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REGIONAL HIPPOCAMPAL ALTERATION ASSOCIATED WITH COGNITIVE DEFICIT FOLLOWING EXPERIMENTAL BRAIN INJURY: A SYSTEMS, NETWORK AND CELLULAR EVALUATION

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Abstract—Cognitive deficits persist in patients who survive traumatic brain injury (TBI). Lateral fluid percussion brain injury in the mouse, a model of human TBI, results in hippocampal-dependent cognitive impairment, similar to retrograde amnesia often associated with TBI. To identify potential substrates of the cognitive impairment, we evaluated regional neuronal loss, regional hippocampal excitability and inhibitory synaptic transmission. Design-based stereology demonstrated an approximate 40% loss of neurons through all subregions of the hippocampus following injury compared with sham. Input/output curves recorded in slices of injured brain demonstrated increased net synaptic efficacy in the dentate gyrus in concert with decreased net synaptic efficacy and excitatory postsynaptic potential-spike relationship in area CA1 compared with sham slices. Pharmacological agents modulating inhibitory transmission partially restored regional injury-induced alterations in net synaptic efficacy. Both evoked and spontaneous miniature inhibitory postsynaptic currents (mIPSCs) recorded in surviving dentate granule neurons were smaller and less frequent in injured brains than in uninjured brains. Conversely, both evoked and spontaneous mIPSCs recorded in surviving area CA1 pyramidal neurons were larger in injured brains than in uninjured brains. Together, these alterations suggest that regional hippocampal function is altered in the injured brain. This study demonstrates for the first time that brain injury selectively disrupts hippocampal function by causing uniform neuronal loss, inhibitory synaptic dysfunction, and regional, but opposing, shifts in circuit excitability. These changes may contribute to the cognitive impairments that result from brain injury.

Key words: head injury, hippocampus, conditioned fear response, GABA, mIPSC, stereology.

Traumatic brain injury (TBI) is the leading cause of death and disability in children and young adults in the United States (Sosin et al., 1995; NIH Consensus Conference, 1999). Brain-injured patients often suffer cognitive deficits, including impaired learning and memory (McAllister, 1992). Pathological examination of post-traumatic human brains has demonstrated specific damage to the temporal lobe and limbic hippocampus (Adams et al., 1989; Graham et al., 1995). Damage to the human hippocampus results in significant impairment of cognitive function (Cave and Squire, 1991; Rempel-Clower et al., 1996; Miller et al., 1998; Bohbot et al., 2000; Deweer et al., 2001). Fluid percussion injury (FPI) in the rodent reproduces many of the pathological features of human TBI, including neuronal loss, gliosis, and metabolic and ionic perturbations (Carbonell and Grady, 1999; Dixon et al., 1987; McIntosh et al., 1989; Hovda et al., 1990; Ginsberg et al., 1997). Post-traumatic pathology following FPI contributes to an enduring cognitive impairment (Pierce et al., 1998). However, the underlying cellular etiologies of TBI-induced cognitive deficits remain elusive.

Based on prior efforts that focused on pharmacology (McIntosh et al., 1998) and cell loss (Baldwin et al., 1997; Raghupathi et al., 2000; Hartman et al., 2002; Grady et al., 2003), the systems approach employed in the present study addresses injury-induced hippocampal dysfunction in animals with demonstrated cognitive deficits. Previously, Hicks et al. (1993) showed a significant correlation between cognitive performance and bilateral loss of hilar neurons 2 days after injury in rats. Kline et al. (2002) showed that post-injury hypothermia decreased area CA3 neuronal loss and improved beam walk and water maze performance. Furthermore, non-specific hilar neuronal loss has been reported to reduce inhibition, resulting in increased dentate gyrus (DG) excitability (Lowenstein et al., 1992; Coulter et al., 1996; Toth et al., 1997; Santhakumar et al., 2000). Conversely, a decrease in area CA1 excitability following FPI in the rat paralleled the inability to induce long-term potentiation (LTP; Reeves et al., 1995;

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Abbreviations: aCSF, artificial cerebrospinal fluid; AP5, D-2-amino-5-phosphono-pentanoic acid; asf, area sampling fraction; BMI, bicuculline methiodide; CE, coefficient of error; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; CV, coefficient of variation; DG, dentate gyrus; DHK, dihydrokainic acid; eIPSCs, evoked inhibitory postsynaptic current; EPSP, excitatory postsynaptic potential; E-S, excitatory postsynaptic potential-spike; fEPSP, field excitatory postsynaptic potential; FPI, fluid percussion injury; HEC, hippocampal–entorhinal cortex; I/O, input/output; LTP, long-term potentiation; mIPSC, miniature inhibitory postsynaptic currents; MWM, Morris water maze; PP, perforant path; PS, population spike; R_S, series resistance; ssf, section-sampling fraction; TBI, traumatic brain injury; TTX, tetrodotoxin.

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D'Ambrosio et al., 1998; Sanders et al., 2000). The basis for changes in excitability may lie in alterations in inhibitory circuits, since GABA-mediated inhibition is critical in initiating, synchronizing, and terminating both normal and pathological neuronal network activity. This crucial activity is due to specific localization of inhibitory synapses coupled with wide divergence in interneuron innervation of pyramidal neurons (Freund and Buzsaki, 1996).

Injury-induced neuronal loss and altered hippocampal regional excitability have been previously studied, but not assessed with injury-induced cognitive deficits in the same animal set. The present study was undertaken to determine cellular, circuit and synaptic alterations in the hippocampus that may contribute to TBI-induced cognitive impairment. To achieve our goal, hippocampal-dependent cognitive impairment was assessed using contextual fear response at 1 week following lateral FPI in the mouse. At the cellular level, injury-induced neuronal loss was quantified in hippocampal subregions using design-based stereology. At the circuit level, regional shifts in excitability were examined by generating extracellular input/output (I/O) curves and excitatory postsynaptic potential (EPSP)-spike (E-S) relationship curves. At the synaptic level, alterations of hippocampal inhibitory activity were confirmed by recording GABAergic inhibitory events. We report that neuronal loss and perturbation of surviving inhibitory neuronal networks change hippocampal excitability. These changes may contribute to injury-induced cognitive dysfunction seen in the clinical setting.

EXPERIMENTAL PROCEDURES

Generation of TBI animals

Adult male C57BL/6J mice (5–7 weeks, 20–25 g; Jackson Laboratory, Bar Harbor, ME, USA) were used in all experiments. All experimental procedures and protocols for animal studies were approved by the University of Pennsylvania and the Children's Hospital of Philadelphia Institutional for Animal Care and Use Committees in accordance with international guidelines on the ethical use of animals. Experiments were designed to minimize the number of animals required and those used were cared for, handled and medicated as appropriate to minimize their suffering. (National Research Council, National Academy Press, Washington, DC, 1996).

Day 1

Each animal was anesthetized using sodium pentobarbital (65 mg/kg, i.p.). The animal was placed in a mouse stereotaxic frame (Stoelting, Wood Dale, IL, USA). The scalp was reflected with a single incision and the fascia scraped from the skull. All of the following procedures were conducted under 0.7–3.5× magnification. An ultrathin Teflon disc, with the outer diameter equal to the inner diameter of a trephine, was glued with Vetbond (3M, St. Paul, MN, USA) onto the skull between Lambda and Bregma, and between the sagittal suture and the lateral ridge over the right hemisphere. A miniature screw was placed into the skull directly above the right olfactory bulb. Using a trephine (3 mm outer diameter), the craniectomy was performed, keeping the dura intact. A rigid Luer-loc needle hub (3 mm inside diameter; Becton Dickinson, Franklin Lakes, NJ, USA) was secured to the skull over the opening with cyanoacrylate adhesive and dental acrylic. The skull sutures were sealed with the cyanoacrylate during this process to ensure that the fluid bolus from the injury remained within cranial cavity. The hub was capped until day 2. The animal was sutured, placed on a heating pad, and returned to the home cage once ambulatory.

Day 2

Each animal was placed under isoflurane anesthesia (2% oxygen in 500 ml/min) via nose cone and respiration was visually monitored. Once the animal reached a surgical plane of anesthesia (one respiration per 2 s), the nose cone was removed, the cap over the hub was removed, and dural integrity was visually confirmed. The hub was filled with isotonic sterile saline and a 32 cm piece of high-pressure tubing from the FPI device (Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA, USA) was attached to the Luer-loc fitting of the hub. The animal was placed onto a heating pad on its left side and, once a normal breathing pattern resumed, before sensitivity to stimulation, the injury was induced by a 20 ms pulse of saline onto the dura. The pressure transduced onto the dura was monitored with an oscilloscope, with injury severity ranging between 2.0 and 2.1 atm. Immediately after injury, the hub was removed from the skull and the animal was placed in a supine position. Previous studies in rats have shown that the duration of unresponsiveness after experimental brain injury correlates with the degree of histopathology (Morehead et al., 1994). In our study, the time elapsed until the animal spontaneously righted was recorded as an acute neurological assessment, and defined as the righting reflex time. Animals in the sham group had mean righting times less than 20 s (n=22). Injured animals were included in the study only if righting times were greater than 250 s (n=22). We selected for a mild to moderate injury by excluding two animals which died after FPI, 13 animals that had insufficient righting times after injury, and one animal that had an excessive righting time after sham surgery. The animal was then anesthetized under isoflurane to suture the scalp. Sham animals received all of the above, with the exception of the fluid pulse. Animals were returned to a heating pad until ambulatory and then returned to the home cage.

We did not measure physiologic variables that play a role in secondary injury such as pH, PCO_2, PO_2, or arterial blood pressure due to technical constraints in the mouse model. Primary (impact) and secondary (hypoxia, ischemia, hypotension) injuries combine to mediate injury-induced pathology. These secondary disturbances play a more significant role in severe TBI than in mild to moderate TBI as defined in the present study.

Contextual fear response

Classical conditioning of a context to fear is hypothesized to be hippocampal-dependent as demonstrated by specific lesion and pharmacological inhibition, which inhibit acquisition or expression of the behavior (Kim and Fanselow, 1992; Phillips and LeDoux, 1992). By using this phrase (hippocampal-dependent) we do not mean to imply that the hippocampus is the exclusive mediator but rather a crucial component of this behavior. It is well accepted that several brain regions are important contributors to the neural circuitry underlying fear conditioning. The hippocampal-dependent contextual fear response paradigm was implemented to classically condition a non-noxious contextual environment (conditioned stimulus=context) with a noxious stimulus (unconditioned stimulus=shock; Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Gerlai, 1998). All animals received 2 days of handling prior to conditioning. During conditioning, animals were trained individually in a conditioning chamber (44×17×24 cm), with metal and Plexiglas walls and a metal grid floor (Med Associates, St. Albans, VT, USA). Each mouse was placed in the chamber for 148 s, before a 1.5 mA 2 s foot shock was delivered. The animal remained in the chamber for an additional 30 s after the shock before being returned to its home cage. The fear conditioning was performed 2 days before FPI or sham surgery.