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Startle suppression after mild traumatic brain injury is associated with an increase in pro-inflammatory cytokines, reactive gliosis and neuronal loss in the caudal pontine reticular nucleus

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ABSTRACT

Mild traumatic brain injury (mTBI) can produce somatic symptoms such as headache, dizziness, fatigue, sleep disturbances and sensorimotor dysfunction. Sensorimotor function can be measured by tests such as the acoustic startle reflex (ASR), an evolutionarily conserved defensive response to a brief yet sharp acoustic stimulus. mTBI produces a long-lasting suppression of ASR in rodents and humans; however, the mechanism of this suppression is unknown. The present study examined whether inflammatory processes in the brainstem (particularly the caudal pontine reticular nucleus, PnC) could account for the suppression of ASR after mTBI, because the PnC is an essential nucleus of the ASR circuit. Furthermore, while inflammation after mTBI is commonly observed in brain regions proximal to the site of impact (cortex and hippocampus), the effects of mTBI in brainstem structures remains largely understudied. The present study demonstrated a suppression of ASR one day after injury and lasting at least three weeks after an mTBI, replicating previous findings. Within the PnC, transient elevations of IL-1β and TNF-α mRNA were observed at one day after injury, while IL-1α mRNA exhibited a delayed increase at three weeks after injury. Reactive gliosis (via IBA-1-ir for microglia and GFAP-ir for astrocytes) were also observed in the PnC, at one day and seven days after injury, respectively. Finally, the number of giant neurons (the major functional cell population in the PnC) was decreased three weeks after injury. The results indicate that glial activation precedes neuronal loss in the PnC, and correlates with the behavioral suppression of the ASR. The results also raise implications for brainstem involvement in the development of post-traumatic symptoms.

1. Introduction

Traumatic brain injury affects nearly 1.7 million Americans each year, and 75% of these injuries are classified as mild (mTBI). In addition to affecting cognition and mood, mTBI causes somatic symptoms such as headache, dizziness, fatigue and sleep disturbances, as well as sensorimotor dysregulation. Dysfunction of sensorimotor processes, such as simple reaction time and the acoustic startle reflex (ASR), may provide sensitive markers of mTBI (Barker-Collo et al., 2015).

The acoustic startle response (ASR) is a simple defensive response with a core neural circuitry located in the pons of the brainstem (Davis et al., 1982; Koch, 1999). Auditory information is relayed to the cochlear nucleus via the eighth cranial nerve (VIII). Information is transmitted from the cochlear nucleus to the caudal pontine reticular nucleus (PnC) before descending to the spinal cord via the reticulospinal tract and synapsing on spinal motor neurons. The PnC is especially important for the sensorimotor integration necessary for ASR (Lingenhohl and Friauf, 1994). The ASR neural circuit is well-conserved through mammalian species including humans (Pissiota et al., 2002) and ASR has been used in humans to investigate changes in emotion in anxiety disorders and after TBI (Saunders et al., 2006; Grillon et al., 1996).

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Following mild TBI or moderate TBI, ASR is suppressed in animals and humans (Saunders et al., 2006; Lu, 2003; Washington et al., 2012; Wiley et al., 1996; Xing et al., 2013; Pang et al., 2015). While previous studies have observed suppressed ASR lasting 30 days after injury in rats (Wiley et al., 1996), humans demonstrate suppressed ASR lasting at least 1 year after TBI (Saunders et al., 2006). Despite the robust and long-lasting suppression of ASR following mild and moderate TBI, the neurobiological changes responsible for the suppression is still unknown.

Because of the importance of the PnC to ASR, the present study investigated changes in this region following mTBI. Besides its importance to ASR, the PnC is located nearby the locus coeruleus, a structure where p-tau and neurofibrillary tangles are observed early in Chronic Traumatic Encephalopathy (CTE) (Stein et al., 2014), a neurodegenerative disorder induced by sustaining multiple concussions over time. Long-lasting suppression of ASR following mTBI may reflect a general dysfunction of pontine nuclei including the locus coeruleus. Furthermore, the brainstem is the locus of key homeostatic and neuromodulatory systems, and the effect of mTBI is largely understudied in this brain region. Thus, understanding the mechanisms by which mTBI alters ASR may have implications for utilizing ASR as a behavioral marker of mTBI, as well as elucidating the progression of brain injury to neurodegeneration in CTE.

It is well established that moderate and severe TBI induces glial activation (Loane and Kumar, 2016). In rodent models, injury increases the pro-inflammatory cytokines, interleukin-1beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), and elevated levels of these mediators result in both cellular dysfunction and neurodegeneration (Shaftel et al., 2008; Gosselin and Rivest, 2007). Increased IL-1β in the brain triggers a cascade in which cyclooxygenase-2 activation facilitates further production of inflammatory mediators such as eicosanoids (Bartfai et al., 2007). Additionally, elevations in TNF-α can activate apoptotic pathways thus promoting neurotoxicity (Longhi et al., 2013). In rodent models of moderate and severe TBI, IL-1β and TNF-α mRNA is detectable up to 6 h post-injury in regions proximal to the site of impact such as the cortex and hippocampus (Fan et al., 1995, 1996; Raghupathi et al., 1995); levels of TNF-α protein are elevated after moderate brain injury in the cortex up to 4 h post-injury (Knoblach et al., 1999). However, Perez-Polo et al. (Perez-Polo et al., 2013) demonstrated that similar effects are also evident in mTBI. In the cortex and hippocampus proximal to the injury site, IL-1β and TNF-α protein levels were increased up to 6 h post-injury, as were levels of reactive gliosis (both microglial and astrocytic). Similar studies using weight-drop models found either a delayed increase of IL-1β, IL-6 and TNF-α 4–6 days after injury with no change during the first 2 days (Holmin et al., 1997), or an elevation of IL-1β mRNA and proteins 1, 3 and 7 days after injury in the cortex (Lv et al., 2014). Therefore, more studies are required to understand the nature of inflammatory processes after mTBI.

The present study was conducted to determine whether glial activation in the PnC, a critical site for ASR, could account for the suppressed ASR following mTBI. On one hand, mTBI-induced glial activation is expected given the previous literature. On the other hand, the PnC is quite remote from the area of impact, and previous studies have reported the enhanced inflammatory responses in brain areas close to the impact zone.

2. Materials and methods

2.1. Subjects

Male Sprague Dawley rats (approximately 3 months of age, 300–350 g at the start of the studies) were housed individually in a room with a 12:12 h light:dark cycle. Food and water were available *ad libitum*. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the IACUC of the Veterans Affairs Medical Center at East Orange, New Jersey.

2.2. Surgery

Rats were prepared for lateral fluid percussion injury, as previously described (Neuberger et al., 2014; Pang et al., 2015). Briefly, animals were anesthetized with a mixture of ketamine and xylazine (60 mg/7 mg per kg, 1 ml/kg, i.p.). The scalp was incised and retracted, and a craniectomy (4 mm, centered at −3.0 mm posterior to and ±3.5 mm lateral to Bregma) was created in the left or right parietal bone plate; left and right locations were counterbalanced across animals. A Luer-Lok connector was glued to the skull surrounding the craniectomy. A plastic cylinder (cut from a 12 ml syringe) surrounded the craniectomy to protect the Luer-Lok connector. Dental cement fixed the plastic cylinder and Luer-Lok connector to stainless steel screws inserted into the skull. A small piece of Kimwipe was inserted into the connector to keep the dura clean of debris.

2.3. Fluid percussion injury

Lateral fluid percussion is a well-established experimental model of TBI that produces a focal injury, modeling a clinical contusion injury without skull fracture (Cernak, 2005; Johnson et al., 2015; Petraglia et al., 2014). Lateral fluid percussion injuries were induced using a computer controlled voice-coil injury device (Abdul-Wahab et al., 2011). Subjects were anesthetized with 5% isoflurane and the Leur-Lok hub was connected to the device. As soon as rats recovered a foot-pinch reflex bilaterally, a fluid pressure pulse was delivered to the dura. After delivery of the impact, duration of apnea was recorded; rats were laid supine and the latency of their righting reflex (in the absence of any tactile stimulation) was measured. SHAM control subjects also underwent surgery and isoflurane anesthesia, and were attached to the device. However, a fluid pressure pulse was not delivered to the dura. Therefore, the only acute measure obtainable in SHAM controls was the latency of the righting reflex, which was a measure of the effects of anesthesia alone.

2.4. Acoustic startle reflex

Assessment of acoustic startle reflex (ASR) was performed as previously described (Servatius et al., 2005). ASR was assessed in individual, sound-attenuated chambers, each equipped with an exhaust fan and two speakers to maintain background noise (68 dB) and delivery of the acoustic stimuli (82, 92, and 102 dB; 100 ms), respectively. A single session consisted of 24 trials, in which the acoustic stimulus was presented immediately after a 250 ms baseline period. Trials occurred every 25–35 s in a pseudo-randomized order (8 trials per acoustic intensity). Subjects were placed under loose restraint on platforms that transduced whole body force (Coulbourn Instruments, Holliston, MA). Movement was considered a startle response if the amplitude exceeded the sum of the maximum amplitude during the baseline period and 4× the standard deviation of baseline activity. Two dependent measures were obtained from the ASR test: sensitivity and amplitude. ASR sensitivity was the occurrence of a startle response to an acoustic stimulus (expressed as a percentage at each stimulus intensity). ASR amplitude was the amplitude of the startle response at each stimulus intensity, and was corrected for the subject's body weight. ASR amplitude was calculated only for those trials in which a startle response was detected. If no responses were