Experimental Neurology 277 (2016) 227–243

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Alterations of functional properties of hippocampal networks following repetitive closed-head injury☆

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ARTICLE INFO

Article history:
Received 3 July 2015
Received in revised form 9 December 2015
Accepted 22 December 2015
Available online 28 December 2015

Keywords:
Traumatic brain injury
Hippocampus
Long-term potentiation
Neuroplasticity
Electrophysiology
Spontaneous excitatory currents
Spontaneous inhibitory currents, fear-conditioning context and cued

ABSTRACT

Traumatic brain injury (TBI) is the leading cause of death for persons under the age of 45. Military service members who have served on multiple combat deployments and contact-sport athletes are at particular risk of sustaining repetitive TBI (rTBI). Cognitive and behavioral deficits resulting from rTBI are well documented. Optimal associative LTP, occurring in the CA1 hippocampal Schaffer collateral pathway, is required for both memory formation and retrieval. Surprisingly, ipsilateral Schaffer collateral CA1 LTP evoked by 100 Hz tetanus was enhanced in mice from the 3× closed head injury (3× CHI) treatment group in comparison to LTP in contralateral or 3× Sham CA1 area, and in spite of reduced freezing during contextual fear conditioning at one week following 3× CHI. Electrophysiological activity of CA1 neurons was evaluated with whole-cell patch-clamp recordings. 3× CHI ipsilateral CA1 neurons exhibited significant increases in action potential amplitude and maximum rise and decay slope while the action potential duration was decreased. Recordings of CA1 neuron postsynaptic currents were conducted to detect spontaneous excitatory and inhibitory postsynaptic currents (sEPSCs/sIPSCs) and respective miniature currents (mEPSCs and mIPSCs). In the 3× CHI mice, sEPSCs and sIPSCs in ipsilateral CA1 neurons had an increased frequency of events but decreased amplitudes. In addition, 3× CHI altered the action potential-independent miniature postsynaptic currents. The mEPSCs of ipsilateral CA1 neurons exhibited both an increased frequency of events and larger amplitudes. Moreover, the effect of 3× CHI on mIPSCs was opposite to that of the sIPSCs. Specifically, the frequency of the mIPSCs was decreased while the amplitudes were increased. These results are consistent with a mechanism in which repetitive closed-head injury affects CA1 hippocampal function by promoting a remodeling of excitatory and inhibitory synaptic inputs leading to impairment in hippocampal-dependent tasks.

1. Introduction

Traumatic brain injury is the leading cause of death and disability worldwide for people under the age of 45 (Faul et al., 2010). In the United States, over 1.7 million new cases of TBI are reported each year (Lucke-Wold et al., 2014), with more than 80% of cases classified as mTBI (Cassidy et al., 2004). However, this classification appears to be used interchangeably with several other brain injuries such as concussion, secondary impact syndrome, and even chronic postconcussion syndrome (Harmon et al., 2013; Hoge et al., 2009) demonstrating the heterogeneity and complexity of mild brain injury.

In addition to civilian injuries, more than 300,000 U.S. combat veterans of the Iraq and Afghanistan wars have been diagnosed with a mTBI (Tanielian and Jaycox, 2008). Treatment plans and therapeutic interventions to improve the neurocognitive outcome of these patients are impeded by the intense debate on mTBI diagnostic criteria and data interpretation of research studies (Hoge et al., 2010; Marion et al., 2011). Furthermore, neurocognitive tests, whose resulting data is subject to varied interpretation, are challenged to address the comorbidity of mTBI with PTSD (Hoge et al., 2009). This study focuses on rTBI and its applicability to combat-related cases of rTBI and in athletes who play contact sports.

Abbreviations: AMPARs, AMPA receptors; AP, action potential; Ca^{2+}, calcium; CHI, closed head injury; EAE, experimental autoimmune encephalomyelitis; fEPSP, field excitatory postsynaptic potential; LTP, long-term potentiation; mEPSCs, miniature excitatory postsynaptic currents; mIPSCs, miniature inhibitory postsynaptic currents; mTBI, mild traumatic brain injury; PPF, paired pulse facilitation; PTSD, post traumatic stress disorder; rTAI, repetitive traumatic axonal injury; rTBI, repetitive traumatic brain injury; sEPSCs, spontaneous excitatory postsynaptic currents; sIPSCs, spontaneous inhibitory postsynaptic currents; TAI, traumatic axonal injury; TARPs, transmembrane AMPA receptor regulatory proteins; TBI, traumatic brain injury; TTX, tetrodotoxin.

☆ Conflict of interest: None.

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http://dx.doi.org/10.1016/j.expneurol.2015.12.019

1.1. Repetitive traumatic brain injury: pathophysiology

In cases of moderate-to-severe TBI, the pathophysiology of secondary injury is characterized by compromise of the blood brain barrier, trauma-induced activation of voltage-gated ion channels, glutamate excitotoxicity, Ca^{2+} imbalance, mitochondrial dysfunction, necrosis, and apoptosis within the CNS (Cernak and Noble-Haeusslein, 2010). However, in the case of mild rTBI, this cascade of deleterious events, likely of neuroinflammatory/electrophysiological origin, does not occur with the intensity and duration as seen in moderate-to-severe TBI (Barkhoudarian et al., 2011). Rather, the aberrations in ionic currents, energy homeostasis, and cytoskeletal integrity caused by the primary injuries are both transient and reversible (Hall et al., 2005). As these pathophysiological events dissipate and eventually are no longer detectable, the cognitive and behavioral deficits that resulted from the pathophysiology may also diminish and eventually disappear. However, in spite of improvement in majority of patients, 15% of patients with a previous diagnosis of mTBI will continue to present with neurological deficits 12 months after the initial trauma (Carroll et al., 2014).

rTBI initiates a heterogeneous and complex array of cellular responses in the brain that contribute to subsequent post-injury neurodegenerative and behavioral disorders (see Smith et al., 2013 for review). When exposed to multiple TBIs, the severity of damage and corresponding behavioral changes are often significantly worsened longitudinally (Smith et al., 2013). Animal models of rTBI have characterized the cognitive and behavioral decline associated with the cumulative damage caused by rTBI (Brody et al., 2015; Johnson et al., 2015; Laurer et al., 2001; Longhi et al., 2005). However, the cellular mechanisms underlying these changes have not yet been elucidated. We suspected that this deteriorating outcome is paralleled by degeneration of the mechanisms underlying activation and regulation of CNS neuronal network plasticity.

The majority of single mild TBI studies observed impaired hippocampal LTP, decreased hippocampal plasticity, excitability and/or increased inhibition (Albensi et al., 2000; Reeves et al., 2005; Schwarzbach et al., 2006; Witgen et al., 2005). Therefore, it would be a reasonable assumption that multiple impacts delivered within a short time interval would result in synergistic or at least additive attenuation of neuronal excitability in CA1 neurons ipsilateral to the injury and impaired hippocampal function (Kim et al., 2005; Weber, 2007). We hypothesized that mild repetitive TBI would result in either the failure to induce LTP or a significant reduction in LTP when compared to the sham-injured group (Reeves et al., 1995). This absent or reduced LTP would be expected to be accompanied by alterations in the properties of CA1 pyramidal neurons consistent with its diminished intrinsic electrical activity. Furthermore, the excitatory postsynaptic currents, action potential-dependent and independent, would be decreased, whereas inhibitory postsynaptic currents, action potential-dependent and independent, would be increased in the triple injury group (Albensi et al., 2000; Miyazaki et al., 1992; Reeves et al., 1995; Weber, 2007). In order to test our hypothesis, we identified changes in CA1 hippocampal LTP following repetitive mild TBI; and determined changes in the intrinsic electrophysiological properties of CA1 neurons, and examined spontaneous excitatory and inhibitory activity to detect any shift(s) between the excitatory and inhibitory CA1 networks. Intriguingly, our results showed augmented LTP accompanied by abnormal intrinsic and synaptic hippocampal activity, contrary to our initial hypothesis. However, as previously reported in mouse models of repetitive concussive TBI (Berberian et al., 1990; Creeley et al., 2004; DeFord et al., 2002; Longhi et al., 2005; Meehan et al., 2012; Mouzon et al., 2014; Petraglia et al., 2014), we found that 3× CHI mice are impaired in hippocampal-dependent behavioral tasks.

2. Methods

2.1. Animals

Groups of male C57Bl/6 (NCI, Poolesville/Frederick, MD) mice were subjected to single (1× CHI) and triple (3× CHI) closed-head injuries, along with their respective sham controls (1× Sham and 3× Sham). All mice were 6–7 weeks old on delivery to model the age of young service members. After delivery all mice were acclimated for one week and were kept on a reversed light cycle in regular animal rooms throughout the surgeries and until the time of sacrifice for electrophysiological or behavioral experiments. All experiments were approved by the Uniformed Services University of the Health Sciences Institutional Animal Care and Use Committee.

2.2. Closed-head injury surgery

The CHI model of rTBI was created by employing an electromagnetically controlled cortical impact device (Leica Impact One™ Stereotaxic CCI Instrument, Buffalo Grove, IL), which provides high reproducibility of closed-head injury (Brody et al., 2007). The repetitive CHIs were performed as follows. Each surgery was carried out at the same time of the day, with one CHI administered every 24 h for 3 consecutive days. Under a 2% isoflurane/oxygen mixture of anesthesia, a rostral to caudal incision was made to retract the scalp. The impact coordinates from the bregma were (in mm): X (lateral): 3.0, Y (posterior): −2.0, Z (ventral): −1.5 (Fig. 1). The angle of the 5 mm diameter impactor tip was set at 15° so that the impactor tip was approximately parallel to the surface of the skull. The CHI was delivered with a velocity of 4 m/s and a dwell time of 100 ms. These coordinates and impact parameters define the neocortical peri-infarct site (Fig. 1). The CCI coordinates and parameters were selected to achieve minimal or histologically non-detectable damage to the ipsilateral hippocampus throughout the repetitive injury. After each surgery, the scalp was closed with sutures and the mouse was kept on a heated pad (36 °C) during anesthesia. The mice were returned to the clean home cage (maximum of four mice per cage) with food and water ad libitum and the cage was kept on a heated pad (38 °C). After each injury all mice were closely observed for ~1–2 h by the investigator to detect any abnormal behavior. The cage was then moved to the regular animal room where the animals are monitored by laboratory and veterinary staff for any abnormal status. The Sham mice underwent all handling like the CHI mice, with anesthesia, opening of the scalp, and suturing of the skin, except the impactor tip was not released. Three repetitive injuries and/or sham surgeries were performed for three consecutive days approximately at the same time of the day. The handling of the mice and duration of anesthesia were the same for TBI and sham injured mice to minimize confounding factors related to repetitive use of anesthesia and mouse handling (Statler et al., 2006; Yurdakoc et al., 2008).

2.3. Methods

2.3.1. Histopathology

For histopathological analysis mice were perfused intracardially with PBS (pH 7.4) followed by PBS and 4% PFA (wt/vol). The extracted brains were then fixed in PFA for 12 h at 4 °C followed by overnight incubation in 20% sucrose (wt/vol) solution and then were sent to

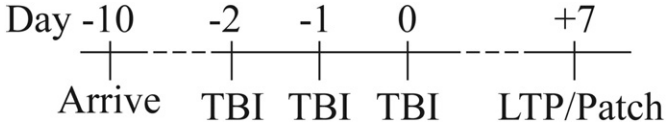

Fig. 1. Model of repetitive TBI closed head injury. The timeline diagram depicts timing of the experiments from arrival of mice at the animal facility through the rTBI protocol and electrophysiological experiments.