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14. ABSTRACT We pinpointed the hyperexcitable "hot spots" in the brain responsible for epileptic activities after TBI. These are located in the anterior portion of the injured neocortex in the S1 somatosensory cortex and in the functionally connected somatosensory portion of the thalamus. We found two anatomical and physiological biomarkers of the hot spots: 1) a massive upregulation of the C1q molecule, and 2) a reduced synaptic inhibition in these hot spots. These discoveries we made during the first funded year of the award pinpoint the neural circuits that we can now target to test two treatments in parallel. One treatment will consist in blocking the C1q effects by using the anti-C1q ANX005 drug; and the second treatment will consist in enhancing synaptic inhibition by human stem cell transplants in the "hot spots". We showed the feasibility of these two approaches and are starting to perform chronic recordings to determine the disease-modifying efficacy of the treatments. The results we will obtain during the next two years may lead to two treatments for preventing and/or treating the post-traumatic epilepsy after TBI. The long-term impact of this work will be to prevent, control, and cure post-traumatic epilepsy with no side effects, in contrast with the systemic treatment currently provided by anticonvulsants.

15. SUBJECT TERMS Traumatic brain injury, Post-traumatic Epilepsy, Seizures, Neural Circuits, Inflammation, Gliosis, C1q molecule, Electrophysiology, Optogenetics, In vivo recordings during free behavior, Chronic EEG

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

   This proposal addresses significant gaps in the understanding of the pathophysiology of post-traumatic epileptogenic changes in the brain. The vision of this study is to determine the causative links between TBI and development of epileptic activities. The scope of this study is to understand the mechanisms underlying post-traumatic epilepsy, especially in service members and veterans. After traumatic brain injury (TBI), there is a latent period between the injury and the onset of spontaneous seizures in post-traumatic epilepsy (PTE). Although no one knows what leads to development of PTE, during the latent period the brain is thought to undergo a number of changes that predispose it to epilepsy, a process known as epileptogenesis. We received an Epilepsy Research Program award to study specific neural circuits that may be most vulnerable to changes that may lead to PTE. We seek to understand where and when epileptogenesis takes place, in the hope that this knowledge will lead to new therapeutic approaches. Our work so far has pinpointed hot spots of inflammation and neural network hyperexcitability located in the cortex around the site of injury and in the somatosensory thalamus, a part of the brain that senses touch and pressure. Our team has discovered that those hot spots form before the onset of PTE, and persist chronically. We have also shown that C1q, an immune molecule involved in regulating synaptic connectivity, is an important marker of these hot spots, and that blocking C1q with a drug can prevent chronic inflammation and reduce aberrant increases in cortical power after TBI. We hope that our research will lead to interventional strategies that harmonize immune-neuronal interactions after TBI to prevent PTE.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

   Traumatic brain injury, post-traumatic epilepsy, seizures, neural circuits, inflammation, gliosis, C1q molecule, electrophysiology, optogenetics, *in vivo* recordings during free behavior, chronic EEG.

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

   There were no changes in the project or its direction.

   What were the major goals of the project? *List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.*

   Major Goals of the project as stated in the approved SOW:

Major Task 1: Determine the role of corticothalamocortical hyperexcitability in seizures after TBI.	Months
Aim 1a: Determine if TBI results in hyperexcitability in CTC circuits	1-7
Aim 1b: Determine if the thalamus can bi-directionally control PTE seizures in freely behaving animals.	1-7
Milestone(s): unveil the epileptic circuit hot spots and specific cells that are causally involved in PTE.	7
Major Task 2: Determine the role of C1q in seizures after TBI.	
Aim 2a: Determine if C1q is upregulated in CTC "hot spots" after TBI	1-7
Aim 2b: Determine if blocking C1q action prevents PTE	1-12
Aim 2c: Determine if blocking C1q cures PTE	8-17
Milestone(s): Reveal the role of the immune response involving C1q in circuit plasticity after TBI, validate a new biomarker (C1q and thalamic gliosis) for the epileptogenesis in PTE, and determine if the drug ANX005 that blocks the effects of C1q is efficient in preventing and curing PTE.	17
Major Task 3: Determine if transplanting inhibitory neurons into CTC "hot spots" of hyperexcitability prevents and cures PTE.	
Aim 3a: Determine if inhibitory transplants in cortex and thalamus prevent circuit hyperexcitability in cortical and thalamic slices, respectively	6-12
Aim 3b: Determine if transplanted cells prevent PTE in behaving animals.	13-24
Aim 3c: Determine if transplanted cells cure PTE in behaving animals.	24-36
Milestone(s): Test the efficacy of a novel therapeutic approach involving mouse and human cell transplants for preventing PTE and cure PTE.	36

What was accomplished under these goals? *For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.*

Below we describe for each Major Task the specific objectives, activities, findings, conclusions (both positive and negative), and other achievements.

1. Major Task 1: Determine the role of cortico-thalamo-cortical hyperexcitability in seizures after TBI.

1.1 Specific objectives:

-Aim 1a: Determine if TBI results in hyperexcitability in CTC circuits.
-Aim 1b: Determine if the thalamus can bi-directionally control PTE seizures in freely behaving animals.

1.2 Major activities and findings:

1.2.1 Secondary C1q expression coincides with chronic inflammation, neurodegeneration, and synaptic dysfunction in the thalamus.

To determine the secondary, long-term effects of TBI, we induced a mild cortical impact injury to the right primary somatosensory cortex (S1) of adult mice (Figure 1A), and assessed the impact on their brains three weeks later. This period corresponds to the latent phase in humans, when the brain is undergoing adaptive and maladaptive changes. We determined neuron count and gliotic inflammation in the corticothalamic circuit by immunofluorescent staining of coronal brain sections with markers of neurons (NeuN) and of glial inflammation (C1q, complement pathway; GFAP, astrocytes; Iba1, microglia/macrophages) (Figure 1C-E). Three weeks post-surgery, TBI mice had significantly higher GFAP, C1q, and Iba1 expression in the peri-TBI S1 cortex, the functionally connected ventrobasal thalamus (VB), and the reticular thalamic nucleus (nRT) than sham mice (Figure 1B-E). Inflammation occurred within 24 hours after injury in the cortex, while the functionally connected nRT and VB displayed glial changes around five days later (not shown), suggesting secondary thalamic inflammation. We also saw increased expression of similar inflammatory markers in thalamic tissue from human TBI patients, confirming that thalamic inflammation is a consequence of TBI in humans too (Figure S1). We conclude that a chronic inflammatory process, secondary to the injury and characterized by C1q expression, occurs in the thalamus.

Glial inflammation was associated with significant neuronal loss in the thalamic region, particularly in the nRT (Figure 1D-E, Figure 2A), which receives the majority of its glutamatergic inputs from the cortex. The nRT of TBI mice had significantly fewer neurons than sham mice, particularly in the region of the nRT that receives most of its excitatory inputs from the injured somatosensory cortex (Figure 2B-C). This result suggests that the inflammation, which may be initiated by retrograde axonal degeneration, follows the long-range, corticothalamic circuit, and marks its two ends: the injured cortex and the connected thalamus.

To test whether C1q might mark functional damage in this circuit, we performed whole-cell patch-clamp recordings in the cortex and thalamus in brain slices at chronic stages of TBI (three to six weeks). We recorded layer-5 pyramidal neurons and fast-spiking GABAergic interneurons in the peri-TBI S1 cortex, glutamatergic neurons in the VB, and GABAergic neurons in the nRT. The neurons' intrinsic membrane electrical properties and the spontaneous excitatory and inhibitory postsynaptic current (sEPSC and sIPSC) properties were similar between sham and TBI mice in both the peri-TBI cortex and the VB thalamus (see Table 1 for details). However, in the nRT, TBI led to a reduction in the frequency of sIPSCs (Figure 2D-E). Furthermore, nRT sEPSCs were smaller in amplitude, and trended toward a lower frequency (Figure 2F-G). Immunofluorescence staining for GFP in Thy1-

GCaMP6f mice, a marker of neuronal calcium levels in corticothalamic neurons, revealed reduced corticothalamic fluorescence in the thalamus after TBI (Figure 2H-I), suggesting that this circuit is indeed impaired.

Figure 1.

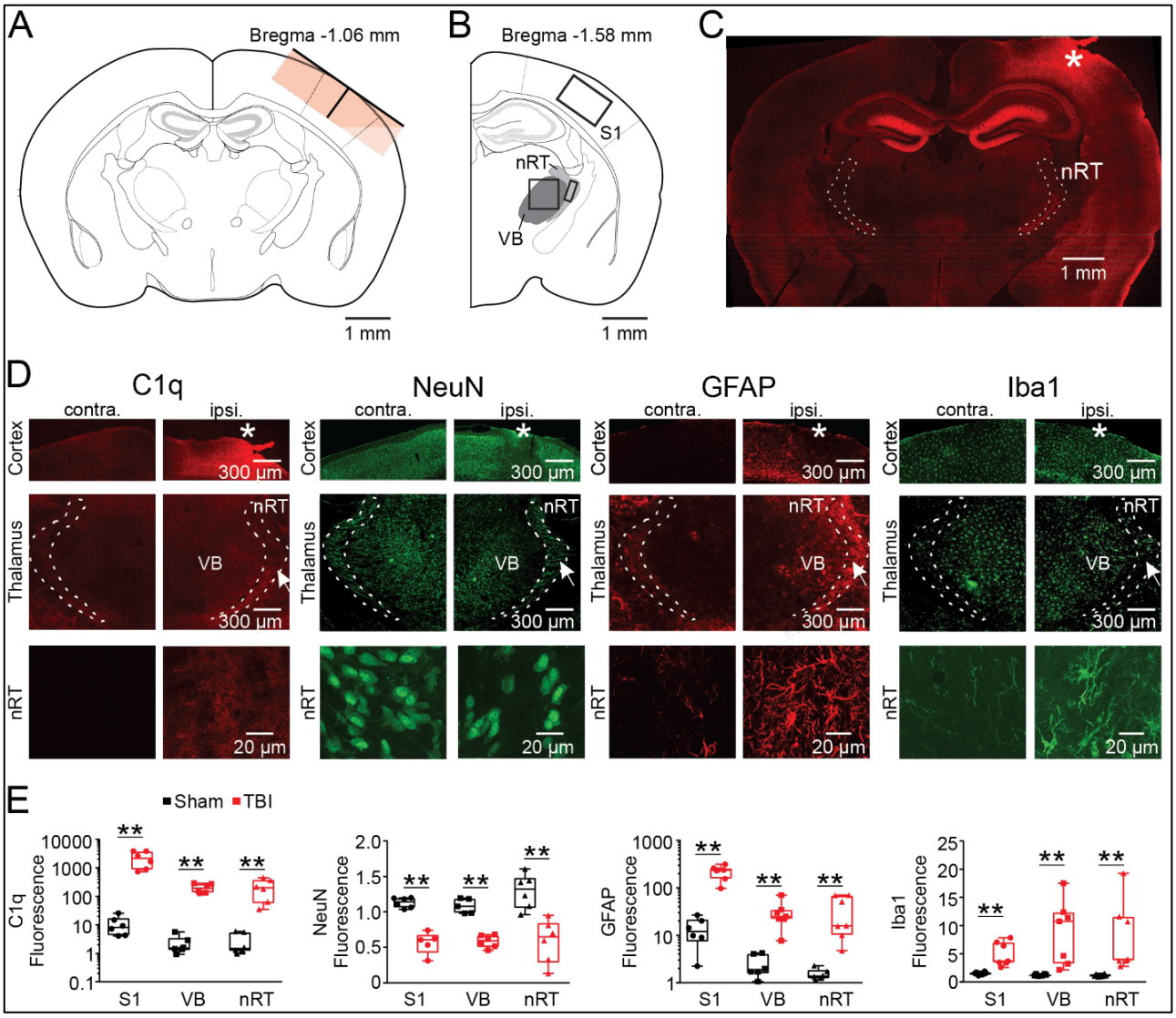


Figure 1. The injured cortex and functionally connected thalamus show chronic inflammation and neuron loss three weeks after TBI. A, B) Schematic of a mouse brain coronal section showing the site and depth of the controlled cortical impact (A) and the location of the S1 cortex and nRT and VB thalamic regions (B). The impactor has a diameter of 3 mm and the impact was delivered at a depth of 0.8 mm to the right somatosensory cortex. C) Representative coronal brain section from a TBI mouse stained for C1q. C1q expression in the hippocampus is typical of physiological conditions. D) Close-up images of S1 (top), VB and nRT (middle), and confocal images of nRT (bottom) stained for C1q, neuronal marker NeuN, GFAP, a marker for astrocytes, and Iba1, a marker for microglia/macrophages. Injury site in the right S1 cortex is marked by an asterisk. Arrow in nRT indicates location of confocal image. Scale bars, 300 µm (top/middle) and 20 µm (bottom). E) Quantification of fluorescence ratios between ipsilateral and contralateral regions in sham and TBI mice. Data represent all points from min to max, with a Mann-Whitney test and $\alpha = 0.05$ (*$p < 0.05$, **$p < 0.01$). Analysis includes between five and seven mice per group (n = three sections per mouse, one image per region).

Figure S1.

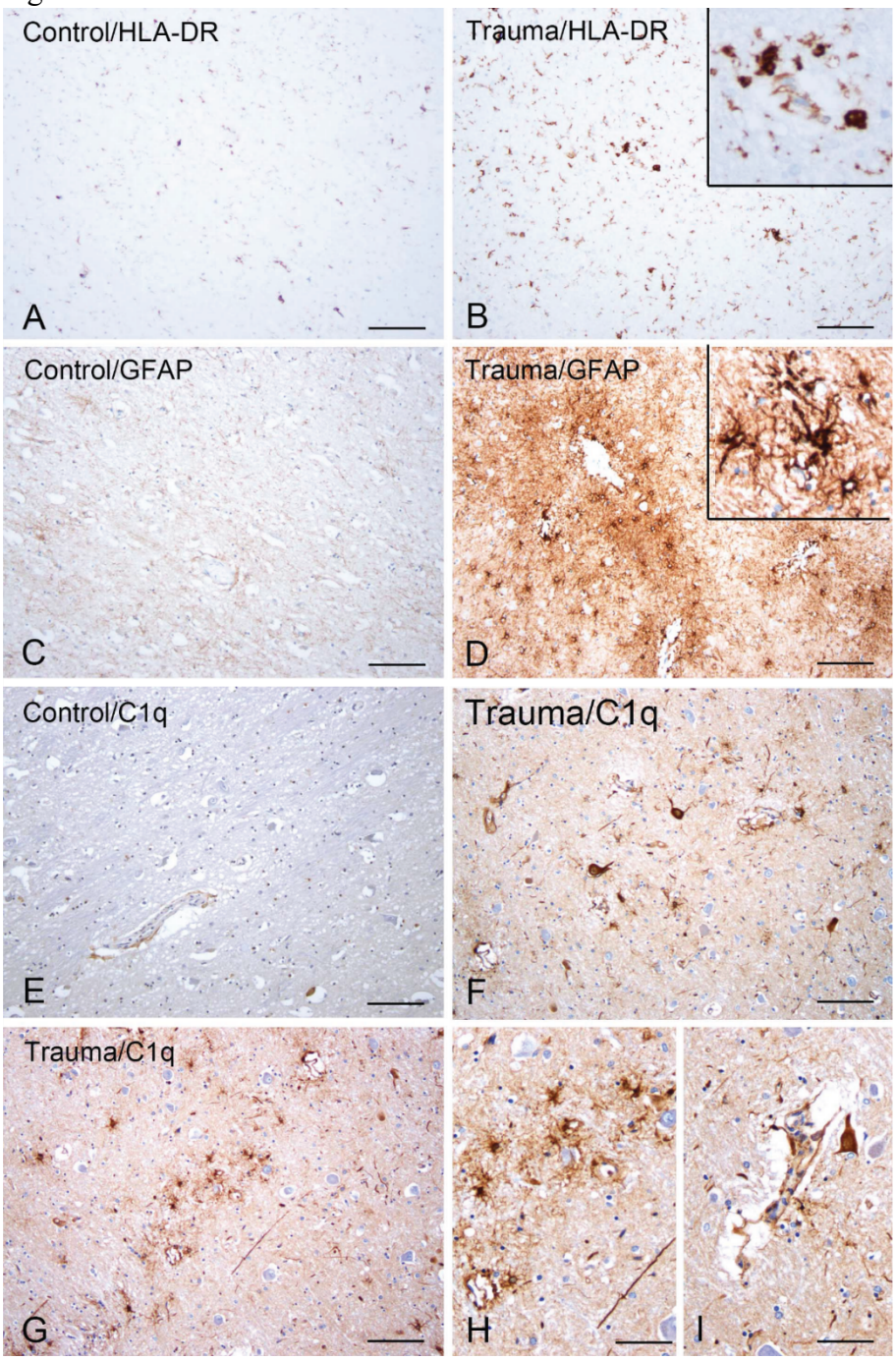


Figure S1. Postmortem brain tissue from a patient with TBI shows chronic inflammation eight days after TBI. A) Postmortem brain tissue from one control patient stained for HLA-DR, a marker for an MHC class II cell surface receptor that is expressed in microglia and macrophages. Case information: male, age 78. Scale bar, 1 cm. B) Postmortem brain tissue from one TBI patient stained for HLA-DR. Case information: male, age 79; fall accident, Injury Severity (GCS): moderate, CT: cerebral edema; no epilepsy (post-TBI: eight days); no history of neurological diseases and without evidence of cognitive decline, based on the last clinical evaluation; no evidence of primary neurodegenerative pathology, evidence of trauma-induced diffuse axonal damage. Scale bar, 1 cm. C) Same as (A) but stained for GFAP. Scale bar, 1 cm. D) Same as (B) but stained for GFAP. Scale bar, 1 cm. E) Same as (A) but stained for C1q. Scale bar, 1 cm. F-I) Same as (B) but stained for C1q. Scale bars, 1 cm (F-G) and 40 μm (H-I). This work was performed in our collaborator's laboratory (Dr. Eleonora Aronica's laboratory).

Figure 2.

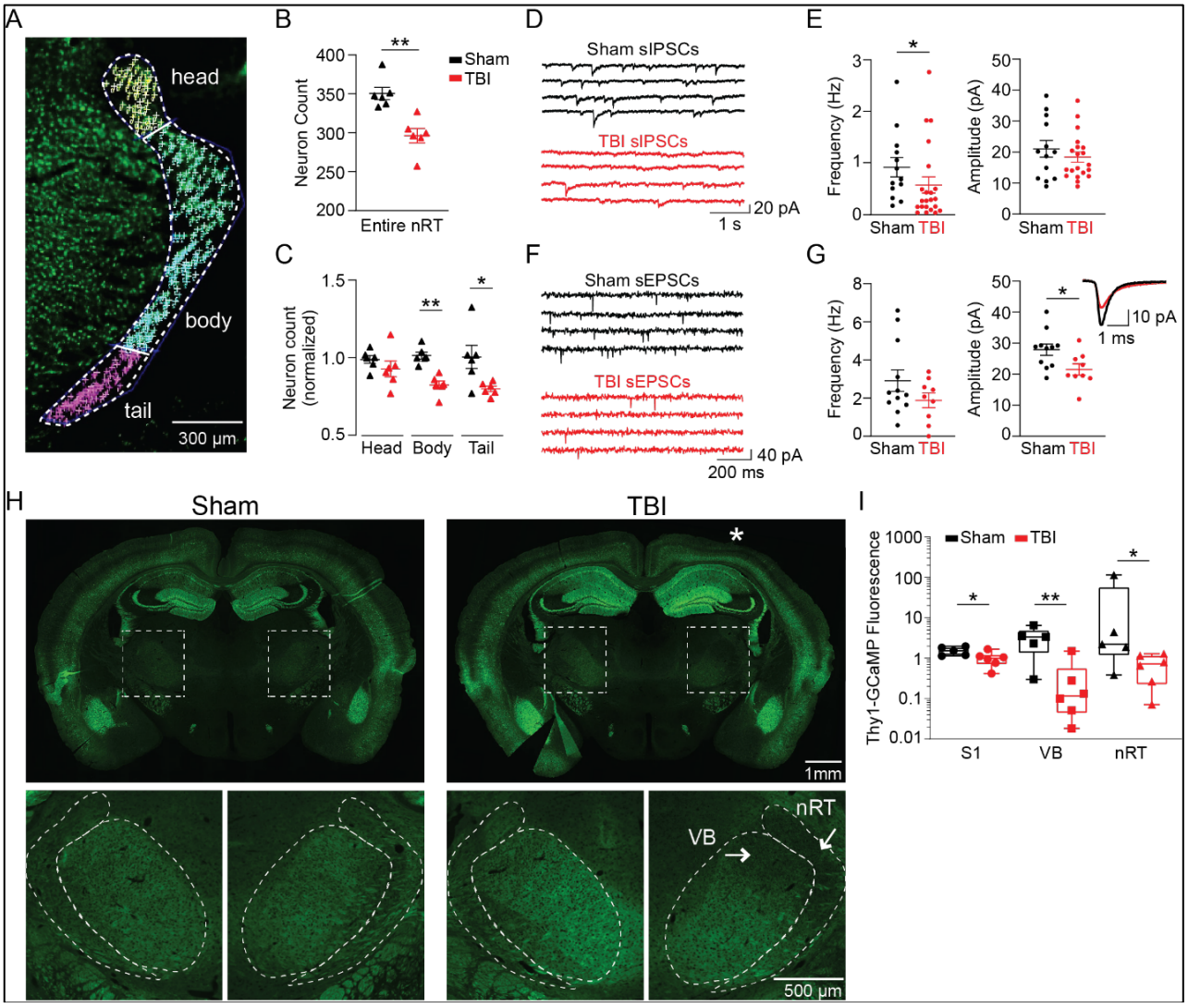


Figure 2. The nRT ipsilateral to the injured cortex shows neuron loss and altered IPSC and EPSC properties three weeks after TBI. A-C) High-magnification coronal image of the nRT showing divisions into "head", "body", and "tail" (*50*), and quantification of neuron counts across the entire ipsilateral nRT (B) or per subdivision, normalized to the median value from the sham group (C). Neuron count data represent mean ± SEM, with a Mann-Whitney test and $\alpha = 0.05$ (*$p < 0.05$, **$p < 0.01$). Analysis includes six mice per group (n = three sections per mouse, averaged). D, E) Spontaneous IPSC recordings (D) from representative nRT neurons in sham and TBI mice, and frequency and amplitude distributions (E) in 13 posterior nRT neurons from four sham mice and 22 posterior nRT neurons from six TBI mice. IPSC data represent mean ± SEM analyzed with a Mann-Whitney test and $\alpha = 0.05$ (*$p < 0.05$). F, G) Spontaneous EPSC recordings (F) from representative nRT neurons in sham and TBI mice, and frequency and amplitude distributions (G) in 11 posterior nRT neurons from six sham mice and nine posterior nRT neurons from seven TBI mice. Inset shows averaged EPSC traces from single nRT neurons from sham and TBI mice, plotted on the same scale. EPSC data represent mean ± SEM analyzed with a Mann-Whitney test and $\alpha = 0.05$ (*$p < 0.05$). H) Representative images of coronal brain sections from Thy1-GCaMP6f mice with sham surgery (left) and TBI (right) (injury site marked by asterisk). Bottom panels show projection terminals from the cortex to VB and nRT. Scale bars, 1 mm (top) and 500 µm (bottom). Reduction in projection terminals from the cortex to VB and nRT (marked by arrows) were observed in n = six TBI mice. I) Quantification of Thy1-GCaMP fluorescence ratios between ipsilateral and contralateral regions in sham and TBI mice. Data represent all points from min to max, with a Mann-Whitney test and $\alpha = 0.05$ (*$p < 0.05$, **$p < 0.01$). Analysis includes five sham mice and six TBI mice (n = three sections per mouse, one image per region).

Table 1.

Intrinsic features	Cm (pF)	Vm (mV)	Rin (MOhm)	Tau (ms)	Rheobase (pA)	AP Thr. (mV)	AP Dur. (ms)	AP Amp. (mV)	cells	slices	mice
L5 pyr.											
sham	94 ± 9.5	-79 ± 2.2	388 ± 22	33 ± 2.0	55 ± 7.1	-55 ± 0.7	4.2 ± 0.2	71 ± 1.2	21	9	6
TBI	91 ± 9.7	-71 ± 2.4	421 ± 43	36 ± 4.8	54 ± 9.2	-51 ± 1.1	4.1 ± 0.3	60 ± 2.4	21	10	6
MW p-value	ns	0.03	ns	ns	ns	0.01	ns	0.0001			
L5 FS											
sham	58 ± 6.0	-70 ± 2.5	473 ± 71	33 ± 5.2	40 ± 8.8	-54 ± 0.4	1.7 ± 0.2	58 ± 2.4	9	6	6
TBI	54 ± 7.3	-72 ± 2.0	573 ± 91	27 ± 3.1	34 ± 5.7	-57 ± 1.2	1.7 ± 0.2	57 ± 2.2	14	9	6
MW p-value	ns	ns	ns	ns	ns	0.03	ns	ns			
VB											
sham	166 ± 18	-62 ± 1.2	198 ± 32	28 ± 3.2	158 ± 17	-50 ± 0.9	2.7 ± 0.2	51 ± 2.9	21-22	7	6
TBI	169 ± 12	-65 ± 1.2	200 ± 19	27 ± 2.6	170 ± 13	-51 ± 0.9	2.3 ± 0.1	49 ± 2.6	28-33	8	8
MW p-value	ns	ns	ns	ns	ns	ns	ns	ns			
nRT											
sham	91 ± 13	-74 ± 3.1	402 ± 58	27 ± 2.8	42 ± 5.5	-53 ± 1.3	1.3 ± 0.1	55 ± 2.2	10	7	5
TBI	82 ± 10	-60 ± 3.5	523 ± 114	44 ± 11	69 ± 26	-50 ± 1.5	1.4 ± 0.1	45 ± 3.2	9	6	5
MW p-value	ns	0.009	ns	ns	ns	ns	ns	0.03			

EPSCs	Frequency (Hz)	Charge (pA x ms)	Amplitude (pA)	Half-width (ms)	Rise time (ms)	Decay time (ms)	Decay Tau (ms)	cells	slices	mice
L5 pyr.										
sham	0.2 ± 0.1	117 ± 7.5	25 ± 1.3	2.2 ± 0.2	1.1 ± 0.1	5.2 ± 0.4	4.0 ± 0.2	21	9	6
TBI	0.6 ± 0.2	107 ± 9.1	27 ± 2.4	2.1 ± 0.2	0.9 ± 0.1	5.5 ± 0.4	3.0 ± 0.2	16	10	6
MW p-value	ns	ns	ns	ns	ns	ns	0.01			
L5 FS										
sham	1.4 ± 0.5	56 ± 2.4	26 ± 0.8	1.0 ± 0.1	0.4 ± 0.02	3.0 ± 0.4	1.6 ± 0.1	8	6	6
TBI	1.2 ± 0.4	55 ± 6.3	29 ± 3.6	1.1 ± 0.2	0.4 ± 0.04	3.0 ± 0.6	1.5 ± 0.2	11	10	6
MW p-value	ns	ns	ns	ns	ns	ns	ns			
VB										
sham	0.9 ± 0.2	54 ± 4.4	22 ± 1.6	1.4 ± 0.2	0.4 ± 0.03	4.2 ± 0.7	1.9 ± 0.2	15	7	7
TBI	1.0 ± 0.4	50 ± 4.6	21 ± 1.6	1.2 ± 0.1	0.5 ± 0.1	3.6 ± 0.5	1.8 ± 0.2	13	8	7
MW p-value	ns	ns	ns	ns	ns	ns	ns			
nRT										
sham	2.9 ± 0.6	32 ± 2.3	28 ± 1.8	0.7 ± 0.1	0.3 ± 0.03	1.7 ± 0.2	0.7 ± 0.1	11	6	6
TBI	1.9 ± 0.4	30 ± 2.0	22 ± 1.8	0.9 ± 0.1	0.3 ± 0.1	2.3 ± 0.3	1.0 ± 0.2	9	7	7
MW p-value	ns	ns	0.04	ns	ns	ns	ns			

IPSCs	Frequency (Hz)	Charge (pA x ms)	Amplitude (pA)	Half-width (ms)	Rise time (ms)	Decay time (ms)	Tau (ms)	cells	slices	mice
L5 pyr.										
sham	1.2 ± 0.2	471 ± 49	39 ± 2.7	6.1 ± 0.3	1.1 ± 0.1	21.1 ± 1.0	10.0 ± 0.4	19	8	6
TBI	1.3 ± 0.3	397 ± 36	36 ± 2.7	5.6 ± 0.3	1.2 ± 0.1	18.0 ± 1.2	8.4 ± 0.4	16	6	6
MW p-value	ns	ns	ns	ns	ns	0.04	0.02			
VB										
sham	2.4 ± 0.8	884 ± 360	47 ± 5.6	5.4 ± 0.4	1.2 ± 0.1	20 ± 3.8	12 ± 4.7	10	6	5
TBI	1.9 ± 0.4	566 ± 127	42 ± 5.1	6.6 ± 0.8	1.1 ± 0.1	24 ± 6.6	10 ± 2.9	11	5	4
MW p-value	ns	ns	ns	ns	ns	ns	ns			
nRT										
sham	0.9 ± 0.2	836 ± 103	21 ± 2.7	16 ± 2.1	1.3 ± 0.1	69 ± 9.0	55 ± 6.0	13	5	4
TBI	0.6 ± 0.2	1144 ± 141	18 ± 1.6	30 ± 2.5	2.7 ± 0.4	79 ± 7.9	73 ± 3.5	22	9	6
MW p-value	0.02	ns	ns	0.0003	0.0002	ns	0.04			

Table 1. Summary of intrinsic properties, EPSC, and IPSC data recorded from S1 cortex, VB, and nRT. Mice were recorded between three and six weeks post-TBI, and recording conditions are described in the patch-clamp electrophysiology section of the methods. A Mann-Whitney test was performed for statistical analysis. The recordings were performed as described in the preprint of our publication Holden et al., 2020 BioRxiv. doi: https://doi.org/10.1101/2020.05.29.120220.

1.2.2 TBI leads to long-term changes in cortical states and excitability in freely behaving mice

We next investigated the longitudinal impact of the cortical injury, using brain rhythms as a readout of corticothalamic circuit function *in vivo.* To this end, we implanted chronic wireless electrocorticographic (ECoG) devices into sham and TBI mice during the craniotomy/TBI induction surgery, returned mice to their home cages for chronic recording, and analyzed changes in ECoG power over time (Figure 3A-H). We observed a chronic increase in broadband power in TBI during both light epochs (Figure 3C-H) and dark epochs (data not shown).

Given that severe TBI has been shown to lead to epileptogenesis over time, we investigated whether mild TBI also resulted in epileptogenesis. We quantified different types of epileptic activities including epileptiform spikes, epileptic discharges, spike-and-wave discharges, and spontaneous focal or generalized seizures at 24 hours and three weeks post-TBI using previously reported classification. In the first 24 hours, 3 out of 16 TBI mice, but none of the 8 sham mice, showed generalized tonic-clonic seizures (GTCSs, Table 2). None of the mice showed GTCSs at later time points (up to three weeks) (Table 2). However, at three weeks post-TBI, we saw more epileptiform spikes in TBI mice (n=9) than in sham mice (n=5), suggesting an increase in excitability (Table 2). Similarly, in another recording setup using simultaneous ECoG and multi-unit thalamic recordings, we observed that TBI mice have spontaneous epileptiform events that include synchronized thalamic bursting and increased normalized theta power, as early as one week and up to three weeks post-TBI (Figure 4). The various types of epileptic activities we observed are described in Figures 5 and 6.

Figure 3

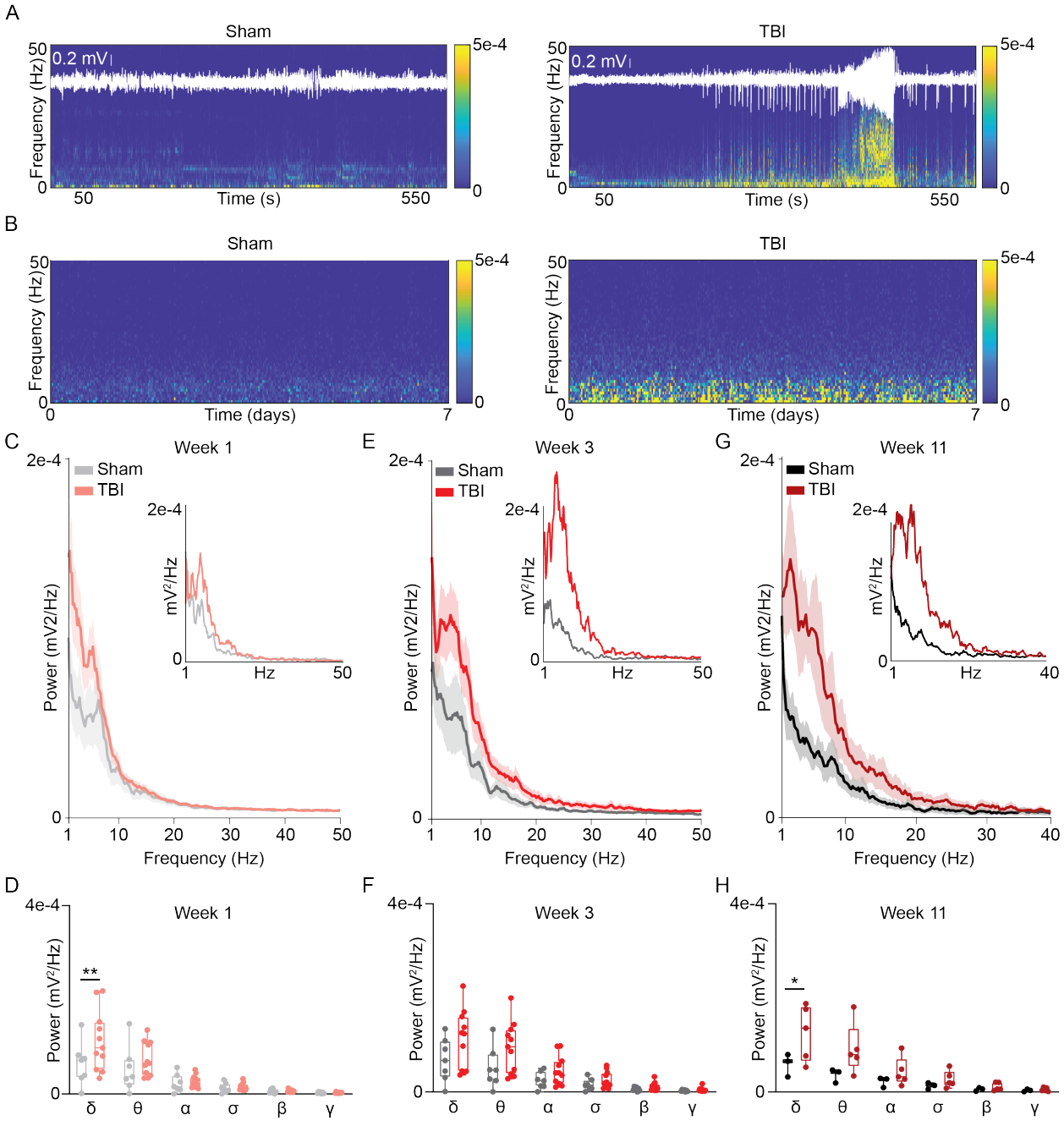


Figure 3. Chronically recorded TBI mice show altered power across different ECoG frequency bands. A) Example 10-minute spectrograms from a sham mouse (left) and TBI mouse (right) taken from the same time point within the first 24 hours of TBI, overlaid with ECoG traces from ipsilateral S1. The TBI spectrogram shows an example of an electrographic seizure, while the sham spectrogram shows normal ECoG activity. Color bar represents power (mV^2/Hz). B) Example seven-day spectrograms from a sham mouse (left) and TBI mouse (right) showing power across different frequency bands two to three weeks post-TBI. Power bands are sampled every 30 minutes. Color bar represents power (mV^2/Hz). C) Power spectral density of ECoG activity from sham and TBI cohorts averaged across the first week post-TBI. Inset shows example power spectral density plots from a representative sham and TBI mouse. See methods for details. D) Two-way ANOVAs of average power across frequency bands for the first week post-TBI. Each dot represents power for one mouse. E) Same as C) but at three weeks post-TBI. F) Same as D) but at three weeks post-TBI. G) Same

as C) but at 11 weeks post-TBI. H) Same as D) but at 11 weeks post-TBI. Data represent all mice recorded, analyzed with a two-way ANOVA (*p < 0.05, **p < 0.01), even if they died or if the battery ran out before the experimental endpoint. n = eight sham mice, 16 TBI mice. One mouse died within two days post-TBI. The remaining mice were recorded for the first week post-TBI, then recorded for alternating weeks until eleven weeks post-TBI. Delta = 1-4 Hz, theta = 5-8 Hz, alpha = 9-12 Hz, sigma = 13-15 Hz, beta = 16-30 Hz, gamma = 31-50 Hz.

Figure 4.

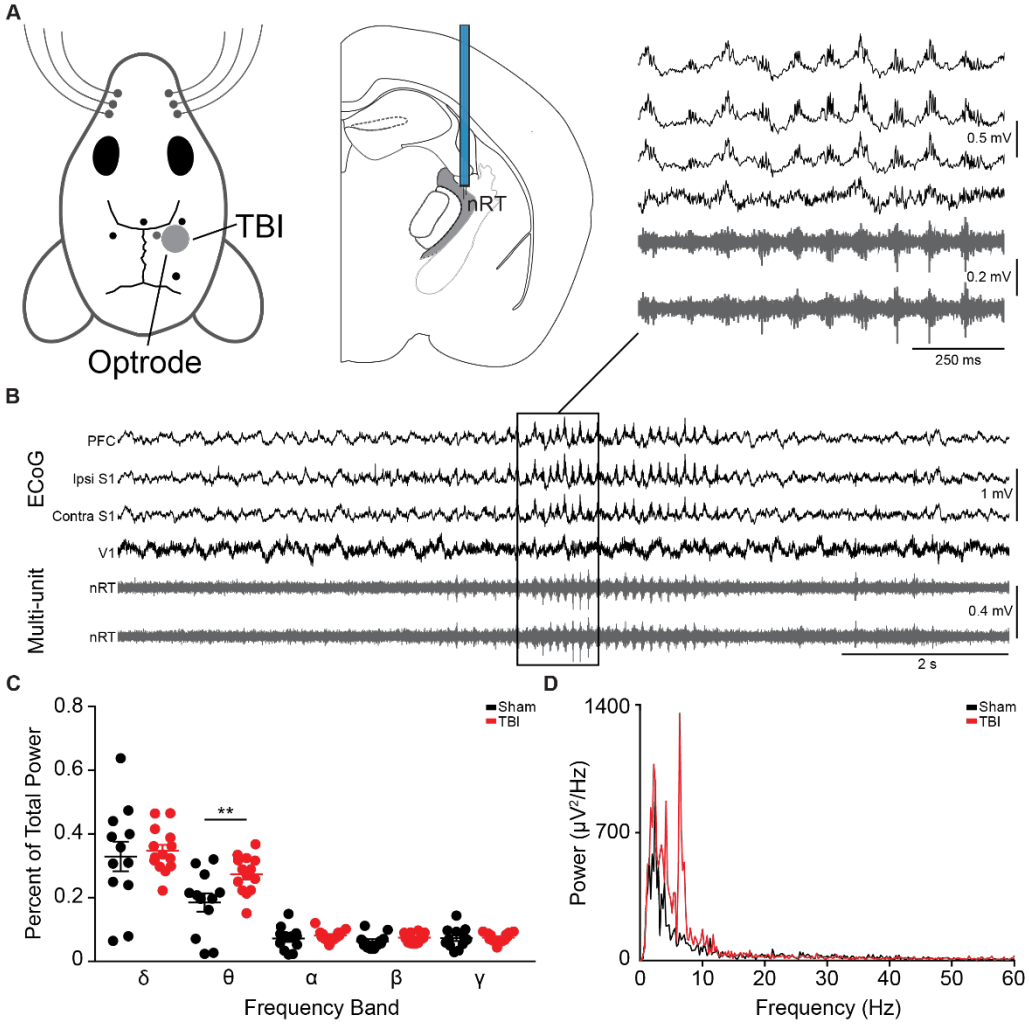


Figure 4. Mice with TBI have spontaneous seizure-like events in the theta to theta-alpha frequency range that are time-locked with thalamic bursting. A) Diagram of recording locations for *in vivo* experiments. Left, ECoG recording sites and TBI location are shown on the mouse skull. Right, approximate location of tungsten depth electrodes implanted unilaterally in the nRT. B) Representative ECoG traces from cortical recording sites and multi-unit traces from nRT showing a spontaneous seizure-like event. C) Power spectral analysis showing the average power across different frequency bands in the first 15 minutes of baseline ECoG signal from the ipsilateral S1 cortex in sham and TBI mice. D) Periodogram showing the power across frequencies taken from the first 15 minutes of baseline ECoG signal from the ipsilateral S1 cortex in a representative sham and TBI mouse. Data represent mean ± SEM analyzed with a Mann-Whitney test and α = 0.05 (*p < 0.05, **p < 0.01). Analysis includes between 12 and 14 mice per group.

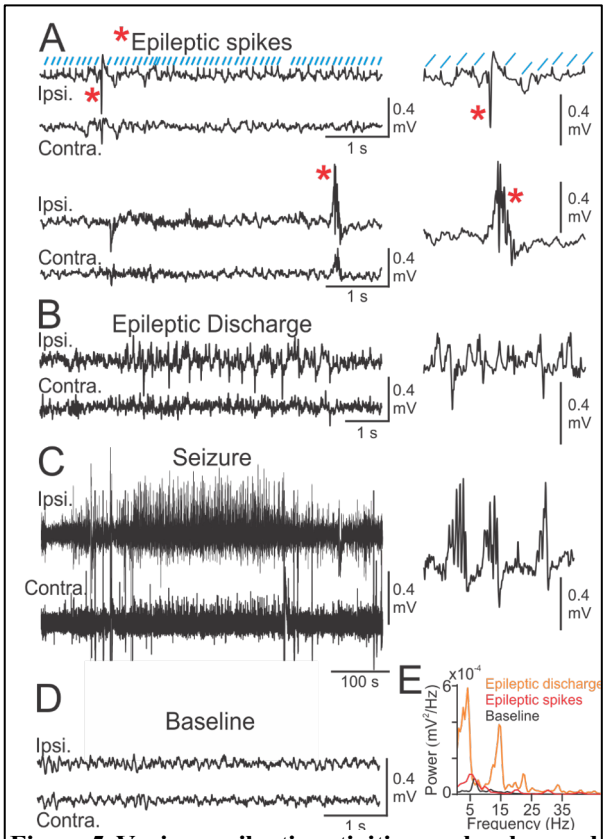


Figure 5. Various epileptic activities can be observed in the same mouse with PTE (three months post-TBI). Representative recordings of chronic EEG from the peri-TBI S1 cortex (Ipsi., Ipsilateral), and the corresponding contralateral cortex (Contra.) from the same mouse. **A-D,** Left: various epileptic activities observed in the same mouse (**A-C**). Right: Zoom on the left ipsi. EEG shows epileptic spikes. **A,** Epileptic spikes (asterisk) were either single (top) or complex (bottom). Note that epileptic spikes were nested in a background spiking activity in the theta/alpha frequency range (~8-10 Hz). **B,** Epileptic discharges (typically 5s – long, peak ~2-5Hz) composed of a cluster of epileptic spikes, predominant in the ipsilateral cortex. **C,** Seizures (~1-5 min-long, characterized by a complex rhythmic spiking activity, peak at 5-6Hz, harmonics at 10-12Hz). A-C, Note the higher amplitude of epileptic activities in ipsi. vs. contra EEG. **E,** Overlay of power spectra corresponding to the ipsi. traces depicted on the left. These results are representative of all the mice described in Figure 3.

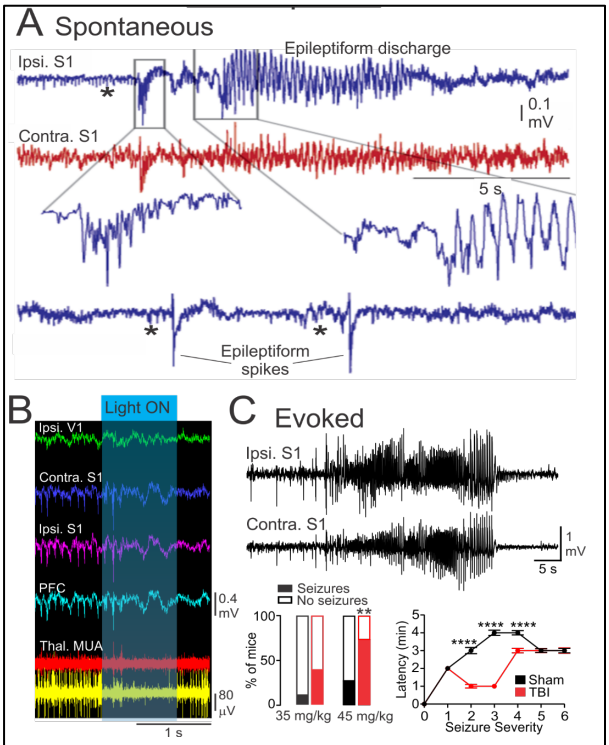


Figure 6. Spontaneous epileptiform events and seizure susceptibility. A, Representative epileptiform spikes and discharges in peri-TBI S1 cortex (ipsi.) and the corresponding contralateral (contra.) cortex. Note that epileptiform events in the ipsi. cortex were nested in low amplitude oscillatory activities (asterisks). **B,** Such epileptiform events could be interrupted by optogenetic activation of ChR2-expressing nRT neurons, suggesting thalamocortical output involvement in early epileptiform events (representative of four mice out of four in which opsin expression and location were verified). **C,** Top: Representative generalized tonic-clonic seizure (GTCS) in a TBI mouse subjected to the PTZ challenge (45 mg/kg, i.p.). Bottom left: Proportion of 21 sham and 26 TBI mice with GTCS after PTZ injection (**p<0.01, Chi-square test). Bottom right: Latency to reach each stage of seizure severity after PTZ injection. Stage 2-4 seizures occurred more rapidly in TBI mice (****p<0.0001, multiple t-tests).

1.3 Conclusions

We conclude that the major long-term effect of TBI on corticothalamic circuits involves disruption of synaptic transmission in the nRT, which coincides with increased C1q expression, reduced cortical inputs, and local neuronal loss. In contrast, neurons in the peri-TBI cortex and the VB appear normal at chronic stages post-TBI (Table 1), suggesting that inflammation - in particular, increased C1q expression - in these regions is not associated with long-term dysfunction in neuronal excitability or synaptic function. Furthermore, we show that in our mouse model the increased ECoG power in low frequency bands is a biomarker of injury. Notably, TBI mice developed various types of epileptic activities 3 weeks post-TBI but did not show delayed full-blown generalized tonic-clonic seizures.

2. Major Task 2: Determine the role of C1q in seizures after TBI

2.1 Specific Objectives:

Milestone(s): Reveal the role of the immune response involving C1q in circuit plasticity after TBI, validate a new biomarker (C1q and thalamic gliosis) for the epileptogenesis in PTE, and determine if the drug ANX005 that blocks the effects of C1q is efficient in preventing and curing PTE.
Aim 2a: Determine if C1q is upregulated in CTC "hot spots" after TBI
Aim 2b: Determine if blocking C1q action prevents PTE
Aim 2c: Determine if blocking C1q cures PTE

2.2 Major findings

2.2.1 Blocking C1q function reduces chronic glial inflammation and neuron loss

Increased C1q expression persisted four months post-TBI (Figure 7A-B). To test C1q's causal involvement in the inflammation and neuronal loss observed three weeks post-TBI, we used an antibody that specifically binds to C1q and blocks its downstream activity. Mice were given i.p. injections of the C1q antibody or a mouse IgG1 isotype control 24 hours after TBI or sham surgery, followed by twice weekly treatments for three weeks (see methods for more details).

TBI mice treated with the anti-C1q antibody showed a strong reduction in inflammation and reduced neuronal loss (Figure 8A-C) relative to control-treated TBI mice, and on average had the same number of nRT neurons as antibody-treated sham mice (Figure 8C). TBI mice treated with the control still showed inflammation and neuron loss three weeks after TBI (Figure 8). As an alternative approach to the antibody treatment, we repeated the study using C1q -/- mice and found that TBI C1q -/- mice also exhibited reduced chronic inflammation and reduced neuron loss in the nRT (Figure 9).

To confirm presence and effects of the anti-C1q antibody in the brain, we measured free drug, free and total C1q, C1s, and albumin levels in naïve, sham and TBI brains after two doses of control or antibody treatment (Figure 10). In plasma from the treated mice, free anti-C1q antibody was observed in both sham and TBI mice treated with the drug (Figure 10). In agreement, we found that total C1q protein was undetectable in drug-treated animals using an assay that is not affected by free drug. These results suggest that drug-bound C1q is fully cleared from the circulation. Free anti-C1q antibody was observed in treated sham and TBI mice: 0.4-8.6 ug/ml in the ipsilateral side and 0.09-3.8 ug/ml in the contralateral side. The sham and TBI injuries led to a significant increase in ipsilateral C1q and small increase in contralateral C1q in untreated mice. In the anti-C1q treated sham and TBI mice, total C1q levels were significantly reduced in the ipsilateral side and showed trends of reduction in the contralateral side. Measurable levels of free anti-C1q antibody were observed, suggesting that C1q was fully saturated, but not fully cleared as in the periphery.

These outcomes indicate that C1q may lead to inflammation and neuron loss in TBI, and that blocking C1q reduces these deleterious effects.

Figure 7.

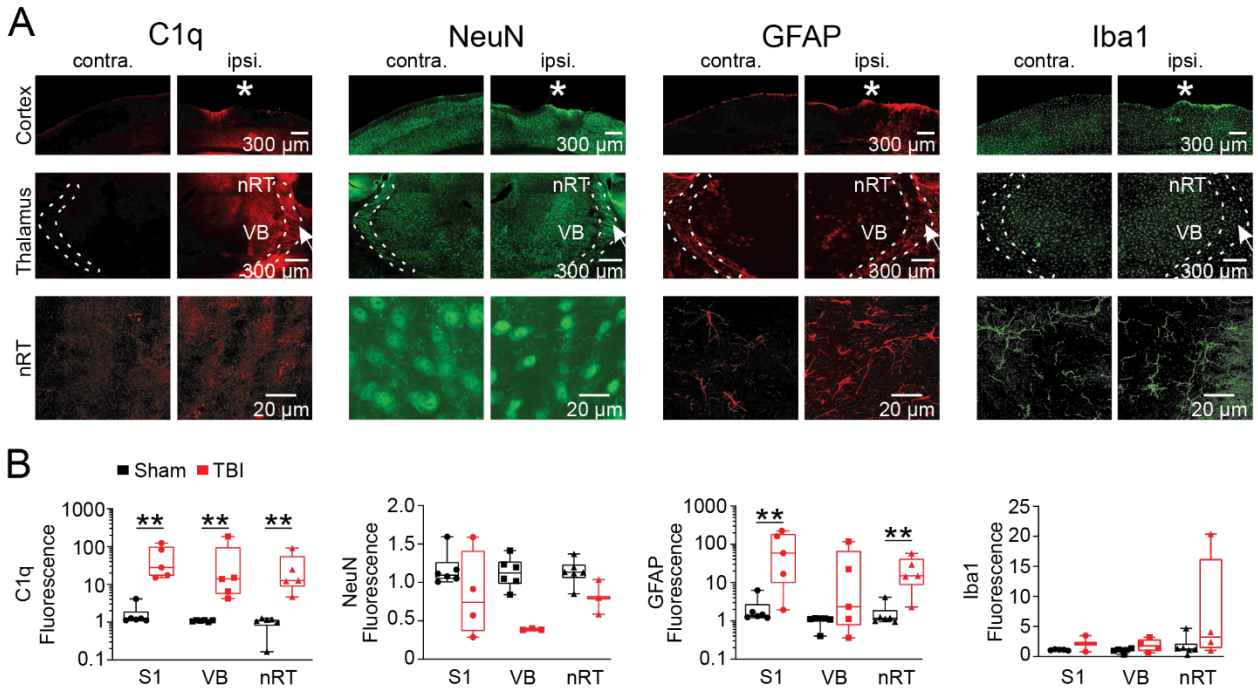


Figure 7. The injured cortex and functionally connected thalamus show chronic inflammation and neuron loss four months after TBI. A) Close-up images of S1 (top), VB and nRT (middle), and confocal images of nRT (bottom), stained for C1q, NeuN, GFAP, and Iba1. Injury site in the right S1 cortex is marked by an asterisk. Arrow in nRT indicates location of confocal image. Scale bars, 300 µm (top/middle) and 20 µm (bottom). B) Quantification of fluorescence ratios between ipsilateral and contralateral regions in sham and TBI mice. Data represent all points from min to max, with a Mann-Whitney test and $\alpha = 0.05$ (*$p < 0.05$, **$p < 0.01$). Analysis includes between four and six mice per group (n = one to three sections per mouse, one image per region).

Figure 8

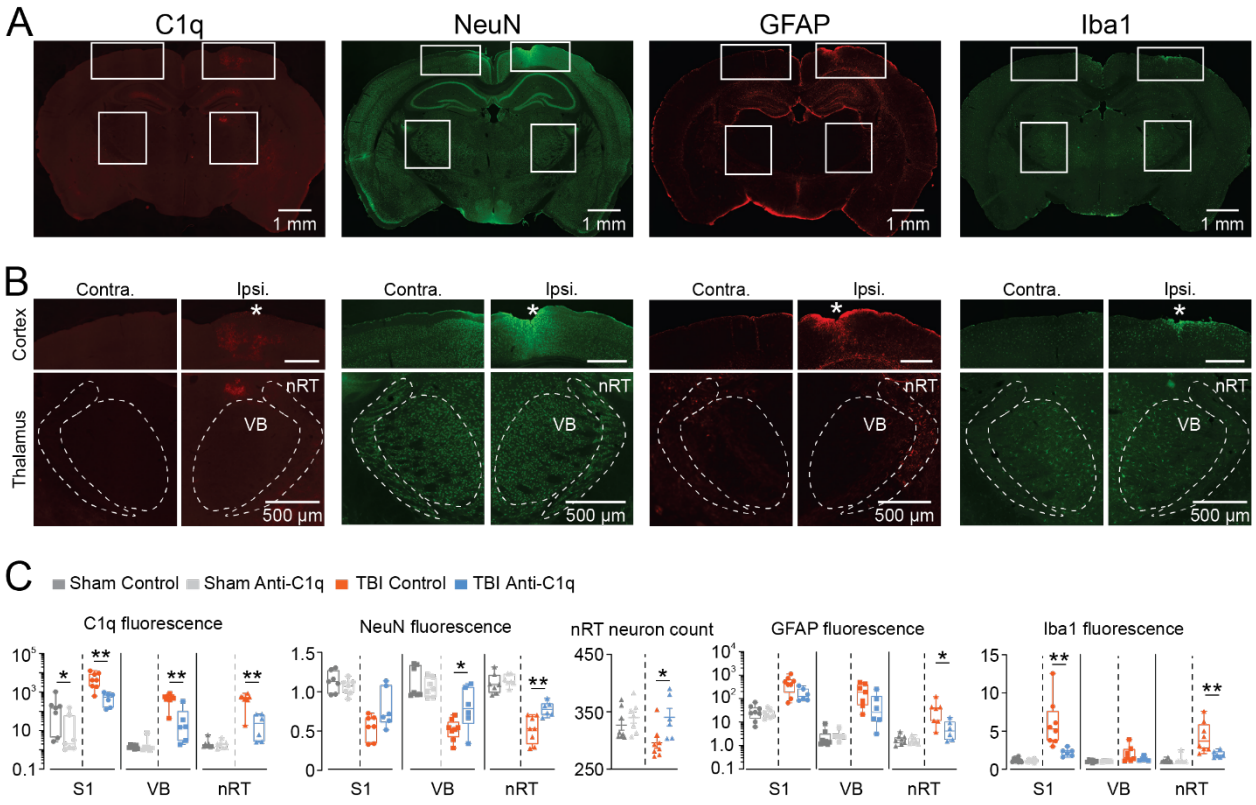


Figure 8. Anti-C1q antibody reduces chronic inflammation and neuron loss three weeks after TBI. A, B) Representative coronal brain sections (A) and close-ups (B) of S1 (top), VB and nRT (bottom) from TBI mice treated with anti-C1q antibody and stained for C1q, NeuN, GFAP, and Iba1. Injury site in the right S1 cortex is marked by an asterisk. Scale bars, 1 mm (A), 500 µm (B). C) Quantification of nRT neuron counts and fluorescence ratios between ipsilateral and contralateral regions in control and antibody-treated sham and TBI mice. Data represent all points from min to max, with a Mann-Whitney test and $\alpha = 0.05$ (*$p < 0.05$, **$p < 0.01$). Analysis includes between six and eight mice per group (n = three sections per mouse, one image per region).

Figure 9

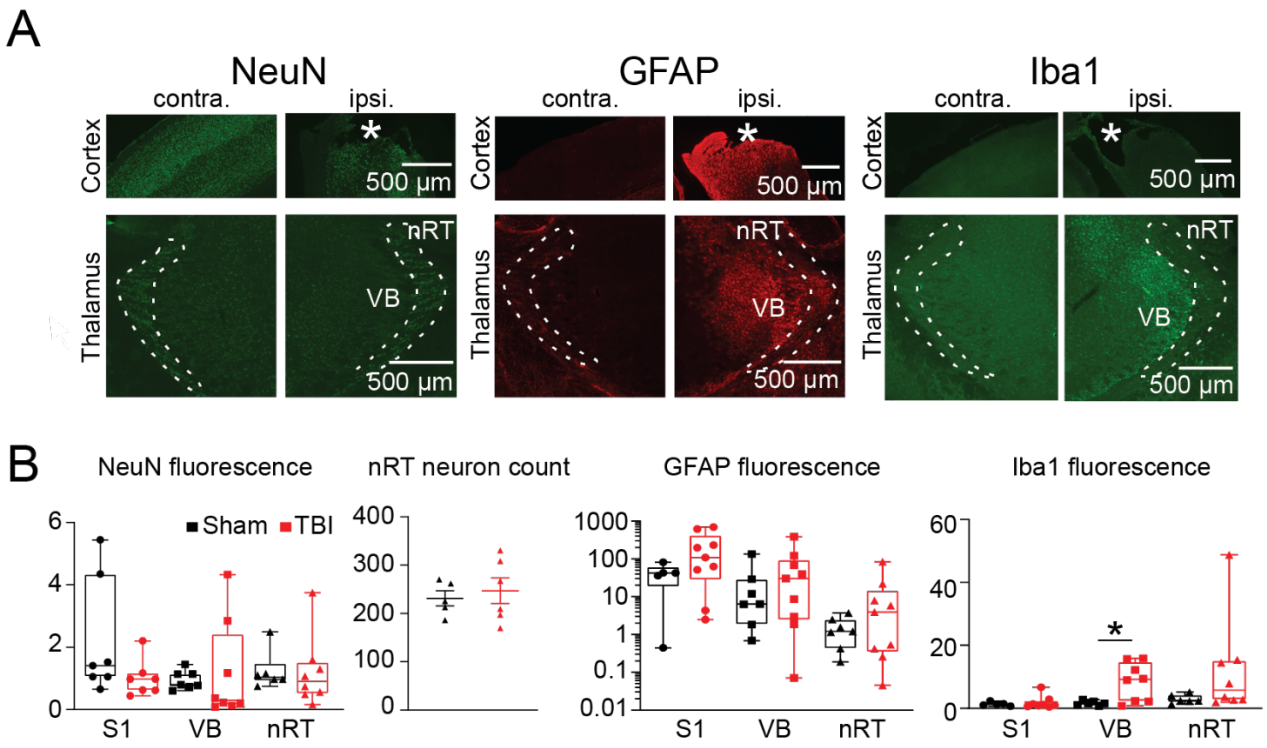


Figure 9. C1q -/- mice show reduced inflammation and neuron loss three weeks after TBI. A) Close-up images of S1 (top), VB and nRT (bottom) stained for NeuN, GFAP, and Iba1. Injury site in the right S1 cortex is marked by an asterisk. Scale bars, 500 µm. B) Quantification of fluorescence ratios between ipsilateral and contralateral regions and nRT neuron counts in sham and TBI C1q -/- mice. Data represent all points from min to max, with a Mann-Whitney test and $\alpha = 0.05$ (*$p < 0.05$, **$p < 0.01$). Analysis includes between four and six mice per group (n = one to three sections per mouse, one image per region).

Figure 10

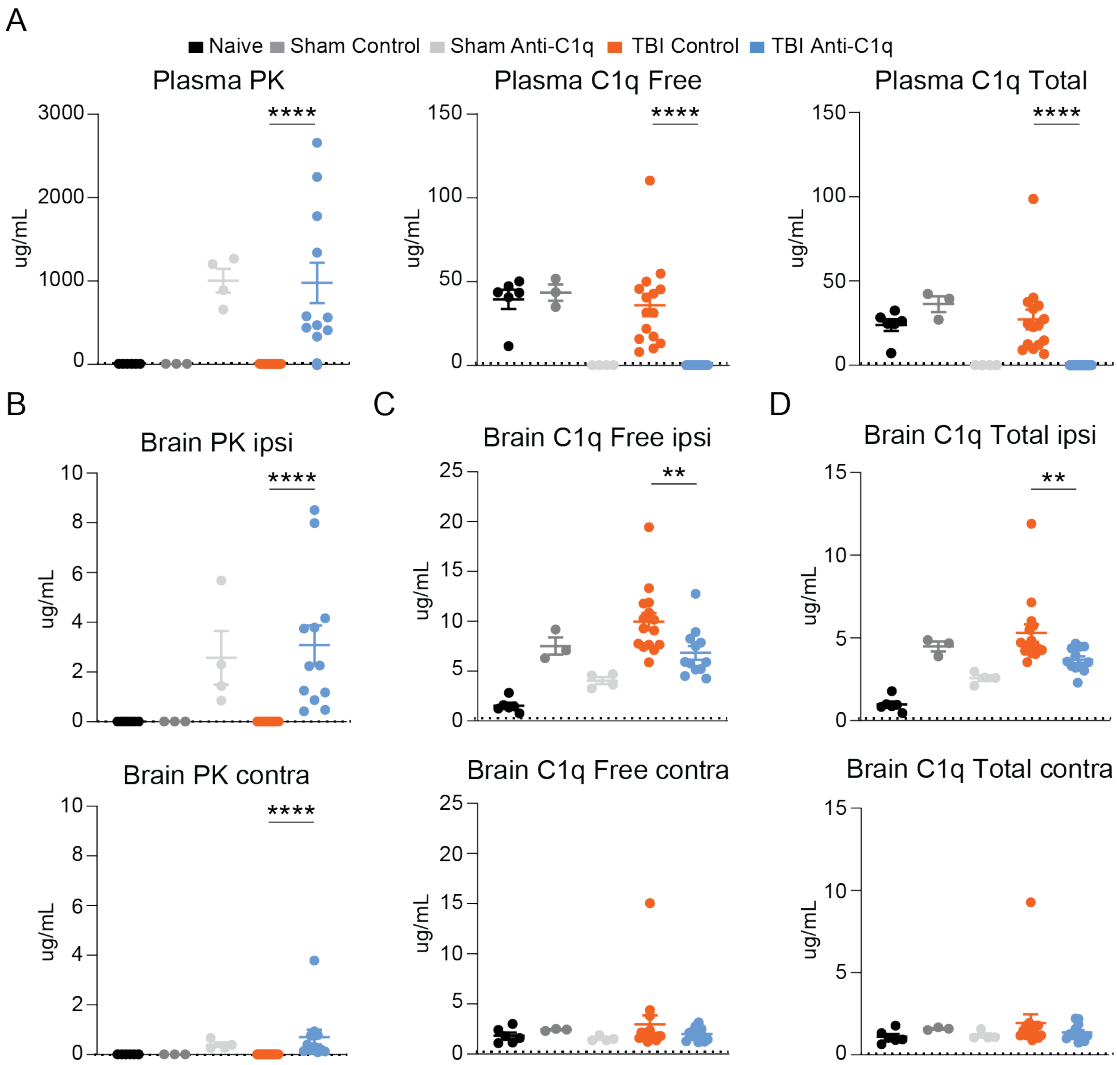


Figure 10. Plasma and brain PK/PD show presence of free drug and reduced C1q in anti-C1q drug-treated sham and TBI mice. A) Plasma levels of free drug, C1q-free, and C1q-total were measured using sandwich ELISAs after TBI and sham mice were treated with two doses of 100 mg/kg anti-C1q or isotype control antibodies. Dotted line shows lower limit of quantification. B-F) Levels of free drug (B), C1q-free (C), and C1q-total (D) were measured in brain lysates in the ipsilateral (top) and contralateral (bottom) sides using sandwich ELISAs. Naïve mice were negative controls. Dotted line shows lower limit of quantification. Data represent all points from min to max, with a Mann-Whitney test between TBI control and TBI anti-C1q, and $\alpha = 0.05$ (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$). Analysis includes between three and 15 mice per group.

2.2.2 Effects of the anti-C1q antibody on chronic cortical states in mice with TBI

To determine whether blocking C1q could rescue changes in cortical states, we treated mice with the anti-C1q antibody or isotype control for five weeks, starting 24 hours post-TBI, while maintaining ECoG recordings for up to 9-15 weeks post-TBI (Figure 11A, Figure 12. While the ECoG spectral features were similar within the first week of anti-C1q antibody or control treatment (Figure 11B-C, Figure 12B), analysis of the combined cohorts show that the anti-C1q group trended toward reduced power across most frequency bands at three weeks (Figure 11D-E, Figure 12C).

Notably, epileptiform activities were not affected by the anti-C1q antibody (Table 2). Three weeks post-TBI, we saw no GTCSs and no differences in the frequency of epileptic events between control-treated and antibody-treated TBI mice (Table 2).

Figure 11

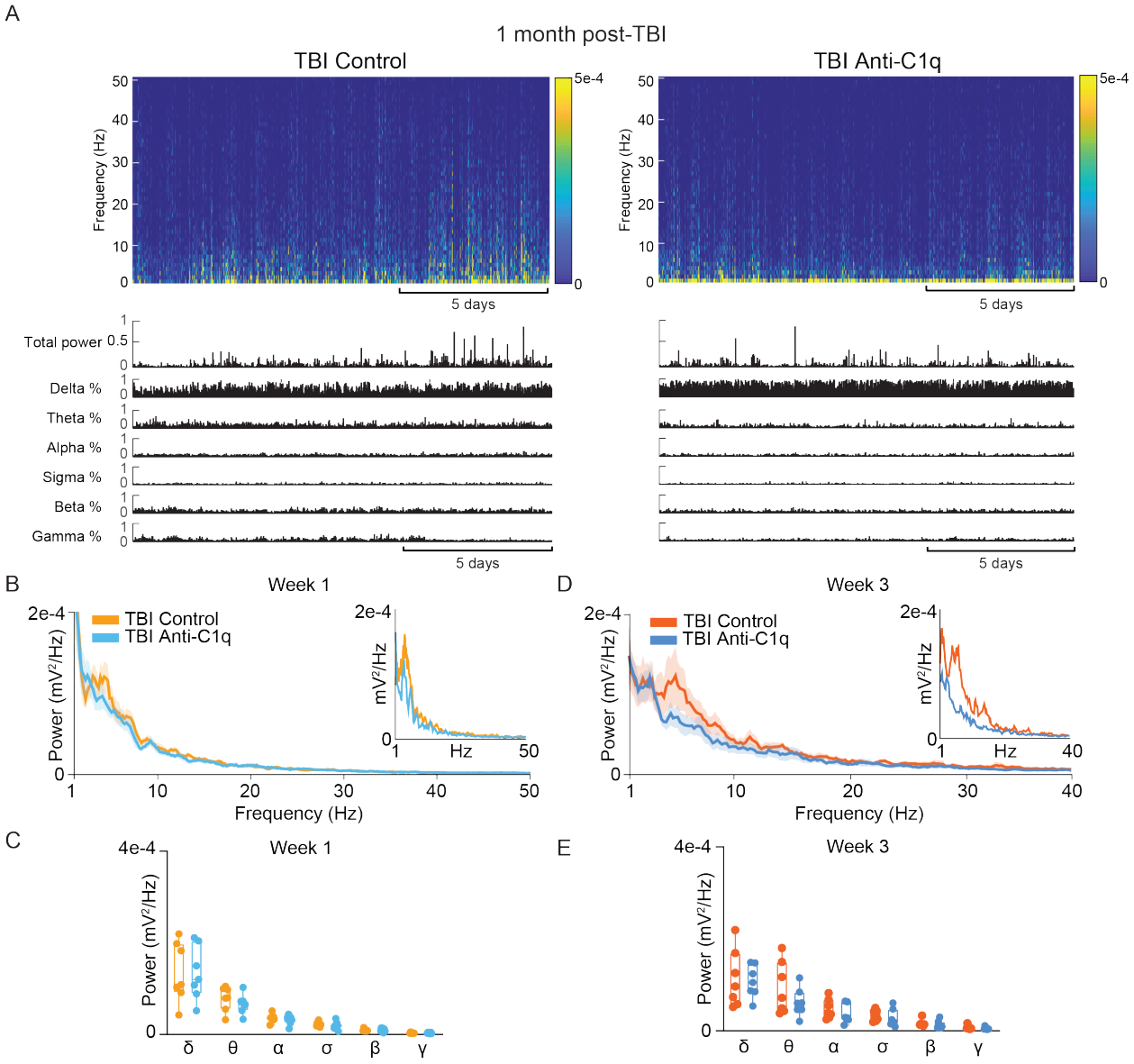


Figure 11. Effects of anti-C1q antibody on ECoG spectral features in mice with TBI. A) Example spectrograms (top) and histograms (bottom) from a control-treated mouse (left) and antibody-treated mouse (right) showing power across different frequency bands one month post-TBI. Power bands are sampled every 30 minutes. Color bar represents power (mV^2/Hz). B) Power spectral density of ECoG activity from control-treated and antibody-treated TBI cohorts averaged across the first week post-TBI. Inset shows example power spectral density plots from a representative control-treated TBI mouse and an antibody-treated TBI mouse. See methods for details. C) Two-way ANOVAs of average power across frequency bands for the first week post-TBI. Each dot represents power for one mouse. D) Same as B) but at three weeks post-TBI. E) Same as C) but at three weeks post-TBI. Data represent all mice recorded, analyzed with a two-way ANOVA, even if they died before treatment ended. n = seven control-treated mice, seven antibody-treated mice. Delta = 1-4 Hz, theta = 5-8 Hz, alpha = 9-12 Hz, sigma = 13-15 Hz, beta = 16-30 Hz, gamma = 31-50 Hz.

Figure 12

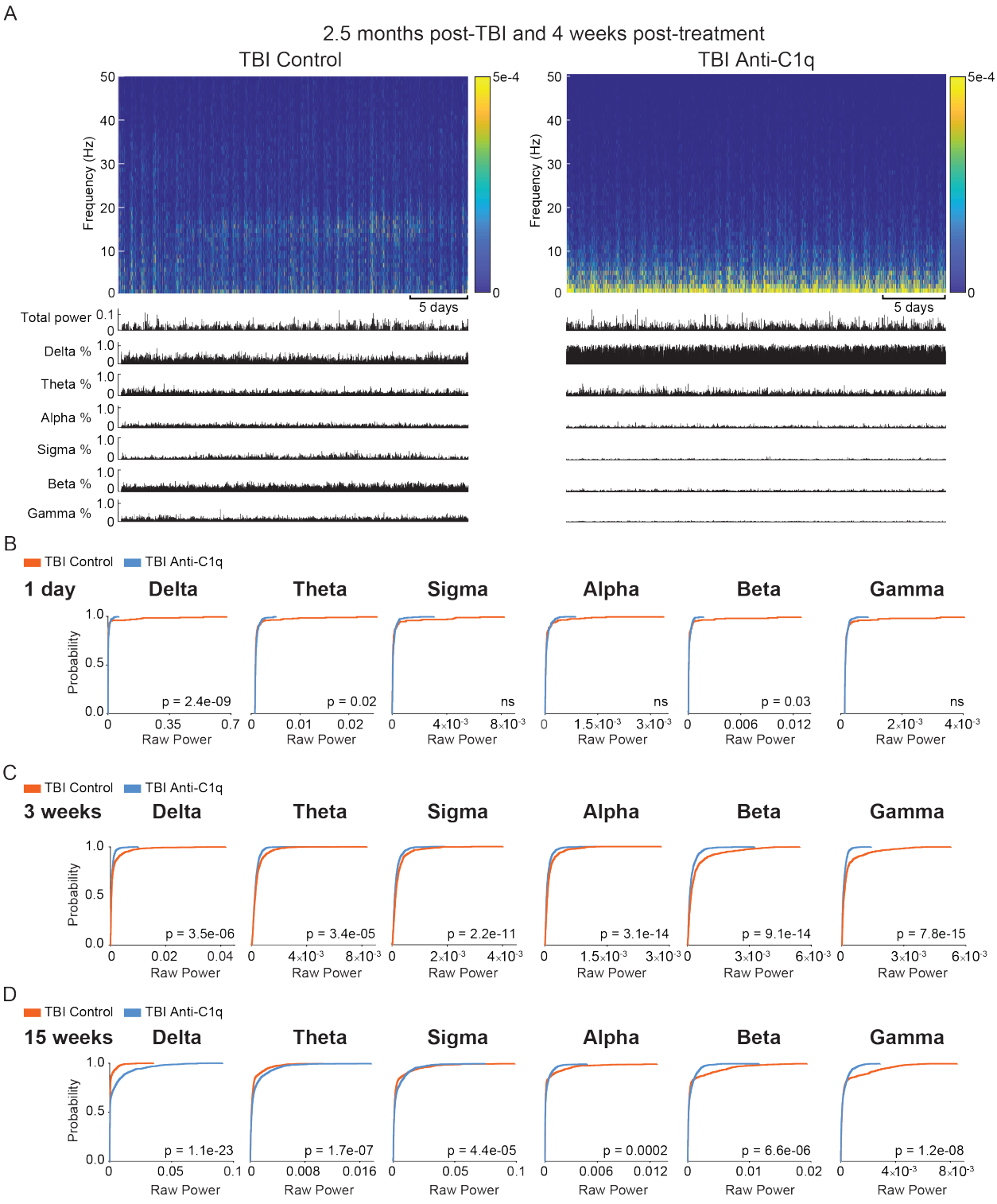


Figure 12. Anti-C1q antibody has chronic disease-modifying effects on ECoG power in mice with TBI. A) Example spectrograms (top) and histograms (bottom) from a control-treated mouse (left) and antibody-treated mouse (right) showing power across different frequency bands 2.5 months post-TBI, which was four weeks after the treatment ended.

Power bands are sampled every 30 minutes. B) Cumulative distribution functions for control-treated and antibody-treated cohorts sampled across different frequency bands in the first day post-TBI. We sampled 48 points from the first 24 hours within the start of each recording. C) Same as B, but at three weeks post-TBI. We sampled 232 points between 15.25-20.1 days from the start of each recording. D) Same as B, but at 9-15 weeks post-TBI. We sampled 296 points between 104.6 to 110 days from the start of each recording. Data represent all mice recorded, even if they died before treatment ended. One control-treated mouse and one antibody-treated mouse died within three weeks post-TBI, two control-treated mice died within six weeks post-TBI, and the remaining mice were recorded for at least nine weeks post-TBI. At 24 hours, n = seven control-treated mice, seven antibody-treated mice. At three weeks, n = seven control-treated mice, seven antibody-treated mice. At 9-15 weeks n = six control-treated mice, four antibody-treated mice. Delta = 1-4 Hz, theta = 5-8 Hz, alpha = 9-12 Hz, sigma = 13-15 Hz, beta = 16-30 Hz, gamma = 31-50 Hz. ns = $p > 0.05$.

Table 2

First 24 hours	Epileptiform spikes	Epileptiform discharges	Spike-and-wave discharges	Generalized tonic-clonic seizures	Acute post-injury mortality
Sham	8/8 (100%)	6/8 (75%)	0/8 (0%)	0/8 (0%)	0/8 (0%)
TBI	16/16 (100%)	13/16 (81%)	4/16 (25%)	3/16 (19%)	0/16 (0%)
Drug study					
TBI Vehicle	7/7 (100%)	5/7 (71%)	0/7 (0%)	2/7 (28%)	0/7 (0%)
TBI anti-C1q	7/7 (100%)	5/7 (71%)	0/7 (0%)	1/7 (14%)	0/7 (0%)

3 weeks	Epileptiform spikes	Epileptiform discharges	Spike-and-wave discharges	Generalized tonic-clonic seizures
Sham	7/7 (100%)	1/7 (14%)	0/7 (0%)	0/7 (0%)
TBI	11/11 (100%)	3/11 (27%)	1/11 (9%)	0/11 (0%)
Drug study				
TBI Vehicle	7/7 (100%)	2/7 (28%)	0/7 (0%)	0/7 (0%)
TBI anti-C1q	7/7 (100%)	3/7 (43%)	0/7 (0%)	0/7 (0%)

	Epileptiform spikes	Epileptiform discharges	Spike-and-wave discharges	Generalized tonic-clonic seizures	mice
Sham – 24h	234 ± 62	4 ± 2	0	0	8
TBI – 24h	452 ± 178	8 ± 6	1 ± 0.6	0.4 ± 0.2	16
Sham – 3wk	66 ± 38	0.7 ± 0.7	0	0	7
TBI – 3wk	292 ± 114	2 ± 1	0.09 ± 0.09	0	11
Mixed-effects analysis	ns	ns	ns	ns	
Drug study					
TBI Vehicle – 24h	278 ± 79	4 ± 1	0	0.6 ± 0.4	7
TBI anti-C1q – 24h	137 ± 55	1 ± 0.5	0	0.3 ± 0.3	7
TBI Vehicle – 3wk	300 ± 92	0.3 ± 0.2	0	0	7
TBI anti-C1q – 3wk	274 ± 50	1 ± 0.8	0	0	7
Mixed-effects analysis	ns	ns	ns	ns	

Table 2. Summary of epileptiform activity analysis in sham, TBI, control-treated TBI, and antibody-treated TBI mice. Mice were recorded continuously starting the day of the TBI up until several weeks post-TBI. Surgical and recording conditions are described in the methods section titled "Surgical implantation of devices for chronic ECoG recordings". Analysis was performed on the first 24 hours post-TBI, and across a 48 hour window at three weeks post-TBI. A repeated measures mixed-effects ANOVA was performed for statistical analysis.

2.3 Conclusions

Our findings indicate that C1q may lead to the secondary inflammation and neuron loss in TBI, and that blocking C1q reduces these deleterious effects. Furthermore, we show that in our mouse model

the increased ECoG power in low frequency bands is a biomarker of injury, and that anti-C1q antibody tends to counteract the increased low power in ECoG. Indeed, while the ECoG spectral features were similar within the first week between TBI mice treated with anti-C1q antibody or vehicle, the anti-C1q group trended toward reduced power across most frequency bands at three weeks post-TBI (Figure 11D-E, Figure 12C). Notably, TBI mice developed various types of epileptic activities 3 weeks post-TBI, and the anti-C1q treatment tended to reduce these. However, the mice did not show full blown generalized tonic-clonic seizures. Therefore, it remains unknown if anti-C1q treatment would have completely prevented this particular type of seizures, which will be investigated in future studies.

Notably, although this was not a milestone in the original SWO, given that we were able to obtain a large amount of excellent quality chronic ECoG data from mice with TBI, we are currently analyzing the sleep dysfunction after TBI, and to what extent the anti-C1q treatment prevents sleep abnormalities. Given that thalamocortical circuits are implicated in sleep, which is known to be affected in patients in TBI, and to be associated with seizures, our future analysis that directly results from this study may uncover if C1q could be a target for treating co-morbidities like sleep.

Major Task 3: Determine if transplanting inhibitory neurons into CTC "hot spots" of hyperexcitability prevents and cures PTE

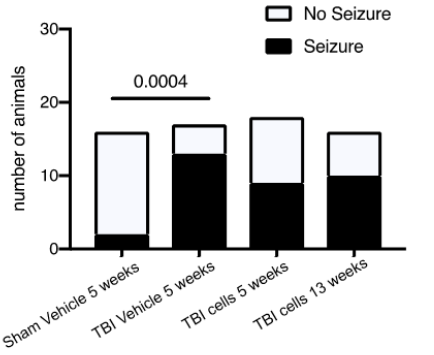
3.1 Specific objectives:

Aim 3a: Determine if inhibitory transplants in cortex and thalamus prevent circuit hyperexcitability in cortical and thalamic slices, respectively
Aim 3b: Determine if transplanted cells prevent PTE in behaving animals
Aim 3c: Determine if transplanted cells cure PTE in behaving animals

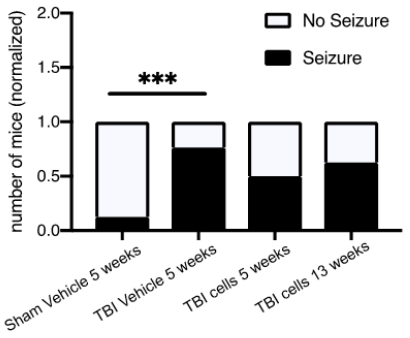
3.2 Major Findings:

We induced TBI or sham surgeries as described in previous aims above, and transplanted human cells in the peri-TBI cortex. We transplanted cells in the deep layers of the peri-injured cortex. To ensure wide targeting of the peri-injured cortex, we transplanted cells in three areas around the TBI lesion: 1) 0.5 mm anterior to the anterior edge of the TBI lesion, 2) 0.5 mm medial from the medial edge of the TBI, 3) 0.5 mm posterior to the posterior edge of the TBI lesion. One week after transplants, we implanted ECoG electrodes in these mice as described in Figure 4. One week after the ECoG implants, we assessed ECoG activity and susceptibility to seizures. We found that cell transplants did not affect the hyperexcitability and seizure susceptibility. These results suggest that restoring inhibition in the cortex is not sufficient to repair cortical abnormalities if the inflammation is still present, and pinpoint the neuroinflammation as a more promising therapeutic target for repairing the function of cortico-thalamo-cortical circuits.

Figure 13



	# mice with seizures	# mice with no seizures
Sham Vehicle 5 weeks	2	14
TBI Vehicle 5 weeks	13	4
TBI PD108 5 weeks	9	9
TBI PD108 13 weeks	10	6



	# mice with seizures	# mice with no seizures
Sham Vehicle 5 weeks	0.125	0.875
TBI Vehicle 5 weeks	0.76470588	0.23529412
TBI PD108 5 weeks	0.5	0.5
TBI PD108 13 weeks	0.625	0.375

Figure 13. Effect of cortical cell transplants on seizures in TBI mice. Number (left) and proportion (right) of mice with and without generalized tonic-clonic seizure (GTCS) in mice subjected to the PTZ challenge (45 mg/kg, i.p.). Mice received injections of cells or vehicle in the peri-TBI cortex. ECoG and seizures were assessed five weeks post-TBI/sham surgery. TBI mice with cell transplants were also recorded 13 weeks post-TBI. (***$p<0.001$, Fischer test). Note that cell transplants did not reduce hyperexcitability/GTCSs.

3.3 Conclusions

We conclude that cell transplants in the peri-TBI cortex are not sufficient to reduce hyperexcitability and seizures. We speculate that cell transplants do not rescue cortical activity because the inflammation is still present, and we propose that neuroinflammation and in particular C1q are more promising therapeutic targets for repairing the function of cortico-thalamo-cortical circuits after TBI.

OVERALL SUMMARY AND CONCLUSIONS

In this study, we set out to understand the role of the C1q pathway in post-TBI secondary injury to the corticothalamic circuit in a mechanistically tractable and highly reproducible mouse model of cortical injury. This model allows us to identify factors such as therapeutic windows, inflammatory phenotypes, and degree of secondary damage, which have been postulated to be important for designing targeted approaches in the treatment of post-TBI outcomes.

Our study pioneers the use of electrophysiological approaches to study the entire somatosensory corticothalamic circuit after TBI. One powerful tool we employ is chronic ECoG recordings to study the progression of post-traumatic changes in cortical rhythms up to four months post TBI. Using electrophysiological approaches at the cellular and circuit levels, we showed that TBI alters the synaptic properties of nRT neurons and is associated with increased C1q accumulation that might mediate pathological states in the corticothalamic circuit, including increased broadband activity.

The nRT as a locus of long-term, secondary impairments post-TBI: Overall, our findings suggest that the major long-term impact of mild TBI is in the thalamic end of the cortico-thalamo-cortical loop.

C1q: good or bad? A question of timing: We chose to test the importance of one specific inflammatory pathway, the classical complement pathway, using a pharmacological tool to block C1q in TBI mice. C1q has a well-documented role in normal brain function such as synaptic pruning during development, as well as its involvement in several neurological diseases. In addition, we had observed that C1q expression was highly increased in the corticothalamic circuit for up to four months after TBI. Although our TBI mice did not develop chronic GTCSs to determine if blocking C1q could cure epileptogenesis, we did observe many other protective effects of the anti-C1q antibody, including reduced inflammation and neurodegeneration. Based on these observations we speculate that C1q plays both good and bad roles but at different stages of pathology. At the time of the injury, C1q plays a "beneficial" role, perhaps by aiding with the formation of the glial scar that limits the size of the injury within the primary site of the cortex. However, at the chronic phase, C1q increase plays a maladaptive role in promoting chronic inflammation and secondary neurodegeneration in the nRT.

The cortex also exhibits an increase in C1q, but it does not appear to have a damaging role at this site, or may play a counterbalancing initial protective role since, unlike in the thalamus, the neuronal physiology is similar in the cortex of sham and TBI mice at chronic time points. Our findings suggest the existence of a time window during which the anti-C1q treatment might prevent secondary damage to the thalamus without impairing homeostatic recovery at the cortex.

In conclusion, our study pinpoints C1q as a potential disease modifier that could be targeted for treating devastating outcomes of TBI within a certain time window (in this study, beginning treatment 24 hours post-injury). C1q might also serve as a biomarker to help identify those individuals likely to develop long-term, secondary injuries. Our study also motivates further investigation of the molecular mechanism by which C1q causes neuronal death in nRT, beyond its well-known role in synaptic pruning in health and disease. In addition, by showing that the thalamus is chronically affected by TBI, we identify a potential cause for many TBI-related disabilities such as altered sensory processing, sleep disruption, and epilepsy, and a novel target for post-TBI treatments.

Other achievements:

The PI (Jeanne Paz) received the prestigious Vilcek Prize 2019 that rewards the individuals who made long-lasting contributions to the American society in arts and sciences. Furthermore, this study resulted in multiple awards for the students in the Paz lab who worked on this project. Two graduate students in the Paz laboratory – Stephanie Holden and Frances Cho – received awards that allowed them to present their work at multiple national and international conferences. These awards include the ARCS Fellowship (2016 and 2017 to SH), the Doctoral Career Development Award from the Society for Neuroscience (to SH), travel awards from the UCSF Graduate Division (to SH and FC), the UCSF Discovery Fellowship (FC), the Ford Dissertation Fellowship (SH), the National Research Scientific Achievement Award (NRSA to FC), the National Science Foundation graduate fellowship (NSF to FC), the Gladstone Distinguished Achievement in Science Award (to SH and FC), and the Dorman Outstanding Student Prize (to FC).

The results of the study funded by this DoD award were presented at multiple international, national and regional conferences:

Conference abstracts (peer-reviewed prior to acceptance):

1. Holden SS, Aboubakr O, Andrews-Zwilling Y, Sankaranarayanan S, Yednock T, **Paz JT** (2019) C1q mediates chronic secondary gliotic inflammation and neurodegeneration in a mouse model of post-traumatic epilepsy. American Epilepsy Society annual meeting, Baltimore, MD, USA. **Abstract selected for a Platform presentation.**
2. Chang A, Tsang C, Higashikubo B, **Paz JT** (2019) Optical interrogation of neurovascular dynamics in seizure progression and epileptogenesis. American Epilepsy Society annual meeting, Baltimore, MD, USA. **AC selected as American Epilepsy Society Fellow.**
3. Cho F, Vainchtein I, Morningstar A, Anink J, Van Vilet E, Aronica E, Molofsky A, **Paz JT** (2019) Reactive astrocytes mediate hyperexcitability in thalamocortical circuits by dysregulating extrasynaptic GABA. American Epilepsy Society annual meeting, Baltimore, MD, USA.
4. Cho FS, Vainchtein IL, Alcauter JA, Morningstar AR, Molofsky AV, **Paz JT** (2018) Transcriptomic analysis of reactive astrocytes and microglia in the thalamus reveals functional deficits linked to circuit excitability. Passwell Symposium, Israel. **Abstract Selected for a talk.**
5. Holden S, Morningstar AR, **Paz JT** (2018) Thalamocortical function after traumatic cortical injury. Passwell Symposium, Israel.
6. Holden S, Morningstar AR, Higashikubo B, **Paz JT** (2018) Thalamocortical function after traumatic cortical injury. Gordon Research Conference, Tuscany, Italy.
7. Holden S, **Paz JT** (2017) Deconstruction of thalamic circuits in a mouse model of post-traumatic epilepsy. Society for Neuroscience, Washington DC, USA.
8. Cho F, **Paz JT** (2017) "Assessing the effect of astrogliosis on thalamic circuit excitability". Young Generation Technical and Leadership Conference 2017 (YGTLC). Hosted by the Korean-American Scientist and Engineers Association, Korea. **Abstract selected for a talk.**
9. Holden S, **Paz JT** (2016) "The Role of the thalamus in focal traumatic brain injury". ARCS Symposium 2016, Menlo Park, CA, USA
10. Holden S, **Paz JT** (2016) "Thalamic excitability after traumatic cortical injury". GRC-Mechanisms of Epilepsy & Neuronal Synchronization conference, Girona, Spain
11. Cho F, Makinson S, Holden S, Tager D, **Paz JT** (2016) "Selective astrogliosis increases thalamic circuit excitability and oscillations" GRC-Mechanisms of Epilepsy & Neuronal Synchronization conference, Girona, Spain. **Abstract selected for a talk.**
12. Holden S, **Paz JT** (2016) "Thalamic function and traumatic cortical injury". Gordon research conference on Thalamus. Ventura, CA, USA.
13. Cho F, Makinson S, Holden S, **Paz JT** (2016) Assessing the effect of astrogliosis n thalamic circuit excitability". Gordon research conference on Thalamus. Ventura, CA, USA.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

This project provided multiple opportunities for training and professional development of numerous trainees in the Paz lab (see list below). Indeed, the following trainees worked on the project or were involved in the activities supported by the project:

-Two graduate (Neuroscience PhD) students: Stephanie Holden and Frances Cho
-Four postdoctoral fellows: Agnieszka Csieleska, Stefanie Ritter-Makinson, Andrew Chang, Bryan Higashikubo
-Research Associates (Staff): Irene Lew, Alex Urry, Scott Brovarney, Juan Alcauter (Latino under-represented minority), Dale Tager
-Visiting and rotation graduate students: Chenyu Wang, Morgane Leroux, Henna Mohabbat (under-represented minority), Francisco Aparicio (Latino under-represented minority), Marie Burkart, Deanna Necula, Charell Sherman (African American under-represented minority).

Participation of these trainees in conferences, workshops, and seminars is listed under major activities.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

The results of the study funded by this DoD award were presented at multiple international, national and regional conferences:

Conference abstracts (peer-reviewed prior to acceptance):

1. Holden SS, Aboubakr O, Andrews-Zwilling Y, Sankaranarayanan S, Yednock T, **Paz JT** (2019) C1q mediates chronic secondary gliotic inflammation and neurodegeneration in a mouse model of post-traumatic epilepsy. American Epilepsy Society annual meeting, Baltimore, MD, USA. **Abstract selected for a Platform presentation.**
2. Chang A, Tsang C, Higashikubo B, **Paz JT** (2019) Optical interrogation of neurovascular dynamics in seizure progression and epileptogenesis. American Epilepsy Society annual meeting, Baltimore, MD, USA. **AC selected as American Epilepsy Society Fellow.**
3. Cho F, Vainchtein I, Morningstar A, Anink J, Van Vilet E, Aronica E, Molofsky A, **Paz JT** (2019) Reactive astrocytes mediate hyperexcitability in thalamocortical circuits by

dysregulating extrasynaptic GABA. American Epilepsy Society annual meeting, Baltimore, MD, USA.

4. Cho FS, Vainchtein IL, Alcauter JA, Morningstar AR, Molofsky AV, **Paz JT** (2018) Transcriptomic analysis of reactive astrocytes and microglia in the thalamus reveals functional deficits linked to circuit excitability. Passwell Symposium, Israel. **Abstract Selected for a talk.**
5. Holden S, Morningstar AR, **Paz JT** (2018) Thalamocortical function after traumatic cortical injury. Passwell Symposium, Israel.
6. Holden S, Morningstar AR, Higashikubo B, **Paz JT** (2018) Thalamocortical function after traumatic cortical injury. Gordon Research Conference, Tuscany, Italy.
7. Holden S, **Paz JT** (2017) Deconstruction of thalamic circuits in a mouse model of post-traumatic epilepsy. Society for Neuroscience, Washington DC, USA.
8. Cho F, **Paz JT** (2017) "Assessing the effect of astrogliosis on thalamic circuit excitability". Young Generation Technical and Leadership Conference 2017 (YGTLC). Hosted by the Korean-American Scientist and Engineers Association, Korea. **Abstract selected for a talk.**
9. Holden S, **Paz JT** (2016) "The Role of the thalamus in focal traumatic brain injury". ARCS Symposium 2016, Menlo Park, CA, USA
10. Holden S, **Paz JT** (2016) "Thalamic excitability after traumatic cortical injury". GRC-Mechanisms of Epilepsy & Neuronal Synchronization conference, Girona, Spain
11. Cho F, Makinson S, Holden S, Tager D, **Paz JT** (2016) "Selective astrogliosis increases thalamic circuit excitability and oscillations" GRC-Mechanisms of Epilepsy & Neuronal Synchronization conference, Girona, Spain. **Abstract selected for a talk.**
12. Holden S, **Paz JT** (2016) "Thalamic function and traumatic cortical injury". Gordon research conference on Thalamus. Ventura, CA, USA.
13. Cho F, Makinson S, Holden S, **Paz JT** (2016) Assessing the effect of astrogliosis n thalamic circuit excitability". Gordon research conference on Thalamus. Ventura, CA, USA.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Nothing to Report because this is the final report.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

This proposal addressed significant gaps in the understanding of the post-traumatic neural plasticity, which are central to the ERP's vision and mission. Tangible intellectual gains from these studies include novel therapeutic targets for traumatic brain injury (TBI) outcomes including brain regions, circuits and cells that have been characterized in terms of their *in vitro* and *in vivo* physiological relevance. Our study identified potential brain regions to target in TBI therapeutics using neuroanatomical and high-throughput electrophysiological assays (*Aim 1*). We targeted these regions in a translational context relevant for preclinical studies with the drug ANX005, which blocks a specific inflammatory pathway by binding to the C1q molecule (*Aim 2*). We also targeted specific brain regions using human cell transplants (*Aim 3*) to reverse or prevent epilepsy after TBI, but found that this strategy fails preventing hyperexcitability and seizures, suggesting that it needs to be combined with targeted anti-inflammatory treatments.

Our work motivates a future body of research that will aim to determine the role of the C1q pathway in human patients with PTE.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Our study is of interest to multiple disciplines given that it has revealed the role of the C1q-mediated complement pathway in neural circuit plasticity and in modulating post-TBI cortical states. C1q has been implicated in several other diseases that have a high comorbidity with epilepsy, such as Alzheimer's disease and stroke, and could be a biomarker for chronic circuit abnormalities that lead to the development of epilepsy. Furthermore, our study will motivate to determine whether blocking the C1q pathway could also prevent chronic neurodegeneration in other neurological disorders that involve neurodegeneration.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

N/A

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

This study could lead to disease-modifying treatments for post-TBI outcomes such as post-traumatic epilepsy, which would enhance the quality of life and reduce the social and economic burden associated with deadly intractable seizures and associated cognitive deficits.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

N/A

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals

N/A

Significant changes in use of biohazards and/or select agents

N/A

PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state Nothing to report*

Nothing to report

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  We have a manuscript in revision, and are planning to submit it in the upcoming weeks. Given that the peer-review process and revisions take a significant amount of time due to COVID19 shutdown, we posted the preprint of several results resulting from this study on BioRxiv to share our findings with the public and the scientific community to accelerate research before our manuscript is published in a peer-reviewed journal.

  Stephanie S Holden, Oumaima Aboubakr, Bryan Higashikubo, Frances S Cho, Andrew H Chang, Allison Morningstar, Sethu Sankaranarayanan, Yaisa Andrews-Zwilling, Jasper Anink, Eleonora Aronica, Ted Yednock , and Jeanne T Paz. Complement factor C1q mediates chronic neuron loss and cortical rhythm changes post- brain injury. Preprint on BioRxiv.
  doi: https://doi.org/10.1101/2020.05.29.120220

  Acknowledgement of federal support: YES.

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Cho FS, Clemente A, Holden S, **Paz JT** (2017) Thalamic models of seizures in vitro. In *models of seizures and epilepsy* (Pitkänen A, Buckmaster P, Galanopoulou AS, Moshé SL, eds.) 2nd Ed., pp. 273–284, Academic Press.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

The results of the study funded by this DoD award were presented at multiple international, national and regional conferences:

Conference abstracts (peer-reviewed prior to acceptance):

1. Holden SS, Aboubakr O, Andrews-Zwilling Y, Sankaranarayanan S, Yednock T, **Paz JT** (2019) C1q mediates chronic secondary gliotic inflammation and neurodegeneration in a mouse model of post-traumatic epilepsy. American Epilepsy Society annual meeting, Baltimore, MD, USA. **Abstract selected for a Platform presentation.**
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13. Cho F, Makinson S, Holden S, **Paz JT** (2016) Assessing the effect of astrogliosis n thalamic circuit excitability". Gordon research conference on Thalamus. Ventura, CA, USA.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  N/A

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

  N/A

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  N/A. We may apply for a patent in the future.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

  N/A

6. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

1. Name: **Jeanne Paz**
Project Role: PI
Research identifier: ORCID ID: 0000-0001-6339-8130
Nearest person month worked: 8 months during reporting period
Contribution to Project: Provided technical and conceptual guidance on all aspects of the project: cortical lesions/optrode and EEG device design and surgical implants/data collection/data analysis/data interpretation/project management.
Funding Support: This DoD grant

2. Name: **Byan Higashikubo**
Project Role: Postdoctoral fellow
Nearest person month worked: 2 months during reporting period
Contribution to Project: Dr. Higashikubo was actively involved in purchasing and setting up the equipment for chronic EEG recordings from freely behaving mice, in surgical implants of chronic EEG devices in mice with TBI, in data acquisition, and in analysis. Bryan's programming skills from his PhD training at MIT are an important asset for managing and analyzing the large data sets that we obtain from chronic recordings.
Funding Support: This DoD grant

3. Name: **Andrew Chang**
Project Role: Postdoctoral fellow
Nearest person month worked: 5 months during reporting period
Contribution to Project: Mr. Chang performed experiments relevant to all aims including immunohistochemistry to determine the longitudinal effects of TBI on gliosis and neurons.
Funding Support: This DoD grant.

4. **Stefanie Ritter-Makinson**

Project Role: Postdoctoral fellow
Nearest person month worked: 2 months during reporting period
Contribution to Project: Mrs. Ritter-Makinson was involved in all the initial immunohistochemical, and slice/EEG experiments that aimed to determine the effect of the anti-C1q antibody on the inflammation.
Funding Support: This DoD grant.

5. Name: *Stephanie Holden*
Project Role: Graduate student
Nearest person month worked: 9.00 months during reporting period
Contribution to project: Ms. Holden performed all surgeries involving TBI induction, implants, EEG recordings and electrophysiology, as well as data analysis.
Funding Support: Mainly this DoD grant, the ARCS graduate fellowship, and the Ford graduate fellowship.

6. Name: **Scott Brovarney**
Project Role: Research Associate I
Nearest person month worked: 3.00 months during reporting period
Contribution to project: Mr. Brovarney took care of the mouse colonies, animal and lab safety protocols, prepared EEG implants, and helped with anti-C1q and vehicle treatments of mice (in Aim 2).
Funding Support: This DoD grant.

7. Name: **Juan Alcauter**
Project Role: Research Associate I
Nearest person month worked: 4.00 months during reporting period
Contribution to project: Mr. Alcauter took over Mr. Brovarney's job when Mr. Brovarney was promoted to a different position at UCSF. Additional job responsibilities of Mr. Alcauter included preparation of EEG electrodes, anti-C1q and vehicle treatments of mice (in Aim 2), and cell transplant surgeries and EEG recordings (Aim 3).
Funding Support: This DoD grant.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report.

7. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

N/A

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

N/A

8. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

We attached the preprint of our manuscript in revision.