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Research Paper

Diminished amygdala activation and behavioral threat response following traumatic brain injury

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ABSTRACT

Each year, approximately 3.8 million people suffer mild to moderate traumatic brain injuries (mTBI) that result in an array of neuropsychological symptoms and disorders. Despite these alarming statistics, the neurological bases of these persistent, debilitating neuropsychological symptoms are currently poorly understood. In this study we examined the effects of mTBI on the amygdala, a brain structure known to be critically involved in the processing of emotional stimuli. Seven days after lateral fluid percussion injury (LFPI), mice underwent a series of physiological and behavioral experiments to assess amygdala function. Brain-injured mice exhibited a decreased threat response in a cued fear conditioning paradigm, congruent with a decrease in amygdala excitability determined with basolateral amygdala (BLA) field excitatory post-synaptic potentials together with voltage-sensitive dye imaging (VSD). Furthermore, beyond exposing a general decrease in the excitability of the primary input of the amygdala, the lateral amygdala (LA), VSD also revealed a decrease in the relative strength or activation of internuclear amygdala circuit projections after LFPI. Thus, not only does activation of the LA require increased stimulation, but the proportion of this activation that is propagated to the primary output of the amygdala, the central amygdala, is also diminished following LFPI. Intracellular recordings revealed no changes in the intrinsic properties of BLA pyramidal neurons after LFPI. This data suggests that mild to moderate TBI has prominent effects on amygdala function and provides a potential neurological substrate for many of the neuropsychological symptoms suffered by TBI patients.

1. Introduction

Traumatic brain injury (TBI) afflicts approximately 3.8 million people annually in the United States alone, with at least 5.3 million or 2% of the US population suffering persistent TBI-related disability (Faul and Coronado, 2015; Langlois et al., 2006; Rutland-Brown et al., 2006). Even mild to moderate traumatic brain injury (mTBI i.e. concussion), the most common form of TBI, results in debilitating symptoms and cognitive dysfunction. Whereas much of the animal research performed on mTBI has focused on cognition and memory (Xiong et al., 2013), cognitive deficits and memory impairment represent only a few of the debilitating neuropsychological symptoms suffered by mTBI patients. Many other prominent symptoms including, anxiety, aggression/irritability/rage, depression/anhedonia, apathy, and poor impulse control indicate emotional destabilization following mTBI (Bazarian et al., 2009; Jorge et al., 2004; Malkesman et al., 2013; Rao et al., 2009; Riggio, 2010).

Functional alterations in the amygdala, a brain region critically involved in the conscious and autonomic response to emotional stimuli, are associated with many if not all of the aforementioned symptoms (Cardinal et al., 2002; Duvarci and Pare, 2014; Ledoux, 2000; Phelps and LeDoux, 2005). Furthermore, if concussion induces acute physiological alterations in the amygdala, this could represent a potential substrate for the substantial comorbidity of traumatic brain injury and post-traumatic stress disorder (PTSD) (Riggio, 2010). While a limited number of human studies linking changes in amygdala size and diffusivity (diffusion tensor imaging) to TBI have been performed (Depue et al., 2014; Juranek et al., 2011), there exists a dearth of studies concerning amygdala physiology and dysfunction following mTBI. Understanding the effects of mTBI on amygdala function is critical for the development of therapeutic interventions to treat mild to moderate TBI and further our understanding of how mTBI relates to PTSD.

Using a well-established mouse model of mTBI, lateral fluid percussion injury (LFPI) (Thompson et al., 2005; Xiong et al., 2013), physiological and behavioral experiments were performed to determine the effects of mTBI on amygdala function. As the amygdala is a critical component of threat response (freezing behavior), our studies began by testing animals for the acquisition and expression of threat response in a cued fear conditioning paradigm. Next, extracellular recording together with voltage-sensitive dye (VSD) imaging was conducted to examine network activity and circuit function across multiple amygdala

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sub-regions, in brain slices from sham and brain-injured animals. VSD imaging allowed us to determine how brain injury affects local responses in the stimulated amygdala sub-regions, and further determine how this activation propagated to other amygdala nuclei; thus revealing potential changes in amygdala circuit dynamics. Finally, intracellular recordings were performed to assess intrinsic properties of amygdala neurons following LFPI. Through the combined use of physiological and behavioral techniques we have identified circuit level dysfunction in the amygdala corresponding to deficits in threat response in brain-injured animals.

2. Materials and methods

2.1. Animals

All experiments were performed on 7 to 12 week-old, male C57BL/J6 mice (The Jackson Laboratory). All animals were group housed with free access to food and water. All procedures were performed in accordance with the guidelines published in the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and approved by the Children's Hospital of Philadelphia Institutional Animal Care and Use Committee. Behavioral and physiological experiments were performed on separate cohorts of animals.

2.2. Lateral fluid percussion injury

LFPI is a well-established model of mild to moderate traumatic brain injury that mimics many aspects of human TBI pathology and symptomatology (Carbonell et al., 1998; Thompson et al., 2005; Xiong et al., 2013). Animals were randomly assigned into three groups; naïve animals that received no surgery or injury, but were treated otherwise identically; LFPI (surgery + injury); or sham (surgery only). Surgery and injury were performed on consecutive days, for full details see (Cole et al., 2010; Witgen et al., 2005). Briefly, on day one mice were anesthetized with a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg) and placed in a stereotaxic frame (Stoelting). A 3 mm outer diameter trephine was then used to perform a craniectomy of the right parietal bone lateral of the sagittal suture between lambda and bregma, without disrupting the intact dura. A Luer-loc needle hub (3 mm inner diameter) was secured to the skull surrounding the craniectomy with adhesive and dental cement. The needle hub was filled with sterile saline solution and sealed overnight. On the following day, LFPI animals were anesthetized with isoflurane and connected to the LFPI device (Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA). The LFPI device was then triggered, delivering a 10–15 ms fluid pulse (peak pressure 1.5–1.8 atm) onto the intact dura of the brain to generate a mild to moderate TBI. The needle hub was removed and the incision sutured. Any animals with injuries resulting in dural breach or herniation were excluded from the study. Sham animals underwent an identical procedure without receiving the fluid pulse injury. Since no significant differences between sham and naïve animals were evident, these two groups were pooled for analysis and referred to as the sham group.

2.3. Cued fear conditioning

It is well established that acquisition and expression of a threat response, that is freezing, to a light and/or tone cue, paired with an aversive stimuli, are dependent upon physiological processes within the basolateral and central amygdalae (Dębiec et al., 2010; Duvarci and Pare, 2014; Phillips and Ledoux, 1992; Tronson et al., 2012). Fear conditioning paradigms pair an emotionally neutral stimulus or context (conditioned stimulus or CS) with an aversive stimulus (unconditioned stimulus or US), leading to the expression of a threat response to presentation of the neutral CS alone. As noted, both the context and cue become predictive of the US and elicit a threat response. For these experiments the context was altered between training (context A) and testing (context B) to isolate the light/tone (CS) cued response from the hippocampal dependent contextual response.

The cued fear conditioning paradigm used in this study was modified from experiments described in Newton et al. (2004) and Wolff et al. (2014)). The CS consisted of simultaneous auditory (75 dB, white noise, 20 s) and light stimuli (yellow light pulses, 20 s, flickering at 4 Hz) generated by built in audio and light stimuli generators (Med Associates, St. Albans, VT, USA). The US consisted of a footshock (1.05 mA, 1.5 s) delivered through the metal grid floor. During CS–US pairings, the US was delivered immediately following the cessation of the CS. All behavioral experiments were performed in an isolated behavioral suite under low light.

On days 4 and 5 after injury or sham operation, each animal was handled for 3 min by the experimenter. On day 6 animals underwent fear conditioning training in context A, a rectangular conditioning chamber (21.6 cm × 17.8 cm × 12.7 cm) with Plexiglas and metal walls, and a metal grid floor (Med Associates, St. Albans, VT, USA). Animals were allowed to freely explore the chamber for 1 min before experiencing 3 CS–US pairings (15 s–105 s interstimulus interval, mean 60 s). The mean of the first and second interstimulius intervals (ISI) was consistently 60 s, with the actual duration of ISI 1 vs ISI 2 assigned pseudo-randomly within a group, but consistent across groups such that each ISI pair used in a sham animal was used in a subsequent LFPI animal. One minute after the final CS–US pairing (5 min total), mice were removed from context A and placed back in their home cage (see Fig. 1 for schematic). Twenty four hours later (day 7) animals underwent behavioral testing to measure threat responses in context B, a custom made triangular conditioning chamber with black striped Plexiglas walls and a smooth, opaque black plastic floor, scented with organic vanilla extract. Mice were allowed to freely explore the chamber for 1 min before experiencing 3 presentations of the CS alone (60 s, interstimulus interval). Again, animals were removed from context B after a total of 5 min. During testing, freezing behavior was scan sampled every 5th second from the onset of the first CS presentation to the end of the trial (4 min total). Freezing was defined as a total lack of movement aside from respiration at the instant of every 5th second. The total instance of freezing was then divided by total observations to generate a freezing percentage per animal. Behavioral data was collected from 26 animals (17 sham; 9 LFPI).

2.4. Electrophysiology

All electrophysiological experiments were performed on days 7–8 after injury or sham surgery. Slice preparation was performed as previously described (Johnson et al., 2014). Briefly, animals were anesthetized with isoflurane, the brain was dissected out and placed in ice-cold oxygenated (95% O_2/5% CO_2) sucrose-containing artificial cerebrospinal fluid (aCSF) containing (in mM): sucrose 202, KCl 3, NaH_2PO_4 2.5, $NaHCO_3$ 26, glucose 10, $MgCl_2$ 1, and $CaCl_2$ 2. Coronal slices 300 μm thick were cut on a vibratome (VT1200S, Leica Microsystems, Buffalo Grove, IL, USA) and transferred to 33–37 °C oxygenated (95% O_2/5% CO_2) control aCSF containing (in millimolar): NaCl 130, KCl 3, NaH_2PO_4 1.25, $NaHCO_3$ 26, glucose 10, $MgCl_2$ 1, $CaCl_2$ 2. Slices were allowed to incubate for at least 60 min before recording. VSD imaging and field potential recordings were performed in an interface chamber, intracellular recordings were performed in a submersion chamber, both with a flow rate of approximately 2.0 ml/min and maintained at 27–30 °C. Brain slices for recording were consistently selected from the same rostral–caudal region of the amygdala that exhibited a pear shaped basolateral amygdala (BLA) contiguous with an ovoid central amygdala (CeA), as seen in Figs. 2 & 3. This rigid criterion resulted in 1–2 brain slices per animal. Brain slices were hemisected prior to recording and only slices from the hemisphere ipsilateral to the site of injury were used. Our laboratory has previously demonstrated that contralateral slices are altered by LFPI and thus do not serve as an appropriate control for the injured