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TITLE: Deconstruction and Control of Neural Circuits in Posttraumatic Epilepsy

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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:

This proposal addresses significant gaps in the understanding of the pathophysiology of post-traumatic epilepsy. The **vision** of this study is to determine the causative links between TBI and epilepsy and prevent post-traumatic epilepsy. The **scope** of this study is to understand the mechanisms underlying the 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 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 the injury and in the part of the brain that senses touch and pressure called the "somatosensory thalamus". Our team has discovered that those hot spots form before the onset of PTE, and persist during PTE. 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 after TBI. We are now testing whether blocking the C1q pathway after TBI can prevent PTE. If successful, new interventional strategies could be designed that harmonize immune-neuronal interactions after TBI to prevent PTE.

## 2. KEYWORDS:

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 Officer 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.*

Below we report the percentage of completion for the period relevant for this yearly report.

| **Major Task 1: Determine the role of corticothalamocortical hyperexcitability in seizures after TBI.** | Months | Percentage of completion |
|---|---|---|
| Aim 1a: Determine if TBI results in hyperexcitability in CTC circuits | 1-7 | 100% |
| Aim 1b: Determine if the thalamus can bi-directionally control PTE seizures in freely behaving animals. | 1-7 | 98% |
| 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 | 100% |
| Aim 2b: Determine if blocking C1q action prevents PTE | 1-12 | 95% |
| Aim 2c: Determine if blocking C1q cures PTE | 8-17 | 30% |
| 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 | 70% |
| Aim 3b: Determine if transplanted cells prevent PTE in behaving animals. | 13-24 | _20% |
| 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.*

**1) Major activities:**

-During the second finding year, we made a significant progress by performing electrophysiological recordings in slices and *in vivo*, chronic EEG recordings with the DSI system in mice treated with anti-C1q drug, optogenetic manipulation of hot spots, and immunohistochemistry.

-We completed immunohistological analysis which allowed us to determine a sequence of changes in the cortico-thalamic circuits. We showed the following sequence of events:
a) Within hours post-TBI: GFAP, C1q, Iba1 are upregulated in the peri-TBI cortex.
b) Within 1 week post-TBI: GFAP, C1q, Iba1 are upregulated and NeuN is downregulated in the thalamus which is functionally connected to the injured cortex.
c) GFAP, C1q and Iba1 upregulation is persistent in both peri-TBI cortex and thalamus and can be observed up to 4 months post-TBI which is the latest time point we studied.
d) Within 3 weeks post-TBI: the neurons in the reticular thalamus degenerate resulting in a significant reduction in GABAergic inhibition in the thalamus.
e) The intrinsic and synaptic membrane properties in cortical and thalamic neurons are similar to shams.

The regions of interest analyzed in all mice were peri-TBI somatosensory cortex, ventrobasal somatosensory thalamus (containing excitatory glutamatergic thalamocortical cells) and the reticular thalamic nucleus (RT) containing GABAergic neurons. The three regions of interest showed persistent gliotic inflammation marked by GFAP, Iba1 and C1q upregulation.

-We identified the hyperexcitable hot spots and brain targets for preventing maladaptive plasticity after TBI, and preventing PTE.

-We identified the acute and chronic roles of C1q complement pathway after TBI.

-We performed anti-C1q treatments after TBI to assess effects on epileptic activities that develop within weeks after TBI.

-We purchased a software “Neuroscore” to analyze the chronic EEG data to determine the timeline of PTE development after TBI in various treatment groups.

-We hired a new postdoctoral fellow who has a major expertise in stem cell transplants in rodents, inflammation, neurodegeneration and in longitudinal studies. These skills will be a key asset for a successful completion of Aim 3 during the next quarterly period.

-We disseminated our findings at multiple conferences, meetings and departmental, regional, national and international formal seminar series.

-We showed that optogenetic modulation of reticular thalamic neurons modulates epileptic activties in real-time, thus pinpointing the importance of this thalamic nucleus in post-traumatic epileptic events.

-We validated that the human cell transplants survive in the inflamed peri-injured cortex and planned the transplants for determining whether the human cells can prevent the development of epilepsy after TBI.

The detailed significant results are summarized below.

**2) Specific objectives:** The objectives of the experiments performed during this funding period are listed below:
1. Determine if blocking C1q action prevents "hot spots" identified in the previous aims (i.e. prevents neurodegeneration, chronic inflammation, and loss of inhibitory post-synaptic currents in the hot spots).
2. Determine the optimal timing of anti-C1q treatment.
3. Determine if blocking C1q action after TBI prevents the development of epileptic seizures after TBI.
4. Determine if optogenetic manipulation of thalamic neurons in "hot spots" can bi-directionally modulate epileptic events.

**3) Significant results:**

1. We were able to locate the circuit "hot spots" in the cortex and thalamus in TBI mice during the latent period (i.e. prior to the development of post-traumatic epilepsy). Surprisingly, we found that during the latent epileptogenic period (1 month post-TBI) the reticular thalamic GABAergic neurons are the most affected by TBI, although these are not directly connected to the injured cortex. We found that TBI induces neuronal death in the reticular thalamus and a long-term reduction or loss in $GABA_A$-receptor mediated inhibition within the reticular thalamus which could be a key mechanism in epileptogenesis because loss of intra-RT inhibition has been implicated in thalamic hypersynchronicity and seizures in previous studies (Huntsman et al, Science 1999 PMID:9915702; Sohal and Huguenard J Nsci 2001 PMID:14523100; Sohal et al 2000 J Neurophysiol PMID:10684875).

2. We found that mice lacking C1q (C1q knock-out mice) exhibited a dramatic increase in the cortical lesion size marked by significant loss of the cortical tissue although the TBI induction protocol was the same in in sham mice. These results suggest that C1q upregulation **during** TBI is necessary for containing the TBI lesion size. Therefore, the C1q blockade during TBI is not beneficial as it worsens the TBI outcomes (increased neurodegeneration and inflammation).
Interestingly, we found that C1q upregulation >24 hours after TBI is maladaptive, and that its blockade >24h after TBI prevents chronic gliotic inflammation in all regions of interest, prevents neurodegeneration and the loss of inhibition in the reticular thalamus and reduces epileptic events.

This result is groundbreaking as it implicates that C1q is required for maladaptive neurodegeneration and chronic inflammation after TBI. These results suggest that blocking the C1q upregulation >24 hours **after** TBI could be anti-epileptogenic.

**4) Other achievements**
-This study resulted in multiple awards to the students who work on this project in my lab. Two graduate students in my 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 for two years in a row (2016 and 2017), the Doctoral Career development Award from the *Society for Neuroscienc*e as well as travel awards from the UCSF graduate

division and the UCSF Discovery fellowship to attend conferences and present the work on the mechanisms underlying post-traumatic epileptogenesis.

-The results of the study funded by this award were presented this work at multiple conferences:

Conference abstracts (peer-reviewed prior to acceptance):

· F.S. Cho, I.L. Vainchtein, J.A. Alcauter, A.R. Morningstar, A.V. Molofsky, J.T. Paz (2018) Transcriptomic analysis of reactive astrocytes and microglia in the thalamus reveals functional deficits linked to circuit excitability. Passwell Symposium, Israel.

· S. Holden, A. Morningstar, J. Paz (2018) Thalamocortical function after traumatic cortical injury. Passwell Symposium, Israel.

· Stephanie S. Holden, Allison R. Morningstar, Bryan Higashikubo, Jeanne T. Paz. (2018) Thalamocortical function after traumatic cortical injury. Gordon Research Conference, Tuscany, Italy.

· Holden S, Paz JT (2017) Deconstruction of thalamic circuits in a mouse model of post- traumatic epilepsy. Society for Neuroscience, Washington DC, USA

· 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

· Holden S, Paz JT (2016) "The Role of the thalamus in focal traumatic brain injury". ARCS Symposium 2016, Menlo Park, CA, USA

· Holden S, Paz JT (2016) "Thalamic excitability after traumatic cortical injury".GRC-Mechanisms of Epilepsy & Neuronal Synchronization conference, Girona, Spain

· 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

· Holden S, Paz JT (2016) "Thalamic function and traumatic cortical injury". Gordon research conference on Thalamus. Ventura, CA, USA.

· 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.

**Awards and Honors for the relevant funding period:**

- Vilcek Prize 2019 for Creative Biomedical Research, awarded to Jeanne Paz
- CURE Selected Speaker 2018, Citizens United for Research in Epilepsy
- *Top reviewer*, Nature Publishing Group for 2 years in row, awarded to Jeanne Paz
- Achievement Awards for College Scientists (ARCS) Graduate Scholarship for two years in a row in 2016 and 2017, awarded to Stephanie Holden
- GradSlam 2017 finalist, the University of California San Francisco. Video: "The Epileptic Brain: Rewiring After Injury" ; Stephanie Holden
- Trainee Professional Development Award 2017, Society for Neuroscience (SfN), awarded to Stephanie Holden
- Excellence award at Gladstone Institute of Neurological Disease, awarded to Stephanie Holden (only 1-2 PhD students per institute receive this award per year)
- Moritz-Heyman Discovery Fellowship 2017, awarded to Frances Cho for her leadership potential, excellence in research, community- mindedness, and communications skills
- McNair Scholarship 2017, awarded to Trinidad Arceo
- Scholarship to present findings at the SACNAS conference 2017, awarded to Allison Morningstar
- Scholarship to present findings at the SACNAS conference 2018, awarded to Trinidad Arceo

- GradSlam 2018 finalist, University of California San Francisco, Frances Cho
- Young Investigator award 2018, American Epilepsy Society, awarded to Frances Cho (only 20 awards out of 1300 applications)
- Early Career Fellowship award 2018, American Epilepsy Society, awarded to Bryan Higashikubo

**Future outcomes expected from this work:**
Patents (related to anti-C1q drug for treating epilepsy)
Publications in peer-reviewed journals.

**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.*

I require each of the trainees in my lab to complete a written Individual Development Plan (IDP) each year, and we meet formally at the end of the year to discuss their overall development. In addition, I meet with each trainee weekly to discuss progress during the week. For the first 2 years of a graduate student's tenure in my lab, I advise them to use "My Annual Plan" (MAP), which was created by UCSF's Graduate Division and focuses the student's efforts on broad training goals and accomplishments. Postdoctoral fellows participate in Gladstone's annual performance goal setting and appraisal program, which includes a thorough discussion of training milestones and advancement. Both graduate students and postdoctoral fellows are encouraged to use Science Careers' *myIDP* which provides more structure for considering a broad array of anticipated career outcomes and encourages productive goal setting toward the pursuit of a chosen career. Postdocs are also advised to use *myIDP* throughout their tenure at Gladstone.

I mentored three minority trainees in my laboratory who were involved in post-injury epilepsy studies: a graduate student (Alexandra Clemente-Perez), a research associate (Juan Alcauter), and a summer interns –Trinidad Arceo – from the *Promoting Underrepresented Minorities Advancing in the Sciences (PUMAS)* internship program. Trinidad's abstract was accepted at the SACNAS conference in 2018.

This award also provided a training opportunity for an undergraduate student from the University of Rochester (Allison Morningstar) whose abstract was accepted at a SACNAS conference in 2017.

Two graduate students in my laboratory - Stephanie Holden and Frances Cho – whose work focuses on this DoD-funded project, received multiple awards that allowed them to present their work at multiple national and international conferences that gather the experts in the field which created important training and networking opportunities for the students. These awards include fellowships, scholarhsips, career development awards, excellence awards, and travel awards to attend conferences and present our work on the mechanisms underlying post-traumatic epileptogenesis. Stephanie and Frances presented their work at multiple conferences:

1. Frances S. Cho, Allison R. Morningstar, Juan Alcauter, Jeanne T. Paz (2018) Selective astrogliosis enhances thalamic circuit excitability by altering GABA(A)R-mediated tonic inhibition. Gordon Research Conference, Tuscany, Italy.
2. F.S. Cho, I.L. Vainchtein, J.A. Alcauter, A.R. Morningstar, A.V. Molofsky, J.T. Paz (2018) Transcriptomic analysis of reactive astrocytes and microglia in the thalamus reveals functional deficits linked to circuit excitability. Passwell Symposium, Israel.
3. S. Holden, A. Morningstar, J. Paz (2018) Thalamocortical function after traumatic cortical injury. Passwell Symposium, Israel.
4. Stephanie S. Holden, Allison R. Morningstar, Bryan Higashikubo, Jeanne T. Paz. (2018) Thalamocortical function after traumatic cortical injury. Gordon Research Conference, Tuscany, Italy.
5. Holden S, Paz JT (2017) Deconstruction of thalamic circuits in a mouse model of post- traumatic epilepsy. Society for Neuroscience, Washington DC, USA
6. Clemente A, Makinson SL, Higashikubo B, Cho F, Urry A, Holden S, Wimer M, Fenno L, David C, Acsady L, Deisseroth K, Paz JT (2017) Distinct thalamic reticular cell types differentially modulate normal and pathological cortical rhythms. Society for Neuroscience, Washington DC, USA
7. 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
8. Holden S, Paz JT (2016) "The Role of the thalamus in focal traumatic brain injury". ARCS Symposium 2016, Menlo Park, CA, USA
9. Holden S, Paz JT (2016) "Thalamic excitability after traumatic cortical injury".GRC-Mechanisms of Epilepsy & Neuronal Synchronization conference, Girona, Spain

- **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 obtained during the relevant funding period were disseminated to communities of interest through presentations at conferences:

1. Holden S, Paz JT (2017) Deconstruction of thalamic circuits in a mouse model of post- traumatic epilepsy. Society for Neuroscience, Washington DC, USA.
2. 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 (selected for a talk).
3. Cho FS, Allison R. Morningstar, Juan Alcauter, Jeanne T. Paz (2018) Selective astrogliosis enhances thalamic circuit excitability by altering GABA(A)R-mediated tonic inhibition. Gordon Research Conference, Tuscany, Italy.
4. Cho FS, Vainchtein IL, Alcauter J, 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.

5. S. Holden, A. Morningstar, J. Paz (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. Clemente A, Makinson SL, Higashikubo B, Cho F, Urry A, Holden S, Wimer M, Fenno L, David C, Acsady L, Deisseroth K, Paz JT (2017) Distinct thalamic reticular cell types differentially modulate normal and pathological cortical rhythms. Society for Neuroscience, Washington DC, USA
9. 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

**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.*

We plan 1) to complete the work proposed in Aim 1 and finish the analysis of the results, 2) to continue the work proposed in Aim 2, and 3) to start working on Aim 3. Briefly, this year our work pinpointed the "hot spots" of neural dysfunction after TBI. These are located in the peri-injured cortex and the functionally connected somatosensory thalamus. We used histological and electrophysiological approaches in brain slices and in vivo to locate the "hot spots" of circuit dysfunction and characterize their evolution in time and space. Our results suggest that the chronic upregulation in C1q and the reduced of synaptic inhibition are the biomarkers of the "hot spots". We also purchased and set up a chronic EcoG recording setup which will allow us to monitor the ECoG for multiple mopnths after TBI. Furthermore, we validated the penetration of the ANX005 anti-C1q drug in the brain regions of interest, and we found the optimal conditions for the treatment (dose and frequency of administration). Thanks to the progress we made during year 1, we are starting the experiments that will allow us to assess the efficacy of two novel treatments for the post-traumatic epileptogenesis: 1) anti-C1q drug (ANX005) treatment (Aim 2), and 2) stem cell treatment (Aim 3). We hypothesize that these two treatments will prevent post-traumatic epileptogenesis by reducing inflammation and restoring the inhibition/excitation balance.

*4.* **IMPACT:**

- **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 addresses significant gaps in the understanding of the pathophysiology of post-traumatic epilepsy, which are central to the ERP's vision and mission.

**Short-Term Impact:** Tangible intellectual gains from these studies will include novel therapeutic targets for post-traumatic epileptogenesis (PTE) and effective PTE seizure control (regions, cells, and circuits) that have been thoroughly characterized in terms of their *in vitro* and *in vivo* physiological relevance. Our study identified the hyperexcitable "hot spots" using neuroanatomical and high-throughput electrophysiological assays (*Aim 1*). We start to target these regions in a

translational context relevant for preclinical studies with the drug ANX005 *(Aim 2*), and human interneuron cell transplants (*Aim 3*) to reverse epilepsy after TBI or prevent PTE from developing after injury.

**Long-Term Impact: Our ongoing study aims to develop novel treatments to prevent, control, and cure PTE with no side effects, in contrast with the systemic treatment currently provided by anticonvulsants.** We expect that a better understanding of the underlying pathophysiological mechanisms of PTE will lead to development of novel treatments that target relevant brain circuits identified in our anatomical screen. Thus, this proposal will provide insight into long-term strategies that might represent a more focal therapeutic intervention for people suffering from epilepsy. The current gold standard for epilepsy treatment, regardless of its origin, is anticonvulsant drugs. These drugs are ineffective in 30% of patients with epilepsy and cause a variety of negative side effects. Short-term side effects include tremors, sedation, and loss of coordination. Long-term effects include a higher incidence of birth defects, the generation of liver and heart disease, and additional seizures and other neuropsychiatric symptoms, such as psychosis (Center for Epilepsy and Seizure Education). The novel preventive (ANX005 drug in ***Aim 2***) or focal treatments (human cell transplants in ***Aim 3***) would diminish the activation of off-target tissues, possibly reducing negative side effects.

- **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 has a fundamental interest given that it will reveal the role of C1q complement pathway in neural circuit plasticity. We find a pathological upregulation of C1q in the thalamus of a rat model of cortical thrombotic stroke, suggesting that C1q upregulation could represent a biomarker for chronic circuit abnormalities that lead to the development of epilepsy.

- **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-traumatic epilepsy that will enhance quality of life and reduce the social and economic burden associated with deadly intractable seizures and associated cognitive deficits.

*5.* **CHANGES/PROBLEMS:** *The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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:*
Nothing to report

**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.*

An error in the IACUC office in February 2015 gave a three-year expiration on a protocol that was approved months earlier with a June 2017, 3-year expiration. That has now been resolved and IACUC has approved the February 2015 review as a de novo protocol that re-started the 3- year approval cycle. Once the error was discovered we stopped all animal work until it was resolved. This delayed progress on aims 1b, 2b and 3a. This delay was minor and will not affect the progress of the other aims. We are planning to complete all the Aims during the upcoming year.

- **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**

**6. PRODUCTS:** ***List any products resulting from the project during the reporting period. If there is*** **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).*
- N/A

- **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 F, Clemente A, Holden S, Paz JT (2017) Thalamic Models of Seizures In Vitro. In: Models of Seizures and Epilepsy, 2nd edition. Eds. Pitkänen A, Buckmaster P, Galanopoulou AS, Moshe SM. Chapter 19.

- **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 obtained during the first year of the award were presented at multiple national and international conferences:

1. Holden S, Paz JT (2017) Deconstruction of thalamic circuits in a mouse model of post- traumatic epilepsy. Society for Neuroscience, Washington DC, USA.
2. 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 (selected for a talk).

3. Cho FS, Allison R. Morningstar, Juan Alcauter, Jeanne T. Paz (2018) Selective astrogliosis enhances thalamic circuit excitability by altering GABA(A)R-mediated tonic inhibition. Gordon Research Conference, Tuscany, Italy.
4. Cho FS, Vainchtein IL, Alcauter J, 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.
5. S. Holden, A. Morningstar, J. Paz (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. Clemente A, Makinson SL, Higashikubo B, Cho F, Urry A, Holden S, Wimer M, Fenno L, David C, Acsady L, Deisseroth K, Paz JT (2017) Distinct thalamic reticular cell types differentially modulate normal and pathological cortical rhythms. Society for Neuroscience, Washington DC, USA
9. 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

**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

**a. Technologies or techniques**

*Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.*

- N/A

**b. Inventions, patent applications, and/or licenses**

*Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. 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

**c. 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.*

- N/A

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

### d. What individuals have worked on the project?

*i. 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: | *Dr. Jeanne Paz* |
| Project Role: | *Principle Investigator* |
| 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
Nearest person month worked: 4
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.

2. Juan Alcauter
Project Role: Graduate Student
Nearest person month worked: 2
Contribution to Project: Mr. Alcauter has carried out the local field potential recordings in acute neocortical brain slices from mice with TBI and the littermate controls, and was involved in analyzing the EEG from chronically implanted TBI and sham mice.

3. Bryan Higashikubo
Project Role: Postdoctoral fellow
Nearest person month worked: 2

Contribution to Project: Bryan 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 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.

4. Andrew Chang
Project Role: Postdoctoral fellow
Nearest person month worked: 3
Contribution to Project: Mr. Chang performed experiments relevant to all aims: immunohistochemistry to determine the longitudinal effects of TBI on gliosis and neurons.

5. Stephanie Holden
Project Role: Graduate Student
Nearest person month worked: 6
Contribution to Project: Ms. Holden Holden performed all surgeries involving TBI induction, implants, EEG recordings and electrophysiology, as well as data analysis.

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

*i. If there is nothing significant to report during this reporting period, state "Nothing to Report."*
*ii. 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.*

N/A

**f. What other organizations were involved as partners?**

*i. If there is nothing significant to report during this reporting period, state "Nothing to Report."*

Nothing to Report.

*ii. 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.*

Nothing to Report.

1. **Organization Name: N/A**
2. **Location of Organization:** *N/A*
3. **Partner's contribution to the projec**t *N/A*
   a. **Financial support;**
   b. **In-kind support** N/A
   c. **Facilities** N/A
   d. **Collaboration** N/A
   e. **Personnel exchanges** *N/A*

**8. SPECIAL REPORTING REQUIREMENTS**
**COLLABORATIVE AWARDS:** *N/A*

**QUAD CHARTS:** *N/A*

**9. APPENDICES*: N/A***

# Deconstruction and Control of Neural Circuits in Epilepsy

Log #EP150038
W81XWH-16-1-0576

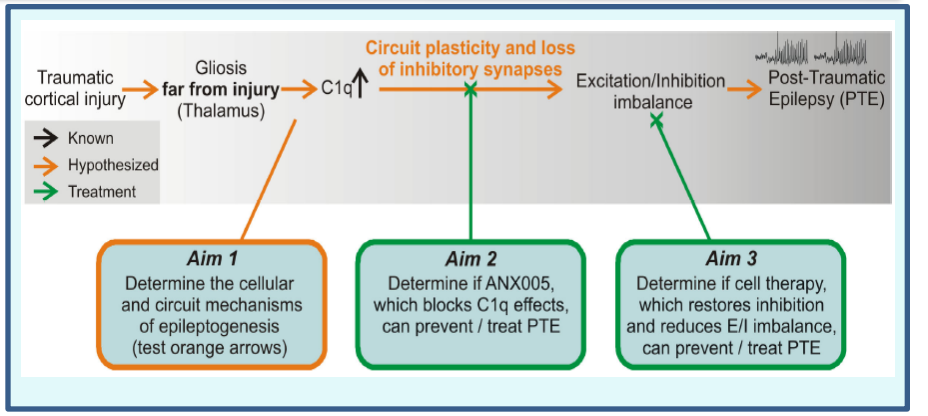
**PI:** Dr. Jeanne Paz **Org:** The J. David Gladstone Institutes **Award Amount:** $875,289

## Study/Product Aim(s)

- Aim 1: Determine the role of corticothalamocortical hyperexcitability in seizures after TBI.
- Aim2: Determine the role of C1q in seizures after TBI.
- Aim 3: Determine if transplanting inhibitory neurons into CTC "hot spots" of hyperexcitability prevents and cures PTE.

## Approach

To achieve these goals we use a mouse model in which post-traumatic epilepsy develops after a traumatic cortical injury induced by a controlled cortical impact method. To understand how epilepsy develops after TBI and to test two new disease-modifying treatments, we combine cellular and circuit electrophysiology *in vitro* and in freely behaving mice, optogenetic approaches, pharmacology, and chronic electrocorticogram (EcoG) recordings.




Accomplishment: We located the neural "hot spots" responsible for the generation of epileptic activities after TBI. We characterized the hot spots and found their "biomarkers": local gliotic inflammation, upregulation of the C1q molecule and a reduced synaptic inhibition. Using chronic EcoG recordings, we found biomarkers of post-traumatic epileptogenesis. Notably, we were able to prevent or reduce the hot spots by blocking the chronic upregulation of the C1q complement pathway.

## Timeline and Cost

| Activities | CY16 | CY17 | CY18 | CY19 |
|---|---|---|---|---|
| Aim 1: Determine the role of corticothalamocortical hyperexcitability in seizures after TBI. *Milestone(s): unveil the epileptic circuit hot spots and specific cells that are causally involved in PTE.* | | | | |
| Aim 2: Determine the role of C1q in seizures after TBI. *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 3: Determine if transplanting inhibitory neurons into CTC "hot spots" of hyperexcitability prevents and cures PTE. *Milestone(s): Test the efficacy of a novel therapeutic approach involving mouse and human cell transplants for preventing PTE and cure PTE.* | | | | |
| **Estimated Budget ($K)** | **$97** | **$355** | **$252** | **$171** |

## Goals/Milestones (Example)

CY16 Goal
☑ Acquire new equipment for inducing the controlled cortical impact and optimize the cortical lesions to obtain lesions (reproducibility and size).
☑ Acquire new TDT equipment for assessing circuit excitability in slices and in vivo
CY17 Goal
☑ Determine the role of corticothalamocortical hyperexcitability in seizures after TBI. Locate the hot spots of hyperexcitability after TBI.
☑ Determine if C1q is upregulated in CTC "hot spots" after TBI
☐ Determine if blocking C1q action prevents PTE
☐ Determine if inhibitory transplants in cortex and thalamus prevent circuit hyperexcitability in cortical and thalamic slices, respectively
CY18-19
☐ Determine if transplanted cells prevent PTE in behaving animals.
☐ Determine if transplanted cells cure PTE in behaving animals
Budget Expenditure to Date
Projected Expenditure: $646,915
Actual Expenditure: $651,443

**Updated:** Oct 25th 2018