Published in final edited form as:

Conf Proc IEEE Eng Med Biol Soc. 2018 July ; 2018: 5414–5417. doi:10.1109/EMBC.2018.8513500.

Craniectomy, Alterations in fMRI Functional Connectivity and Cognitive Performance in Developmental Model of TBI*

Saman Sargolzaei [Member IEEE],

David Geffen School of Medicine (DGSOM), UCLA, Los Angeles, CA 90095, USA.
(ssargolzaei@mednet.ucla.edu)

Yan Cai,

David Geffen School of Medicine (DGSOM), UCLA, Los Angeles, CA 90095, USA

Melissa J. Walker,

David Geffen School of Medicine (DGSOM), UCLA, Los Angeles, CA 90095, USA

David A. Hovda,

David Geffen School of Medicine (DGSOM), UCLA, Los Angeles, CA 90095, USA; UCLA Department of Molecular and Medical Pharmacology, Los Angeles, CA 90095, USA

Neil G. Harris, and

David Geffen School of Medicine (DGSOM), UCLA, Los Angeles, CA 90095, USA

Christopher C. Giza

David Geffen School of Medicine (DGSOM), UCLA, Los Angeles, CA 90095, USA; University of California Los Angeles Mattel Children's Hospital, Los Angeles, CA 90095, USA.
(cgiza@mednet.ucla.edu)

Abstract

Experimental models have been proven to be valuable tools to understand downstream cellular mechanisms of Traumatic Brain Injury (TBI). The models allow for reduction of confounding variables and tighter control of varying parameters. It has been recently reported that craniectomy induces pro-inflammatory responses, which therefore need to be properly addressed given the fact that craniectomy are among the control procedures for TBI experimental models. The current study aims to determine whether craniectomy procedure induces alterations in Resting State Network (RSN) in a developmental model. Functional Magnetic Resonance Imaging (fMRI) data-driven RSN show clusters of peak differences (left caudate putamen, somatosensory cortex, amygdala and piriform cortex) between craniectomy and control group, four days post-craniectomy. The Novel Object Recognition (NOR) task revealed impaired cognitive memory in the craniectomy group, accompanied by alterations in the RSN. The evidence supports craniectomy-induced neurological changes which need to be carefully addressed, considering the inclusion/exclusion of craniectomy as control procedures for developmental models of TBI.

*Research supported by NIH grant R01NS27544, University of California Los Angeles (UCLA) Research Safety and Animal Welfare Administration (RSAWA), UCLA Eastern Labs for Brain Injury, UCLA Steve Tisch BrainSPORT program, and UCLA Brain Injury Research Center (BIRC).

I. INTRODUCTION

Debates exist over the proper design for control groups, interchangeably referred to it as sham groups, when Traumatic Brain Injury (TBI) mechanisms are studied via experimental models. To control for the effect of surgery, craniectomy procedures have largely been cited as the sham procedure in conjunction with Fluid Percussion Injury (FPI) and Controlled Cortical Impact (CCI) [1] models of TBI. This sham procedure requires skin incision, skull drilling and bone flap removal. While the majority of studies report the craniectomy procedure as part of the sham injury procedure [2]–[4], there are studies replacing it with less invasive procedures such as sham anesthesia, uni- and bi-lateral drilling without bone removal, and bone thinning sham procedures [5]–[9].

Recent investigations on the adult rat brain report craniectomy induced cellular and motor related behavioral alterations such as upregulated expression of endothelial tyrosine kinase (Etk) [10], PDK1-2 isoenzyme mRNA expression level enhancement [11], altered seizure susceptibility [12], impaired sensory and motor response in conjunction with significant increase of KC-GRO and IFN- γ cytokines [13]. Activation of proinflammatory cytokines pathways has shown to be caused by peripheral trauma, leading into chronic learning and memory impairments [14]. These studies, although limited, are in agreement about the possibility of alterations in cellular and circuitry pathways resulting from craniectomy procedures.

The aim of the current observational study was two-fold: 1. to determine the effect of craniectomy procedure on the resting state brain networks when measured by low frequency fluctuations of Blood Oxygenation Level Dependent (BOLD) functional Magnetic Resonance Imaging (fMRI); and 2. To study the subsequent effects of craniectomy on cognitive performance using the Novel Object Recognition (NOR) task.

II. MATERIALS AND METHODS

Male, Sprague Dawley (SD) rat pups (postnatal age 19 days, body weight 46.3 ± 3.7 g; Charles River, MD) were randomized into Craniectomy and Control groups ($n=5/\text{group}$) following the study protocol depicted in Figure 1. All procedures performed were reviewed and approved by the University of California Los Angeles (UCLA) Chancellor's Animal Research Committee (ARC protocol number: 2014-099-11), and were in conformity with the National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals [15]. Animals were maintained on a 12-h light/dark cycle with food and water given *ad libitum*.

A. Craniectomy/ Control Procedure

All rats were anesthetized with isoflurane (induction: 3-4%; maintenance: 2.5-3% vaporized in oxygen flowing at 0.61/min), maintained at 37°C with a homeostatic temperature controlled blanket, monitored for respiration, muscular relaxation and toe pinch response. After the head was shaved, all rats were immobilized in a stereotaxic surgical frame, and the surgical site was then prepared using aseptic technique, including three alternating preparations with betadine and alcohol. The craniectomy group received a midline incision, followed by a craniectomy (diameter: 6mm carefully drilled by an experienced surgeon and

under intermittent saline flush), centered +3 *mm* posterior to and –6 *mm* lateral to bregma, scalp closure, and topical antibiotic application. Control group rats received a similar level and duration anesthesia but without a skin incision and craniectomy. All rats were then transferred to a temperature-controlled recovery to a normal, awake state before being returned to their home cage.

All animals were monitored daily for their weight, activity level, and scalp wound (craniectomy group). All rats were weaned on postnatal day 20.

B. Novel Object Recognition (NOR)

The Novel Object Recognition (NOR) task [16] was completed over four days beginning the day after craniectomy/control procedure day. The task included a habituation phase (postnatal days 20, 21, and 22) when each animal is allowed to explore the test arena freely. NOR task was then followed on post-natal day 23 by first introducing the animal to two similar objects (the object memory formation phase) over a period of five minutes. The animal is then returned to their home cage for 20 minutes (the inter-trial interval). The final test phase of the task is to return the animal to the arena for five minutes after one object is replaced with a novel one, and to determine the amount of time engaged with each object. Studies have demonstrated that a healthy animal naturally shows a significantly time preference for interacting with the novel object during the test trial [17]. Object interaction was defined as the physical presence of animal to within 4 *cm* circular proximity to the object, and constrained to the times when the rat snout was pointing toward the object. Times when the rat was leaning, grazing, and climbing the object (unless poking the top with snout) were excluded. Quantification of the time was determined by averaging scores from multiple rater who were blinded to the object category (old, Novel). Novel object type and placement within the arena (Figure 1.) was randomized within the study group to prevent bias.

C. Resting state functional MRI Data Acquisition and Analysis

Upon completion of the NOR study, all rats were transferred to UCLA Brain Mapping Center to conduct imaging using a Bruker 7T spectrometer. Animals were initially anesthetized with isoflurane (3%) to allow intraperitoneally injection of 0.05 *mg/kg* dexmedetomidine, after which they were positioned in a perspex cradle that enabled the head to be secured with a bite bar and ear bars. Temperature was continuously monitored and maintained at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ (Small Animal Instruments, Inc., NY, USA2). During scanning, anesthesia was maintained using 0.5% isoflurane (vaporized in oxygen) via a nasal cone, and continuous subcutaneous injection of dexmedetomidine at the rate of $0.016 \frac{\mu\text{l}}{\text{min}}/\text{kg}$.

The combined low dose of isoflurane and dexmedetomidine was shown to result in a stable measurement across rodent resting state functional MRI scans and with a high test-retest reliability [18], [19]. Two dimensional anatomical images were acquired with 24 coronal slices using a Rapid Acquisition with Relaxation Enhancement method (RARE factor: 8; TE/TR: 14/6020.5 ms; flip angle: 180°; 128×128 matrix size in a 30×30*mm* field of view; slice thickness: 0.75*mm*). BOLD fMRI data were acquired using a single-shot echo planar

gradient recalled echo sequence over 18 coronal slices (TE/TR: 20/1000ms; 300 repetitions; 30×30mm field of view encoded in 128×128 matrix; slice thickness: 1mm).

Anatomical volumes were manually masked to segment brain from non-brain tissue. Brain image volumes were then registered to a digital rat brain MRI template [20] using FSL software package [21], [22]. Affine transformation matrices were later used to align preprocessed functional image volumes to the MRI template for group-wise analysis. The preprocessing pipeline for the fMRI data was implemented in AFNI [23], and included the removal of the first 10 volumes to include volumes at steady-state, slice time correction, movement correction, followed by General Linear Model (GLM) based deconvolution with estimated motion parameters and average Cerebrospinal fluid (CSF) signals and a second degree polynomial baseline trend. Band-pass filtering (0.01-0.1 Hz) was embedded within the deconvolution.

Root mean square (R_i) of pairwise correlation coefficients between i th voxel and all other voxels were calculated as described in equation (1), and (2).

$$r_{ij} = \frac{\sum_{i,j} (x_i - \bar{x}_i)(x_j - \bar{x}_j)}{\sqrt{\sum_{i,j} (x_i - \bar{x}_i)^2 (x_j - \bar{x}_j)^2}} \quad i, j = 1, 2, \dots, n \quad (1)$$

$$R_i = \sqrt{\frac{1}{n} \sum_j r_{ij}^2} \quad (2)$$

where r_{ij} presents the Pearson correlation coefficient among pairs of voxels associated with time-series x_i and x_j . n represents the number of voxels on the intersection mask computed from all rats in the analysis. Atlas-registered individual R_i connectomes were then tested for craniectomy versus group difference.

III. RESULTS AND DISCUSSIONS

Age-related weight gain was not significantly different between craniectomy and control groups for the postnatal days 19-23 (1.08±0.01 and 1.09±0.01 grams per day for craniectomy and control group, respectively). Figure 2. (a) shows sagittal and coronal images group difference (craniectomy-control contrast) map, error corrected for clusters of 10 voxels or more at $p < 0.05$. No apparent histological damage was observed following the visual inspection of group anatomical T2-weighted imaging (Figure 2. (b)). The data shows higher synchronous low-frequency oscillation in the craniectomy group at the following regions: caudate-putamen, somatosensory cortex, amygdala, piriform cortex, and hippocampus-subiculum, all ipsilateral to the craniectomy. Side of the induced alterations, ipsilateral to the craniectomy, was in agreement with previously reported molecular observations[10], [13]. One possible explanation for the observed alterations could be the prolonged effect caused by acute mechanical stress of drilling followed by chronic dural irritation caused by the bone powder after drilling [13]. Caudate-putamen and Amygdala are

recognized for their role in stress and mood disorders [24]. Contribution of somatosensory cortex in upregulation of proinflammatory cytokines signaling [13], [25] and the recognition of caudate-putamen and amygdala for their role in stress and mood disorders [24] could possibly explain the network alterations after craniectomy.

In addition to the circuit-based effects, memory performance in the NOR task (Figure 3.) was also affected. Within the group “Tukey” test for difference in adjusted means (percent interaction time with Novel vs percent Interaction time with Old) showed that control animals spent greater amount of time with the novel object ($t_{Novel-Old} = 2.25$, $p = 0.08$), while animals with craniectomy ($t_{Novel-Old} = 1.03$, $p = 0.36$) did not show any preference on exploring novel object over familiar one.

These observations suggest that Craniectomy induces alterations at both levels of BOLD contrasted synchronous activation during rest and cognitive performance mirrored previous reports of craniectomy effects on molecular and circuitry pathways. Peripheral immune-related events and their remote contribution to brain function [14], along with craniectomy induced RSN and NOR task alterations emphasize an underlying mechanism that needs be controlled for. Controlling for the craniectomy effect asks for a study with larger sample size, including a molecular pro-inflammatory study as well as electrophysiological probing of the induced alterations over various phases of brain maturation in developmental models. Reported findings, when joined with recent evidences of TBI association with hyperconnectivity [27]–[30], suggests that a precise justification needs to be accompanied prior to inclusion/exclusion of craniectomy as a control group when developmental model of TBI is experimentally studied.

Acknowledgment

The authors would like to acknowledge the support provided by Ms. Sima Ghavim, and Ms. Kathryn Rasco from UCLA BIRC, and student volunteers, Srija Bhaduri, Umair Khan, and Shauna Perigo.

References

- [1]. LIGHTHALL JW, DIXON CE, and ANDERSON TE, “Experimental Models of Brain Injury,” J. Neurotrauma, vol. 6, no. 2, pp. 83–97, Jan. 1989. [PubMed: 2671392]
- [2]. Hsieh T-H, Lee HHC, Hameed MQ, Pascual-Leone A, Hensch TK, and Rotenberg A, “Trajectory of Parvalbumin Cell Impairment and Loss of Cortical Inhibition in Traumatic Brain Injury,” Cereb. Cortex, Nov. 2016.
- [3]. Thomas TC, Ogle SB, Rumney BM, May HG, Adelson PD, and Lifshitz J, “Does time heal all wounds? Experimental diffuse traumatic brain injury results in persisting histopathology in the thalamus,” Behav. Brain Res, vol. 340, pp. 137–146, Mar. 2018. [PubMed: 28042008]
- [4]. Wright DK, Johnston LA, Kershaw J, Ordidge R, O’Brien TJ, and Shultz SR, “Changes in Apparent Fiber Density and Track-Weighted Imaging Metrics in White Matter following Experimental Traumatic Brain Injury,” J. Neurotrauma, vol. 34, no. 13, pp. 2109–2118, Jul. 2017. [PubMed: 28152648]
- [5]. Damar U et al., “Alterations in the Timing of Huperzine A Cerebral Pharmacodynamics in the Acute Traumatic Brain Injury Setting,” J. Neurotrauma, vol. 35, no. 2, pp. 393–397, Jan. 2018. [PubMed: 29054135]
- [6]. Zheng F et al., “Plasma metabolomics profiles in rats with acute traumatic brain injury,” PLoS One, vol. 12, no. 8, p. e0182025, 2017. [PubMed: 28771528]

- [7]. Teng SX and Molina PE, "Acute alcohol intoxication prolongs neuroinflammation without exacerbating neurobehavioral dysfunction following mild traumatic brain injury.," *J. Neurotrauma*, vol. 31, no. 4, pp. 378–86, Feb. 2014. [PubMed: 24050411]
- [8]. Gatson JW et al., "Estrone is neuroprotective in rats after traumatic brain injury.," *J. Neurotrauma*, vol. 29, no. 12, pp. 2209–19, Aug. 2012. [PubMed: 22435710]
- [9]. Schober ME, Block B, Requena DF, Hale MA, and Lane RH, "Developmental traumatic brain injury decreased brain derived neurotrophic factor expression late after injury.," *Metab. Brain Dis*, vol. 27, no. 2, pp. 167–73, Jun. 2012. [PubMed: 22527999]
- [10]. Wu JC-C et al., "Different sham procedures for rats in traumatic brain injury experiments induce corresponding increases in levels of trauma markers.," *J. Surg. Res*, vol. 179, no. 1, pp. 138–44, Jan. 2013. [PubMed: 23122667]
- [11]. Xing G, Ren M, O'Neill JT, Verma A, and Watson WD, "Controlled cortical impact injury and craniotomy result in divergent alterations of pyruvate metabolizing enzymes in rat brain.," *Exp. Neurol*, vol. 234, no. 1, pp. 31–8, Mar. 2012. [PubMed: 22193111]
- [12]. Forcelli PA, Kalikhman D, and Gale K, "Delayed effect of craniotomy on experimental seizures in rats.," *PLoS One*, vol. 8, no. 12, p. e81401, Dec. 2013. [PubMed: 24324691]
- [13]. Cole JT et al., "Craniotomy: True Sham for Traumatic Brain Injury, or a Sham of a Sham?," *J. Neurotrauma*, vol. 28, no. 3, pp. 359–369, Mar. 2011. [PubMed: 21190398]
- [14]. Femenia T et al., "Disrupted Neuroglial Metabolic Coupling after Peripheral Surgery.," *J. Neurosci*, vol. 38, no. 2, pp. 452–464, Jan. 2018. [PubMed: 29175959]
- [15]. Nih, Od, Oer, and Olaw, "Guide for the Care and Use of Laboratory Animals, 8th edition National Academies Press."
- [16]. Ennaceur A and Delacour J, "A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data.," *Behav. Brain Res*, vol. 31, no. 1, pp. 47–59, Nov. 1988. [PubMed: 3228475]
- [17]. Becker G, Bolbos R, Costes N, Redoute J, Newman-Tancredi A, and Zimmer L, "Selective serotonin 5-HT 1A receptor biased agonists elicit distinct brain activation patterns: a pharmacMRI study," *Nat. Publ. Gr*, 2016.
- [18]. Brynildsen JK, Hsu L-M, Ross TJ, Stein EA, Yang Y, and Lu H, "Physiological characterization of a robust survival rodent fMRI method," *Magn. Reson. Imaging*, vol. 35, pp. 54–60, Jan. 2017. [PubMed: 27580522]
- [19]. Hsu L-M et al., "Constituents and functional implications of the rat default mode network.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 113, no. 31, pp. E4541–7, Aug. 2016. [PubMed: 27439860]
- [20]. Schwarz AJ et al., "A stereotaxic MRI template set for the rat brain with tissue class distribution maps and co-registered anatomical atlas: Application to pharmacological MRI," *Neuroimage*, vol. 32, no. 2, pp. 538–550, Aug. 2006. [PubMed: 16784876]
- [21]. Jenkinson M and Smith S, "A global optimisation method for robust affine registration of brain images.," *Med. Image Anal*, vol. 5, no. 2, pp. 143–56, Jun. 2001. [PubMed: 11516708]
- [22]. Jenkinson M, Bannister P, Brady M, and Smith S, "Improved Optimization for the Robust and Accurate Linear Registration and Motion Correction of Brain Images," *Neuroimage*, vol. 17, no. 2, pp. 825–841, 2002. [PubMed: 12377157]
- [23]. Cox RW, "AFNI: software for analysis and visualization of functional magnetic resonance neuroimages.," *Comput. Biomed. Res*, vol. 29, no. 3, pp. 162–73, Jun. 1996. [PubMed: 8812068]
- [24]. Delgado y Palacios R, Verhoye M, Henningsen K, Wiborg O, and Van der Linden A, "Diffusion Kurtosis Imaging and High-Resolution MRI Demonstrate Structural Aberrations of Caudate Putamen and Amygdala after Chronic Mild Stress," *PLoS One*, vol. 9, no. 4, p. e95077, Apr. 2014. [PubMed: 24740310]
- [25]. Chamaa F, Chebaro M, Safieh-Garabedian B, Saadeh R, Jabbur SJ, and Saadé NE, "Transcriptional expression of inflammatory mediators in various somatosensory relay centers in the brain of rat models of peripheral mononeuropathy and local inflammation," *J. Neuroimmunol*, vol. 297, pp. 81–91, Aug. 2016. [PubMed: 27397080]
- [26]. Khazipov R et al., "Atlas of the Postnatal Rat Brain in Stereotaxic Coordinates.," *Front. Neuroanat*, vol. 9, p. 161, 2015. [PubMed: 26778970]

- [27]. Harris NG, Verley DR, Gutman BA, Thompson PM, Yeh HJ, and Brown JA, "Disconnection and hyper-connectivity underlie reorganization after TBI: A rodent functional connectomic analysis," *Exp. Neurol*, vol. 277, pp. 124–138, Mar. 2016. [PubMed: 26730520]
- [28]. Dobryakova E, Boukrina O, and Wylie GR, "Investigation of Information Flow During a Novel Working Memory Task in Individuals with Traumatic Brain Injury," *Brain Connect*, vol. 5, no. 7, pp. 433–441, Sep. 2015. [PubMed: 25490432]
- [29]. Hillary FG et al., "The Rich Get Richer: Brain Injury Elicits Hyperconnectivity in Core Subnetworks," *PLoS One*, vol. 9, no. 8, p. e104021, Aug. 2014. [PubMed: 25121760]
- [30]. Caeyenberghs K, Verhelst H, Clemente A, and Wilson PH, "Mapping the functional connectome in traumatic brain injury: What can graph metrics tell us?," *Neuroimage*, Dec. 2016.

Figure 1.

Study protocol. Sprague-Dawley rat pups were randomized to into Craniectomy/Control groups at 19 post-natal days. Resting state functional MRI was accompanied by a memory test (Novel Object Recognition - NOR) on post-natal day 23.

Figure 2.

(a) Data-driven construction of resting state brain network of craniectomy-control contrast map error corrected for the cluster size of 10 voxels or more at $p < 0.05$ overlaid on the MRI template. The clusters were highlighted with circle. Atlas regions were marked from the atlas of postnatal rat brain in stereotaxic coordinates[26]. (b) Group maps for anatomical T2 weighted image volumes.

Figure 3.

Novel object recognition test performance for (left) control group and (right) craniectomy group. “Old” and “Novel” referred to the object type during NOR test trial.