Short communication

Behavioral, electrophysiological, and histopathological consequences of mild fluid-percussion injury in the rat

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Abstract

Metabolic dysfunction in the relay nuclei of the rat vibrissa circuit follows traumatic brain injury (TBI). This study examined the effects of mild (1.4–1.5 atm) parasagittal fluid-percussion injury on the electrophysiology of this circuit. TBI caused significant reductions in slope and increases in latency of vibrissa-evoked field potentials 3 days after injury. Assessment of open-field swimming revealed an increase in thigmotaxis 2 days after injury. TBI caused mild selective cortical damage and limited axonal swelling at the injury site. Thus mild injury disrupts somatosensory electrophysiology and exploratory behavior.

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Experimental traumatic brain injury (TBI) is known to result in a perturbation of cerebral metabolism [3,5,8,11,20]. Experimental brain injury results in depression in local cerebral glucose utilization (local cerebral metabolic rate for glucose, or IC\textsubscript{M}R\textsubscript{glu}) within the rat vibrissa somatosensory circuit [3]. Moderate TBI results in a disturbance in the ability of whisker stimulation to activate metabolically all three nuclei involved in transmitting sensory information to the cortex.

To date, no investigation has examined the effects of trauma on the electrophysiology of the vibrissa response. The present study represents an initial effort to examine functional activation of the cortex in light of the existing data on acute metabolic activation following TBI. It is hoped that the present data lays the foundation for future studies of plasticity following injury. This analysis may allow for the eventual delineation of neural circuitry underlying behavioral recovery following injury. The vibrissa system is ideal in this respect, as the peripheral receptors are known to map precisely to functional units, called ‘barrels,’ in the cortex [19].

Injured animals perform poorly on simple sensorimotor tasks [1] and tasks of ‘cognitive’ function. Rats show deficits in both the eight-arm radial maze and Morris water maze after fluid percussion injury, where TBI causes retrograde and anterograde amnesia for spatial information [1,9,12,13,15,16]. Investigators have not examined the effects of trauma on sensorimotor aspects of these tasks. The present study therefore aims to investigate, using quantitative electrophysiologic methods, whisker barrel circuit function following mild TBI and to make a preliminary investigation into the effects of trauma on open-field swimming behavior.

Eleven male Sprague-Dawley rats were subjected to mild TBI (n = 5) or sham procedures (n = 6). All surgical procedures were conducted under the guidelines of the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Miami Animal Care and Use Committee.
Injury was induced under normothermic conditions as reported previously [4]. Fluid-percussion injury was delivered by brief introduction of saline into the extradural space through a round craniotomy (4.8 mm diameter) over the right parietal cortex, with center coordinates of 3.8 mm posterior and 2.5 mm lateral to bregma. A microcomputer recorded the injury pressure in a voltage deflection that was later converted to atmospheres (atm) for analysis. Pressure levels ranged from 1.4 to 1.5 atm. Sham animals underwent all surgical procedures excluding attachment to the fluid percussive device and delivery of the pressure pulse.

Two days after surgery, each animal was tested in an open-field water maze task. When placed in an open field, rats tend to run or swim along the edge (thigmotaxis). The open water maze was used to assess the extent of thigmotaxis and bias in using the vibrissae on one side of the face or the other. We hypothesized that lateralized damage to the vibrissa system would result in lateralized use of the vibrissae during exploration. Twenty-four hours after behavioral assessment, each animal was evaluated for activation of the barrel field under urethane anesthesia (1.5 g/kg). A small craniotomy was placed at 1.5 mm posterior to bregma, at the right temporal ridge (well lateral to the injury site). Potentials were recorded with glass pipettes pulled to open tip diameters of approximately 5–10 μm. Stimulation was achieved by collectively twitching the left (contralateral) vibrissae. The group of vibrissae were glued to a balsa probe (approximately 15 mm from the face) attached to the coil of a 3.5 in., 8 ohm speaker (from which the sound cone had been removed). Three-millisecond pulses were delivered, at 0.5 Hz, through an audio amplifier to produce probe deflections of approximately 2 mm. All stimulation, recording, and analysis were performed with the Experimenter’s Workbench software package (DataWave Inc., Longmont, CO, USA).

Immediately after the collection of the electrophysiological measures, animals were sacrificed for histopathological assessment. Semi-serial coronal sections were obtained at a level 3.8 mm posterior to bregma, corresponding to the focus of the injury site. Slices were mounted on microscope slides and stained with Hematoxylin and Eosin for assessment of neuronal and axonal damage in cortical and subcortical brain regions.

The repeated measures analysis of thigmotaxic swimming revealed that injured animals swam significantly greater distances along the perimeter of the pool during the 2 min test \(F(1,10) = 6.75, P = 0.0266; \text{Fig. 1A}\). However, neither group of animals exhibited any bias for swimming in one direction or the other \((P > 0.1)\).

Field potentials from injured animals exhibited significantly smaller slopes \(t(9) = 2.488, P = 0.0345; \text{Fig. 2A}\) as well as significantly longer peak latencies \(t(9) = 2.770, P = 0.0218\). Potentials did not differ between groups on measures of amplitude or area \((P > 0.2)\).

Light microscopic analysis of the cortical barrel field demonstrated relatively mild selective neuronal damage within the cortex of injured animals. Damaged neurons containing pyknotic nuclei and eosinophilic cytoplasm were seen scattered among normal-appearing cells. Damaged neurons were seen mostly in deeper cortical layers, including layers 5 and 6. The external capsule appeared relatively unremarkable. However, evidence of mild axonal swelling and a mild inflammatory response was seen in the majority of rats. Evidence of severe white matter damage, including the presence of retraction bulbs, was an uncommon finding.

The major finding of the present work is that mild TBI is sufficient to disrupt vibrissa stimulation-induced activation in the barrel field cortex. Slopes of field potentials were reduced and latencies were lengthened in injured animals. The cellular basis of these effects is a matter of
speculation. The threshold for activation in individual neurons may shift after trauma. In such a case, careful single-unit recording should be combined with systematic manipulation of individual vibrissae. Autoradiographic 2-deoxyglucose studies of moderate injury in this preparation show altered metabolic activation at both the trigeminal medullary complex and the thalamus [3], and damage or dysfunction at any point in the circuit could result in altered latency of activation and/or failure in activating individual neurons in the cortex. Additional study will be necessary to examine the electrophysiology of trigeminal and thalamic nuclei after trauma in order to pinpoint the exact site(s) of circuit dysfunction. Additionally, investigation must be made of barrel field activation following moderate and severe injury. While the current study reveals reduced responsiveness following mild TBI, previous metabolic study of moderate injury revealed a loss of activation.

A small number of studies, to date, have examined somatosensory evoked potentials after TBI in rodents [10,14]. A study by Shaw [14] examined the latencies of potentials evoked by forepaw stimulation after closed head injury. Components of the potential showed reduced amplitude and increased latency. The current results extend these findings and indicate dysfunction in a separate, trigeminal system after mild TBI. The scarcity of studies on evoked potentials in experimental TBI is surprising, as evoked potentials are a potent tool for investigating disturbances in cerebral function in the clinic. In a mixed population of head trauma and ischemia patients, qualitative gradations of median-nerve SEPs predicted scores on the Glasgow Outcome Scale [17]. Additionally, a multiple regression analysis, constructed with multiple components of the median-nerve SEP, significantly predicted outcome [2]. Studies of evoked potentials in animal models of TBI should be conducted in the future to determine whether such electrophysiological measures predict performance on behavioral and cognitive tasks. Study of the vibrissa response after injury can provide for analysis of injury-induced cortical plasticity, as the vibrissae of the rat are known to project in a highly stereotyped fashion to groups of cells in the cortex known as barrels [18]. By mapping the barrel fields in injured and uninjured animals (or by constructing maps before and after trauma), cortical plasticity engendered by trauma can be investigated more thoroughly. In the present study, mild TBI caused structural damage that was limited primarily to cortex where damaged cells were scattered infrequently among normal
cells. The thalamic relay of the barrel field circuit appeared intact and only mild swelling was seen in cortical axons. The present findings likely result from the combined effects of cell loss, axonal perturbation or other currently unidentified subtle consequences of mild TBI.

Following mild TBI, maze naive rats showed increased thigmotaxic swimming in an open-field swimming pool. The exact deficit found in this study likely is not related directly to lateralized damage in the barrel cortex, as asymmetric swimming was not evident. Nonetheless, injured animals showed a propensity to swim in the periphery of the pool. This type of apparatus typically is used to execute the spatial version of the ‘Morris water maze’ procedure [1,6,7], which requires the animal to swim to a submerged platform from which it escapes the water. Acquisition requires an effective search strategy and discovery of the submerged platform. Although enhanced thigmotaxis is not indicative of a specific learning deficit, thigmotaxis may affect performance by impinging on the ability of the injured rat to learn the procedural and spatial aspects of the task. At a minimum, the presence of this thigmotaxic behavior might complicate an analysis of hidden-platform acquisition and performance. Future studies might include a non-spatial pretraining phase before hidden platform training to minimize the effects of non-spatial behavioral deficits. In this manner, the effects of traumatic injury on spatial learning may be studied selectively.

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