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Transient cognitive deficits are associated with the reversible accumulation of amyloid precursor protein after mild traumatic brain injury

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Abstract

Mild traumatic brain injury (MTBI) may frequently cause transient behavioral abnormalities without observable morphological findings. In this study, we investigated neuropathological mechanisms underlying transient cognitive deficits after MTBI. *Mongolian gerbils* were subjected to experimental MTBI. At various time points after injury, behavioral changes were evaluated by the open-field test and T-maze test, and immunohistochemistry of microtubule-associated protein (MAP2) and amyloid precursor protein (APP) was performed to examine disruptions of the neuronal cytoskeleton and axonal transport, respectively. Transient cognitive deficits were observed after MTBI. Sustained MAP2 loss was found within the cortical impact site, but not the hippocampus. Transient APP accumulation at the same time as transient cognitive deficits occurred in the ipsilateral hemisphere, particularly in the subcortical white matter. These results suggest that the axonal dysfunction indicated by the reversible APP accumulation in the white matter, but not the sustained neuronal cytoskeletal damage reflected by the cortical MAP2 loss confined to the impact site, is responsible for the transient functional deficits after MTBI.

Keywords: Axonal injury; Microtubule-associated protein2; *Mongolian gerbil*; Neuronal cytoskeleton

Mild traumatic brain injury (MTBI) represents 70% to 90% of all human traumatic brain injuries (TBI) [6]. Even without observable morphological brain damage, MTBI patients frequently suffer from cognitive deficits, emotional difficulties, and behavioral disturbances [19,25]. The pathological mechanisms underlying these functional impairments and the recovery process are not fully understood.

Diffuse axonal injury is one of the most important types of brain damage that can occur after MTBI [1]. Clinical studies have shown that axonal injury may cause significant learning and memory dysfunction [1,10]. Since disruption of axonal transport results in amyloid precursor protein (APP) accumulation, APP has been used as a sensitive marker of axonal injury [10,27].

The disruption of the neuronal cytoskeleton is a prominent pathological finding in acute experimental MTBI, and the degradation or loss of microtubule-associated protein (MAP2) serves as a reliable marker of damage to the neuronal cytoarchitecture [24]. In addition, alterations in MAP2 immunoreactivity appear to be associated with functional recovery after brain injury [5].

Using *Mongolian gerbils* for studies on cerebral ischemia have proved that this model is reliable and can induce predictable neuropathological and behavioral outcomes in both short-term and long-term investigations [18,16]. Previously in our laboratory, we produced transient or prolonged hyperlocomotion and working memory deficits following experimental TBI induced by mild or moderate lateral fluid percussion injury (LFPI) in *Mongolian gerbils* [21]. However, the processes mediating the functional recovery in this model are unclear. Here we performed MAP2 and APP immunohistochemistry after mild LFPI in *Mongolian gerbils* to examine post-injury neuronal cytoskeleton and axon changes, and their potential involvement in the transient behavioral abnormalities after MTBI.

Male *Mongolian gerbils* ranging in age from 22 to 28 weeks and in weight from 65 to 80 g were housed in groups of three or four and maintained on a 14/10-h light/dark cycle with unlimited access to food and water. All animal procedures were approved

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by the Animal Experiment Committee of Tokyo Medical and Dental University, in compliance with the guidelines for animal experimentation of the National Institute of Heath.

Animals were randomly divided into a sham-operated group (SHAM; $n = 11$) and an MTBI group (MTBI; $n = 23$). LFPI was induced as previously described [21]. Briefly, each animal was anesthetized with ketamine hydrochloride (50 mg/kg, i.m.), supplemented as necessary. All wounds were infiltrated with 2.0% lidocaine hydrochloride during the surgical preparation and throughout the experiment. When using rats and gerbils for experimental TBI, the application of ketamine hydrochloride in combination with topical injection of 2.0% lidocaine hydrochloride has been proved a reliable anesthetic method with fewer side effects [21,23]. The animals were allowed to breathe spontaneously throughout all surgical procedures. The animal was placed in a stereotaxic frame, and a round craniotomy (3.5 mm in diameter) was made on the right parietal cortex, with center coordinates midway between the bregma and lambda and 2.5 mm lateral to the midline. LFPI of mild severity (0.7–0.9 atm) was induced. SHAM animals received anesthesia and underwent all of the surgical procedures except the delivery of the LFPI. The animals were placed on heating pads to maintain normothermia during the surgical procedure and for 2 h after injury.

Animals (MTBI-7 d, $n = 8$; SHAM-7 d, $n = 8$) were tested in open-field and T-maze tests before injury and at 6 h, 24 h, and 3, 5, and 7 days after injury. We used the open-field test to evaluate spontaneous locomotor activity. Animals were placed individually in an open field apparatus (85 cm × 85 cm at the bottom) and allowed to start from one of the four corners selected randomly by the experimenter. The total distance moved (cm/10 min) by each animal was analyzed as spontaneous locomotor activity by using a video-tracking system and Smart software (Bio Research Center, Nagoya, Japan). The T-maze spontaneous alternation task has been used to test exploratory behavior and working memory [21,11]. Each animal was allowed to alternate between the left and right goal arms of a T-shaped maze (60 cm [stem] × 25 cm [arm] × 10 cm [width]) throughout a 15-trial continuous alternation session. The spontaneous alternation rate (SAR) was calculated as the ratio of the alternating choices to the total number of choices (50%, random choice; 100%, alternation at every trial; 0%, no alternation) [11].

Animals were anesthetized and perfused with 4% paraformaldehyde at 6 h (MTBI-6 h, $n = 5$; SHAM-6 h, $n = 1$), 24 h (MTBI-24 h, $n = 5$; SHAM-24 h, $n = 1$), Day 3 (MTBI-3 d, $n = 5$; SHAM-3 d, $n = 1$), and Day 7 (MTBI-7 d, $n = 8$; SHAM-7 d, $n = 8$; used in Behavioral Evaluations above). The brain was cut into six serial coronal sections every 2.0 mm from the level of the anterior pole of the caudate nucleus to the posterior pole. The sections were embedded in paraffin. Each coronal section was sliced to the thickness of 4 μm and processed for the immunohistochemical detection of neuronal cytoskeletal disruption (MAP2, mouse monoclonal antibody, Sigma, St. Louis, MO, USA) and injured axons (APP, mouse monoclonal antibody 22C11, Chemicon, Temecula, CA, USA). Briefly, after blocking with 5% normal horse serum, sections were incubated with primary antibodies (MAP2: 1:1000; APP: 1:500) overnight at 4 °C. After further rinsing, biotinylated secondary antibody was applied. The avidin–biotin complex method (Vector, Burlingame, CA, USA) was used for antibody detection, with 3,3′-diaminobenzidine (DAB) as the chromogen. Following the reaction with DAB, slides were washed, dehydrated, and coverslipped. Omission of primary antibodies served as negative controls.

Alterations in MAP2 immunoreactivity were quantified in the ipsilateral cortex and hippocampus. Areas with negative MAP2 immunostaining in six coronal sections were traced on an image of each scanned section with NIH Image software. The total volume of MAP2 loss (indirect lesion volume) of the ipsilateral hemisphere on each section was calculated as a percentage of the volume of the contralateral hemisphere [26]. To assess the APP immunoreactivity, predefined regions rich in axons were selected in the ipsilateral subcortical white matter, and APP immunoreactivity for each animal was evaluated semi-quantitatively [27,15]. Briefly, ipsilateral corpus callosum, external capsule, internal capsule, and caudate putamen were selected as predefined regions for APP score. Each coronal section was then analyzed for APP by light microscopy and a semi-quantitative rating was given: 0 for no APP, 1 for only scattered APP accumulation, 2 for a moderate amount of APP accumulation (no more than 50% of the total axons), and 3 for large amounts of APP accumulation (more than 50% of the total axons). The total APP accumulation score for each animal was the sum of the scores for the six coronal levels.

Data are presented as means ± S.E.M. Statistical analyses were performed using two-way analysis of variance (ANOVA). All *post-hoc* comparisons between groups used Fisher's PLSD test. A difference was considered statistically significant at $P < 0.05$.

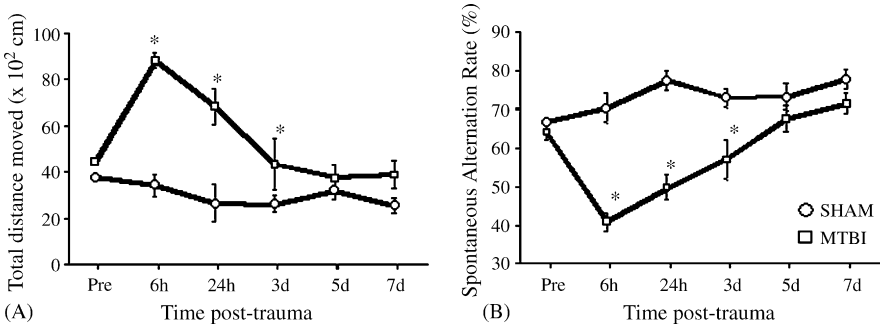

Fig. 1. Results of behavioral tests. (A) Total distance moved in the open-field test; (B) spontaneous alternation rate in the T-maze test. * $P < 0.05$.