, which delayed progress and necessitated expending a fraction of the funds to support personnel in transition (i.e. while waiting for IACUC approval without being able to perform experiments). We plan to apply for additional funding in the future to pursue our observations, particularly the mechanism by which micro-seizure foci emerge, and to study more comprehensively VIP+ cells as well as other types of interneurons.

Personnel Changes:

Personnel changes from reporting period to reporting period were reported in prior reports. As the project is finishing no further personnel changes are expected.

6. PRODUCTS

AES presentation 12/2019: **J. F. Meyer, Z. Hao, S. Smirnakis,** "Local disinhibition via tetanus toxin injection reshapes neural activity and EEG patterns in mouse neocortex", abstract #3.007, American Epilepsy Society Annual meeting, Baltimore, 2019.

Boston VA Research Week Conference, May 2018. Z Hao, JF Meyer, J Lombardo, A Palagina, S Smirnakis. "Network-level Interrogation of Cortical Seizures in a Mouse Model of Focal Epilepsy"

Manuscript in Preparation to be submitted ~12/2020

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

Name: Stelios Smirnakis
Project Role: Principal Investigator
Researcher Identifier: orcid.org/0000-0002-1929-2811
Nearest person month worked: 2 months
Contribution to Project: Conceive and Design the project. Participate in experimental planning and analysis.

Name: Jochen Meyer
Project Role: instructor
Researcher Identifier: orcid.org/0000-0002-3976-3334
Nearest person month worked: no change (as reported previously)
Contribution to Project: Participate in training, experiments and analysis, though a subcontract at Baylor College of Medicine. His sub-contract came to an end on 9/30/2018.

Name: Ganna (Anna) Palagina
Project Role: Postdoctoral Associate.
Researcher Identifier: https://orcid.org/0000-0001-9857-9062
Nearest person month worked: no change (as reported previously)
Contribution to Project: Chief responsibility is to perform data analysis, and help Dr Lombardo with experiments.

Name: Joseph Lombardo
Project Role: Postdoctoral Associate.
Researcher Identifier: orcid.org/0000-0003-4806-0849
Nearest person month worked: no change (as reported previously)
Contribution to Project: Conduct Experiments and perform data analysis with the help of Dr Palagina.

No other organizations were involved as partners. During the new period of this report (10/30/2019-9/29/2020), Dr Meyer was continuing to perform analysis supported by other funds at Baylor College of Medicine.

8. SPECIAL REPORTING REQUIREMENTS:

None.

9. APPENDIX OF ANIMALS IMAGED

experimenter	mouse ID	animal # for paper	ca indicator	on computer?	tdtomato to interneurons?	seizures?	before	1 hr	D1	D2-7	D10+/-	D30+/-	D45+/-	D60+/-	D90+/-	L4 data present?	Patching, LFP recordings	Analyzed with CNMF?
	for paper (new since last progress report)																	
tetanus-toxin 1x dose																		
ZH	zh83G6BN						Ca, EEG	EEG	EEG	Ca, EEG	Ca, EEG							
ZH	zh96_Thy1_BN	1	Thy1-Gcamp6S	JFMEYER-DT-01			Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG					
ZH	zh99_Thy1_2272		Thy1-Gcamp6S				Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG							
ZH	zh117_Thy1_2268		Thy1-Gcamp6S				Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG (D20)	EEG	EEG					
ZH	zh117_Thy1_2289	2	Thy1-Gcamp6S	JFMEYER-DT-01			Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG					
ZH	zh129_Thy1_2296		Thy1-Gcamp6S	JFMEYER-DT-01			Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG					
ZH	zh152_Thy1_2417		Thy1-Gcamp6S				EEG	EEG	EEG	EEG	EEG	EEG						
ZH	zh153_Thy1_2418		Thy1-Gcamp6S				EEG	EEG	EEG	EEG	EEG	Ca, EEG						
JM	Tet_mouse_021616		AAV-G6M				Ca, EEG	Ca, EEG			Ca, EEG							
JM	Tet-Som_cre_mouse 5571	3	AAV-G6M	Jochenfmeyer-PC	SOM+		Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG						patching D16	
JM	Tet_Som-cre_mouse_1	4	AAV-G6M	Jochenfmeyer-PC	SOM+		Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG							
JM	Tet_PV-Cre_091416	5	AAV-G6M	NA208-DT-02	PV+		Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG							yes
JM	Tet_PV-Cre_092016	6	AAV-G6M	NA208-DT-02	PV+		Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG							
JM	WT_Dlx_3	7	AAV-G6M	JFMEYER-DT-01	Dlx+		Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG		Ca, EEG				yes
JM	Tet_Ai96-Syn-cre_mouse_1	8	Ai96/syn-cre	Jochenfmeyer-PC			Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG				yes
JM	Tet_PV-Cre_N424		AAV-G6M	Jochenfmeyer-PC	PV+								Ca, EEG					
JM	Tet_PV-Cre_N425	9	AAV-G6M	Jochenfmeyer-PC	PV+		Ca, EEG	Ca, EEG					Ca, EEG	Ca, EEG				yes
	TeT_mouse_PV-Cre_021616		AAV-G6M	Jochenfmeyer-PC	PV+		Ca, EEG	Ca, EEG		Ca, EEG								
total Ca							**11**	**11**	**8**	**8**	**9**	**5**	**5**	**3**				
total EEG							**11**	**11**	**8**	**8**	**9**	**5**	**5**	**3**				
tetanus-toxin 33x dose																		
ZH	zh154_Thy1_2423		Thy1-Gcamp6S				EEG	EEG	EEG	EEG	EEG	EEG						
ZH	zh154_Thy1_2424		Thy1-Gcamp6S				EEG	EEG	EEG	EEG	EEG	EEG						
ZH	zh137_Thy1_2425	10	Thy1-Gcamp6S	JFMEYER-DT-01			Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG		Ca, EEG					
ZH	2096_G6						EEG	EEG	EEG	EEG	EEG		EEG					
ZH	zh41_G6_BR						Ca, EEG	EEG	EEG	EEG	EEG	EEG	EEG					
JM	Tet_PVCre_mouse_N606	11	AAV-G6M	NA208-DT-02	PV+	no	Ca, EEG	Ca, EEG				Ca, EEG		Ca, EEG		yes	LFP D30	yes
JM	Tetx_mouse_N673	12	Ai96/syn-cre	Jochenfmeyer-PC			Ca, EEG	Ca, EEG				Ca, EEG		Ca, EEG		no	LFP D30	yes
JM	Tetx_mouse_N516	13	Ai96/syn-cre	JFMEYER-DT-01			Ca, EEG	Ca, EEG		Ca, EEG		Ca, EEG		Ca, EEG		no	LFP D37	now
JM	Tetx_mouse_N574	14	AAV-G6M	JFMEYER-DT-01	PV+		Ca, EEG	Ca, EEG				Ca, EEG						
JM	Tetx_mouse_N785	15	AAV-G6M	JFMEYER-DT-01	PV+		Ca, EEG	Ca, EEG	Ca, EEG									
JM	Tetx_mouse_N763	16	AAV-G6M	NA208-DT-02	PV+		Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG		Ca, EEG		yes	LFP D35	yes
JM	Tetx_mouse_N666	17	Ai96/syn-cre	4HHP1HO			Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG	no	patching D125	now
JM	Tetx_mouse_N748	18	Ai96/syn-cre	JFMEYER-DT-01			Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG	no	patching D115	
JM	SST180604MO_N	19	AAV-G7F	JFMEYER-DT-01	SOM+		Ca, EEG	Ca, EEG			Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG		yes	patching D46	
JM	SST180710_MM_N	20	AAV-G7F	JFMEYER-DT-01	SOM+		Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	yes	patching D40	yes
JM	SST180710_MM_L	21	AAV-G7F	JFMEYER-DT-01	SOM+		Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG			Ca, EEG	yes	patching D40	yes
JM	N_1112	22	Thy1-Gcamp6S	4HHP1HO			Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	no		yes
JM	N_1113	23	Thy1-Gcamp6S	4HHP1HO			Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	yes	patching D43	yes
total Ca							**14**	**14**	**10**	1	**9**	**12**	**5**	**10**	**6**			
total EEG							**14**	**14**	**10**	1	**9**	**12**	**5**	**10**	**6**			
BSA																		
ZH	zh83G6BL						EEG	EEG	EEG	EEG	EEG							
ZH	zh84_G6						EEG	EEG	EEG	EEG	EEG	EEG						
ZH	zh117_Thy1_2269		Thy1-Gcamp6S	JFMEYER-DT-01			Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	EEG	EEG					
ZH	zh117_Thy1_2292		Thy1-Gcamp6S	JFMEYER-DT-01			Ca, EEG	Ca, EEG	Ca, EEG	EEG	EEG	EEG	EEG					
ZH	zh153_Thy1_2416		Thy1-Gcamp6S				EEG	EEG	EEG	EEG	EEG	Ca, EEG						
ZH	zh170_Thy1_2385		Thy1-Gcamp6S				EEG	EEG	EEG	EEG	EEG	EEG						
JM	Tet_PVCre_mouse_N606		AAV-G6M	NA208-DT-02	PV+		Ca, EEG	Ca, EEG		Ca, EEG								yes
JM	Tetx_mouse_N673		Ai96/syn-cre	Jochenfmeyer-PC			Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG						yes
JM	Tetx_mouse_N516		Ai96/syn-cre	JFMEYER-DT-01			Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG	Ca, EEG						
JM	Tetx_mouse_N574		AAV-G6M	JFMEYER-DT-01	PV+		Ca, EEG	Ca, EEG		Ca, EEG								yes
JM	Tetx_mouse_N785		AAV-G6M	JFMEYER-DT-01	PV+		Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG							
JM	Tetx_mouse_N763		AAV-G6M	JFMEYER-DT-01	PV+		Ca, EEG	Ca, EEG			Ca, EEG							yes
JM	Tetx_mouse_N666		Ai96/syn-cre	JFMEYER-DT-01			Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG	Ca, EEG						yes
JM	Tetx_mouse_N748		Ai96/syn-cre	JFMEYER-DT-01			Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG						
JM	SST180629_MN_N		AAV-G7F	JFMEYER-DT-01	SOM+		Ca, EEG	Ca, EEG			Ca, EEG							
JM	SST180710_MM_L		AAV-G7F	JFMEYER-DT-01	SOM+		Ca, EEG	Ca, EEG			Ca, EEG	Ca, EEG						yes
JM	SST180604MO_N		AAV-G7F		SOM+		Ca, EEG	Ca, EEG			Ca, EEG							
JM	SST180710_MM_N		AAV-G7F	JFMEYER-DT-01	SOM+		Ca, EEG	Ca, EEG			Ca, EEG							yes
total Ca							**14**	**14**	**5**	**6**	**11**	**6**	0	0				
total EEG							**18**	**18**	**9**	**11**	**16**	**10**	2	0				

<u>Completed Set of Experiments. PV+:</u> parvalbumin positive interneurons express Td Tomato and can therefore be identified and analyzed; *<u>SOM+:</u>* Somatostatin positive interneurons express Td-Tomato. *<u>EEG:</u>* electro-encephalogram has been obtained. *<u>Ca:</u>* 2-photon calcium imaging of

neural activity has been obtained. *BSA:* Control animals injected with bovine serum albumin. *CNMF:* Algorithm used for data pre-processing (based on an algorithm devised by E. Pnevmatikakis). *Patching:* Animals that underwent patch-clamp experiments. Note that some animals were injected first with BSA and monitored for 30-60 days and then injected with TeT. This allowed them to serve as their own controls.