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PERSISTENT WORKING MEMORY DYSFUNCTION FOLLOWING TRAUMATIC BRAIN INJURY: EVIDENCE FOR A TIME-DEPENDENT MECHANISM

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Abstract—The prefrontal cortex is highly vulnerable to traumatic brain injury (TBI) resulting in the dysfunction of many high-level cognitive and executive functions such as planning, information processing speed, language, memory, attention, and perception. All of these processes require some degree of working memory. Interestingly, in many cases, post-injury working memory deficits can arise in the absence of overt damage to the prefrontal cortex. Recently, excess GABA-mediated inhibition of prefrontal neuronal activity has been identified as a contributor to working memory dysfunction within the first month following cortical impact injury of rats. However, it has not been examined if these working memory deficits persist, and if so, whether they remain amenable to treatment by GABA antagonism. Our findings show that working memory dysfunction, assessed using both the delay match-to-place and delayed alternation T-maze tasks, following lateral cortical impact injury persists for at least 16 weeks post-injury. These deficits were found to be no longer the direct result of excess GABA-mediated inhibition of medial prefrontal cortex neuronal activity. Golgi staining of prelimbic pyramidal neurons revealed that TBI causes a significant shortening of layers V/VI basal dendrite arbors by 4 months post-injury, as well as an increase in the density of both basal and apical spines in these neurons. These changes were not observed in animals 14 days post-injury, a time point at which administration of GABA receptor antagonists improves working memory function. Taken together, the present findings, along with previously published reports, suggest that temporal considerations must be taken into account when designing mechanism-based therapies to improve working memory function in TBI patients. **© 2009 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: Golgi staining, neuronal morphology, prelimbic cortex, delayed alternation T-maze, delay match-to-place, GAD.

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Abbreviations: DLPFC, dorsolateral prefrontal cortex; GAD67, glutamic acid decarboxylase 67 kDa; mPFC, medial prefrontal cortex; PFC, prefrontal cortex; PL, prelimbic cortex; TBI, traumatic brain injury; WM, working memory; 6-OHDA, 6-hydroxydopamine.

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Cognitive and behavioral dysfunctions are pervasive among people with traumatic brain injury (TBI). While these deficits often normalize within a year for many with mild TBI, they persist in approximately 10%–15% of mild, 50% of moderate, and a large percentage of severe TBI victims (Kraus and Chu, 2005). One of the prominent cognitive deficits in individuals with TBI is working memory (WM) impairments, the ability to hold information on-line for subsequent integration and manipulation in order to guide goal-directed behavior (Baddeley, 1992; McAllister et al., 2001, Finley et al., 2005, Dash et al., 2007). Since WM is critical for many high level cognitive functions, patients with WM deficits have difficulties with executive function, such as one's ability to organize and execute complex processes like planning. In addition, information processing speed, language, memory, attention and perception, all require some degree of WM (Baddeley, 1992; Arnsten, 1997). A number of functional imaging studies in humans and electrophysiological/pharmacological experiments in non-human primates have demonstrated that dysfunction of the dorsolateral prefrontal cortex (DLPFC) is the main cause for WM deficit (Fuster and Alexander, 1971; D'Esposito, 2000). Consistent with this, recent functional imaging studies have indicated that people with mild TBI show more activation of the DLPFC at low WM load, and less activation of the same region at high WM load, as compared to healthy volunteers (McAllister et al., 2001). Given the major role of the prefrontal cortex (PFC) and its circuitry in mediating WM, it has been somewhat perplexing that persistence of post-injury WM deficits often arises in the absence of overt damage to this structure. Therefore, an understanding of the cellular and molecular mechanisms underlying TBI-associated WM deficits in acute, sub-acute, and chronic stages of injury are required for designing temporally-appropriate pharmacological treatments.

As with humans, rodents exhibit WM deficits following TBI (Hamm et al., 1996; Kline et al., 2002). Recent rodent studies have demonstrated that lateral cortical impact injury causes WM dysfunction in the absence of neuronal cell death in the prelimbic cortex (PL) region of the medial prefrontal cortex (mPFC) (Kobori et al., 2006), a structure anatomically and functionally analogous to the primate DLPFC (Kolb, 1984). Mechanistically, these studies have identified excess GABA-mediated inhibition of prefrontal neuronal activity as a major contributor to the observed TBI-associated WM deficits in these animals (Kobori and Dash, 2006). This conclusion was based upon observations that the level of the rate-limiting enzyme for GABA

synthesis, glutamic acid decarboxylase 67 kDa (GAD67), is enhanced in the PFC for up to 1 month post-injury, and that administration of $GABA_A$ receptor antagonists (bicuculline, carboline) improves WM function in injured rats. It has, however, not been examined if these biochemical changes, or WM dysfunction, persist beyond 4 weeks after TBI.

In the present study, we examined if rats subjected to lateral cortical impact injury still display WM deficits 4 months after injury. In these animals, we measured the levels of GAD67 in the PL cortex, and examined the effect of bicuculline on their WM. We also examined if TBI alters the morphology of prelimbic pyramidal neurons and if these changes occur concurrent with, or subsequent to, the previously observed increase in GABAergic signaling.

EXPERIMENTAL PROCEDURES

Animals

All experiments involving the use of animals were carried out under protocols approved by the Institutional Animal Care and Use Committee in compliance with the National Institutes of Health guidelines outlined in Guide for the Care and Use of Laboratory Animals. Protocols were designed in order to minimize pain and suffering during the procedures. Every effort was made to minimize the number of animals required, with only the minimum number of animals required to obtain statistical significance used. Male Sprague–Dawley rats (≥300 g) were purchased from Harlan Sprague Dawley (Indianapolis, IN, USA). Rats were housed in pairs and maintained on a 12-h light/dark cycle with *ad libitum* access to food and water.

Controlled cortical impact injury

A controlled cortical impact device (CCI) was used to initiate a unilateral brain injury as described previously (Dixon et al., 1991; Smith et al., 1995). Briefly, animals maintained under anesthesia (4% isoflurane and 2:1 mixture of N_2O/O_2) were placed in a stereotaxic frame while a 7 mm craniotomy (halfway between bregma and lambda, 3.5 mm lateral to midline) was performed. A heating pad was used to maintain body temperature at 37 °C. Using a 6-mm-diameter impact tip, a single impact (1.7 mm deformation, 6 m/s) was delivered to the parietal association cortex at an angle of 10° from the vertical plane, such that the impact was orthogonal to the cortex surface. These parameters produce a moderate to severe injury without detectable neuronal loss within the mPFC, as assessed previously with histopathological measures (Kobori and Dash, 2006). Although a unilateral craniectomy was performed, this magnitude of injury causes bilateral responses and neuronal dysfunction (Kline et al., 2001; Giza et al., 2002; Verbois et al., 2003; Chen et al., 2005; Enomoto et al., 2005). For this reason, sham-operated animals who received all surgical procedures except the impact, were used as controls for the behavioral, biochemical and morphological measures.

Intra-mPFC cannulae placement and drug infusion

Rats were bilaterally implanted under isoflurane anesthesia with sterile stainless steel guide cannulae aimed at the dorsal border of the prelimbic area using a stereotaxic device (bregma 3.2 mm, lateral ±0.75, and depth −2.5 mm). A bilateral infusion was performed as this has been previously employed to examine the functional consequences of intra-mPFC infusion of muscimol in prefrontal-dependent tasks (Amat et al., 2005; Blum et al., 2006; Jo et al., 2007). For drug administration, the infusion needles were inserted into awake, moving animals. The infusion needles extended 1.5 mm beyond the end of the guide cannulae, giving a total depth of 4.0 mm. Drugs were dissolved in saline and infusions were performed at a rate of 0.25 μl/min. for 2 min. Following infusion, the needles were left in place for 2 min to allow for diffusion of the drug. For examination of the prefrontal dependency of the water version of the delayed alternation task, muscimol (1 μg/side) was infused into the PL 15 min prior to testing. Following the completion of the studies, cannula placement was assessed in a representative group of animals. All infusion needle tracks examined terminated within the PL cortex.

WM testing

All behavioral tests were performed by an experimenter blind to treatment conditions.

Delay match-to-place water maze. Approximately 120 days after injury, animals were trained in the delay match-to-place version of the Morris water maze (Hamm et al., 1996; Kline et al., 2002). The maze consisted of a circular (1 m in diameter) tank in which a submerged platform (10 cm in diameter) was located. The water was maintained at 24 °C and made opaque by the addition of a non-toxic water-soluble paint. Rats were initially given five pairs of trials to familiarize them with the task and the extramaze cues. Testing consisted of a “location” trial, in which animals were given a maximum of 60 s to find the hidden platform. If an animal failed to find the platform, it was led there by the experimenter. Once on the platform, rats were allowed to rest for a period of 10 s after which time they were removed from the maze. After a 5 s delay, animals were returned to the maze and allowed to again search for the hidden platform. After each “location-match” pair of trials, the platform was moved to a different location and a new “location-match” pair tested. An intertrial interval of 4 min separated each pair of “location-match” trials. Each animal was tested in five “location-match” pairs with each pair having a novel platform location and random start site.

Delayed alternation water T-maze. Rats were trained in a delay alternation task using a version of the standard T-maze adapted for use in water. Water effectively motivated animals to explore the T-maze and find an escape platform located at the end of each arm. Training consisted of 7 days’ exposure to the task, during which time the animals were examined for their ability to swim within the maze, and for their ability to remain on the target platform once found. All animals used in the current study were capable of navigating the T-maze and recognized the hidden platform as an escape. For the first trial of each testing session, platforms were placed in both ends of the cross-piece of the T-maze such that the animal was allowed to choose either direction after swimming down the stem. Once the animal reached the platform, it was to remain there for 15 s. After a 10 s delay, the animal was returned to the maze and allowed to choose either arm, but only the arm opposite from the one chosen in the immediately preceding trial had a platform. Consequently, if an animal re-entered the arm chosen in the immediately preceding trial, he was confined to that arm with no platform for 15 s and that trial was scored as incorrect. A total of 10 consecutive trials were performed for each daily session, with a total of 12 days of testing performed on each animal. The percent correct choices were calculated for each session.

Western blotting

Animals were killed and brains were dissected and submerged under ice-cold artificial cerebrospinal fluid (10 mM Hepes pH 7.2, 1.3 mM NaH_2PO_4, 3 mM KCl, 124 mM NaCl, 10 mM dextrose, 26 mM $NaHCO_3$, and 2 mM $MgCl_2$). The PL ipsilateral to the side of impact was quickly removed and snap-frozen on dry ice. Sham-operated animals were used as controls. The mPFC brain tissue was homogenized in a lysis-buffer containing 10 mM Tris pH 7.4,

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