Suppressed acoustic startle response in traumatic brain injury masks post-traumatic stress disorder hyper-responsivity

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An exaggerated acoustic startle reflex (ASR) is a clinical indicator of anxiety disorders, such as post-traumatic stress disorder (PTSD). Given the prevalence of PTSD following traumatic brain injury (TBI), we studied the effects of TBI on ASR. Adult Sprague Dawley rats exposed to moderate controlled cortical impact injury model of TBI displayed suppression of ASR intensity and sensitivity. As patients with PTSD have been shown to display hyperactive startle responses, the present discrepant observation of TBI-induced suppression of ASR has clinical implications, in that the reduced, instead of elevated, startle response in patients with comorbid TBI/PTSD could be owing to a masking effect of TBI. *NeuroReport* **29:939–944 Copyright © 2018 The Author(s). Published by Wolters Kluwer Health, Inc.**

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Introduction

With civilian and military treatment protocols for traumatic brain injury (TBI) improving drastically, patients are surviving brain injuries, which would have previously been fatal. As a result, a growing population of patients are living with the chronic effects of TBI [1]. In the USA alone, TBI accounts for ~1.1 million emergency room visits annually [2]. Moreover, since 2000, more than 300 000 American service members have been diagnosed with TBI, resulting in TBI being named the signature injury of the wars in Iraq and Afghanistan [3]. Of increasing concern, mild, moderate, and severe TBI are all associated with a dynamic and progressive pathology that manifests chronically with a myriad of cognitive and behavioral deficits. The long-term ramifications of TBI have been observed to coincide with anxiety and post-traumatic stress disorder (PTSD)-like symptoms, particularly in returning combat veterans who are often exposed to chronic stressors at the time of TBI [4–7]. Among these symptoms are increased anxiety, irritability, sleep disturbances, mood alterations, and attention deficits [8,9]. Owing to symptom overlap, distinguishing post-TBI symptoms from genuine PTSD can be difficult, and the underlying cause for the comorbidity of TBI and PTSD is not understood.

To assists in the accurate diagnoses of psychiatric disorders such as PTSD, strict adherence to standardized diagnostic protocols is advised. One such tool is the Diagnostic and Statistical Manual of Mental Disorders, which notes exaggerated startle response as an indicator of PTSD [10]. Indeed, this symptom is common among patients with PTSD and may help facilitate its diagnosis. The startle response can be reliably evaluated by measuring the acoustic startle reflex (ASR) [11], a brain stem-mediated defensive response to alarming auditory stimuli that is conserved in rodents and humans [12]. ASR intensity has been used as a metric for tracking anxiety levels in rodents [13,14] and has similarly been implicated in the pathogenesis, diagnosis, and tracking of anxiety disorders such as PTSD in humans [15–18]. Although the ASR has been well studied, its presentation in chronic TBI past 28 days remains unexplored.

Here, we present data demonstrating that moderate TBI caused a long lasting and stable suppression in the intensity of the ASR up to 98 days after injury, whereas other components of the ASR (i.e. latency and sensitivity) gradually recovered. Accompanying this behavioral dysfunction, degeneration was observed within the caudal pontine reticular nucleus (PnC), a brain stem nucleus distal to the site of injury which is pivotal to ASR [19]. These findings have clinical implications for physicians in the diagnosis of PTSD, as the suppression of the ASR following TBI may mask the physiological increase in the startle response observed in many patients with PTSD. Moreover, the chronic suppression of this reflex implies

that human PTSD symptom presentation (i.e. exaggerated startle reflex) may be blunted in patients who experienced TBI years before. We believe that a recognition of the possible masking effect of TBI on the ASR of patients with PTSD may guide physicians in their differential diagnosis of patients with chronic TBI.

Materials and methods

Materials

Experimental procedures were approved by the University of South Florida Institutional Animal Care and Use Committee. All animals were housed under ambient conditions (20°C, 50% relative humidity, and a 12-h light/dark cycle), and necessary precautions were undertaken throughout the study to minimize pain and stress associated with the experimental treatments. All studies were performed by personnel blinded to the treatment conditions.

Surgical procedures

Two-month-old male Sprague Dawley rats ($n=16$) were subjected to TBI using a controlled cortical impactor (CCI; Pittsburgh Precision Instruments Inc., Pittsburgh, Pennsylvania, USA) or sham surgery ($n=4$). Deep anesthesia was achieved using 2% isoflurane, and it was maintained using a gas mask. All animals were fixed in a stereotaxic frame (David Kopf Instruments, Tujunga, California, USA). After exposing the skull, coordinates of -0.2 mm anterior and $+0.2$ mm lateral to the midline were used to impact the brain at the frontoparietal cortex with a velocity of 6.0 m/s reaching a depth of 1.0 mm below the Dura matter layer and remained in that position for 150 ms. The impactor rod was angled 15° vertically to maintain a perpendicular position in reference to the tangential plane of the brain curvature at the impact surface. A linear variable displacement transducer (Macro Sensors, Pennsauken, New Jersey, USA), which was connected to the impactor, measured the velocity and duration to verify consistency. An electric drill was used to perform the craniectomy of ~2.5-mm radius with coordinates calculated from the bregma at -0.2 anterior and $+0.2$ mm lateral right. Sham surgery consisted of animals exposed to anesthesia, craniectomy, and wound closure. An automated thermal blanket pad allowed maintenance of body temperature within normal limits. All animals were closely monitored postoperatively with weight and health surveillance recording as per University of South Florida Institutional Animal Care and Use Committee guidelines. Rats were kept hydrated at all times, and the analgesic Carprofen was administered after TBI surgery and as needed thereafter. Before and after TBI, rats were fed regular rodent diet (Harlan Laboratories, Indianapolis, Indiana, USA).

Acoustic startle reflex

All rats were subjected to acoustic startle response evaluation before TBI surgery, and then on day 1, day 7, day 14, day 21, day 28, day 42, day 56, day 84, and day 98. Measurements or ASR intensity and latency were obtained in the following manner: in test 1, rats were acclimated to a constant 68-dB background noise for 5 min in an SR-LAB startle response system (San Diego, California, USA). Then, a 110-dB white nose burst (acoustic stimuli) lasting 250 ms was introduced 30 times over an ~25-min period in pseudorandom intervals ranging from 30 to 45 s, and the physical response was recorded using the SR-LAB acoustic startle system software. The point of most intense response, whereby the SR-LAB Startle Response System detects maximum subject amplitude of movement in arbitrary units relative to their basal prestimulus movement, was recorded as V_{max}. Additionally, the latency (T_{max}) of the response was measured as the delay between the stimuli and V_{max}.

Directly thereafter, rats were exposed to a second series of auditory cues to gauge their sensitivity to various auditory stimuli. Measurements or ASR sensitivity were obtained in a similar manner: in test 2, rats were also acclimated to a constant 68-dB background noise for 5 min. Then, acoustic stimuli of 0, 80, 90, 100, or 110 dB lasting 250 ms were introduced five times each in a pseudorandom order over ~20-min (five presentations of five stimuli, for a total of 25 trials), with pseudorandom intervals ranging from 30 to 45 s. A genuine startle response was scored if movement exceeded a response threshold during the 250-ms window starting at the onset of the stimulus. This response threshold was calculated as five times the SD of the baseline activity during the same 250-ms window. Thus, for each acoustic stimulus presentation, a score of response positive or response negative was recorded.

Nissl staining

Under deep anesthesia, rats were killed at 4 months after TBI surgery, and perfused through the ascending aorta with 200 ml of ice cold PBS, followed by 200 ml of 4% paraformaldehyde in PBS. Brains were removed and post-fixed in the same fixative for 24 h followed by 30% sucrose in PBS for 1 week. Coronal sectioning was carried out at a thickness of 40 μm using a Leica Cryostat CM 1950 (Leica Biosystems Inc., Buffalo Grove, Illinois, USA). Cresyl Violet Nissl staining was performed on one of every six consecutive coronal sections spanning the PnC, beginning at coordinates AP 9.0 mm and ending at AP 10.4 mm from bregma, to evaluate cell death within the PnC. Cells were manually analyzed for morphological characteristics of surviving neurons and quantified based on previously described methods [20]. In summary, neuron size, shape regularity, border continuity, singular nucleolus staining, and color contrast were considered when distinguishing viable neurons. Sections were examined with a Nikon Eclipse 600 microscope at ×20.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance with repeated measures in GraphPad Prism 6

for Windows (GraphPad Software, La Jolla, California, USA). In addition, Student's *t*-test was used to determine cell death within the PnC of TBI animals versus sham controls. All data are presented as mean values ± SEM. Statistical significance was set at *P* value less than 0.05 for all analyses.

Results

Traumatic brain injury reduces acoustic startle response intensity

TBI drastically reduced the intensity of the ASR intensity (Fig. 1a and b). An 88.8% reduction was observed in the average V_{max} on day 1 compared with baseline ($P < 0.0001$), and remained suppressed through day 98 ($P < 0.0001$). Moreover, individual analysis shows large variability in baseline V_{max} intensities, but this variability is greatly reduced following TBI (Fig. 1b).

Traumatic brain injury increases acoustic startle response latency

TBI significantly increased the latency of ASR (T_{max}) at acute and subacute time points. Average latency increased 20.96% from baseline on day 1 ($P < 0.01$), 18.04% on day 7 ($P > 0.05$), and 18.46% on day 14 ($P < 0.05$); however, a trend towards normalization was seen from day 21 to day 98 (Fig. 1c). Greater variation in individual values was noted in rat latency following TBI (Fig. 1d).

Traumatic brain injury suppresses acoustic startle response sensitivity

The probability of ASR response at 80 dB was significantly reduced at all time points compared with baseline ($P < 0.0001$), indicating a stable loss of ASR at low stimulus intensity (Fig. 2a). The probability of response was also drastically reduced at 90 dB through day 42, yet showed a trend toward normalization through day 98 (Fig. 2b). Similar patterns were observed at 100 and 110 dB, with significant initial loss of sensitivity showing a trend toward recovery by day 98 (Fig. 3c and d). At 110 dB, a near-total return of ASR was recorded by day 28, indicating a normalization of rat sensitivity to the stimulus used for ASR intensity (V_{max}) recordings discussed in TBI reduces acoustic startle response intensity. This verifies that the loss in V_{max} intensity (TBI reduces acoustic startle response intensity) was not because of an absence of response.

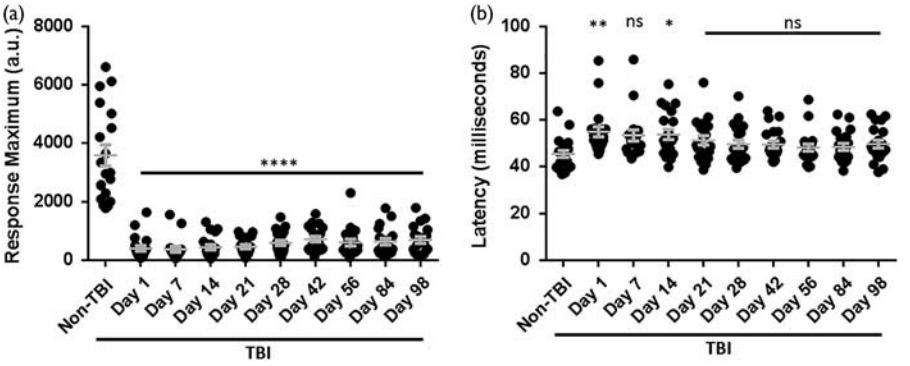
Traumatic brain injury induces brain stem degeneration

Nissl staining of the PnC in chronically brain-injured rats revealed a significant bilateral loss of surviving neurons in the PnC ($t = 7.015$; df = 18; $P = 0.0011$), a distal brain stem nucleus associated with ASR processing, when compared with sham controls (Fig. 3). No significant difference was observed in cell death of ipsilateral versus contralateral TBI animals (data not shown).

Discussion

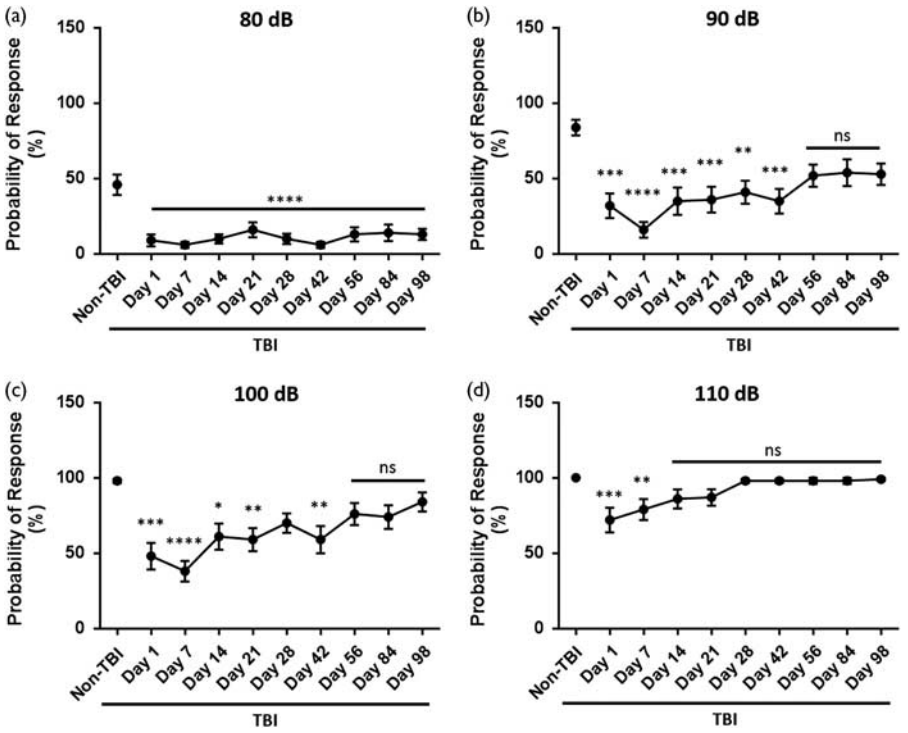
To our knowledge, the present study is the first to characterize the ASR in a moderate TBI model and report a long lasting and stable suppression of ASR intensity. We report a profound reduction in ASR intensity up to 98 days, whereas other metrics of the ASR (latency and sensitivity) trended toward normalization over the same period. That the latency and sensitivity of the ASR show clear signs of recovery indicates that the underlying circuitry still successfully transmits the necessary sensorimotor signals to produce the ASR, and that the intensity of response (V_{max}) is a uniquely effected metric of the ASR. Moreover, the detection of brain stem degeneration indicates underlying pathological alterations likely contributing to the observed behavioral abnormalities. To this end, the CCI model of TBI is a widely accepted and reproducible method of TBI modeling, which recapitulates many pathological manifestations of penetrating TBI seen in the clinic.

Fig. 1



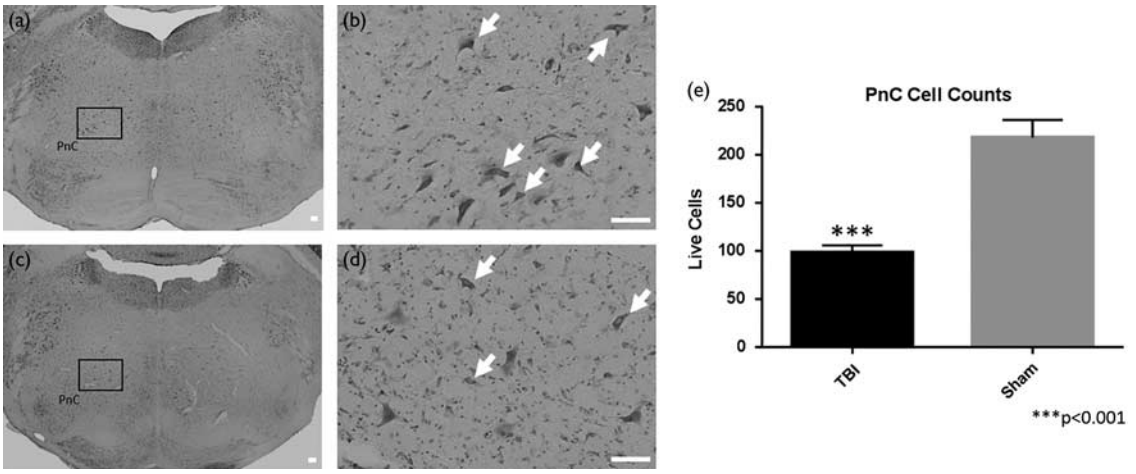
Chronic effects of moderate TBI on ASR intensity (a) and latency (b). All significance calculated relative to baseline values. *$P < 0.05$; **$P < 0.01$; ****$P < 0.0001$. ASR, acoustic startle reflex; a.u, arbitrary units; TBI, traumatic brain injury.

Fig. 2



Chronic effects of moderate TBI on ASR sensitivity. All significance calculated relative to baseline values. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$. ASR, acoustic startle reflex; TBI, traumatic brain injury.

Fig. 3



Brain stem degeneration accompanies ASR alterations. Nissl staining revealed cell death in PnC of chronically brain-injured rats (c, magnified in d) compared with sham controls (a, magnified in b). ***$P < 0.001$. Scale bar: 50 μm. ASR, acoustic startle reflex; PnC, caudal pontine reticular nucleus; TBI, traumatic brain injury.

Accordingly, this technique was selected based on high likelihood of translational application of the laboratory findings to clinical scenarios. Additionally, the CCI model, while producing a primary focal cortical injury, is accompanied by a secondary cell death in remote areas [20], such as the PnC detected here. Moreover, the primary focal nature of this TBI model – as opposed to more diffuse modes of injury infliction such as that produced

by the fluid percussion injury model – highlights the indirect secondary cell death as the likely underlying mechanism of the observed pathological manifestations, assuaging concerns that the TBI model used could be suppressing ASR intensity simply through direct auditory pathway injurious pathways.

Multiple steps were taken to reduce the effects of short-term habituation and long-term habituation of ASR, including pseudorandom interstimulus intervals to reduce short-term habituation, and spacing of ASR testing by 7 days to prevent long-term habituation. Indeed, habituation typically reduces ASR intensity by ~50% [21], which is significantly less than the ~90% decline observed here. Furthermore, the slight increase in V_{max} from day 1 to day 98 also supports a lack of habituation, as habituation would present a reversed trend of progressive decline in V_{max} [22].

Our study showing reduced ASR parallels reports of similar suppression of ASR. Adult rats subjected to mild TBI using the fluid percussion model exhibited reduced ASR as early as day 1 and persisting up to 21 days [23]. In concert, rats exposed to fluid percussion-induced moderate TBI displayed severe reduction in sensorimotor reactivity to acoustic stimuli at 8 days after injury, which lasted for more than 30 days after injury [24]. Moreover, rats exposed to blast injury, with corresponding tinnitus and hearing loss, displayed hyperactivity at 1 month after injury, but developed hypoactivity by 3 months after injury [25]. The present results showed reduced ASR up to at least 98 days after TBI, suggesting long lasting suppression of ASR that may correlate with a stable dysfunction of brain stem neural circuits. Indeed, this notion of brain stem-associated ASR alteration is supported by the present observation of brain stem degeneration even at months after TBI. Notwithstanding some varying levels in ASR and transient hyperactivity and hypoactivity, which may be because of the TBI model and severity, the need for long-term observation of TBI rats is warranted to provide a better understanding of parameters that will closely approximate TBI-induced PTSD.

Clinically, assessing the intensity of a patient's ASR is a valuable tool in the diagnosis of anxiety disorders such as PTSD. Therefore, an awareness of ASR suppression subsequent to TBI is valuable for clinicians as PTSD is a recognized comorbidity of TBI. Here, we suggest for the first time that chronic TBI may exert a masking effect on the typically hypersensitive ASR of patients experiencing comorbid PTSD. As a result, a hypersensitive startle response as indicated by the Diagnostic and Statistical Manual-V should be a symptom considered with caution when diagnosing patients with a history of TBI.

In an effort to reveal the underlying mechanisms of the PnC degeneration, we performed histological analysis for major histocompatbility complex-II positive cells – an established inflammatory marker – hypothesizing that aberrant inflammation may have propagated through the brain, eventually resulting in the observed distal cell death in the PnC. However, our analysis showed no change in major histocompatbility complex-II positive cell counts within and around the PnC in TBI animals compared with sham controls (data not shown), indicating that alternative noninflammatory pathways of cell death are likely mediating these pathological manifestations. Additional in-depth investigations are needed to uncover the true pathological mechanisms at work.

Although this report demonstrates a stable suppression in the ASR, much remains to be learned about which aspect of TBI pathology is responsible for its suppression. Indeed, whether this reduced ASR intensity is accompanied by a parallel decrease in the sympathetic output associated with the ASR (i.e. fear, tachycardia, tachypnea, and anxiety) should be a topic of future exploration. Uncovering these correlates, or lack thereof, may facilitate a better understanding of the underlying TBI pathology which suppresses the ASR and could aid in the development of novel therapeutic targets for TBI and PTSD. Moreover, whether this TBI masking effect is unique to the ASR, or is true of the visual or tactile-stimulated startle response, remains to be explored. Finally, the effects of various TBI models on ASR should be investigated, as the current model produces consistent initiation of cell death predominantly in the cortex and progressively extends to remote areas, which we showed here spreads to the PnC. The disease severity and its primary and secondary cell death mechanisms may allow a closer approximation of ASR toward better diagnosis and treatment for TBI and PTSD.

Conclusion

We report here for the first time that moderate TBI induces a stable suppression of the ASR intensity up to 98 days, whereas the latency and sensitivity trend toward normalization. This observation has clinical implications in how we diagnose patients with chronic TBI with suspected PTSD.

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Conflicts of interest

There are no conflicts of interest.

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