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TITLE: Reduced GABAergic Tonic Inhibition as a Shared Mechanism of Post-Traumatic Sleep Disorders and Epilepsy

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Abstract: TBI is the signature injury in Veterans from recent wars and over 1.5 million Americans sustain TBI each year. Whether from a mild, moderate, severe, or repetitive insult, TBI may lead to a series of complications including PTE, SWD, and MD such as post-traumatic stress disorder (PTSD) and depression. Across the spectrum of TBI severity, PTE occur in up to 40%, SWD 30-70% and MD in 35-40% of patients. In many patients, PTE and SWD occur simultaneously and chronically, further contributing to mood and cognitive disorders, and a negative impact on quality of life. *From a clinician's perspective, there is a large literature gap in understanding the time-course for development of and the pathogenic mechanisms of TBI sequelae*. The hypothesis of the proposal was that *persistently reduced GABAergic tonic inhibition (GTI) in the thalamus and hippocampus contributes to sleep disruptions and network hyper excitability and pathogenesis of PTE respectively*. While still being analyzed, our results show that: a) ~21% (9 out of 41) of the animals exhibited seizures that started anywhere from week 1 to 6 months following a controlled cortical impact (CCI) whereas 80% had epileptiform activity in the form of interictal spikes or spike trains; b) sleep-wake disturbances (SWD) consisted of "hypersomnia" in acute recordings and "insomnia" in chronic recording after CCI; c) animals treated with DORA-22, but not THIP, had markedly less seizures as well as other epileptiform activity compared to untreated groups; d) preliminary analysis of patch clamp electrophysiology showed that after TBI, there was a reduction in mIPSC amplitude and frequency suggesting altered GABAergic signaling, whereas DORA-22 rescued the amplitude and frequency of mIPSCs in CCI groups; and e) behavioral studies demonstrated a depression phenotype post-TBI (with no sex differences) and PTSD phenotype seen more in males compared to females post TBI.

Introduction:

Traumatic brain injury (TBI) is a common problem in civilians and military personnel alike. According to CDC, there are over 5 million survivors with TBI related disability in the United States. It is also the signature injury in Veterans from OEF/OIF/OND. TBI can lead to several sequelae that include sleep disorders, post-traumatic epilepsy (PTE), cognitive and motor deficits and even post-traumatic stress disorder[1]. Of these sequelae, epidemiological data shows that PTE can develop immediately or years after the injury, in up to 20% of patients and may be even higher in military injuries. Many of these patients remain intractable to conventional anti-epileptic drugs as well. Similar to PTE, post-traumatic sleep disruptions can be a chronic sequela of TBI and persist long after sustaining the injury in humans and animal models. In humans 20-50% of patients report post-traumatic sleep disruptions that range from insomnia to hypersomnia.

Gaps in TBI research: While TBI and its sequelae remain a huge problem, currently there is no way to predict who gets these complications, why they get it and how to prevent them. Studies that translate from the bench to the bedside or the clinic are lacking. If sequelae of TBI have a common or shared mechanism, perhaps similar treatments may work for both.

Focus of our research: We proposed to focus on GABAA receptor mediated functions which are known to be affected in the lesion core, thalamus, amygdala and hippocampus following TBI. Reductions in GABAergic tonic inhibition (GTI) and changes in GABA receptor subunits that favor phasic inhibition had been shown in models of TBI. Moreover, reduced GTI is known to occur in hippocampus in models of epilepsy and reduced GTI, especially in thalamus had been shown to be associated with sleep disruptions. Taken together, we hypothesized that *persistently reduced GTI in the hippocampus and thalamus contributes to persistent sleep disruptions, network hyper excitability and pathogenesis of PTE*

Experimental Aims:

Aim 1: To measure sleep disruptions and seizures followed by electrophysiological measures of GTI, at 1 week, 1 month and 3 months following a temporal-parietal CCI or sham control.

Aim 2: To measure if a) GTI can be rescued; b) sleep disruptions and network excitability restored and c) seizures prevented, with a selective agonist of δ subunit-containing GABAA receptors gaboxadol OR a dual orexin antagonist.

Study Endpoints:

1. Following CCI, compared to sham injury, what percentage of animals develop seizures (PTE) and sleep disturbances? Do we see one or both and in what percentage of animals?

2. How are electrophysiological measures such as GTI, mIPSCs and EPSCs altered in hippocampus and thalamus after TBI and what is the association or correlation of these changes to PTE or post-traumatic sleep disruptions (PSD)

3. Do drugs that alter GTI (Gaboxadol) or drugs that are Orexin antagonists (eg: almorexant) restore PSD; restore electrophysiological changes and prevent development of PTE?

The Unique nature of our hypothesis:

In other words, does “normalizing sleep and its homeostasis” following TBI prevent development of PTE??? If our hypothesis is proven to be true, administration of one the above or related drugs may potentially prevent some of the sequelae of TBI.

Key words: TBI; Post-traumatic epilepsy; sleep disturbances, tonic inhibition

SOW:

ACUC and ACURO approvals	**June to September 2017**	
Hiring of Post Doc (Paulo Rodrigues, PhD) and training set up	**Sept to Nov 2017**	
Obtaining equipment for CCI and training/trouble shooting	**Oct to Dec 2017**	
Research Aim: Specific Aim 1: Sleep, EEG recording and slice electrophysiology following TBI and no drugs	**Dates**	**Number of animals**

Animal recording count

	Week 1	Month1	Month 2	Month 3	Month 4	Month 6
TBI	15	6	20	25	6	23
Sham injury	10	6	-	-		
TBI- Dora	12	9	5	5	No recordings	
TBI-Vehicle	11	9	5	5		
TBI-THIP	12	11	8	8		
TBI-Vehicle	14	9	7	5		

Electrophysiology:

TBI with no drug: We attempted recording from 8 animals and we were able to patch about 10 cells. Experiments consisted of tonic current recordings from about 8 cells and input output curves from about 10 cells.

Sham with no drug: We attempted recording from 8 animals and we were able to patch about 10 cells. Experiments consisted of tonic current recordings from about 8 cells and input output curves from about 10 cells.

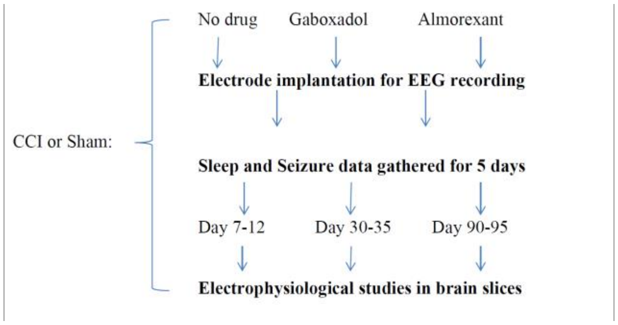
TBI with DORA: We attempted recording from 3-4 animals and we were able to patch about 6-7 cells.

TBI with THIP: We attempted recording from 3-4 animals and we were able to patch about 6-7 cells.

Behavioral studies: Behavioral studies including tail suspension (test of depression) and contextual and cued fear conditioning (test for PTSD) were performed about 3 months in animals (of both sexes) following CCI or sham injury.

Accomplishments:

Experimental Design Schematic:



1. ACUC and ACURO approvals in September 2017.
2. Modification of protocol and switching from Sprague-Dawley rats to CD-1 mice done in Sept 2017.
3. Obtained CCI machine for performing TBI, recruitment of a Post-Doc.
4. Experiments began around Nov, 2017. First 2 months was a learning phase.
5. First 6 months- was recording and troubleshooting EEG recordings in TBI and Sham injury animals.
6. Number of animals recorded in each group thus far:

   a. No Drug TBI: 6 cohorts of ~4-8 animals each. Recordings were done at week 1(Day 0-7after TBI); Month 1 (days 30-35 after TBI); Month 2 (days 60-63 after TBI); Month 3 (day 90-95 after TBI) and month 6 (day 180-185 after TBI). We have Week 1 recordings for all cohorts; Month 1, month 2 and month 3 recordings are available for 4 cohorts and month 6 recordings are available for 2 cohorts of animals. Total number of animals recorded is 41.
   b. No Drug Sham injury: 3 cohorts of ~8 each. Recordings completed at week1 (day 0-7 after sham injury); Month 2 (day 60-65 after sham injury) and month 3 (day 90-95 after sham injury)
   c. Drug 1 (DORA) or vehicle after TBI: Animals were recorded in 4 cohorts, with a total of 12 animals that received TBI+ Vehicle; 11 TBI+DORA 22; 10 for Sham+ Vehicle and 10 for Sham + DORA 22. Recordings completed at week 1(day 0-7 after TBI); month 1 (Day 30-35 after

TBI); month 2 (days 60-65) and at month 3 (days 90-95). Total number of animals recorded was 43.

d. Drug 2 (THIP) or vehicle after Sham injury: Animals were recorded in 4 cohorts with a total of 14 each for TBI+THIP and TBI+Vehicle; 14 for Sham + THIP and 12 for Sham injury + Vehicle. Recordings were completed at week 1 (day 0-7 after sham injury); month 1 (day 30-35 after sham injury); month 2 and month 3 (days 60-65 and 90-95 respectively). Total number of animals recorded was 54.

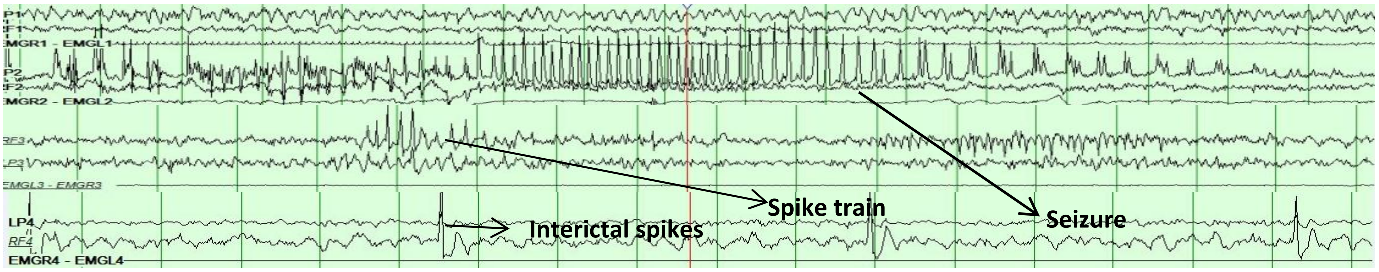


Figure 1. EEG recording from 4 CD-1 mice after CCI with right frontal (RP), left parietal electrodes (LP) and EMG electrodes is shown. Top Panel shows example of a seizure in animal 2 where buildup of rhythmic spike wave discharges were seen which on video were Racine scale 2-3; Middle Panel shows example of spike trains or clusters that last from 1-3 sec without any clear behavioral change; Bottom Panel shows example of interictal spikes. Preliminary analysis showed that about 80% (42 out of 55) of the animals had epileptiform activity in the form of interictal spikes and spike trains or clusters (Figure 1 middle and bottom). 16 out of the 55 TBI mice had electro-clinical seizures, consisting of behavioral arrest, often with automatisms of the head and neck.

In all, between different treatment groups a total of 138 mice were recorded. It should be noted that not all animals have recordings at month 1, month 2, or month 6 in the above groups as some were sacrificed in between for electrophysiological studies.

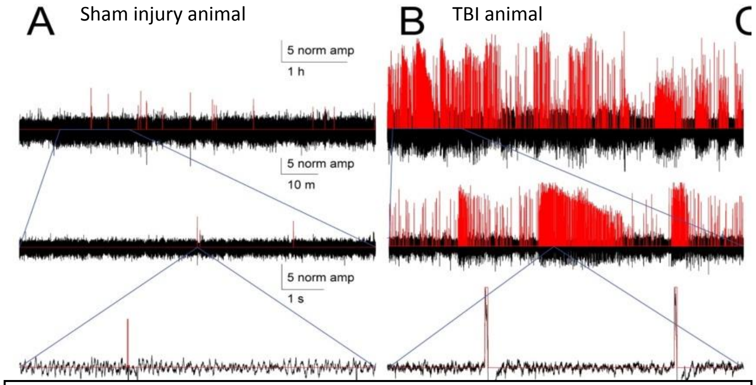


Figure 2: Examples of interictal spikes in sham and TBI groups

Results to date: <u>1. Seizures or Ictal events</u>: 9 out of 41 animals in the TBI+ no treatment group had seizures. A total of 25 seizures were recorded in the 9 animals. Among DORA (or vehicle) group 5 out of 23 animals had seizures with all seizures recorded in week 1 only. Among the THIP (or vehicle) treated group, 6 out of 26 animals had seizures but seizures occurred anywhere between week 1 to month 3. None of the sham injury animals had seizures. On video recording, seizures were of Racine class II-III where mice were observed to have behavioral arrest, often with automatisms of the head and neck. 50% of the seizures progressed to a Racine class V with generalized tonic-clonic activity. The electrographic characteristics of the seizures demonstrate a progression from a focal/lateralized electrographic seizure that is initially seen over the frontal cortex ipsilateral to the site of injury, and later are seen bilaterally (Figure 1).

<u>2. Interictal phenomena</u>: Several different types of interictal phenomena were observed including: isolated spikes; spike clusters and brief rhythmic ictal discharges. These were seen in about 70% of

the TBI animals and though some spike-like discharges were also see to a lesser extent in the sham injury group (Figure 1).

<u>3. Interictal Spike Scoring</u>: As animals have a large number of inerictal spikes, we are in the process of utilizing automated scoring methods for interictal spikes and spike clusters. In the attached figure 3, we show interictal spikes like events which are of high amplitude, occurring in a TBI vs sham injury animal. We have not completed this analysis, but the scripts were developed to eventually determine: a) what percentage of TBI animals have these epileptiform abnormalities? b) what is the time course of development of these epileptiform abnormalities ? c) what is the effect of drugs that are Orexin antagonists or positive allosteric modulators of GABAergic tonic inhibition on frequency of the interictal events or ictal events?

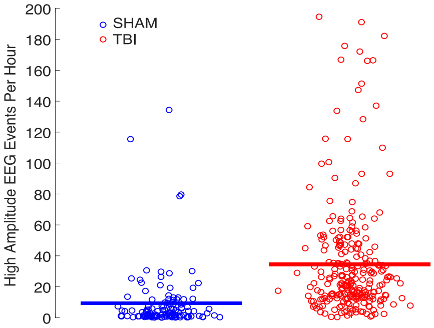


Figure 3: High amplitude events (identified using the method as described by our group[15] from the EEG of CD-1 mice with either sham injury or CCI TBI.

<u>4. Sleep disturbances following TBI</u>: Along with seizures we recorded sleep-wake patters in TBI and sham injury animals. Following TBI or sham injury with no drugs, we have recorded sleep-wake patters at first week and month 2 so far where data had been analyzed. We found that while there were no big differences in overall time spent in awake or sleep (Non-REM vs REM) patterns, closer examination showed that the ratio of time spent in sleep to time spent in awake was different in TBI animals compared to sham injury groups. At week 1 TBI animals spent lot more time in sleep compared to wake (ratio of sleep:wake being higher) at week 1 and the same ratio being much smaller in TBI animals compared to a sham injury animals. This is similar to “hypersomnia” reported acutely and “insomnia” reported chronically after TBI in humans. We also examined the cumulative distribution frequency of sleep and wake bouts in different groups and we found that after TBI, in the first week, they have longer bouts of sleep compared to sham injury animals and shorter bouts of sleep at month 2. When sleep bouts were compared in TBI animals at week 1 and month 2, we found that in the first week they have much longer bouts of sleep compared to the second month. Please see attached figure for these data.

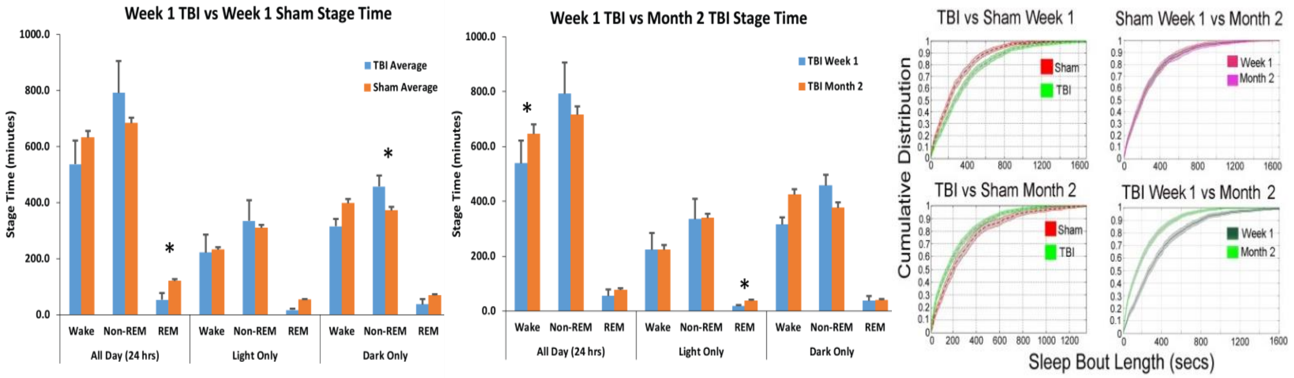


Figure 4. Sleep wake analysis showed that at week 1 (Left) TBI mice have less total REM sleep time but during dark period they spend more time in NREM sleep suggestive of daytime sleepiness. Compared to week 1 TBI group spend more time awake in month 2, suggestive of “insomnia” (middle). Cumulative distribution frequency analysis (Right) of sleep bouts showed that TBI group had longer sleep bouts at week 1 and shorter sleep bouts at month 2 compared to sham suggestive of fragmentation of sleep chronically.

Sleep data following treatment with DORA-22 or THIP are being analyzed at this time.

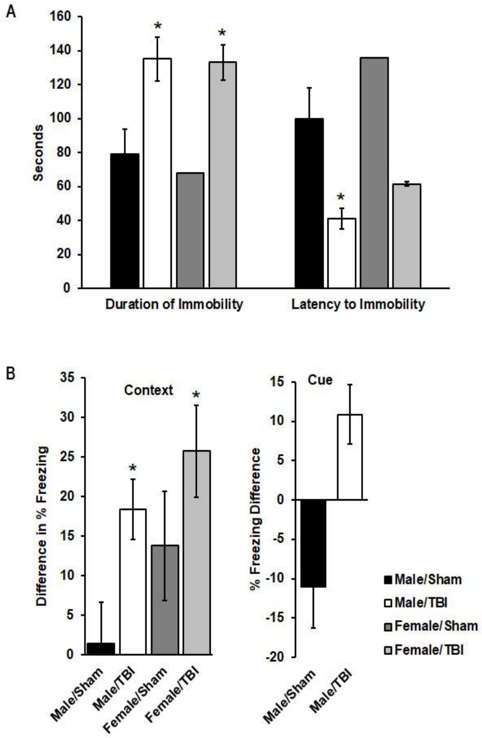


Figure 5: Preliminary behavioral data was collected in approximately 6 months post-TBI. (**A**) Tail suspension: The duration of immobility was increased in male and female TBI mice compared to their sham controls [male/sham=79±15, n=7; male/TBI=135±13, n=12, *p*=0.009; female/TBI=133±10, n=4, female/sham=68, n=1; *p*=0.03]. The latency to the first episode of immobility was significantly decreased in male/TBI but not in female/TBI mice compared to sham control [male/sham=100±18, n=7; male/TBI=43±6, n=12, *p*=0.002; female/TBI=62±1, n=4, female/sham=136, n=1; *p*=0.15]. (**B**) Fear conditioning (contextual and cued paradigms): An increased freezing was seen in male/TBI and female/TBI mice compared to male/sham in the context [male/sham=1.5±5.2, n=3; male/TBI=18.4±3.8, n=6, *p*=0.036; female/sham=13.8±6.9, n=3, *p*=0.23]; female/TBI=25.75±6.9, n=4, *p*=0.047; but not cued [male/sham = -11.1±10.4, n=3; male/TBI=10.9±7.9, n=6, *p*=0.15) conditions. Because of the low sample size, no difference was seen with the females in the Cued paradigm task (data not shown). Overall, the data are indicative of increased depression and PTSD phenotype in the TBI mice with the latter being worse in males compared to females (15 fold vs 2 fold difference compared to respective sham).

1. <u>Behavioral Studies</u>: We performed tail suspension test and contextual/cued fear conditioning for TBI and sham injury groups as tests of depression and PTSD respectively (Figure 5) approximately 6 months post-TBI. In the tail suspension test, each mouse was suspended by its tail approximately 60 cm above the benchtop, and struggle response was monitored for 5 min by video recording. Latency, frequency and duration of immobility was measured. These outcomes were taken as an index of depression status. Fear conditioning (contextual and cued paradigms) was employed in sham and TBI groups. The difference in the percent freezing between the control day and test day was graphed. Increased freezing under each condition was taken as an index of PTSD phenotype. Our results suggest PTSD phenotype is worse in males compared to female mice with TBI.

2. Electrophysiologic experimental data: Following TBI and EEG recording, mice were sacrificed and voltage-clamp recordings were performed, from dentate granule cells in brain slices (Figure 6), to evaluate the effects of CCI on synaptic inhibition. Figure 6 illustrates mIPSCs from Sham-treated mice (n=8 slices from 5 animals) and mice that received TBI either without (vehicle) or treatment with a dual orexin antagonist (DORA-22) (n=8 slices from 5 animals each) (note that these recordings were performed ≥1 week after cessation of DORA-22 treatment). TBI significantly reduced the amplitude and frequency of mIPSCs compared to Sham, whereas DORA-22 elevated mIPSC amplitude compared to both Sham and TBI. Therefore, some of the consequences of TBI may be mediated by disrupted GABAergic inhibition, and may be partially rescued by DORA-22 treatment. While experiments with another sleep aid Gaboxadol are nearly complete, data

is yet to be analyzed. Furthermore, tonic current data and input-output curves were obtained and they are yet to be analyzed.

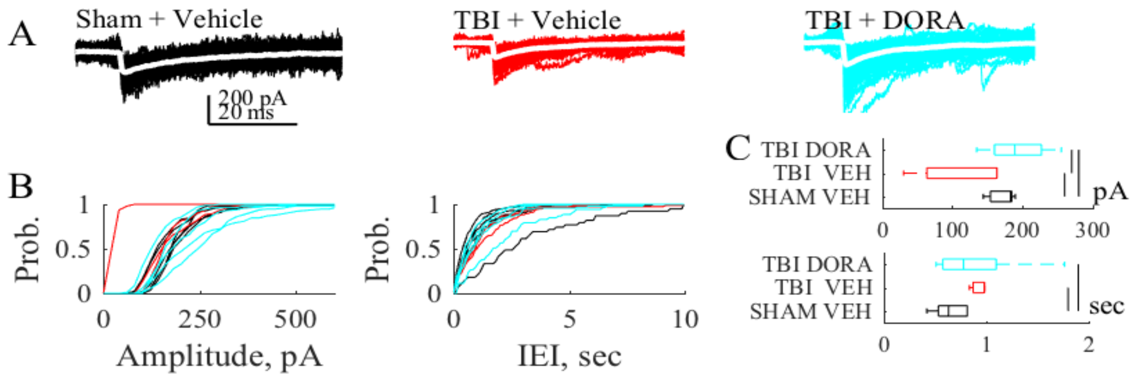


Figure 6. TBI effects on mIPSCs are partially rescued by a dual orexin antagonist (DORA-22) (**A**) Example mIPSCs from dentate granule cells. White overlays are the averages. (**B**) Cumulative amplitude and inter-event interval (IEI) distributions. Same color scheme as in A. (**C**) Box-and-whisker plots of median amplitudes (top) and IEIs (bottom). Vertical black bars indicate pairs of significant differences (p≤0.05, Kruskal-Wallis test).

3. Imaging data: Imaging study was performed at UW small imaging facility. Formalin fixed mouse brains were CT scanned with a Siemens Inveon microCT (Siemens Medical Solutions USA, Inc., Knoxville, TN). All scans were acquired with the following parameters: 80 kVp, 1000 µA current, 700ms exposure time, 220 rotation steps with 600 projections, medium-high magnification, and binning factor of 2. Raw data were reconstructed with filtered back-projection applying the Shepp-Logan filter using the high-speed COBRA reconstruction software (Exxim Computing Corporation, Pleasanton, CA) yielding isotropic voxels of 31.5 microns. Analysis was conducted with Inveon Research Workplace (Siemens Medical Solutions USA, Inc., Knoxville, TN). We compared whole brain, lesion size (from TBI and from epidural electrode placement) between sham and TBI groups. Analysis showed a trend toward smaller whole brain volumes (minus size of lesions from TBI and epidural screws) for the TBI group but sample size was small (Figure 7).

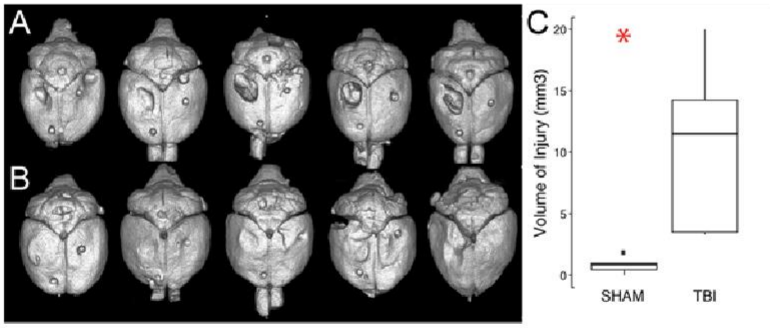


Figure 7: **(A, top row)** five brains from TBI animals. Notice the lesion produced by TBI on the right hemisphere (left side of image). **(B, bottom row)** five brains from Sham animals that received a craniotomy without TBI. **(C)** differences in volume of lesion at the TBI/craniotomy site is statistically significant. (Sham: 0.82 ± 0.68; TBI: 10.49 ± 7.2 mm^3; p = 0.039)

8. RTP-PCR data: We wanted to compare differences in GABA-A receptor subunits in ipsilateral and contralateral hippocampus, thalamus and cortex between sham injury and TBI groups as well as in animals with no craniotomy as well. This data is currently being collected.

Future grants:
Further understand if same mechanisms apply to repeated mild TBI and other models of TBI.
Do sleep disruptions impact neurobehavioral complications of TBI?
Other Funding agencies interested in TBI and its complications: NINDS; CDC

Training/Professional Development: There are 2 Post-Doctoral Fellows on the grant with both having learned new techniques and new methods of analysis. Our automated analysis of interictal spike scoring was developed by Jesse Pfammatter, PhD and will be publishing on this. Paulo Rodrigues, PhD who is another post-doctoral fellow had mastered the technique of EEG electrode implantation, performing the TBI (controlled cortical impactor) and in performing patch clamp electrophysiology.

How are results to be disseminated? : In the form of publications in the future. There is nothing to report now.

Impact:

Nothing to report

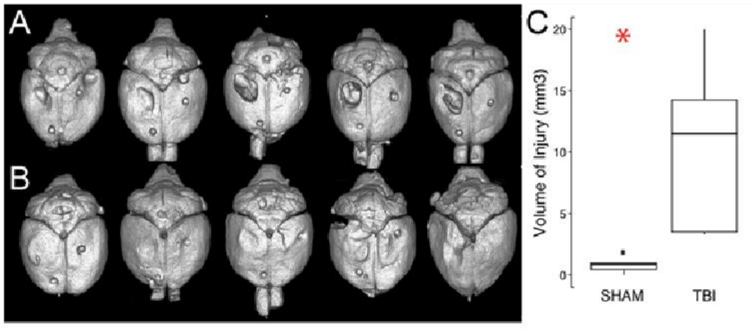
Changes/Problems: The only change we made is that initially we planned to do the experiments in Sprague-Dawley rats that are fast kindlers. However, we switched to mice, specifically CD-1 mice based on other available Literature. However we submitted the change in this approach as well as reasons for it to ACURO as well as our ACUC and obtained permissions. Apart from this there were no other protocol changes.

Products: Nothing to report

Participants and other collaborating organizations:

Individuals that have worked for the project:

a. Paulo Rodrigues, PhD:
   Project Role: Post-doctoral fellow



Contribution to the project: Paulo was the person performing many of the TBI surgeries, recording data for seizures and sleep (converting all acquired EEG data to sleep scoring formats); monitoring all animals as well as performing the patch clamp electrophysiology.

b. Jesse Pfammater, PhD:
Project role: Post-Doctoral fellow
Contribution: Jesse is responsible for analysis of data using automated soft wares or for statistical analysis. He was also responsible for fine tuning and preparing/running an old Electrophysiology rig to run patch clamp experiments. He also assisted Paulo in performing and designing the experiments.

c. Sruthi Reddy Konduru:
Project Role: Lab Tech
Contribution: Sruthi learned and performed some TBI and sham surgeries as EEG electrode implantation. In addition she assisted in scoring some of the sleep data
Funding source: None

d. Undergraduate students:
Project role: Students for data analysis and lab experience
Contribution: Students for primarily responsible for scoring all sleep files which is a very labor intensive process.
Funding source: UW Medical Foundation- in part.

Has there been a change in the active “other support” of PD/PI?

Nothing new to report

Special reporting requirements: None

Appendices: Abstracts submitted to Society for Neuroscience, 2019

Sleep spindles and its role as a potential biomarker for the development of post-traumatic epilepsy

Konduru SSR, Pfammatter JA, Rodrigues PV, Jones MV, Maganti RK

1. Department of Neurology; 2. Department of Neuroscience, University of Wisconsin School of Medicine and Public Health

Epidemiological data shows that post-traumatic epilepsy (PTE) is seen in about 10-20% of patients with Traumatic Brain Injury(TBI). It can be as high as 50% in injured military personnel. Currently, it is uncertain as to who develops PTE after TBI. Sleep-Wake disturbances are very common in TBI patients and there is a close relationship between sleep disorders and seizures. Here, we analyzed sleep spindles,(a hallmark of NREM sleep) in mouse models of TBI as a potential biomarker for the

development of PTE. We analyzed EEG data sets from 3 cohorts of CD-1mice. 1) TBI cohort (n=40) underwent severe TBI (controlled cortical impact) to the right parietal lobe. 2) Sham cohort (n=22) underwent craniotomy without TBI. TBI and sham cohorts were recorded at week 1, month 1,2,3,6 after surgery. 3) The third set of mice underwent surgery without craniotomy (n= 6). EEG was obtained at week 1 from this cohort to determine the baseline for CD-1 mice. All the obtained EEG signals were 1) Manually scored for sleep-wake patterns in 4s epochs (Sirenia sleep) and artifactual EEG was excluded 2) Manually scored for sleep spindles after bandpass filtering between 9- 15hz (1hour NREM segments) to compare with automated method. 3) TBI cohort manually scored for seizures (Natus Neuroworks). We used an automated spindle detection method based on Ferrarelli et al(2007) in Wonambi, Python. Automated analysis was done using multiple detection and selection thresholds and correlated with manual spindle scoring. The best possible match (~50%) was identified when the detection and selection thresholds were set to 3 and 2. The method will be further reviewed and validated to yield higher predictive value and F1 score. Other parameters used for spindle detection are frequency (9-15hz) and duration(0.5-2s). We compared spindle results from automated detection across groups, TBI with seizures (~25% of TBI recorded), TBI without seizures and Sham. Preliminary analysis showed a difference in mean peak amplitude and power between sham (108.35+/-28.06; 10.43+/-4.74) and TBI groups (84.95+/-17.39; 6.67+/-2.59). Within the TBI groups, there was no difference in average peak amplitude and power between TBI animals with seizures (84.99+/-23.96; 6.37+/-3.26) and TBI animals without seizures (84.91+/-10.82; 6.97+/- 1.92). Normalized spindle density/hour/animal showed an increase in spindle density in TBI animals with seizures (47.58) and TBI animals without seizures (43.24) compared to sham (25.23). There was no difference in the mean frequency(hz) and duration of the spindles(sec) across groups. Further analysis of our data is being done to determine the significance of these findings.

2. **Does treatment with sleep aids following TBI delay development of post-traumatic epilepsy?**

Rodrigues PV, Konduru SSR, Pfammatter JA, Jones MV, Maganti RK

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Traumatic Brain injury (TBI) leads to several sequelae including post-traumatic epilepsy (PTE), sleep-wake disturbances (SWD) and even mood disorders. Currently there are no preventive treatments for these sequelae. However, it is well established that lack of sleep triggers seizures, but it is unclear if SWD leads to PTE. We hypothesized that post-TBI SWD is a necessary and sufficient condition for the development of PTE. Here, we aimed to investigate whether sleep aids-dual orexin antagonist (DORA-22) or Gaboxadol (THIP- an agonist of delta subunit containing $GABA_A$ receptor), when used early after TBI, will restore sleep and prevent development of PTE.

We performed severe TBI (Controlled cortical impact-CCI) or sham injury (craniotomy only with no injury) in CD-1 mice and implanted epidural EEG electrodes in the right frontal and left parietal areas along with nuchal EMG under isoflurane anesthesia. After TBI/sham injury and EEG implantation animals were treated with either DORA-22, THIP, or control (vehicle control or no treatment) via oral gavage for 1 months from the day of surgery. We then conducted week long video-EEG recordings in week 1, and months 1, 2, 3 and 6. Acquired EEG data was transferred to a sleep scoring software (Sirenia Sleep) and was scored in 4 sec epochs. We then analyzed epileptiform events (seizures and interictal spikes) and sleep-wake patterns across time.

Seizures occurred in ~ 25% of TBI animals whether untreated (9/40) or treated (3/12-for DORA-22 and 3/12 for THIP group). Normalized seizure counts (seizures per animal per hour of recording) indicate consistent seizure frequency across time in the untreated TBI animals(1.21 at week 1, 2.78 at month 2, 1.88 at month 3, and 2.08 at month 6). Interestingly, TBI mice treated with DORA-22 has no recorded seizures after the first week (2.78 at week 1and 0 at other time points)and we observed an increase in seizure rate of THIP animals as compared to controls (8.33 at week 1, 4.17 at month 1, 4.69 at month 2, and 0 at month 3). Seizures consisted of behavioral arrest, head nodding and hind limb stretching that sometimes progressed to Racine class V seizures. SWD consisted of “hypersomnia” in the first week, but “insomnia” at month 2 after TBI. Our preliminary results suggest no differences in sleep-wake patterns between DORA-22, THIP, or control treated TBI animals. A full analysis of seizures, interictal spikes (~80% of animals had interictal spikes), and sleep parameters is ongoing and further analysis is focused on understanding relationship between sleep disruptions, epileptiform events with or without treatment.