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TITLE: Dissecting Neuronal Participation to Focal Epileptic Events in Vivo

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14. ABSTRACT

Focal epilepsy is prevalent among Veterans conferring significant morbidity. To develop rational approaches to therapy we need to understand how individual neurons of different types participate in and modulate epileptic events. During this annual period, we used the two-photon, "optical micro-encephalogram" strategy to study how individual cortical neurons of identified type engage in seizure and epileptiform events in the chronic Tetanus Toxin model of focal epilepsy. In this 2^{ns} annual report we met our goals to: 1) re-optimize our setup, re-hire and train two postdoctoral fellows after transferring the award to the JP VA, 2) Optimized the TeT injection model of chronic focal epilepsy to make it amenable to analysis via 2-photon imaging (i.e. ensure it has a sufficient rate of ictal events), 3) We imaged 23 TeT injected and 10 control animals from pre-injection up to day 60 post-injection and mapped how pyramidal neurons and PV+, SOM+ interneurons engage during EEG-seizures as well as during EEG-silent "mini-seizure" events observed only on calcium imaging. We are currently preparing our first manuscript, on track to complete the goals outlined in the SOW.

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.

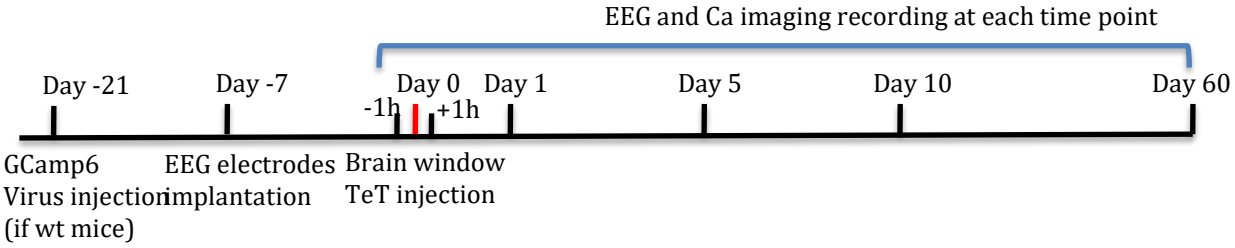

Figure 1 The experimental time line.

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: Brigham and Women's 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.

PERIOD COVERED BY THIS REPORT: 30 SEP 2017 - 29 SEP 2018

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 a 1-year 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 Hospital
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 Hospital
SA#1,2 proceed in parallel, studying the pyramidal neurons. Originally anticipated to take 16 months (from month 4 to month 20)	In progress. Months 5,6,7 were completed at BCM before laboratory transferred to JP VA . Work on this aim restarted on 8/2017 following re-optimization of our protocols after the ACURO approval to do experiments at the JP VA was granted (6/1/2017). We expect to finish this work on 12/2018, ~ taking 16 months as originally planned.	BCM and JP VA Hospital
Studying PV+ interneuron cohorts. Originally planned from month 9 to 24.	In progress. Work on this aim started at JP VA on 10/2017 and we expect to finish it by 2/2019.	JP VA Hospital
Milestone(s) To Achieve:		

Write a first manuscript. Originally planned from month 16 to 24.	Manuscript is in progress. We expect to submit it for publication by	JP VA Hospital
Study SOM+ and VIP+ interneuron cohorts		
Have SA#1,2 proceed in parallel studying SOM + interneurons. Originally planned from month 16-30.	Work is in progress. We expect to finish by 6/2019.	JP VA Hospital
Have SA#1,2 proceed in parallel studying VIP+ interneurons. Originally planned from month 24-34.	We expect to start this work on 11/2018 and finish by 9/30/2019.	JP VA Hospital
Write 1-2 additional manuscripts. Originally planned from month 30-36.	We expect to start this work on 5/2019 and finish by 9/30/2019	JP VA Hospital
Milestone(s) Achieved: 1. 1-2 Manuscripts	By the end of the no-cost extension	JP VA Hospital

What Was Accomplished in the period covered by the progress report :

In the prior progress report we anticipated that during the next reporting period we would: "

1) Optimize the identification of abnormal EEG patterns in collaboration with Drs Noebels and Maheshwari,
2) Optimize the TeT delivery to maximize animal survival and development of focal epilepsy
3) Obtained 2-photon recordings from the pyramidal neurons of an initial cohort of ~5 animals with TeT focal epilepsy
4) Initiate 2-photon experiments to record from the PV+ interneurons of TeT injected animals "

We accomplished these goals. We describe below one-by-one what was accomplished during this period:

1) Two postdoctoral fellows (Dr Anna Palagina, and Dr Joseph Lombardo) new to this project were trained to perform 2-photon imaging experiments with simultaneous EEG recordings after TeT injection.

2) We have established the homozygous C57BL/6J-Tg(Thy1-GCaMP6s)GP4.3Dkim/J (GP4.3) mouse colony that expresses the calcium-indicator Gcamp6 in pyramidal neurons in Boston.

3) TeT injection experiments were optimized to model chronic focal epilepsy with a sufficient number of ictal events to make it amenable to analysis via 2-photon imaging. We tested different doses of TeT toxin and determined that increasing the dose of TeT injection yields more reliable and frequent seizure events, compared to the original proposal by Mainardi et al. (Epilepsia 53 (7): e132-6, 2012). This yields sufficient number of events per session, to make it feasible to extract information from calcium imaging data (see # 4 below).
   Optimized Surgery and Injection protocol: 7-14 days prior to the Tetanus toxin injection, animals are implanted with Electroencephalogram (EEG) electrodes and a cranial window as previously described (Meyer J et al, Nat Comms 2018). In Briefly,

mice were anesthetized by inhalation of Isoflurane (4-5% in pure O_2 for induction and 1% - 2% during surgery). EEG electrodes (Teflon coated silver wire with a diameter of 0.005 inch,) were inserted into the epidural space through cranial burr holes and secured by dental cement (Lang Dental, Wheeling, IL). Recording electrodes were placed bilaterally at 2.7 mm lateral and 3.5 mm rostral to lambda, and reference electrodes were placed at 2 mm lateral and 2 mm posterior to lambda. A 3 mm in diameter craniotomy (centered at 2.7 mm lateral and 1.5 rostral to lambda) was drilled and covered with cover glass. A headpost is attached to the skull with dental cement for imaging.

Tetanus toxin injection: Using a glass micropipette, Tetanus toxin solution (Sigma-Aldrich, Inc.,USA, 200nl, 5 ng in phosphate buffered saline containing 2 % bovine serum albumin) is injected to the visual cortex at depth of 500 µm through a hole in the cover glass under 2-photon microscope guidance or through a burr hole next to the cover glass. The depth of the injection was controlled by micromanipulator (MPC-200, Sutter Instrument Company, San Francisco, CA). The solution volume was controlled by an automatic nanoliter injector (Nanojector II, Drummond Scientific Company, Broomall, PA)

Figure 2 illustrates data from 3 animals that have 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.

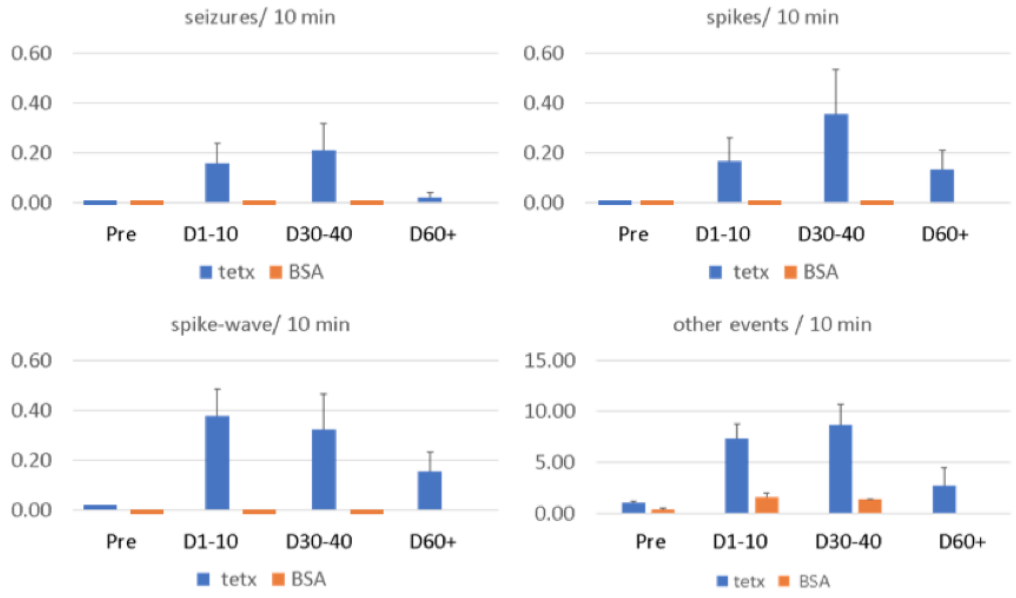

Figure 2: ***Pre:*** prior to injection. ***D1-10*****:** first 10 days post injection; ***D30-40:*** days 30-

40 post injection; ***D60+:*** days 60-80 post injection. ***Y-axis:*** Event frequency (number of events/10min).

4) We recorded from 10 animals with simultaneous EEG and Calcium imaging following the new and improved TeT injection protocol. A further 13 animals have been processed and recorded from using a protocol identical to that used by Mainardi et al. (Epilepsia 53 (7): e132-6, 2012) . Ten control (BSA injected) animals have also been processed. We are in the process of completing the data collection from these animals to 60+ days post-injection. We appended an addendum with a detailed reporting of how many animals have been imaged per time point at this time. We expect to finish the collection of the remaining animals by the end of November. Analysis will proceed in parallel and we anticipate to have the draft of a manuscript ready for submission by December 2018 / January 2019.

Fig. 3 illustrates a typical calcium event recorded from layer 4 of area V1 in an animal injected with the high dose of TeT injection. We observed multiple calcium events evolving over several seconds as indicated in the figure. Such events were never noted in control animals injected with BSA. Note that the EEG spectrum has increased power in the range of frequencies 20-60Hz, when the calcium signal is increased (**fig. 3B**). There are also times, when abnormal calcium events are seen that have no obvious correlate on the EEG (mini-seizures). This animal had its PV+ interneurons labeled with Td+ so they look orange when they are double labeled with the green GCamp6s. **Fig. 3C** shows how the calcium signal evolves during the event shown in **panel 3A** in 2 pyramidal (#1,2) and two PV+ (#3,4) interneurons. Note that signals largely co-vary, likely reflecting the engagement of PV+ interneurons by the increased activity in the pyramidal circuits. We are in the process of analyzing the relative strength of the response seen in pyramidal cells versus PV+ interneurons, to try to understand whether a relative failure of PV+ interneurons to follow pyramidal cell activity contributes to ictal generation in this model.

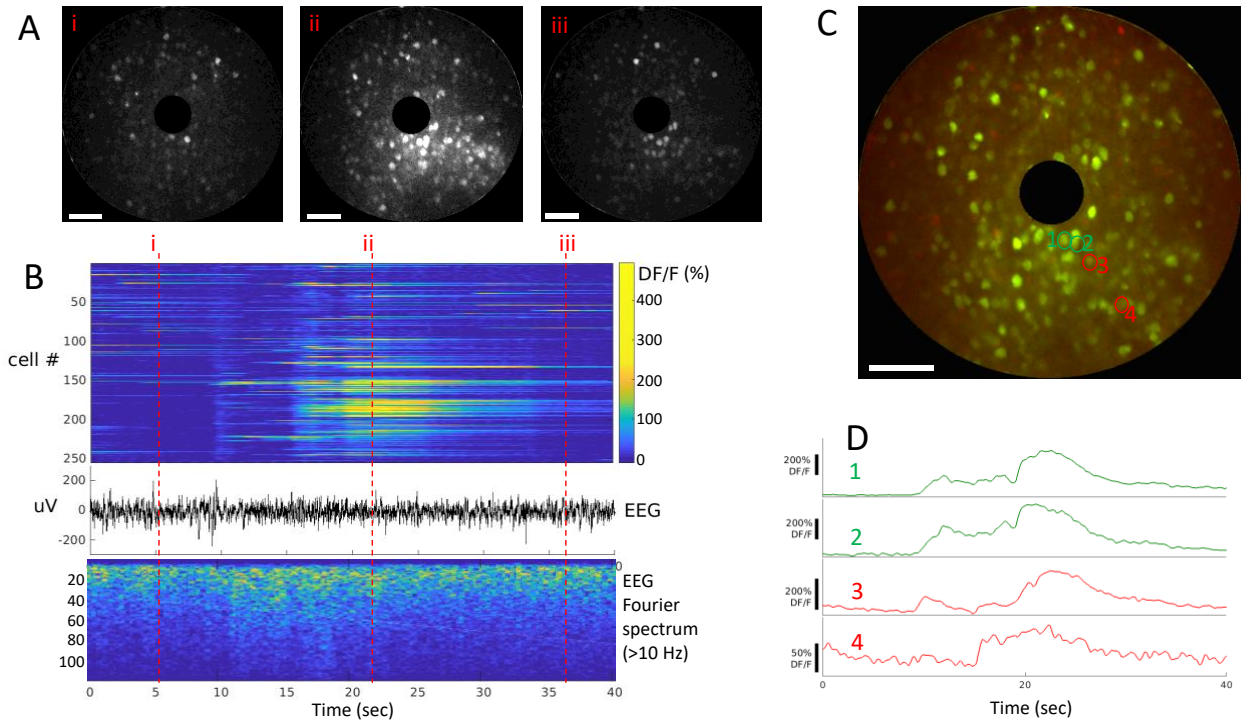

Figure 3. A Calcium mini-seizure event recorded on day 30 post TeT injection: A) Layer 4 FOV (depth 480 um below dura), 600x600 µm, green channel only, spiral scan @8.3 Hz. ***i)*** frame at time point i in B. ***ii)*** frame at time point ii in B, ***iii)*** frame at tie point iii in B. Scale bar = 100 µm. **B)** *Top:* 40 sec of calcium DF/F over time for 255 neurons and 10 neuropil patches (last 10 rows). *Middle:* Ipsilateral EEG (re-referenced to contralateral side), sampling rate 5 kHz. *Bottom:* Fourier spectrum after high-pass filtering the EEG trace (> 10Hz). Note that the EEG spectrum power in the range of frequencies 20-60Hz increases when the calcium signal is increased. **C)** Composite FOV (green = GCamp6M, red = tdTomato in PV+ interneurons). 1-2: exemplary putative pyramidal cells, 3-4: exemplary PV+ cells. **D)** DF/F traces of the cells 1-4 from C (2 pyramidal, 2 PV+), showing the same 40 sec of activity from B.

Patch clamp recordings have also started to be performed in select animals to further validate the calcium results and to better delineate the temporal sequence of engagement of pyramidal neurons to EEG burst events across cortical layers. Please see the appendix, where patch clamp and LFP recordings are also listed.

We expect to adhere to the timeline stated in the modified SOW (see above). We are in the process of writing the first manuscpript, which we expect to be ready for submission around 12/2018).

Opportunities for training and professional development:

i) Prior to her departure, Dr. Meyer trained Dr. Hao for mouse surgery, virus injection, EEG recording, Ca imaging and basic Matlab programming.

ii) Drs Meyer, Hao and Smirnakis training Dr Lombardo and Palagina in performing Calcium imaging and simultaneous EEG recordings in TeT injected animals, and how to perform EEG analysis.

Results Disseminated to communities of interest:

We have modified the TeT injection strategy, 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 on this at the Boston VA Research Week Conference, May, 2018.

Plans for the next reporting period:

We plan to continue the experiments and meet the remaining goals as stated in the updated version of the SOW (appended above).

4. IMPACT:

We have started preparing a manuscript for publication, which we expect to submit for review ~12/2018, as stated in the SOW. We expect the impact of our work to extend further there.

The impact of our project on:
1) the development of the principle discipline: We have modified the TeT injection strategy, 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 on this at the Boston VA Research Week Conference, May, 2018.
2) other disciplines: nothing to report
3) technology transfer: nothing to report
4) society beyond science and technology: nothing to report

5. CHANGES / PROBLEMS

No-Cost Extension: 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 have been resolved. Given these delays we have obtained a 1-year no cost extension, and submitted an amended SOW that was approved on 9/2018. The goals of the research remain the same.

Personnel Changes:

1) In June 2018 Dr ZhaoZhe Hao returned to Houston for family reasons (her husband did not relocate to Boston) and is no longer working on this project.
2) The existing subcontract to Baylor College of Medicine that supported Dr Jochen Meyer came to an end on 9/30/2018, and further work on the project will be performed entirely in Boston at the Jamaica Plain VA Hospital.
3) Elaine Kuang, a laboratory manager that was hired from other sources of funding, will be able to take care of animal colony work starting ~12/1/2018, allowing postdoctoral

fellows Joseph Lombardo and Anna Palagina, who are currently supported by the award additional time to perform experiments. We are also in the process of advertising to hire a new postdoctoral fellow to replace ZhaoZhe Hao.

4) Anna Palagina and Joseph Lombardo, two postdoctoral fellows have started working on the project.

We expect to meet all originally stated goals at no extra cost. Given the delays incurred by the transfer and change in personnel (>18 months), if by the end of the no-cost extension the SOW goals are still not completely finished, we will ask an additional period of no-cost extension to ensure everything is finished by the end of the project at no additional cost.

6. PRODUCTS

Nothing to report so far.

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: 10
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: Zhaozhe Hao
Project Role: Postdoctoral Associate, appointment ended 6/2018.
Researcher Identifier: orcid.org/0000-0003-3212-1600
Nearest person month worked: 12
Contribution to Project: conduct the experiment, data analysis

Name: Jeff Noebels
Project Role: Consultant/Collaborator (the subcontract ended 9/30/2018).
Researcher Identifier: orcid.org/0000-0002-2887-0839
Nearest person month worked: 0.1 calendar months
Contribution to Project: Provide advice with regards to EEG recordings.

Name: Ganna (Anna) Palagina
Project Role: Postdoctoral Associate.

Researcher Identifier: https://orcid.org/0000-0001-9857-9062
Nearest person month worked: 12
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: 12
Contribution to Project: Conduct Experiments and perform data analysis with the help of Dr Palagina.

No other organizations were involved as partners. During the period of the report, Dr Noebels and Meyer were supported through a subcontract to Baylor College of Medicine. This subcontract came to an end on 9/30/2018 and all research continues now locally, at the Jamaica Plain VA Hospital.

8. SPECIAL REPORTING REQUIREMENTS:

None.

9. APPENDIX OF ANIMALS IMAGED DURING THIS PERIOD

tetanus-toxin, Mainardi Protocol

owner	mouse ID	ca indicator	tdtomato	before	1 hr	D1	D2-7	D10+/-	D30+/-	D45+/-	D60+/-	D90+/-	patch-clamp / LFP recordings
ZH	zh83G6BN			Ca, EEG	EEG	EEG	Ca, EEG	Ca, EEG					
ZH	zh96_Thy1_BN	Thy1-Gcamp6S		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	EEG	EEG			
ZH	zh117_Thy1_2289	Thy1-Gcamp6S		Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG			
ZH	zh129_Thy1_2296	Thy1-Gcamp6S		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	AAV-G6M	SOM+	Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG					4 cells @D20
JM	Tet_Som-cre_mouse_1	AAV-G6M	SOM+										
JM	Tet_PV-Cre_091416	AAV-G6M	PV+	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG					
JM	Tet_PV-Cre_092016	AAV-G6M	PV+	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG					
JM	WT_Dlx_3	AAV-G6M	Dlx+	Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG		Ca, EEG		
JM	Tet_Ai96-Syn-cre_mouse_1	Ai96/syn-cre		Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG		3 cells @D27
JM	Tet_PV-Cre_N424	AAV-G6M	PV+							Ca, EEG			
JM	Tet_PV-Cre_N425	AAV-G6M	PV+	Ca, EEG	Ca, EEG					Ca, EEG	Ca, EEG		
total Ca				13	12	10	9	12	6	5	3		
total EEG				15	15	14	11	14	8	6	3		

tetanus-toxin, high 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	Thy1-Gcamp6S		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	AAV-G6M	PV+	Ca, EEG	Ca, EEG				Ca, EEG		Ca, EEG		LFP @ D20, D30
JM	Tetx_mouse_N673	Ai96/syn-cre		Ca, EEG	Ca, EEG				Ca, EEG		Ca, EEG		LFP @ D28
JM	Tetx_mouse_N516	Ai96/syn-cre		Ca, EEG	Ca, EEG		Ca, EEG		Ca, EEG		Ca, EEG		LFP @ D35
JM	Tetx_mouse_N574	AAV-G6M	PV+	Ca, EEG	Ca, EEG				Ca, EEG				
JM	Tetx_mouse_N785	AAV-G6M	PV+	Ca, EEG	Ca, EEG	Ca, EEG							
JM	Tetx_mouse_N763	AAV-G6M	PV+	Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG		Ca, EEG		LFP @ D30
JM	Tetx_mouse_N666	Ai96/syn-cre		Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG	
JM	Tetx_mouse_N748	Ai96/syn-cre		Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG	
JM	SST180710_MM_L	AAV-G7f	SOM+	Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG		Ca, EEG		
JM	SST180604MO_N	AAV-G7f	SOM+	Ca, EEG	Ca, EEG			Ca, EEG	Ca, EEG		Ca, EEG		
JM	SST180710_MM_N	AAV-G7f	SOM+	Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG		Ca, EEG		
total Ca				13	12	7	1	7	10	1	9	2	
total EEG				16	16	11	5	11	13	3	9	2	

BSA

ZH	zh83G6BL			EEG	EEG	EEG	EEG	EEG					
ZH	zh84_G6			EEG	EEG	EEG	EEG	EEG	EEG				
ZH	zh117_Thy1_2269	Thy1-Gcamp6S		Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	EEG	EEG			
ZH	zh117_Thy1_2292	Thy1-Gcamp6S		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	PV+	Ca, EEG	Ca, EEG		Ca, EEG						
JM	Tetx_mouse_N673	Ai96/syn-cre		Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG				
JM	Tetx_mouse_N516	Ai96/syn-cre		Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG	Ca, EEG				
JM	Tetx_mouse_N574	AAV-G6M	PV+	Ca, EEG	Ca, EEG		Ca, EEG						
JM	Tetx_mouse_N785	AAV-G6M	PV+	Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG					
JM	Tetx_mouse_N763	AAV-G6M	PV+	Ca, EEG	Ca, EEG			Ca, EEG					
JM	Tetx_mouse_N666	Ai96/syn-cre		Ca, EEG	Ca, EEG		Ca, EEG	Ca, EEG	Ca, EEG				
JM	Tetx_mouse_N748	Ai96/syn-cre		Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG				
JM	SST180629_MN_N	AAV-G6M	SOM+	Ca, EEG	Ca, EEG			Ca, EEG					
JM	SST180710_MM_L	AAV-G7f	SOM+	Ca, EEG	Ca, EEG	Ca, EEG			Ca, EEG				
JM	SST180604MO_N	AAV-G7f	SOM+	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG						
JM	SST180710_MM_N	AAV-G7f	SOM+	Ca, EEG	Ca, EEG	Ca, EEG	Ca, EEG						
JM	SST180629_MN_N	AAV-G7f	SOM+	Ca, EEG	Ca, EEG	Ca, EEG		Ca, EEG					
total Ca				15	15	9	8	9	6				
total EEG				19	19	13	12	14	10	2			

PV+: parvalbumin positive interneurons express Td Tomato and can therefore be identified and analyzed; *SOM+:* Somatostatin positive interneurons express Td-Tomato. *EEG:* electro-encephalogram has been obtained. *Ca:* 2-photon calcium imaging of neural activity has been obtained. *BSA:* Control animals injected with bovine serum albumin. *Green:* Animals still under the process of being scanned. 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.