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Binge ethanol in adulthood exacerbates negative outcomes following juvenile traumatic brain injury

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ABSTRACT

Traumatic brain injuries (TBI) are a major public health problem with enormous costs in terms of health care dollars, lost productivity, and reduced quality of life. Alcohol is bidirectionally linked to TBI as many TBI patients are intoxicated at the time of their injury and we recently reported that, in accordance with human epidemiological data, animals injured during juvenile development self-administered significantly more alcohol as adults than did sham injured mice. There are also clinical data that drinking after TBI significantly reduces the efficacy of rehabilitation and leads to poorer long-term outcomes. In order to determine whether juvenile traumatic brain injury also increased the vulnerability of the brain to the toxic effects of high dose alcohol, mice were injured at 21 days of age and then seven weeks later treated daily with binge-like levels of alcohol 5 g/kg (by oral gavage) for ten days. Binge-like alcohol produced a greater degree of neuronal damage and neuroinflammation in mice that sustained a TBI. Further, mice that sustained a juvenile TBI exhibited mild learning and memory impairments in adulthood following binge alcohol and express a significant increase in hippocampal ectopic localization of newborn neurons. Taken together, these data provide strong evidence that a mild brain injury occurring early in life renders the brain highly vulnerable to the consequences of binge-like alcohol consumption.

1. Introduction

Traumatic brain injuries (TBI) are a major public health problem with millions of injuries and tens of thousands of deaths occurring annually in the US alone (Langlois et al., 2006). Further, the long-term health consequences and economic costs in terms of health spending and loss of productivity are staggering (Coronado et al., 2012).

Alcohol use is tightly linked to traumatic brain injuries as alcohol intoxication is linked to, by some estimates, more than half of all traumatic brain injuries (Tagliaferri et al., 2006). Much of the research into this relationship therefore has focused on the role of alcohol as a risk factor for TBI, and intoxication at injury as a variable in functional outcomes and recovery (Berry et al., 2011; Chen et al., 2012; Opreanu et al., 2010; Pandit et al., 2014). However, there is also some experimental and clinical evidence that TBI itself could serve as an independent risk factor for substance abuse issues, particularly when injuries occur early in development (Bjork and Grant, 2009; Corrigan et al., 2013; Weil et al., 2016a). Most epidemiological studies have reported that although there is a population of patients that abstain from alcohol after TBI there is also a substantial number that resume drinking in the months after injury (Bombardier et al., 2003; Kreutzer and Harris, 1990; Ponsford et al., 2007). There is some limited, though controversial, evidence that intoxication at the time of TBI can be neuroprotective (Berry et al., 2011; Chen et al., 2012; Opreanu et al., 2010; Pandit et al., 2014), but alcohol abuse following TBI is clearly problematic as it produces significantly poorer outcomes including: impairing the efficacy of rehabilitation programs; reducing vocational opportunities; and substantially increasing the risk for psychiatric disorders, post-traumatic seizures, and additional injuries (Corrigan, 1995; Corrigan et al., 2014; Jorge et al., 2005; Vaaramo et al., 2014a,b).

Recently, we reported that female, but not male, mice that received a single mild traumatic brain injury at 21 days of age self-administered significantly more alcohol in adulthood than did sham-injured mice or those injured in adulthood (Weil et al., 2016b). These data revealed that injuries occurring at a critical

Abbreviations: TBI, traumatic brain injury; DCX, doublecortin; Iba-1, ionized calcium binding adaptor molecule 1; TLR4, toll like receptor 4; IL-1β, interleukin 1 beta.

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developmental time point in pre-adolescent mice resulted in long-lasting consequences that contributed to voluntary alcohol abuse in adulthood.

Although it is clear that drinking after traumatic brain injury reduces the effectiveness of rehabilitation therapy and produces poorer outcomes, the precise mechanisms that link drinking to impaired neuronal recovery and function in this clinical population are not fully understood. However, there is mounting evidence from both the experimental and clinical literature that heavy or binge-like drinking, even in the absence of other insults, induces neuroinflammation and degeneration (Alfonso-Loeches et al., 2010; Crews et al., 2011; Hayes et al., 2013; Qin and Crews, 2012; Vetreno and Crews, 2012). Further, TBI itself induces long lasting impairments in neuronal structure and function and also induces neuroinflammatory responses. Additionally, inflammatory events early in life appear to prime the neuroimmune system to exhibit greater inflammatory responses to other stimuli, such as high-dose alcohol, later in life (Bilbo et al., 2010). Thus, heavy drinking by TBI survivors may impair functional outcomes, at least in part, by inducing inflammatory responses and further impairing already dysfunctional neuronal circuits.

Our previous study using the self-administration paradigm allowed us to investigate injury-induced alterations in alcohol reward processing, but it did not allow us to investigate the functional consequences of alcohol consumption after injury because sham-injured mice did not voluntarily self-administer high doses of ethanol (Weil et al., 2016a). Thus, in order to determine whether heavy drinking after TBI exacerbates the behavioral, neuropathological, and inflammatory consequences of juvenile injuries in female mice, we administered ten daily binge-like doses of ethanol via oral gavage to adult females that had sustained a TBI or sham injury in early life. The goals of this study were to 1) determine whether juvenile TBI increased the functional deficits and tissue damage induced by binge-like levels of alcohol and 2) to begin to uncover the mechanisms that link TBI, binge-like alcohol, and tissue damage. We hypothesized that binge-like exposure to alcohol would exacerbate tissue damage and functional deficits in previously injured female mice in part by inducing neuroinflammation and impairing neuroplasticity.

2. Methods

2.1. Animals

All procedures were conducted on female Swiss-Webster mice derived from breeders purchased from Charles River (Wilmington, MA) and bred at the Ohio State University. Pups were weaned at 21 days of age into a standard mouse cage (32 × 16 × 12 cm) with *ad libitum* access to food and filtered tap water. All animals were housed in a 14:10 light-dark cycle. All procedures were approved by the OSU Institutional Animal Care and Use Committee, and were conducted in accordance with the National Institute of Health guidelines.

2.2. Traumatic brain injury

On the day of weaning, 21 day-old female mice sustained a mild closed-head injury or a sham injury as described previously (Weil et al., 2016b). All mice were anesthetized with inhaled isoflurane, secured in a stereotaxic frame, and a round 2 mm impactor (Impact One device, Leica Biosystems, Richmond IL) was placed on the surface of the exposed skull (−1 mm AP, −1 mm ML). TBI mice underwent an impact at 3 mm/s (dwell time 30 ms) to a depth of 1 mm, while sham mice were exposed to an equivalent amount of anesthesia in the absence of impact. The skin was closed with nylon suture and mice were returned to their home cages.

2.3. Alcohol administration

Beginning seven weeks following TBI or sham injury, all mice were administered 5 g/kg of a 31.5% solution of ethanol or water control (dosing based on (Bertola et al., 2013)). Ethanol or water was administered via daily oral gavage for 10 consecutive days. Mice were weighed daily to account for body mass fluctuations in the dosing. This paradigm resulted in 4 groups (sham/water, TBI/water, sham/ethanol, TBI/ethanol), which were divided into 3 separate cohorts. One cohort was used for assessment of pathophysiology only (n = 8–10 per group), one for microglial extraction (n = 6–8 per group), and another cohort underwent behavioral testing followed by histological assessment (n = 9–12 per group). See Fig. 1 for experimental timeline.

2.4. Blood ethanol concentration

Blood ethanol concentration (BEC) was assessed via a colorimetric 96-well plate assay (modified from Prencipe et al., 1987). Blood was drawn from the retro-orbital sinus 90 min after the final oral gavage. Blood samples were centrifuged and serum stored at −80 °C until use. A BEC assay buffer (100 mM KH_2PO_4; 100 mM K_2HPO_4; 0.7 mM 4-aminoantopyrine; 1.7 mM chromotropic acid disodium salt; 50 mg/L EDTA; 50 mL/L triton X-100) was used to dilute ethanol standards and serum and all wells were assessed in duplicate. A reaction mixture of alcohol oxidase (Sigma A2404) and peroxidase from horseradish (Sigma P8375) diluted in BEC assay buffer was added to all wells and the plate was read at 600 nm.

2.5. Histology

Axon damage was assessed via silver staining using the NeuroSilver kit (FD Neurotechnologies, PK301) per manufacturer's instructions. Mice were overdosed with sodium pentobarbital (200 mg/kg) and transcardially perfused with 4% paraformaldehyde. Following an overnight postfix and cryopreservation, brain sections throughout the forebrain were sliced on a cryostat, (40 μm) and collected into a 24-well culture plate for free-floating immunohistochemistry and silver staining. For immunohistochemistry, tissue was washed with 0.1 M phosphate-buffered saline, quenched in hydrogen peroxide, and incubated in primary antibody overnight (rabbit anti-Iba1, Wako 019-19741; goat anti-doublecortin, Santa Cruz sc-8006). Biotinylated secondary antibodies (goat anti-rabbit and donkey anti-goat, Vector Laboratories BA-1000 and BA-5000) were applied the following day, and staining was visualized using the avidin biotin-diaminobenzidene staining method (Vector Laboratories PK-6100 and SK-4100).

2.6. Histological analysis

Axonal degeneration (silver staining) was assessed qualitatively as previously reported (Weil et al., 2014). Briefly, representative tissue throughout the entire forebrain were assessed and scored on a 4-point scale (0 = few axonal profiles, 3 = dense axonal degeneration throughout white matter tracts bilaterally).

Microglial cells (Iba1-positive staining) were assessed in the prefrontal cortex. Morphological analysis was conducted using Neurolucida software (MicroBrightfield). Briefly, 2–3 cells per hemisphere from each brain were selected using strict criteria as previously reported (Karelina et al., 2016) and traced for subsequent sholl analysis. Using NeuroExplorer (MicroBrightfield) concentric sholl rings spaced 5 μm apart were centered on the cell body, and the number of intersections, total process length, and cell body surface area were obtained for each cell. Total microglial