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Monitoring Neurocognitive Performance and Electrophysiological Activity after Mild Traumatic Brain Injury

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14. ABSTRACT

Our project aimed to explore a wide variety of objective approaches to identify acute concussion (mTBI) patients and distinguish them from trauma controls that did not suffer head injury. Neuropsychometry, balance, actigraphy, electroencephalography (EEG), event-related potentials (ERPs), magnetoencephalography (MEG), magnetic resonance imagining and spectroscopy (MRI and MRS), and blood biomarkers were evaluated. EEG, MEG, MRI, MRS, blood metalloproteinase-9, and blood fatty acid lipolysis all differed in mTBI, especially 1-5 days after injury, and also differently at 30 days after injury. Preliminary analyses indicate the combination of techniques are even more informative than they are individually, especially combinations of EEG with blood lipids, MMP9, and MRI volumetrics and spectroscopy. The data suggest that further testing of these new objective EEG and blood biomarkers have the greatest potential for widespread utility.

15. SUBJECT TERMS

Traumatic brain injury, mTBI, concussion, magnetoencephalography, MEG, MRI, MRS, ERPs, blood biomarkers, actigraphy

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1. Introduction

Concussion or mild traumatic brain injury (mTBI) is difficult to diagnose, based on a lack of objective tests. Many victims do not even recognize its occurrence. This limits both detection and efforts to understand brain damage. The objective of our project is to investigate if individual or combinations of objective measures acquired acutely (on days 1, 14, and 30 after injury), and later (6-12 months after concussion) will help diagnose and define acute mTBI. We evaluated objective neuropsychometry, balance, actigraphy, electroencephalography (EEG), event-related potentials (ERPs), magnetoencephalography (MEG), magnetic resonance imagining and spectroscopy (MRI and MRS), and blood biomarkers.

2. Keywords

Actigraphy, blood biomarkers, concussion, ERPs, fatty acids, MEG, EEG, MRI, MRS, MMP9, mTBI, trauma controls

3. Overall Project Summary

Our project aimed to explore a wide variety of objective approaches to identify acute concussion (mTBI) patients and distinguish them from trauma controls that did not suffer head injury. Neuropsychometry, balance, actigraphy, electroencephalography (EEG), event-related potentials (ERPs), magnetoencephalography (MEG), magnetic resonance imagining and spectroscopy (MRI and MRS), and blood biomarkers were evaluated. EEG, MEG, MRI, MRS, blood metalloproteinase-9, and blood fatty acid lipolysis all differed in mTBI, especially 1-5 days after injury, and also differently at 30 days after injury. Preliminary analyses indicate the combination of techniques are even more informative than they are individually, especially combinations of EEG with blood lipids, MMP9, and MRI volumetrics and spectroscopy. The data suggest that further testing of these new objective EEG and blood biomarkers have the greatest potential for widespread utility.

4. Key Research Accomplishments

The entire project rested on consenting patients, hence a short description is necessary.

A total of 33 patients consented: 23 mTBI; 10 controls:
23 mTBI and 10 trauma controls were assessed 1-5 days after injury;
17 mTBI and all trauma controls were assessed 2 weeks after injury;
18 mTBI and all trauma controls were assessed 4 weeks after injury;
8 mTBI and 5 controls were assessed 6-12 months after injury.

Classification	Female	Mean Age	SAC Score	Altered consciousness <30min	Altered consciousness <24hrs	Memory loss <24hrs
mTBI (n=23)	60.8%	31.5 ± 9.5	24.8 ± 3.2	2	7	3
Trauma Control (n=10)	40.0%	29.5 ± 5.5	N/A	None	None	None

Description of Injury	mTBI	Non-head Trauma Control
Motor vehicle accident	13	
Fell down stairs	2	
Sports accident	2	
Hit on head	3	
Collapsed and fell	1	
Unspecified head injury	2	
Fracture		1
Sprain		1
Trauma to limb		6
Laceration		2

- **Individual objective biomarkers distinguish the mTBI vs. trauma control groups:**

- Abnormal frontal lobe delta activity on resting state MEG 1-5 days after injury

- Abnormal frontal lobe alpha and beta activity on resting state EEG 1-5 days after injury

- Impaired learning (EEG) on simple (0–back) working memory test 30 days after mTBI

- MRS shows higher total choline in mTBI

- Blood 'LGL' peptide of MMP9 is higher in mTBI 2 - 4 weeks after injury

- Increased lipolysis of fatty acids in blood acutely after mTBI

- **Correlations of individual biomarkers add to the detection of mTBI**

- EEG and MRI volumetric changes in mTBI correlate with each other

- EEG and MRS changes in mTBI correlate with each other

- EEG and blood MMP9 and fatty acids changes in mTBI correlate with each other

5. Conclusions

We report that mTBI patients differ from non-head trauma controls significantly using each of the MEG, EEG, MRI, MRS, and blood biomarker objective measures. Preliminary analyses provide evidence that the discrimination from individual metrics are further enhanced when combined. The data suggest all approaches offer important potential for further research, but suggest the EEG and blood biomarkers offer greater potential to be more cost-effective, simplified, validated, and have a greater potential for more widely available utility.

6. Publications, Abstracts, and Presentations

Publications

Brain activation profiles in mTBI: Evidence from combined resting-state EEG and MEG activity. Lianyang Li, Pagnotta MF, Arakaki X, Tran T, Strickland D, Harrington M, Zouridakis G. Conf Proc IEEE Eng Med Biol Soc. 2015 Aug;2015:6963-6. doi: 10.1109/EMBC.2015.7319994. PMID: 26737894

Brain activation profiles in mTBI: evidence from ERP activity of working memory response. Lianyang Li, Arakaki X, Thao Tran, Harrington M, Padhye N, Zouridakis G. Conf Proc IEEE Eng Med Biol Soc. 2016 Aug;2016:1862-1865. doi: 10.1109/EMBC.2016.7591083. PMID: 28268689

Under revision at PLoS One:
Alpha desynchronization/synchronization during working memory testing is compromised in acute mild traumatic brain injury (mTBI). Xianghong Arakaki, Michael Shoga, Lianyang Li, George Zouridakis, Thao Tran, Alfred N. Fonteh, Jessica Dawlaty, Robert Goldweber, Janice M. Pogoda, Michael G. Harrington

Abstracts

Abstracts presented at 12th World Congress on Brain Injury for March 29 – April 1, 2017 in New Orleans (complete abstracts in Appendix):

a) Thalamocortical dysrhythmia after mild Traumatic Brain Injury: a working hypothesis. Riccardo Zucca[1], Xianghong Arakaki[2], Michael G. Harrington[2], Paul FMJ Verschure[1,3]
b) Source Connectivity Analysis Can Assess Recovery of Acute Mild Traumatic Brain Injury Patients. Lianyang Li[1], Xianghong Arakaki[2], Thao Tran[2], Michael Harrington[2], George Zouridakis[1]
c) Assessing Recovery of Acute Mild Traumatic Brain Injury Patients using Diffusion Tensor Imaging. Esther Mvula[1], Lianyang Li[1], Xianghong Arakaki[2], Thao Tran[2], Michael Harrington[2], George Zouridakis[1]
d) Alpha power during working memory is compromised in acute mild traumatic brain injury. Xianghong Arakaki[1], Michael Shoga[1], Lianyang Li[2], George Zouridakis[2], Jessica Dawlaty[1], Robert Goldweber[3], Michael Harrington[1]

Abstract presented at Society for Neuroscience in Washington DC, 2017

e) Altered dynamics of the thalamo-cortical system following mild Traumatic Brain Injury: a combined experimental and theoretical study. Riccardo Zucca, Xianghong Arakaki, Sock Ching Low, Robert Goldweber, Michael G. Harrington, Paul FMJ Verschure

Abstracts submitted to Experimental Biology for April 21 – April 25, 2018 in San Diego, California

f) Working memory testing reveals neuroplasticity acutely and longitudinally after mild traumatic brain injury (mTBI). Xianghong Arakaki, [1]Ryan Lee, [1]Alfred N. Fonteh, [2]Robert T. Goldweber, [1]Michael G. Harrington.

g) Plasma metalloproteinase-9 (MMP9) changes in acute mild traumatic brain injury (mTBI) and correlates with quantitative EEG. Eric Hubbard, [1]Jessica Dawlaty, [1]Xianghong Arakaki, [1]Soren Cole, [3]Robert Goldweber, [1]Michael G. Harrington.
h) Plasma Lipid Metabolism is altered in Acute Mild Traumatic Brain Injury. Alfred N. Fonteh[1], Katherine Castor[1], Eun Jung Im[1], Xianghong Arakaki[1], Jessica Dawlaty[1], Robert T. Goldweber[2], Michael Harrington[1]

7. Inventions, Patents, and Licenses.
Nothing to report.

8. Reportable Outcomes
Nothing to report beyond the publications.

9. Other achievements
We are strongly justified in pursuing efforts to gain ongoing funding based on the progress in our publications and our ongoing analyses.

10. References
Not applicable

11. Appendices
Publications and poster abstracts

Brain Activation Profiles in mTBI: Evidence from Combined Resting-State EEG and MEG Activity

Lianyang Li, Mattia F. Pagnotta, Xianghong Arakaki, Thao Tran, David Strickland, Michael Harrington, and George Zouridakis, *Senior Member, IEEE*

***Abstract*—In this study, we compared the brain activation profiles obtained from resting state Electroencephalographic (EEG) and Magnetoencephalographic (MEG) activity in six mild traumatic brain injury (mTBI) patients and five orthopedic controls, using power spectral density (PSD) analysis. We first estimated intracranial dipolar EEG/MEG sources on a dense grid on the cortical surface and then projected these sources on a standardized atlas with 68 regions of interest (ROIs). Averaging the PSD values of all sources in each ROI across all control subjects resulted in a normative database that was used to convert the PSD values of mTBI patients into z-scores in eight distinct frequency bands. We found that mTBI patients exhibited statistically significant overactivation in the delta, theta, and low alpha bands. Additionally, the MEG modality seemed to better characterize the group of individual subjects. These findings suggest that resting-state EEG/MEG activation maps may be used as specific biomarkers that can help with the diagnosis of and assess the efficacy of intervention in mTBI patients.**

I. Introduction

Mild traumatic brain injury (mTBI) is defined as a transient change in brain function affecting the mental state and possibly consciousness of patients due to an external force (Menon et al., 2010). mTBI is difficult to diagnose because patients typically lack apparent external injuries and clear pathological findings in conventional computed tomography and magnetic resonance imaging (MRI) scans (Tarapore et al., 2013), although evidence of microscopic MRI-based morphological changes has been recently reported (Pasternak et al., 2014; Sasaki et al., 2014). Symptoms, such as headaches, fatigue, and dizziness (Cassidy et al., 2004) usually emerge on the day of injury and persist for a few days following injury (Boccaletti et al., 2006), but in most patients, symptoms resolve and cognition recovers within three months. However, up to 25% of patients (Sigurdardottir et al., 2009) suffer residual symptoms, long-term impairment, and sometimes disability (Levin 2009). Traumatic brain injury is a major cause of sustained morbidity and disability both in the military and civilian populations (Tarapore et al., 2013), as repeated mTBI can cause a wide range of neurological and cognitive deficits affecting memory, reasoning, language, and emotions (NINDS, 2002).

In the last two decades, a plethora of studies has attempted to characterize the structural and functional effects of mTBI (Eierud et al., 2014). Of particular interest are studies of resting state neurophysiological recordings, obtained using Electroencephalography (EEG) and Magnetoencephalography (MEG), because they require no training or experience with cognitive tasks, and they impose minimal demands on a patient, which is especially important after brain injury. One of the very first studies using resting-state MEG as a possible biomarker for mTBI showed that functional connectivity could be a valuable tool for early detection of mTBI (Zouridakis et al., 2012). Other studies showed abnormal slowing in brain areas affected by TBI (Huang et al., 2014) and reduced overall functional connectivity in TBI patients compared to controls (Tarapore et al., 2013). In particular, resting-state MEG source imaging (Huang et al., 2012) was able to detect abnormalities in mild and moderate TBI with 87% and 100% accuracy, respectively. Furthermore, combining MRI with MEG (Lewine et al., 1999) could discriminate between healthy adults and individuals with resolved mTBI. Compared to healthy controls, mTBI subjects showed reduced complexity in multiple brain areas (Luo et al., 2013). Decreased connectivity in resting-state MEG may persist for years after mTBI (Castellanos et al., 2011), but the abnormally reduced connectivity might improve over time (Tarapore et al., 2013).

Continuing our earlier attempts to understand how mTBI affects communication networks in the human brain (Zouridakis et al., 2012, Pollonini et al., 2010, Dimitriadis et al., 2015), in this study, we employ recordings of resting-state EEG and MEG and power spectrum analysis at the source level to investigate abnormalities in brain activation profiles of mTBI patients.

II. Materials and Methods

A. Subjects

Six mTBI subjects (4 male, 2 female, average age 28.3±7.3) and five orthopedic controls (3 male, 2 female, average age 29.4±7.4), i.e., subjects with minor orthopedic/extremity injuries who did not sustain head injury were recruited for this study. All data were obtained at the Huntington Medical Research Institutes, Pasadena, CA, USA. Exclusion criteria included a personal history of neurological or psychiatric illness, neurological disorders, serious medical condition,

M. Pagnotta and L. Li are with the Biomedical Imaging Lab, University of Houston, Houston TX, USA.

X. Arakaki, T. Tran, D. Strickland, and M. Harrington (mghworks@hmri.org) are with Huntington Medical Research Institutes, Pasadena, CA.

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and drug or alcohol addiction. The protocol was approved by the appropriate institutional review board, and written informed consent was obtained from all participants in the study.

B. EEG and MEG Recordings and Signal Preprocessing

Subjects were asked to remain as still as possible during the recording procedure and keep their eyes open.

Approximately 5 minutes of continuous EEG activity was acquired from each subject using a dry electrode EEG system (Wearable Sensing, San Diego, CA). The system includes 21 channels for EEG and two additional channels to record EOG and EKG activity to monitor eye and cardiac artifacts. Data were collected at a sampling rate of 300 Hz.

Furthermore, 5 minutes of continuous MEG activity was acquired from each subject using a CTF MEG system (MEG International Services Ltd., Coquitlam, BC, Canada). This whole-head system includes 66 axial gradiometer sensors and 31 additional channels that can be used for noise reduction. Data were collected at a sampling rate of 625 Hz and bandpass filtered between 0.1–200 Hz using hardware.

All data analyses were done in MATLAB (The MathWorks, Inc., Natick, Massachusetts, United States) using Brainstorm (Tadel et al., 2011) and in-house software developed in the Biomedical Imaging Lab at the University of Houston. Both the EEG and MEG sensors were co-registered with an anatomical MRI template (Colin27) using six fiducial references, the nasion, left pre-auricular point, right pre-auricular point, anterior commissure, posterior commissure, and an interhemispheric point. All results were visually inspected for accuracy.

A detrending procedure was applied to all EEG and MEG recordings to remove linear trends. Additionally, data were bandpass filtered between 0.1-80 Hz, whereas line noise was removed using a 50 Hz notch filter. Eye blink artifacts were removed using the additional eye channel signals and an automatic eye blink detection procedure based on signal-space projection. The identified eye activity topographies were visually inspected for accuracy. At least 2 minutes of artifact-free EEG/MEG activity was necessary for a subject to be included in final analysis.

C. Intracranial Source Power Analysis

A BEM (boundary element method) head model was used for the EEG/MEG forward calculation. Three layers of tissues representing the scalp, outer skull, and inner skull were extracted from a reference MRI (Colin27), using 1922 vertices per layer and 4 mm of skull thickness. The grid of dipolar sources was defined on the reference MRI cortex surface and consisted of approximately 15,000 vertices. The lead field matrix was computed using the overlapping spheres method (Huang et al., 1999).

Estimation of intracranial sources for each subject was performed using the dynamical Statistical Parametric Mapping (dSPM) procedure (Dale et al., 2000), which is based on whitened minimum norm estimation (wMNE), a depth-weighted linear L2 minimum norm estimation algorithm inspired from the original MNE method (Hämäläinen et al., 1994) and related software[1] (MNE manual, section 6). The dSPM value at each location is equal to the wMNE value divided by the projection of the estimated noise covariance matrix onto each source point. After whitening, the operational noise covariance matrix is by definition the identity matrix, and hence the projection of the noise is equal to the L2 norm of the row vector of the wMNE inverse operator (in the case of fixed dipole orientations).

More specifically, given a set of EEG/MEG surface recordings x(t), the relationship between the intracranial dipole sources s(t) and the EEG/MEG data x(t) is given by the so called *forward solution,*

$$\mathrm{x(t)} = \mathrm{A\,s(t)} + \mathrm{n(t)}, \tag{1}$$

where s(t) denotes a vector of dipole component strengths, A denotes the linear forward matrix operator, and *n(t)* denotes additive noise (Dale and Sereno, 1993). Assuming that the prior information about dipole strength follows a multivariate Gaussian distribution, the maximum a posteriori probability estimate is given by

$$\hat{s}(t) = Wx(t), \text{ with } W = RA^T(ARA^T + C)^{-1}. \tag{2}$$

W is the inverse operator, $C = < n(t)n(t)^T >$ is the data noise covariance matrix, and R is the spatial covariance matrix of the dipole strength vector. The variance of each dipole strength estimate due to the additive noise *n(t)* is given by

$$var(\hat{s}_i) = < (w_i n(t))^2 > = w_i C w_i^T, \tag{3}$$

where $\hat{s}_i$ denotes the i^{th} element of the dipole strength vector $\hat{s}$ and w_i is the i^{th} row of the linear inverse operator W.

In the case of fixed dipole orientations, for each time point t and location i, a noise-normalized activity estimate $\hat{s}_{n,i}(t)$ can be computed by dividing the total dipole strength estimate in location i by the predicted standard error of the estimate due to the additive noise, using the formula

$$\hat{s}_{n,i}(t) = \frac{\hat{s}_i(t)}{\sqrt{w_i C w_i^T}} = \frac{w_i \cdot x(t)}{\sqrt{w_i C w_i^T}}. \tag{4}$$

For source reconstruction, we used the recordings obtained from all sensors. A pre-whitening transformation was applied to the data to pre-scale the channels using the noise covariance matrix. Furthermore, we selected constrained source orientation, which considers that at each vertex of the cortex surface there is only one dipole whose orientation is normal to the surface at this point.

Power spectral density (PSD) analysis based on Welch's method was performed on all source estimates, using a window length of 2 sec with 50% time overlap. The EEG/MEG frequency bands of interest were the following:

[1] http://www.nmr.mgh.harvard.edu/meg/manuals/MNE-manual-2.7.pdf

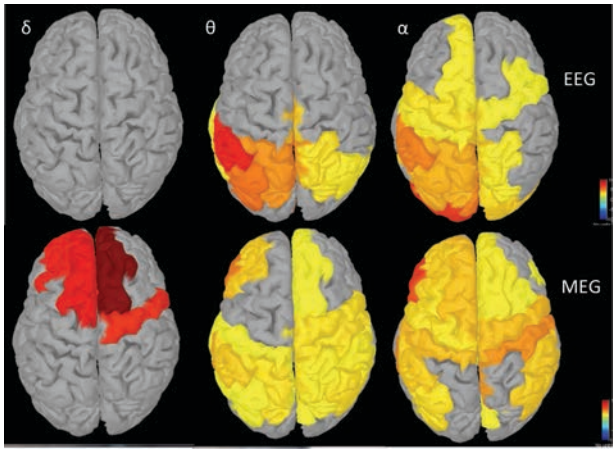

Fig. 1 Statistically significant differences in activation profiles (z-scores) between mTBI and control subjects in the lower frequency bands, for EEG (top) and MEG (bottom).

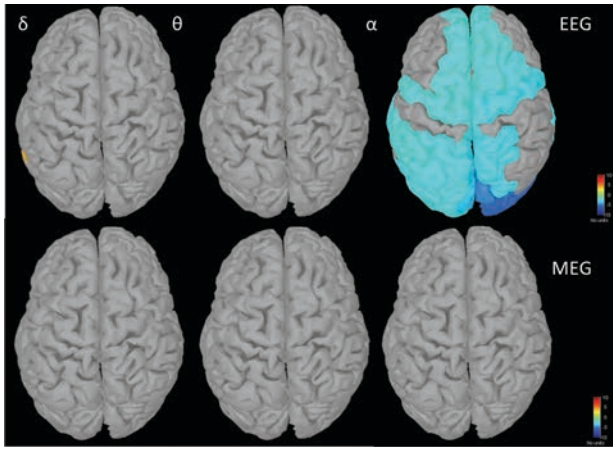

Fig. 2 Statistically significant differences in activation profiles (z-scores) between a control subject and the normative database in the lower frequency bands, for EEG (top) and MEG (bottom).

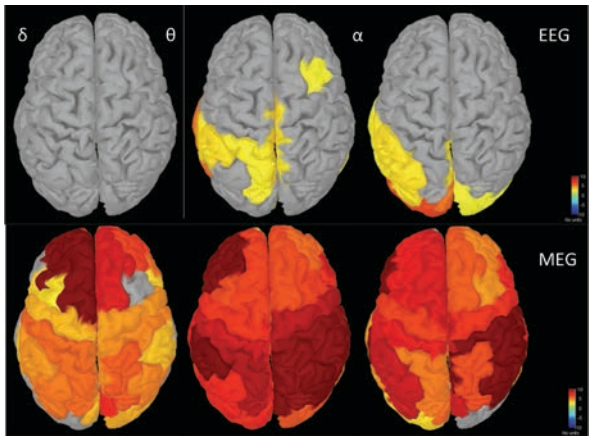

Fig. 3 Statistically significant differences in activation profiles (z-scores) between an mTBI patient and the normative database in the lower frequency bands, for EEG (top) and MEG (bottom).

0.1-4 Hz (delta), 4-8 Hz (theta), 8-10.5 Hz (alpha1), and 10.5-13 Hz (alpha2). For higher frequencies, 13-20 Hz (beta1), 20-30 Hz (beta2), 30-40 Hz (gamma1), and 40-80 Hz (gamma2) were selected.

The Desikan-Killiany atlas (Desikan et al., 2006) defined in the FreeSurfer software was used for common co-registration of sources. This atlas consists of 34 brain regions of interest (ROIs) for each hemisphere and it is available as a free download online[2]. After averaging the power estimates across all vertices belonging to the same ROI, a map of 68 ROIs by 8 frequency bands was obtained for each subject.

D. Normative Database and z-score Maps

We further defined a *normative database* using all five datasets from the control group, separately for the EEG and MEG recordings. Specifically, averaging the power maps across the five control subjects yielded two matrices (68 × 8, ROIs-by-frequency bands), one with the mean values and a second one with the standard deviations, for each frequency band. The same estimation procedure was followed for each mTBI subject.

Each patient was compared to the normative values, and for each ROI assessed, a z score was computed using the following expression:

$$z_{ij} = \frac{P_{ij} - Mean_{ij}^{C}}{SD_{ij}^{C}}, \text{ with i = 1, 2 … 68, j = 1, 2, … 8,} \quad (5)$$

where P_{ij} is the ij element of the power map and $Mean_{ij}^{C}$ and SD_{ij}^{C} are the mean and standard deviation values, respectively, from the two normative database matrices corresponding to the i-th ROI and j-th frequency band (Huang et al., 2012).

E. Statistical Thresholding

To identify statistically significant z-scores we used alpha = 0.05 and false discovery rate correction (Benjamini & Hochberg, 1995) for the 544 (68 × 8) multiple comparisons, resulting in a threshold value of 2.2421. After thresholding, the statistically significant z-scores were projected onto the Desikan-Killiany atlas (Desikan et al., 2006) using different colors for each z-score value to visualize the brain areas that differed significantly between the two groups.

III. Results

When comparing the mTBI and control groups, statistically significant differences in the form of overactivation were seen primarily in the theta (4-8 Hz) and low alpha (8-10.5 Hz) bands for the EEG and the delta (0.1–4 Hz), theta (4-8 Hz), and low alpha (8-10.5 Hz) bands for MEG data, as shown in Fig. 1.

To test the power of the methodology to correctly classify subjects on a single subject basis, we used the first four controls to construct the normative database, while the fifth one was used to construct the activation maps (z scores) shown in Fig. 2. For comparison, Fig. 3 shows the activation maps of one mTBI subject. These figures show that the control subject does not differ significantly from the

[2] http://surfer.nmr.mgh.harvard.edu/

normative database, while the mTBI subject shows significant differences mostly in the lower frequency bands. Additionally, the MEG modality seems to more accurately characterize the individual subject group.

Overall, even though the number of participants is small at this early stage of the study, the results obtained suggest that analysis of resting-state EEG and MEG activation maps is a powerful tool that can help in the diagnosis of and assess the efficacy of intervention in mTBI.

ACKNOWLEDGMENT

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Brain Activation Profiles in mTBI: Evidence from ERP Activity of Working Memory Response

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***Abstract*—In this study we analyzed event related potentials (ERPs) obtained in an N-back working memory test that varied in difficulty from 0- to 2-back. We collected 21 channels of activity from 11 mild traumatic brain injury (mTBI) patients and 7 normal controls, on three different visits, and used the amplitude and latency of the P300 component to characterize the subjects. A preprocessing procedure based on independent component analysis was used first to identify and eliminate electrophysiological noise on a single trial basis. Then to obtain more reliable statistics, the recording electrodes were lumped into five main groups corresponding roughly to frontal, central, parietal, and left and right temporal brain regions. For each subject, the P300 amplitude and latency were measured after averaging the activity of all channels in each group. Group analyses showed that latencies in the central region were significantly shorter in controls, at every visit for the 2-back test. The lack of significant differences across the three visits for the mTBI group indicates that mTBI subjects are not improving at the rate that might have been expected, confirming previous reports that mTBI deficits may persist for years.**

I. Introduction

Mild traumatic brain injury (mTBI) affects the mental state and possibly consciousness level of patients due to an external force (Menon et al., 2010). As typical patients lack apparent external injuries and clear pathological findings in conventional computed tomography and magnetic resonance imaging (MRI) scans (Tarapore et al., 2013), mTBI is often difficult to diagnose. Nevertheless, some evidence of microscopic changes in MRI-based morphology has been recently reported (Pasternak et al., 2014; Sasaki et al., 2014). Symptoms, such as headaches, fatigue, and dizziness (Cassidy et al., 2004) usually emerge on the day of injury and persist for a few days (Boccaletti et al., 2006), but in most patients, symptoms resolve and cognition recovers within three months. However, residual symptoms (Levin 2009), long-term impairment and, occasionally, disability is seen in up to 25% of patients (Sigurdardottir et al., 2009). mTBI can cause a wide range of neurological and cognitive deficits affecting memory, reasoning, language, and emotions (NINDS, 2002) and for that reason it is a major cause of sustained morbidity and disability both in the civilian and military populations (Tarapore et al., 2013).

In our quest to understand how mTBI affects communication networks in the human brain, in our previous studies we used functional connectivity analysis of resting-state magnetoencephalographic (MEG) activity at the sensor level (Zouridakis et al., 2012; Pollonini et al., 2010, Dimitriadis et al., 2015; Antonakakis et al., 2016 and analysis of intracranial source localizations (Li, et al., 2015) to identify reliable biomarkers for mTBI characterization. In this study, we employ event-related potentials (ERPs) obtained in the context of a working memory (WM) paradigm to compare neuronal responses between mTBI patients and normal controls. WM plays a crucial role in temporal retrieval, maintenance, and manipulation of information for a wide range of cognitive functions (Baddeley, 1992, 2003), which we hypothesize can be affected by brain injury.

WM effects have been studied in the context of the N-back paradigm, which requires that subjects indicate whether the current stimulus is identical to the stimulus shown N presentations prior, using functional MRI (Braver et al., 1997; Jaeggi et al., 2007; Manelis and Reder, 2014; Owen et al., 2005) and neurophysiological recordings (Daffner et al., 2011; Gevins and Smith, 2000; McEvoy et al., 2001). The effect of WM demand has been studied by changing the level of difficulty in the N-back task (Braver et al., 1997; Daffner et al., 2011; Jaeggi et al., 2007; McEvoy et al., 2001; Pesonen et al., 2007; Watter et al., 2001). However, only a few studies have assessed ERP changes as a function of working memory capacity (e.g., Dong et al., 2015) or task difficulty across mTBI patients and controls.

II. Materials and Methods

A. Subjects

For this study we recruited 11 mTBI subjects (7 males, average age 25.6) and 7 normal controls (4 males, average age 27.2), all native speakers of English. Data were obtained at the Huntington Medical Research Institutes (HMRI) in Pasadena, CA, USA. Exclusion criteria for the study included a personal history of neurological or psychiatric illness, neurological disorder, serious medical condition, and drug or alcohol addiction. The study protocol was approved by the appropriate institutional review boards at HMRI and

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the University of Houston, and written informed consent was obtained from all participants in the study.

B. ERP Recordings and Signal Preprocessing

In the N-back experiment, subjects were asked to remain as still as possible during the recording procedure and keep their eyes open. Stimuli were delivered using the E-prime 2.0 software. The onset of stimulus presentation was marked with triggers and synchronized with the recording system.

Continuous electroencephalographic (EEG) activity was acquired using a dry electrode system (Wearable Sensing, San Diego, CA) which included 21 EEG channels and three additional channels to record eye movements and heart activity. Electrode impedances were maintained below 10 kΩ. Recordings were digitized at 300 Hz with a 16-bit analog-to-digital converter and referenced to Pz. Later they were re-referenced to linked mastoids for off-line analysis.

Stimuli consisted of 20 orthographically distinct uppercase consonants (B, C, D, F, G, H, J, K, L, M, N, P, Q, R, S, T, V, W, Y, and Z), that were randomized and presented on a computer screen one at a time. Subjects were required to push a "target" button if the stimulus on the screen was the same as the stimulus N trials back, and a "non-target" button otherwise. Each trial started with a 400 ms wait, after which black-colored letters were presented on a white background for 500 ms, followed by a delay of 2000 ms during which the subject was required to respond and terminate. After another 400 ms wait, the next letter would be presented. There were two N-back conditions, 0-back and 2-back, that varied in working memory load. Each condition included 90 trials, 30 "match" and 60 "non-match" stimuli. Each condition consisted of 3 blocks and preceded with a practice session. The experimental session took approximately 25 minutes to complete, depending on subject response speed and requested breaks.

Most mTBI subjects had three recording sessions. The first visit was conducted within one week after the injury, whereas the second and third visits were completed two and four weeks, respectively, after the first visit. Most control subjects completed one or two visits. For all subjects, the accuracy and response time were recorded automatically and used to calculate behavioral measures.

All data analyses were performed using MATLAB (The MathWorks, Inc., Natick, Massachusetts, USA), the EEGALB software package, and in-house software developed in the Biomedical Imaging Lab at the University of Houston. First, ERPs were preprocessed to detect and remove electrophysiological noise, such as muscle activity and eye movements. Preprocessing included filtering between 0.1 and 30 Hz using a third-order bidirectional Butterworth bandpass filter to remove extraneous activity, detrending to remove linear trends, and prestimulus baseline correction to eliminate amplifier drifts. To separate cerebral from noncerebral activity, we employed the extended Infomax algorithm to estimate independent components (ICs) on single trial ERPs separately in each channel. After whitening the IC data, we employed an automated procedure (Antonakakis et al., 2016) based on statistical analysis of the amplitude, variance, and number of zero crossings of individual ICs to identify rejection thresholds to identify artifactual components. More specifically, the kurtosis and skewness of each IC were used to eliminate ocular and cardiac artifacts, respectively, considering as artifacts ICs with more than 30% of their absolute z-score values >2. The remaining components underwent visual inspection, which, along with plotting of each component's surface location, allowed us to identify those components representing "true" brain activity that should be kept. These components were projected back to the time domain to obtain artifact-free ERP responses. All datasets showed clear P100 and P300 peaks, which are typically seen in visual ERPs. As an example of this procedure, Fig. 1 shows the average ERPs across all recording channels before (Fig. 1, top) and after (Fig. 1, bottom) processing, where the large eye-movement artifact around 800 ms is removed revealing a clear P300 component which is typically expected in the interval [300 700] ms poststimulus.

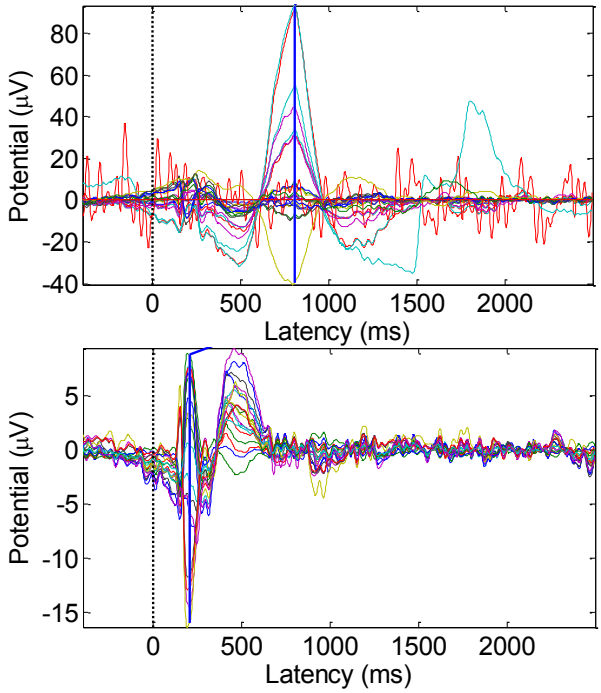

Fig. 1 Average ERPs of all channels before (top) and after (bottom) artifact removal. The large eye movement artifact around 800 ms is removed revealing a clear P300 component that was typically seen in the interval [300 700] ms poststimulus.

C. ERP Measures and Group Analysis

We grouped the cleaned 19 channels into five ROIs: Frontal (F3, Fz, F4), Central (C3, C4, Cz), Parietal (P3, Pz, P4), Left Lateral (F7, T7, P7), and Right Lateral (F8, T8, P8). The Fp1, Fp2, O1, and O2 channels typically contained some residual artifacts, such as muscle activity, and therefore were excluded from further analysis. Then we averaged activity on all channels within a region to obtain a time series representing the activity of that region.

We used a time window between 300 and 700 ms poststimulus within which to identify the P300 component for each subject and then computed the amplitude and

latency of the component in each region for all mTBI and control subjects. The data analyzed include all subjects all visits, for both controls and mTBI patients.

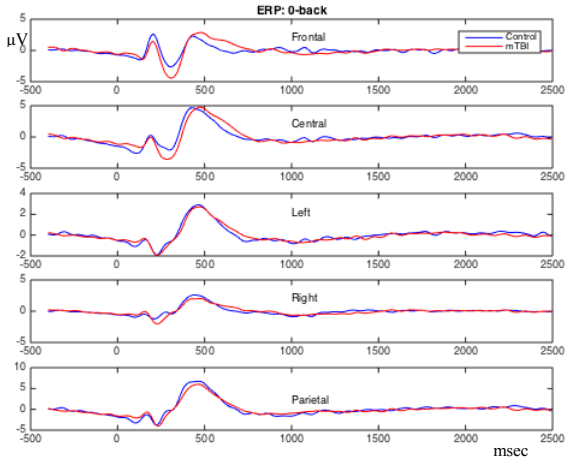

Fig. 2 Average ERPs in the 0-back test for control (blue) and mTBI (red) subjects.

The data structure was quite complicated as there were variables nested within subject (the two tests), groups, repeated measures, various regions, amplitudes and latencies, plus uneven handling of repeated measures in control and treatment groups. Repeated measures can be either in time or brain or locations due to repeated measurements under different experimental conditions. In other words, the various brain regions could be considered to

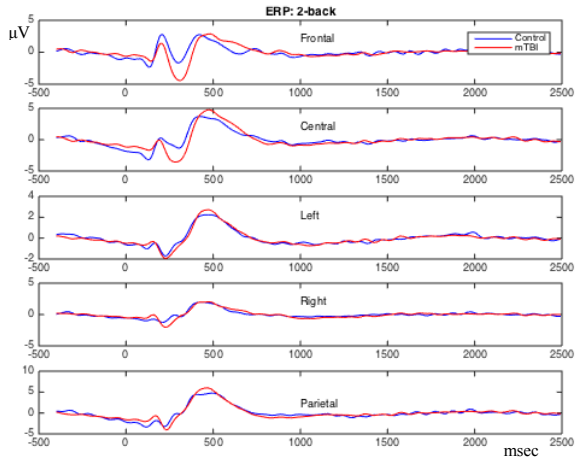

Fig. 3 Average ERPs in the 2-back test for control (blue) and mTBI (red) subjects.

provide repeated measures on the same subject.

We explored linear mixed models (also called multilevel models) that included random intercept per subject and per region nested within subject. This is how they account for correlated data within subject and for random differences found within subjects in the amplitudes and latencies in 5 regions. The fixed effects are quite simple: region, group, and interaction of region and group.

Thus, the data structure was initially set up by treating the regions as if they are repeated measures (or, more correctly, as if they are measurements made in several different conditions, where condition can be simply interpreted as a location of the brain surface), for a specified memory test and a specified visit. Twelve different models were explored under this arrangement.

The data structure was then set up differently, by treating the two memory tests as if they were repeated measures under different conditions, and then analyzing each region of the brain separately for each outcome variable, for a specified visit. Sixty different models were explored under this arrangement.

III. Results

MANOVA on response accuracy and response time showed that mTBI and control groups were significantly different ($F_{(2,184)} = 3.13$, $p = 0.0459 < 0.05$) and that normal subjects were in general faster and more accurate.

Similarly, MANOVA of P300 amplitude and latency across the 5 ROIs showed that mTBI and control groups were significantly different ($F(10, 65) = 2.25$, $p = 0.0251 < 0.05$).

In particular the models showed that latencies were lower in the control group primarily in the central region. This was true at every visit for the 2-back test. Fig. 2 and Fig. 3 show average waveforms for controls (blue tracings) and mTBI patients (red tracings), respectively, for the 0-back and 2-back memory tests. The distribution detail of two groups' central latency (0- or 2-back) are shown in Fig. 4.

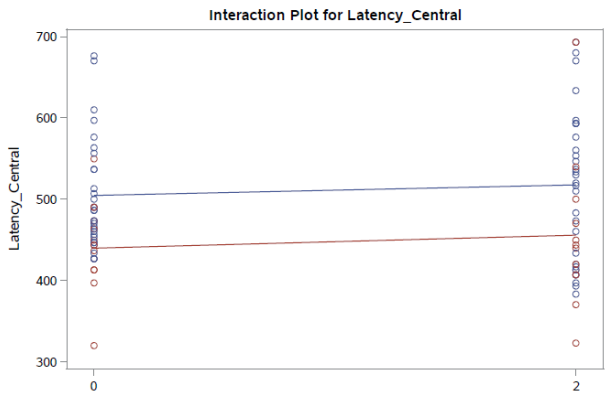

Fig. 4 Control (red) and mTBI (blue) group. Latency distribution plotted separately for 0 back and 2 back. Blue and red lines show the average value of the distributions. The latency of mTBI group is larger in both cases.

Additionally, longitudinal analysis per group and per memory test showed a time effect in the amplitudes, but not latencies, only for the 0-back memory test. However, confidence in it was low because the model coefficients were not significant. It looked like changes in time were minor, if at all present.

IV. Discussion and Conclusion

The above results combined point to the latency being the most interesting measure. Controls in general respond faster than mTBI patients, especially during the more difficult task (2-back). The fact that the mTBI patients are not improving at the rate that we might have expected is consistent with the literature and confirms previous findings that deficits in mTBI patients may persist for year (e.g., Castellanos et al., 2011). It also suggests that the repeat sessions in our study may be too close to one another to be able to see a P300 component latency recovery in the mTBI group.

Acknowledgment

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Alpha desynchronization/synchronization during working memory testing is compromised in acute mild traumatic brain injury (mTBI)

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Abstract

Diagnosing and monitoring recovery of patients with mild traumatic brain injury (mTBI) is challenging because of the lack of objective, quantitative measures. Diagnosis is based on description of injuries often not witnessed, subtle neurocognitive symptoms, and neuropsychological testing. Since working memory (WM) is at the center of cognitive functions impaired in mTBI, this study was designed to define objective quantitative electroencephalographic (qEEG) measures of WM processing that may correlate with cognitive changes associated with acute mTBI. First-time mTBI patients and mild peripheral (limb) trauma controls without head injury were recruited from the emergency department. WM was assessed by a continuous performance task (N-back). EEG recordings were obtained during N-back testing on three occasions: within five days, two weeks, and one month after injury. Compared with controls, mTBI patients showed abnormal induced and evoked alpha activity including event-related desynchronization (ERD) and synchronization (ERS). For induced alpha power, TBI patients had excessive frontal ERD on their first and third visit. For evoked alpha, mTBI patients had lower parietal ERD/ERS at the second and third visits. These exploratory qEEG findings offer new and non-invasive candidate measures to characterize the evolution of injury over the first month, with potential to provide much-needed objective measures of brain dysfunction to diagnose and monitor the consequences of mTBI.

Key words: concussion; qEEG; n-back; working memory; alpha desynchronization/synchronization.

Introduction

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The incidence of mild traumatic brain injury (mTBI), or concussion, is estimated to be over 6 per 1,000 people each year [1]. Patients with mTBI are predisposed to other injuries, particularly before all symptoms resolve [2]. Repeated mTBI increases the risk for subsequent neurological diseases, such as dementia, depression, and migraine [3]. The economic burden of mTBI rivals that of moderate and severe brain injuries due to loss of work productivity and forced early retirement [4]. It is difficult for physicians to rigorously diagnose mTBI, mainly from lack of objective markers to identify and quantify the injury.

Frontal lobe executive dysfunction is almost universally present in acute mTBI and usually persists for several hours or longer [5], but impaired executive function is hard to recognize. In attempts to detect dysfunction, abnormal electroencephalogram (EEG) or magnetoencephalogram (MEG) features have been described in the frontal lobe [6-8] shortly after injury. Challenges that involve or impact executive function activate a combination of top-down and bottom-up information processing pathways. When an external stimulus (eg. an image or sound) elicits perceptual representation (sensation), bottom-up processing occurs. When the cognitive process is influenced by higher mental functions such as motivation or expectation, top-down processing occurs [9, 10]. Top-down activities are mediated by alpha oscillations, and can be assessed by task-related executive functions [11, 12].

Working memory (WM) as a core executive function refers to the cognitive ability to transiently store and manipulate information in real time [9]. WM can be easily assessed by (visual) N-back testing, whereby, for example, letters are displayed on a computer screen and the patient is asked to press a button when a target letter appears (0-back), or if the letter that appears on the screen is the same one presented two screens back (2-back). Brain imaging can reveal the brain networks that are activated during N-back WM tests [10, 11]. Functional magnetic resonance imaging (fMRI) studies have shown that the ability to increase activation in WM circuitry is impaired in mTBI patients [12]. However, the

mechanisms by which brain resources are allocated and integrated to support WM functions, and the extent these processes are compromised in mTBI, are still unclear.

Brain activity, demonstrated by intra- or inter-regional interactions, is thought to result from neuronal synchronization and neural oscillations. EEG recordings during WM testing can identify cerebral oscillatory dynamic changes in the WM network, and so are well-suited to the study of mTBI. Oscillatory activity in the alpha band (8-12 Hz) is the dominant oscillation in human brains and is the only activity that responds to a stimulus with both decrease and increase in power, such that the alpha frequency event-related desynchronization (ERD) is followed by event-related synchronization (ERS) [13]. Alpha ERD is related to memory storage and ERS to memory retention, [14, 15], and so are the focus of our study. Alpha frequency oscillations represent thalamocortical interactions and are essential for information selection and storage functions, including attention and WM tasks [13, 16]. They relate to encoding and manipulation of spatial representations in WM [17] and play an important role in top-down control mechanisms [18]. Pathology can disrupt normal alpha synchronization physiologies in many ways. Alpha ERD during the WM task was reported to be lower in people with a high intelligence quotient, supporting a higher “neural efficiency” [19-21]. Alpha ERD during WM is associated with fronto-parietal network activity, supporting the alpha oscillation relationship to top-down network interactions [22], as shown in concurrent EEG and fMRI recordings [16]. Similar associations have been found in attention deficit/hyperactivity disorder (ADHD) studies [22, 23].

Comparison between evoked and induced activity has been overlooked in previous EEG WM studies [24]. Evoked or phase-locked activity is both time- and phase-locked to the stimulus and is directly driven by the eliciting event. Induced or non-phase-locked activity is time-locked, but not phase-locked to the stimulus and reflects the dynamics that control interactions within or between brain structures [24], representing frontal lobe function or top-down mechanisms [25-28].

Our study aimed to explore how cerebral oscillatory activities change in an acute/subacute mTBI setting. We analyzed evoked and induced EEG activity in a visual N-back WM paradigm to examine

differences in activity changes between mTBI patients and trauma controls. We specifically focused on induced and evoked activity in the 8-12 Hz range in acute mTBI. In addition to overall alpha power comparisons between mTBI and control groups, we also explored alpha power on specific sensors based on symptoms and neurometabolic changes at different stages after mTBI as reported in the literature [2, 29-33]. We show that induced and evoked alpha ERD or ERS are abnormal at different sensors or brain regions at different times during the month after mTBI.

Materials and methods

We designed the study to investigate the neural correlates of mTBI symptom evolution that we would expect during the first month after injury [2, 29-34]. The first time point, within 5 days of injury, was selected to measure WM performance during the acute phase of cognitive deficit, when changes in symptom scales, balance and neurocognitive testing [29], and neurometabolism [32, 33] would be expected. The second time point, 2 weeks after injury, was chosen to measure WM performance when most cognitive functioning begins to normalize [29, 32]. The third time point, one month after injury, was chosen to measure the expected continuing resolution of the mTBI-induced neurocognitive symptoms and to assess any possible residual learning impairment (compared to previous assessments and to peripheral trauma controls) [2, 33, 34].

Patients

The experimental protocol and informed consent documents were approved by an Institutional Review Board (Quorum Review IRB). All patients signed informed consents before participating in the study. Trauma patients between 18-50 years of age with either mTBI (diagnosed by emergency department

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physicians) or non-head mild traumatic injury (controls) were recruited from the emergency department of Huntington Hospital in Pasadena, CA. All mTBI patients had no evidence of skull fracture, brain laceration, or intracranial hemorrhage by computed tomography (CT) scan. Controls had minor non-head trauma not requiring surgery beyond skin sutures and dressings and had the ability to comply with the study protocol. Exclusion criteria included previous TBI, any significant major end-organ pathology such as heart disease or cancer; pregnancy, illicit drug use, sedative medications, alcohol abuse, and injuries or conditions that could affect study compliance.

Thirteen mTBI and seven trauma controls were recruited in this pilot study. Patients from the two groups were similar in age, gender distribution, years of education, body mass index (BMI), and handedness (Table 1). Injury type and locations are shown in Table 2. For the mTBI group, causes of injury were vehicle accidents (n=6), fall-related accidents (n=4), sports injuries (n=2), and bumping (n=1). For the control group, causes of injury were vehicle accidents (n=1), fall-related accidents (n=4), sports injuries (n=1), and dog bite (n=1). There were missed visits for some patients due to conflict of scheduling, as shown in detail in later Tables.

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Table 1. Baseline characteristics of patients.

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		mTBI (n=13)	Controls (n=7)	p-value
Mean Age (SD)	Mean (SD)	26.4 (7.0)	27.6 (6.0)	0.68*
Gender [n (%)]	Female	7 (54%)	4 (57.1%)	0.89#
	Male	6 (46%)	3 (42.9%)	
Mean Education (SD) (yrs)		14.2 (2.8)	13.9 (1.2)	0.74*
Mean BMI (SD) (kg/m^2)		29.0 (5.8)	28.40 (4.1)	0.78*
Handedness [n (%)]	R	11 (85%)	6 (86%)	1.00#
	L	2 (15%)	1 (14%)	
SAC score		25 (3.2)	NO SAC scores	

Abbreviations: BMI, body mass index; R/L, right/left; SAC, Standardized Assessment of Concussion; SD, standard deviation. * Two-tailed t-test; # Fisher's exact test.

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Table 2. Injury type and location

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Mild traumatic head injury (mTBI)					Non-head trauma controls				
Pt. ID	M/F	age	injury location	injury type	Pt. ID	M/F	age	injury location	injury type
mTBI007	M	18	head left front (F7/F3/T3/C3)	motor cycle	mTBI015	F	37	right forearm, cast on	fell on floor
mTBI014	M	23	head right front (F4-F8), whiplash	car accident	mTBI016	M	22	left shoulder	skate board
mTBI011	M	36	head, whole right side	fall off stairs, head on concrete	mTBI017	M	25	right ankle sprain 7/10	during playing basketball
mTBI013	F	28	right leg, head	fall off stairs	mTBI019	M	24	left knee and thigh	motor cycle accident
mTBI018	M	21	right side body and head	motorcycle crash	mTBI036[#]	M	30	left arm dog bite	dog bite while protecting his own dog
mTBI020	F	37	head back/left	softball hit	mTBI038	F	25	left foot	run over by car
mTBI031	F	35	back of head	car accident	mTBI040	F	22	both legs	fell and hit on legs
mTBI034	M	21	front left side	skateboard fall	mTBI041	F	36	feet and ankles	dropped log on feet and ankle twisted
mTBI035	F	25	Whiplash	car accident					
mTBI037	M		back right side of head	hit by 2x4 wood					

mTBI039	M	18	whiplash (front and both temporal headache)	car accident					
mTBI042	F	28	tree feel on left side of head	tree fell on head					
mTBI043	F		front left side of head	hit cabinet					

#: patient's head was too big for EEG headset.

Procedures

The brain cognitive challenge, or N-back WM test (N = 0, 2 to reflect the workload level of the task), was administered using E-prime software (Psychology Software Tools, Inc., Sharpsburg PA) on a Dell Precision T5610 with a 20 inch screen. Although different types of stimuli can be used for WM, in this study we used letters [35].

Overall, patients were comfortably seated in front of a computer screen at a distance of approximately two feet, and were instructed and tested for 0-back, then for 2-back. Instructions were given to each patient before each workload. Uppercase letters were displayed on the screen one at a time for 0.5 seconds, separated by a 2.4-second interval. All patients were asked to use the right hand to respond, regardless of handedness. For 0-back, patients were asked to look for the target letter “X”, and press 1 using their index finger when “X” appeared on the screen, or press 2 for all other letters, using their middle finger. For 2-back, patients were required to remember letters they saw previously. If the letter that appeared on the screen was the same as the letter shown two letters ago, patients were required to press 1; otherwise, they were to press 2 using the same fingers as before. Fig 1 illustrates the instructions given to each patient for this WM task. Patients were presented with instructions displayed

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at the start of each workload. First, all patients were required to complete a 1.5-minute practice block that included 30 trials at the beginning of each workload. At the end of practice block, response accuracy feedback was provided, and each patient could choose to redo the practice block, or continue to do task blocks. Second, after the practice block indicated that task instructions were clear and understandable for each patient, they were asked to proceed to do task trials, which included 3 blocks of 30 trials for each block and for each workload. The n-back task took about 12-25 minutes to complete, depending on each patient's performance.

Fig 1. Experimental instructions given to all patients. Letters will flash on the screen one at a time. For 0-back, when you see X, press 1 with index finger; otherwise, please press 2 with middle finger; For 2-back, if the letter that appears on the screen is the same as the letter you saw two letters ago, press 1 with index finger; otherwise, please press 2 with middle finger.

EEG Recordings

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Continuous EEG activity was recorded while patients engaged in the memory challenge tasks, using a 21-sensor, dry electrode system (Quasar Wearable Sensing, DSI-24, San Diego, CA). Sensor arrangement followed the international 10-20 system and were placed approximately at the locations Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, O2, M1, and M2. All activity was referenced to Pz. Sensor impedances were kept below 1 MOhm. EEG signals were sampled at 300 Hz, and bandpass filtered between 0.003–150Hz. Electrooculographic (EOG) and electrocardiographic (ECG) activity was recorded using two pairs of auxiliary sensors. The time of presentation of the letter stimuli, the patients' responses, and the type of test (0- or 2-back) were encoded with electronic pulses, which were saved with the EEG data for off-line analysis.

Data Processing

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The behavioral responses were summarized by accuracy (ACC) and response time (RT). ACC was calculated as the percentage of correct responses out of the total number of trials. RT was defined as the latency between stimulus onset and patient response.

All datasets underwent the same processing regardless of clinical classification of the patient, using EEGLAB version 13.4.3b [36] running in MATLAB R2014a (The MathWorks, USA) and custom software developed in-house. All EEG signals were re-referenced to the mean of two mastoid sensors (M1 and M2). The continuous EEG recordings were segmented into epochs, using the stimulus onset as a reference, including 500 ms before and 2500 ms after the stimulus onset. Individual epochs were baseline-corrected and bandpass filtered between 2 and 30 Hz. Furthermore, independent component analysis (ICA) [36] was used to remove eye blinks and cardiac and other muscle artifacts. Also, epochs that contained large artifacts, i.e., activity greater than three standard deviations (SDs) from the mean of a specific sensor, were rejected.

The epoched EEG data were decomposed into a time-frequency (TF) representation with logarithmic scaling between 2 and 30 Hz from fast Fourier transform and via Morlet wavelet [$e^{i2\pi tf}e^{-t^2/2\sigma^2}$] convolution with the single-trial EEG data performed in the frequency domain, followed by the inverse fast Fourier transform[27, 37]. In order to remove scale differences between individuals, all power values in the TF representation were normalized by decibels to the baseline power computed as the average power from -400 to -100 ms prestimulus at each frequency band [$dB\ power = 10 * \log10(\frac{power}{baseline})$]. Based on the TF plots and published data, alpha ERD (range 200-800 ms, 8-12 Hz) and alpha ERS (range 1000-2500 ms, 8-12 Hz) were then extracted for comparison, including total power, non-phase-locked power (induced power), and phase-locked power (referred to as phase-locked to stimulus onset, or evoked power), which were acquired by the following steps. First, ERP was calculated by averaging all trials.

Second, induced power was calculated as described as above from the differences between each trial and ERP calculated on time domain. Third, evoked power was calculated by subtracting the non-phase-locked (induced) from the total power [27, 37]. This was done separately for each sensor, condition, and patient.

Hypothesis Generation

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We based the following hypotheses on literature describing mTBI symptoms and neurometabolic changes [2, 30, 31, 34, 38-42].

Hypothesis 1: The first 5 days after mTBI is the acute phase of cognitive deficit associated with increased metabolic demands on the brain [30, 31]. Because cognitive function involves the frontal lobe, and reported acute symptoms indicate a “top-down” executive function impairment, we hypothesized that mTBI patients at the initial visit will have altered induced alpha ERD at the Fz sensor (located at the midline of the frontal lobe) during the 0-back task, i.e., even when the work load is minimal. The frontal midline sensor Fz was chosen for “top-down” function assessment based on previous auditory ERP and EEG alpha oscillation on visual facial preference studies [38].

Hypothesis 2: Previous cognitive evaluations and EEG studies indicate learning impairment in mTBI patients [2, 34]. We hypothesized that our longitudinal WM data would show group differences in learning, especially when using the more challenging 2-back task, and that these differences would be greatest 30 days post-injury (i.e., at the third visit). Learning is part of top-down executive function, measured by induced or non-phase-locked activity [39-41]. WM is mediated by the orbitofrontal cortex, an area that can be assessed by Fp1 and Fp2 sensors [42]. If controls, but not mTBI patients, were able to learn over the 30-day study time period, we would expect alpha ERD at the Fp1 and Fp2 sensors to decrease over visits in controls but remain stable in mTBI patients, or alpha ERD at Fp1/Fp2 sensors at the third visit during 2-back test differs between mTBI and control patients.

Statistical methods

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Evoked and induced alpha power measurements were analyzed by averaging individual sensors within and across patients and visits to derive summary statistics for the following variable clusters: frontal (Fz, F3, F4), central (Cz, C3, C4), parietal (Pz, P3, P4), left lateral (F7, T3, T5), right lateral (F8, T4, T6), and occipital (O1, O2). Group comparisons on patient baseline characteristics were done using two-sided t-tests or Fisher's exact tests. Longitudinal analyses were done using general linear mixed models with group (mTBI or Control) and visit (1, 2, or 3) as fixed effects and patient as a random effect. A term for the interaction between group and visit was included to evaluate varying group effects over visit. Group comparisons within visits were done using two-sided t-test. As this was an exploratory, hypothesis-generating study, no adjustments were made for multiplicity. A significant level of 0.05 was used for all tests. Analyses were done using PRISM v6.07 (GraphPad) and SAS v9.4 (SAS Institute, Cary, NC).

Results

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Behavioral Performance (ACC and RT)

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As seen in Table 3, for the 0-back test, neither ACC nor RT was significantly different between the mTBI and control patients. Considering all of the data simultaneously (but ACC and RT separately), there are no statistical differences between mTBI and control patients in ACC for the 2-back test.

Table 3. Mean (SD) response accuracy (ACC) and response time (RT) in N-back WM by visit

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	mTBI			Controls		
	Visit 1	Visit 2	Visit 3	Visit 1	Visit 2	Visit 3
0-back						
N	11	11	13	7	5	5
ACC	0.97 (0.03)	0.97 (0.04)	0.96 (0.05)	0.97 (0.05)	0.97 (0.02)	0.98 (0.02)

RT (ms)	466.4 (48.7)	446.8 (56.8)	444.9 (65.5)	492.3 (100.9)	457.6 (55.5)	488.0 (74.8)
2-back						
N	11	12	13	7	5	5
ACC	0.84 (0.07)*	0.89 (0.07)	0.88 (0.12)	0.91 (0.05)	0.89 (0.12)	0.92 (0.08)
RT (ms)	553.3 (130.6)	513.2 (95.3)	524.7 (154.9)	640.4 (206.4)	523.3 (145.0)	574.0 (189.6)

* p = 0.03. There are missing visits for some patients.

Induced alpha ERD at Fz sensor, 0-back test, first visit

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Fig 2 shows a comparison (mTBI vs. controls) of time frequency plots of mean induced alpha power of EEG at the Fz sensor during the 0-back test at the first visit. Despite "normal" behavioral performance measures (Table 3), total power of alpha ERD appeared to be greater (more negative) in the mTBI group compared to controls, as evidenced in Fig 2, Column 1. This difference is seen to derive from the induced rather than the evoked power (Fig 2 and Table 4; p = 0.08 for interaction between group and power type, p = 0.08 and 0.06 for total power and induced power, respectively). The induced alpha ERD differences between mTBI and controls appeared to differ only marginally (Table 4, p=0.06).

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Fig 2. Time-frequency plots (Fz sensor) of mean 0-back test, first visit. Column 1 shows total power, column 2 induced power, and column 3 evoked power. The rectangles on the induced power plots locate areas of excessive alpha ERD in mTBI (N=13) vs. control (N=7) patients.

Table 4. Comparison of Induced, evoked, and total alpha ERD between mTBI patients and controls during 0-back test at first visit.

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	mTBI (n=11)		Control (n=7)		P value
	Mean	SD	Mean	SD	
Induced power	-1.78	2.14	0.22	1.17	0.06

Evoked power	0.21	0.19	0.16	0.16	0.57
Total Power	-1.57	2.16	-0.05	1.28	0.08

When comparing induced alpha ERD at the Fz sensor during different workloads at the first visit, i.e., 0-back vs. 2-back, control patients' induced alpha ERD was numerically (but not significantly) lower during 2-back (-1.78+/-2.38) compared to 0-back (-0.22+/-1.17), while mTBI patients' induced alpha ERD during 0-back and 2-back was numerically similar (-2.01+/-2.50 during 2-back vs. -1.78+/-2.14 during 0-back).

Induced alpha ERD at Fp1/Fp2 sensors, 2-back test

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Fig 3 compares mTBI and control patients on time frequency plots of mean induced alpha power of EEG at the Fp1 sensor during the 2-back test for each of the three study visits (data for Fp2 sensor was similar, not shown). The figure suggests that controls used less alpha ERD as time passed after trauma while alpha ERD in the mTBI patients remained elevated (more negative) over time, though the effect of time did not statistically differ by group, nor was "visit" a significant main effect within the control group.

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Fig 3. Time-frequency plots (Fp1 sensor) of mean 2-back test, induced power, by visit and group. Excessive alpha ERD (white rectangles) remained in the mTBI group compared to controls.

Analysis by visit revealed a significant difference between groups at the third visit, with alpha ERD power less negative in controls compared to mTBI patients (p = 0.04, Fig 4. Data for Fp2 sensor was similar, not shown).

Fig 4. Mean (SE) induced alpha ERD from Fp1 sensor, 2-back test, by visit and group. Induced alpha ERD at the third visit was significantly different between mTBI and control group. The alpha ERD in the control group appeared to lessen with successive visits, while alpha ERD in the mTBI group appeared to remain elevated (more negative) over all visits.

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Induced alpha power at all sensors, all visits

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Summary of induced alpha ERD and ERS power for all sensors at all visits during 0-back or 2-back are shown in S1-S4 Tables.

Evoked alpha power at all regions, all visits

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For the 0-back test at the parietal location, for ERD there was an interaction between group and visit (p = 0.02). Analysis stratified by visit revealed a group difference at the second visit only (p = 0.03), with controls measuring higher (less desynchronization) than mTBI patients (Table 5), largely due to increased ERD (less desynchronization) in controls compared to mTBI patients (p = 0.04 for "visit" effect).

Table 5. Evoked parietal alpha ERD during 0-back by visit.

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Parameter	------mTBI ---------			---------- Controls----------			Group
	n	Mean	SD	n	Mean	SD	p-value
ERD PARIETAL Visit 1	11	0.14	0.11	7	0.08	0.07	0.20
ERD PARIETAL Visit 2	11	0.09	0.10	5	0.22	0.11	**0.03**
ERD PARIETAL Visit 3	13	0.12	0.12	5	0.10	0.14	0.76

For the 2-back test at the parietal location for ERS, controls were more synchronized than mTBI patients at the third (p = 0.04) visits (Table 6).

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Table 6. Evoked parietal alpha ERS during 2-back by time.

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Parameter	-------- mTBI---------			---------- Controls ----------			Group
	n	Mean	SD	n	Mean	SD	p-value
ERS PARIETAL Visit 1	11	0.01	0.05	7	0.01	0.05	0.84
ERS PARIETAL Visit 2	12	0.00	0.05	5	0.04	0.05	**0.13**
ERS PARIETAL Visit 3	13	0.00	0.04	5	0.04	0.04	**0.04**

There were no significant group differences among all other regions and visits, or for induced alpha power.

Discussion

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Our study observed identifiable qEEG changes between mTBI patients and non-head-trauma controls in a dynamic setting at times coincident with reports of evolving symptoms in the acute and subacute period after injury [2, 29-33]. Our study supports the hypothesis that induced frontal alpha power was excessive within 1 month after mTBI. The most significant difference we observed was that evoked parietal alpha power 2 weeks after injury in mTBI patients was more negative compared to trauma controls. Our findings suggest the potential for non-invasive measures for acute mTBI patients in the clinic. A strength of the study was the nature of the control group, often omitted in mTBI studies.The choice of the control population was purposeful: We used controls who experienced the stress of trauma coupled with an ER visit to minimize the possibility that EEG changes resulted from pain or other symptoms associated with peripheral trauma rather than specifically from head injury. Causes of injury among our mTBI patients were consistent with what has been reported most frequently among adults, namely vehicle accidents and falls [43].

Consistent with clinical acute/subacute symptom evolution [2, 29-33], we observed an improvement of WM behavioral performance after mTBI. N-back behavioral performance (ACC and RT) was similar in mTBI patients and controls one month post-injury, in agreement with other reports [44-46]. There were no significant differences in RT between controls and mTBI patients at any visit, although mean RT tended to be shorter in mTBI patients compared to controls, also consistent with the literature [45, 46]. This might be because of the relatively young age of the cohort we studied, similar to the age range of previous reports [45, 46]. In young age, relatively higher cognitive reserve and WM capacity can compensate impairment from mTBI, therefore behavioral performance remains similar; however, the cognitive reserve and WM capacity declines in older age [47, 48], possibly from reduced distraction control during WM in older adults [49]. Therefore, we can speculate that the behavioral performance in an older population can be significantly different after mTBI because of less cognitive reserve. Although at the present stage of our research we cannot correlate brain regions with WM performance, a

previous fMRI study indicates that right prefrontal cortex appears to be critical for WM network functioning and performance [45].

Two existing hypotheses are supported by our qEEG results and one new hypothesis has been generated. Our data indicate that alpha power, specifically induced and evoked alpha power from N-back WM testing, is different between mTBI and control patients, suggesting that alpha ERD/ERS is potentially useful in the diagnosis of mTBI.

Hypothesis 1: Induced alpha ERD at Fz sensor is marginally different ($p = 0.06$) between mTBI and control groups. For 0-back testing at the first visit, we found that induced alpha ERD during encoding tended to be greater (more negative) in mTBI patients compared to controls, indicating lower neural efficiency and impaired WM capacitiy after mTBI [21, 50]. Because the behavioral responses of mTBI patients during the 0-back task were similar to those of controls, this excessive frontal alpha ERD during a simple task may imply a compensatory attentional response and is a likely indicator of weak top-down control and lack of attention during WM encoding after mTBI. This observation is consistent with published complaints of mTBI patients regarding their inability to focus or “inattention” which, to date, lacks an objective clinical measure [29]. The Fz alpha ERD during 2-back was similar between mTBI patients and controls. The different workload results support that qEEG-based workload assessment can be used to indicate the resilience of the WM network [51]. Further investigation to examine if the workload effect is revealed in other sensors besides Fz may be informative, and will be addressed in future studies.

Hypothesis 2: Induced alpha ERD at Fp1/Fp2 sensors at the third visit during 2-back tasks differs significantly between mTBI and control groups. Although the N-back is used for testing WM rather than learning, our longitudinal data afforded us the opportunity to examine N-back processing changes over time to evaluate learning effects. Alpha ERD during 2-back tended to decrease in controls from the first to the third visit (though not significantly), but seemed to remain unchanged in mTBI patients over this time period; these patterns are consistent with a learning effect in controls, but suggest a learning

impairment in mTBI patients. The greater induced alpha ERD of mTBI patients compared to controls during 2-back at the third visit also indicates lower WM capacity after mTBI, consistent with previous qEEG evidence of greater ERD in people with lower WM capacities [21, 50]. Lower WM capacity could contribute to learning impairment after mTBI, and both might contribute to long-term cognitive deficits (specifically regarding impaired attention and memory) after mTBI [52]. This mTBI-induced learning deficit reflects reduced brain reserve. An important consequence of mTBI impaired learning might be reduced risk aversion, which may contribute to mTBI patients being three times more likely to sustain another mTBI compared to controls [53].

The new hypothesis generated by our analysis is based on our finding that alpha ERD/ERS differs between mTBI and control groups two weeks after injury. In an analysis of evoked power, we observed that alpha ERD in the parietal area of controls was significantly higher than in mTBI patients but only during the 0-back test at the second visit. This difference is largely due to a significant increase of evoked alpha ERD in controls at visit 2. Whether this is a spurious finding or a real group effect during their second visit is worth further study. Analysis of evoked alpha power during 2-back demonstrated that parietal alpha ERS was significantly higher in controls compared to mTBI patients at the third visits, which indicates that a deeper evoked alpha power defect persisted with higher workload for a more prolonged period in these patients.

The most common cognitive symptom after mTBI is feeling “slowed down”, “in a fog”, or “dazed,” [54, 55] indicating abnormal sensory perception assessed by evoked activity [56]. However, there are no published reports about how the “foggy” symptoms evolve after injury, especially in the acute phase.

Evoked alpha power contributes to visual perception [56]. These abnormal parietal evoked alpha ERD/ERS measures after mTBI may correlate with the “dazed” feelings reported after acute mTBI, a symptom that usually resolves within a month after injury [54-56]. The alpha ERD is closely related to memory storage [14], and ERS is associated with retention [15]. Therefore, our results support that

mTBI might impair information storage for a low-load task and impair information retention for a higher-load task 2 weeks post-injury. The information retention deficit for the higher-load task persisted even at 1 month post-mTBI, when behavioral performance is recovered comparable to controls. So, although the mTBI patients' behavioral performance "normalized" at the third visit, they were still using extra effort to compensate for an information retention deficit. It is puzzling that evoked alpha power did not demonstrate a deficit in mTBIs during the first week post-injury. Based on our results, a possible explanation is that the acute injury sets off structural, metabolic, inflammatory, and oxidative processes that affect neurotransmission slowly, peaking a week after injury when they are reflected in the qEEG pattern [57]. Further investigation of this hypothesis will test the possible interpretations of acute/subacute pathophysiologies. In addition, while WM is critical for short-term memory, and short-term plasticity reflects immediate adaptation to temporary environmental changes, it is strongly linked to long-term memory formation from functional and anatomical overlap with alpha and theta oscillation involvement [58-60]. Therefore, this abnormal alpha ERD during 0-back in mTBI patients might also result in the learning impairment seen by the abnormal alpha ERD during the 2-back challenge.

Although not significant in our small study, our results that induced alpha ERD in the control group tended to be greater during 2-back compared to 0-back are consistent with previous findings that alpha ERD increases correspondingly with higher workload [61-63]. Induced alpha power has been demonstrated to increase for internal attention (inhibition of incoming sensory information that requires internal focus, motivation, or expectation, indicating greater top-down control for internal attention than for external attention [64]. However, in our study, induced alpha ERD was not greater during the 2-back task compared to the 0-back among mTBI patients, further implying that the mTBI patients were already challenged by the 0-back task and overtaxed by the 2-back. The 0-back and 2-back were presented with increasing difficulties, as in other studies. Different brain regions can be involved during different workload, which can be influenced by different pathophysiology[65]. For example, higher activation of bilateral inferior frontal gyrus pars triangularis with higher n-back workload were seen in

healthy at risk for major depressive disorder individuals[66]. Further, it will be interesting to know whether or not, and how, the sequence of different workload influences the brain activity, which might be another topic to explore.

Extensive studies on alpha ERD and ERS suggest that event-related modulation of alpha power reflects sensory information gating in the cortex via selective suppression and selection [13, 67, 68]. Alpha oscillatory activities are modulated via frontothalamic loops during WM [69]. These alpha oscillations during WM actively prevent task-irrelevant stimuli from intruding on the WM buffer [70]. Alpha ERD/ERS of the frontoparietal region is known to be critical for WM [22, 71], supporting its role in top-down modulation and attention [71, 72]. Along with alpha ERD and ERS in WM, alpha oscillations of the fronto-parietal region have been demonstrated to reflect intelligence [19-21, 73]. The WM in healthy patients can be enhanced by neurofeedback training of alpha rhythms [74]. Similar neurofeedback training could potentially help mTBI patients in their recovery.

Neurometabolic changes after mTBI: the functional association between WM performance and alpha (not theta) oscillation may be related to decreased cholinergic transmission [75]. Rats subjected to mTBI show increased expression and function of the nicotinic acetylcholine receptor [76]; the acetylcholinesterase inhibitor donepezil reduces neuronal death and cognitive impairment in this model by increasing nicotinic acetylcholine-receptor activation [77]. The relationship between acetylcholine and alpha rhythms during attention/memory tasks indicate that alpha oscillations are involved in temporal coding organizations in sequential tasks similar to those we studied here [75, 78, 79], suggesting a cholinergic mechanism contributing to the learning impairment found in mTBI patients. If validated, the cholinergic mechanisms of impaired function after mTBI could be amenable to pharmacologic intervention. Even more speculative, the alpha ERD differences at the third visit, shown at Fp1 and Fp2, are localized to the orbitofrontal cortex, a location where amyloid and tau pathology is concentrated in Alzheimer's disease (AD) [80-82], another point connecting mTBI to the known increased risk of subsequent AD and general cognitive deficits [3].

There are limitations to our study. First, the study was exploratory and, as such, was not powered for any particular comparison. Second, because of the exploratory nature of our study, multiple statistical tests were performed without adjusted significance levels; therefore, the reported p-values should be considered as support for further research into specific hypotheses rather than as conclusive evidence of associations. This is likely a common problem for complex EEG data processing due to the numerous data points that are collected. Third, it has been demonstrated that preprocessing may distort EEG signals [83]. For example, reference signal can be dynamic and inevitably affects EEG data[84]. We attempted to minimize distortion by using only widely validated pre-processing procedures [36]. Although beyond the scope of the current paper, future studies using reference electrode standardization technique should be explored[85, 86]. Finally, whereas mTBI patients are twice as likely to be male than female [1], we enrolled a similar number of males and females (7 and 6, respectively). Our experience may reflect greater altruism towards research in females and/or a greater proportion of female athletes at increased risk for mTBI [87] in our local population. Given these limitations, the fact that our findings regarding alpha power during WM task performance are consistent with previous publications is reassuring and supports further studies in larger populations over longer time courses with pre-specified hypotheses and control of type 1 error.

Conclusions

In this pilot study, qEEG during a simple WM paradigm revealed that neurofunctionality is compromised in mTBI. The results support our hypotheses and suggest that alpha ERD and ERS differ between mTBI patients and trauma controls throughout the first month after injury. We demonstrated for the first time that frontal induced alpha ERD was marginally greater in mTBI patients during a low-work load task (0-back). Secondly, we found that induced alpha ERD for a higher-load task (2-back) did not normalize by one month after mTBI vs. trauma controls, consistent with a learning impairment reported after mTBI [34, 88]. Third, consistent with commonly reported symptoms of “foggy” or “dazed” feelings,

our data show that parietal evoked alpha ERD/ERS was greater (more negative) in mTBI after two weeks. Our data notably reveals that the mTBI patients are not fully recovered at one month after injury, thus correlating EEG testing on later visits with careful residual symptom assessment may be useful. These results make it interesting to test prospectively if qEEG findings underlie the frequent post-traumatic symptoms. As the natural history and consequences of mTBI remain elusive, our results suggest that qEEG during an executive function paradigm in longitudinal studies will help identify the consequences that arise from mTBI, and have value for the diagnosis and monitoring of patients.

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Deleted: Author Contributions¶
Conceived and designed the experiments: XA MGH. Performed the experiments: XA MGH. Analyzed the data: XA MS LL GZ ANF JP MGH. Wrote the paper: XA MGH. RG led patient recruitment. All authors contributed toward final manuscript. ¶
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Supporting information

S1 Table. Induced alpha ERD during 0-back. Induced alpha ERD from all sensors during 0-back test were listed in the table, by visit and group.

S2 Table. Induced alpha ERS during 0-back. Induced alpha ERS from all sensors during 0-back test were listed in the table, by visit and group.

S3 Table. Induced alpha ERD during 2-back. Induced alpha ERD from all sensors during 2-back test were listed in the table, by visit and group.

S4 Table. Induced alpha ERS during 2-back. Induced alpha ERS from all sensors during 2-back test were listed in the table, by visit and group.

Abstract accepted for 12th World Congress on Brain Injury for March 29 – April 1, 2017 in New Orleans

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Thalamocortical dysrhythmia after mild Traumatic Brain Injury: a working hypothesis

Abstract

Traumatic brain injury (mTBI) is one of the most common neurological disorders affecting up to 500,000-1,000,000 individuals in US and Europe per year. Individuals with mild TBI (mTBI) are characterized by great heterogeneity in terms of etiology, pathology, and severity, with injuries that can be elusive to standard clinical techniques, thus mTBI diagnosis is a challenging problem. mTBI patients may suffer from chronic disabilities that can progress over days, weeks, and even years. Previous electrophysiological studies reported reductions in alpha band activity during resting state in mTBI patients relative to healthy non-head trauma controls that can be observed over extended periods of time after injury (Arakaki, 2016). A reduction in alpha-band power can be attributed to mechanisms affecting the thalamocortical network (Nuwer, 2005), possibly associated with deficits in cholinergic neurotransmission (Tenovuo 2006). Disrupted large-scale intrinsic connectivity has been also observed following TBI (Sharp, 2014). We have recently shown that stroke-related cortical lesions induce pathological alterations to the thalamo-cortical interactions, or thalamocortical dysrhythmia (TCD); this arises by attenuating the cortical drive onto the thalamus and driving it into a low bursting regime, which further propagates to the neocortex through divergent intrathalamic circuits (van Wijngaarden, 2016). This low-frequency TCD dynamics can account for a variety of non-specific symptoms (e.g., post-stroke pain and fatigue, mood-related disorders, etc.) that are apparently dissociated from the lesion's site and temporal onset. Here we investigate whether the same signatures of TCD emerges acutely following mTBI. We examine the resting state EEG recordings of 12 mTBI patients with those of non-head trauma controls within one week of injury, and 14 days and 30 days after the traumatic event. Preliminary results over aggregated data in the acute phase show a pattern of increased delta-band power in the frontal areas relative to healthy controls with no differences in the alpha band. The patterns of spectral power distribution unmasked at the individual level show high heterogeneity that depends on the lesion site and severity, suggesting that diagnostically relevant EEG patterns could be revealed taking into account those specific individualized features.

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Source Connectivity Analysis Can Assess Recovery of Acute Mild Traumatic Brain Injury Patients

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Objectives: In this study we investigated whether source connectivity analysis of resting state Magnetoencephalographic (MEG) activity can detect patients with mild traumatic brain injury (mTBI).

Methods: Thirteen acute mTBI patients 18-50 years of age and eight age- and sex-matched controls with no head injury were recruited for the study. Approximately 5 minutes of continuous MEG was acquired on three different visits, two weeks and four weeks, respectively, after the first recording, using a CTF whole-head system with 66 axial gradiometer sensors. Data were sampled at 625 Hz and bandpass filtered between 0.1– 80 Hz. Linear drifts, line noise, and eye movement artifacts were minimized using a notch filter at 50 Hz, and a blink detection procedure based on signal-space projection. Estimation of intracranial sources for each subject was based on dynamical Statistical Parametric Mapping (dSPM). The Desikan-Killiany atlas, consisting of 68 brain regions of interest (ROIs), was used for common co-registration of sources. Functional connectivity brain networks among the intracranial sources, including connectivity strength and directionality, were measured using Granger causality (GC). After co-registration of all data onto the same atlas, the intracranial sources, their activation, and the resulting connectivity networks were averaged across all subjects of the same group. The two groups were compared using MANOVA with group and recording session as the independent variables and number of ingoing (IN) and outgoing (OUT) connections in each ROI as dependent variables. The level of statistical significance was p=0.05, corrected using the false discovery rate (FDR) method. Our analysis focused on the delta band, source activations between 0.1-4 Hz.

Results & Discussion: We found that mTBI subjects had a larger number of stronger connections compared to controls. The IN connections were significantly different in two regions, (a) the right entorhinal cortex of the medial temporal lobe and the (b) supramarginal gyrus of the left parietal lobe. The OUT connections were significantly different in five regions, (a) the isthmus of right cingulate gyrus, (b) the pars triangularis of the left inferior frontal gyrus, (c) the right precentral gyrus, (d) the right postcentral gyrus, and (e) the precuneus of the superior parietal lobule. These areas are involved with spatial memory, perception of visual space, emotion formation and processing, learning, and memory, regions also known to be affected in Alzheimer's disease.

Conclusions: Our results indicate that GC can detect injury in patients with mTBI during the acute phase and correlates well with clinical symptomatology, and suggest that GC may be used as a reliable biomarker of mTBI that can help with the diagnosis, prognosis, and assessment of treatment effectiveness.

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Assessing Recovery of Acute Mild Traumatic Brain Injury Patients using Diffusion Tensor Imaging

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Background and Objectives: In the present study we investigated whether Diffusion Tensor Imaging (DTI) can be used to assess recovery in patients with mild traumatic brain injury (mTBI).

Methods: Eleven acute mTBI patients 18-50 years of age and seven age- and sex-matched controls with no head injury were recruited from the emergency department of Huntington Memorial Hospital in Pasadena, CA. Images were acquired on three different visits, two weeks and four weeks, respectively, after the first recording, using a 3.0 T scanner for approximately 12 min of total imaging time. Diffusion images were collected along 32 directions with an isotropic voxel size of 2.5 mm3. An additional image with no-diffusion weighting was used as a reference. Image distortions, resulting from susceptibility-induced and by eddy current-induced off-resonance fields, were corrected using routines from the software package FSL. An affine linear registration routine part of FSL was also used to align the 32 images to the reference image. For each DTI dataset, diffusion Fractional Anisotropy (FA), Mean Diffusivity (MD), Apparent Diffusion Coefficient (ADC), and probabilistic tractography were estimated using FSL and the software package MedInria, with an FA threshold of 200, a minimum length for the detected fibers of 20 mm, and volume sampling every 5 voxels. To perform a quantitative analysis across the two groups, we first used the Johns Hopkins University tractography atlas to define 20 regions of interest (ROI), and the scans from the control subjects to create a reference database that included the mean and standard deviation values in each ROI. Then we computed z-scores for each subject's data and compared the groups using MANOVA with p value set at 0.05, corrected for multiple comparisons, considering group and visit as the independent variables.

Results & Discussion: The two groups were significantly different in FA values, but not in ADC or MD. Furthermore, FA values were significantly different only on the first visit, but not the second or third. The ROIs with the largest differences were the left and right superior longitudinal fasciculi. These areas, and their four distinct components, are involved with motor behavior and association tasks, perception of visual space, spatial attention, language articulation, and working memory.

Conclusions: Our results indicate that FA is a sensitive measure to detect injury in patients with mTBI during the acute phase, and it can also quantify improvement over time that correlate well with clinical measures and subjective patient-reported symptomatology. These findings suggest that FA may be used as a reliable biomarker of mTBI that can help with the diagnosis, prognosis, and assessment of treatment effectiveness.

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Alpha power during working memory is compromised in acute mild traumatic brain injury

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Background and Objectives: We aimed to explore alpha power during working memory (WM) processing in a longitudinal study of acute mild traumatic brain injury (mTBI).

Methods: We used quantitative electroencephalography (qEEG) to explore alpha frequency power during WM processing in an ongoing acute mTBI study. Thirteen acute mTBI patients and seven non-head trauma controls, 18-50 years of age, were recruited from the emergency department of Huntington Memorial Hospital in Pasadena, CA. Brain challenges using the N-back (0-back and 2-back) WM test were administered. Behavioral performance and EEG data from 21 recording electrodes were collected at three different recording sessions: within one week, 14 days, and 30 days after injury.

We observed the symptomatic progression of the TBI patients over the three visits and analyzed the EEG alpha power overall (MANOVA) and for specific channels (exploratory approach), relating the analysis results to the patients' symptoms at each visit.

Results & Discussion: During the first visit, mTBI patients did not realize they were not well; they were "a little shaken up," indicating an executive dysfunction. Compared to controls, the 0-back responses of mTBI patients showed excessive alpha event-related desynchronization (ERD) in the frontal areas (Fz) that was not phase locked to the eliciting events, consistent with an executive dysfunction.

At the second visit, mTBI subjects knew they were not well and their experience was discribed "like a dream." The phase-locked alpha power computed over parietal regions was significantly lower in the mTBI group, consistent with the "dream-like" detached perception of the real world.

At the third visit, they knew they were better, but still they were not back to "normal." However, no different alpha powers were shown during 0-back test.

Behavioral responses to the 2-back trials showed initial WM impairment in the mTBI group that improved at the later visits. In response to the 2-back task, the frontal (Fp1) alpha ERD was decreased in the controls between the first and third visits, possibly indicating a learning mechanism. In mTBI patients, however, no decrease was observed over the three visits, indicative of a learning impairment that correlated well with their feelings that they were not back to "normal".

Conclusions: Our results indicate that alpha ERD during WM processing is sensitive and correlates well with the evolution of subjective symptoms occurring in acute mTBI. These results suggest that WM parameters may have significant potential as objective biomarkers that can reliably quantify mTBI symptoms and may also help with the diagnosis, prognosis, and monitoring of treatment efficacy.

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Altered dynamics of the thalamo-cortical system following mild Traumatic Brain Injury: a combined experimental and theoretical study

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Mild traumatic brain injury (mTBI) is one of the most common neurological disorders and one of the most difficult to diagnose. The initial trauma can lead to a cascade of delayed neurodegenerative events, e.g. diffuse axonal injury and/or excitotoxic neuroinflammation, affecting distal brain areas and causing a variety of adverse sensory, motor, cognitive and affective outcomes which can persist for weeks or even months and lead to severe disability due to cumulative damage (Sharp et al. 2014; Arakaki et al, 2016). Because of its diffuse nature, mTBI is difficult to diagnose and no clear biomarkers exist. Electrophysiological methods and computational modelling may shed light on the disruptions in large-scale neural networks involved in the functional deficits following mTBI. Indeed, we have recently shown that stroke-related cortical lesions induce pathological alterations to thalamo-cortical system, or thalamocortical dysrhythmia (TCD). This arises by attenuating the cortical drive onto the thalamus, switching the latter into a low bursting regime, which further propagates to the neocortex through divergent intra-thalamic circuits (van Wijngaarden et al., 2016). TCD low-frequency dynamics can account for a variety of non-specific symptoms that are dissociated from the lesion site itself. Here we investigate whether such a network mechanism is implied in mTBI.

We investigated brain activation profiles in 10 mTBI patients using resting state EEG after 7, 14 and 31 days following injury, as well as a group of age matched control participants. Our results show a pattern of increased delta activation in the frontal electrodes, which is consistent with (Lianyang et al. 2015; Huang et al., 2009) and an attenuation in the beta-band power relative to healthy controls in the acute phase, which gradually recovers after four weeks. At the individual level, the spectral power distributions show high heterogeneity that depends on the lesion site and distribution, suggesting that diagnostically relevant EEG patterns could be revealed taking into account those specific individualized features. To explain the origins of these alterations, we developed a detailed spiking model of the thalamo-cortical circuits to identify the cellular and network mechanisms by which different thalamo-cortical pathways are entrained by means of propagating low-frequency oscillations beyond the restricted region of the diffuse mTBI lesions, giving rise to the associated symptoms.

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Working memory testing reveals neuroplasticity acutely and longitudinally after mild traumatic brain injury (mTBI)

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Background and Objectives: There are increasing concerns about mild traumatic brain injury (mTBI) because of its effects in later life. However, objective markers that help physicians quantify the injury are still understudied. We aim to investigate the underlying neuroplasticity reflected by working memory processing after mTBI.

Methods: We used cognitive brain challenges to explore neuroplasticity in acute mTBI. Brain activities during the N-back working memory (WM) test was investigated using quantitative electroencephalography (qEEG) in an acute and longitudinal mild traumatic brain injury study. mTBI patients (n=22) and controls (n=9) (trauma patients without head injury), 18-50 years of age, were recruited from the emergency department of Huntington Memorial Hospital in Pasadena, CA. Brain challenges were administered using E-prime software. Data were collected from 21 recording head sensors at four visits: within 1 week, 14 days, 30 days, and 6-12 months after injury (with some missing visits). Behavioral performance as well as spectral power were analyzed to compare the two groups. Brain WM processing were also evaluated by event-related potentials (ERPs) P300, and corresponding EEG signal (ir)regularity or "noise" level measured by spectral entropy (SE). High SE means signal is more irregular and "noisier".

Results & Discussion: Behavioral performance during 0-back challenge was similar between the two groups, though mTBI patients had significantly lower accuracy than controls during 2-back at the first visit. qEEG analysis revealed altered brain activities in mTBI group during 0-back: alpha and beta power were lower in mTBI patients than controls at the second and third visit. Further, theta power during 2-back at second visit were significantly higher (P=0.0099 and 0.0018) in controls (0.73+/-0.37 and 0.96+/-0.46) compared to mTBI (0.21+/-0.41 and 0.09+/-0.55) patients at the left and right temporal regions. When comparing regional theta power from first visit to second visit, controls were increased ($p<0.005$), indicating a learning mechanism, while mTBIs were not changed ($p>0.10$). SE analysis of the EEG signals during the P300 time window demonstrated more irregular or noisier brain signals in mTBI patients. These changes indicate neuroplasticity acutely and longitudinally after mTBI, consistent with learning impairment and "noisier" brain after mTBI. The spectral power and SE under WM challenge are sensitive measures of neuroplasticity after injury, and could be potential objective mTBI markers to help diagnosis, prognosis, or treatment management.

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Plasma metalloproteinase-9 (MMP9) changes in acute mild traumatic brain injury (mTBI) and correlates with quantitative EEG

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Background and Objectives: We aim to explore blood plasma levels of matrix metalloproteinase-9 (MMP9) in acute mild traumatic brain injury (mTBI), and test if MMP9 levels correlate with quantitative electroencephalography (qEEG) during working memory (WM) testing.

Methods: Study participants were recruited from the emergency department of Huntington Memorial Hospital in Pasadena, CA, consisting of thirteen acute mTBI civilian patients and seven controls who were trauma patients without head injury ranging between 18-50 years of age. Blood samples were collected from three time points: within 1 week, 14 days, and 30 days after the injury. To study blood-brain-barrier (BBB) integrity, we quantified three MMP9 peptides (SLGPALLLLQK, QLSLPETGELDSATLK, and LGLGADVAQVTGALR) using liquid chromatography and mass spectrometry with stable isotope standards. We also employed qEEG at each visit to investigate alpha frequency power during N-back WM processing.

Results & Discussion: We detected the presence of all three MMP9 peptides in blood plasma. We observed that MMP9 peptide levels in both mTBI and controls were decreasing in abundance in the 2-4 weeks after injury compared to the first week. Further, the MMP9 levels of the LGLGADVAQVTGALR peptide but not the peptides from the pre-protein, were significantly higher in the mTBI group 2-4 weeks after the injury, consistent with known "secondary injury" phenomena. MMP9 levels correlated with alpha power during WM testing, at the first visit for the controls but not for the mTBI patients, at specific brain regions during different WM load.
Elevated MMP9 levels indicate the BBB integrity is compromised acutely after mTBI. The correlations between MMP9 and alpha power during WM that we only found in the trauma controls need further investigation.

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Plasma Lipid Metabolism is altered in Acute Mild Traumatic Brain Injury

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Background and Objectives: Lipids are the source of signaling and inflammatory molecules that may contribute to trauma pathology. Accordingly, we examined levels of lipid species and a lipolytic enzyme activity in a one month longitudinal study of acute mild traumatic brain injury (mTBI).

Methods: We recruited mTBI patients (n=21) and non-head trauma controls (CT, n=9), aged 18-50 years from the emergency department of the Huntington Memorial Hospital (Pasadena, CA). Symptom progression was observed within days of injury (W1), and four weeks thereafter (W4). Plasma lipids collected at W1 and W4 were extracted and unesterified (UFA) and esterified fatty acids (EFA) quantified using negative ion/chemical ionization gas chromatography/mass spectrometry. Plasma glycerophospholipids (GP) and sphingolipids (SP) were analyzed using liquid chromatography tandem mass spectrometry. Plasma phospholipase A_2 (PLA_2) activity was determined using a fluorescence-based activity assay.

Results: mTBI participants' plasma at W1 had higher levels of 24 UFA species that included seven saturated fatty acids (SAFA), seven monounsaturated fatty acids (MUFA), four omega-3, and six omega-6 polyunsaturated fatty acids (PUFA). The sum of all SAFAs, MUFAs, omega-3 PUFAs, omega-6 PUFAs, and PUFAs were higher in mTBI plasma at W1. For EFA at W1, two omega-6 (homo-γ-C20:3n-6, C22:4n-6), one omega-3 (C20:5n-3), the sum of omega-3 PUFAs, and the omega-3 to omega-6 ratios (omega-3 index) were lower in mTBI than in CT. At W4, esterified C19:0 and C22:3n-3 levels were lower while C24:1 was higher in mTBI compared with CT. The UFA to EFA ratio that estimates endogenous lipolysis of fatty acids was higher in mTBI than CT for three SAFAs, two MUFAs, two omega-6, two omega-3, and the ratio of the sum of UFAs to the sum EFAs. Calcium dependent PLA_2 activity was higher in mTBI plasma at W1 but not at W4. mTBI PLA_2 activity at W1 positively correlated with most UFA species except C18:2n-6 and C18:3n-6 but correlated only with two UFA (C20:1, C22:1) in CT. PLA_2 activity negatively correlated with seven EFA species.

Conclusions: Our data showing higher UFA, UFA to EFA ratios, and correlation of UFA with PLA_2 indicate excessive lipolysis in early mTBI. A lower omega-3 index suggest excessive oxidative breakdown of omega-3 in mTBI. We propose that early intervention using strategies that reduce lipolysis may attenuate tissue damage linked to mTBI. Additionally, measurements of fatty acid fluctuations may be useful in discovering new therapies and monitoring resolution of mTBI.

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