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Research report

Training of the impaired forelimb after traumatic brain injury enhances hippocampal neurogenesis in the *Emx1* null mice lacking a corpus callosum

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HIGHLIGHTS

- Performance of the skill reaching task is adversely affected by traumatic brain injury.
- Unilateral traumatic brain injury induces less limb impairment in mice without an intact hemispheric connection compared to wild type mice.
- TBI induces neuroplasticity in the remote brain regions, which may have a wider implication in functional recovery.
- TBI induces neuroplasticity and neurogenesis signals that recapitulate the state during brain development.

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ABSTRACT

Unilateral brain injury is known to disrupt the balance between the two cortices, as evidenced by an abnormally high interhemispheric inhibitory drive from motor cortex $M1_{intact}$ to $M1_{lesioned}$ transmitted transcallosally. Our previous work has shown that the deletion of homeobox gene *Emx1* not only led to the agenesis of the corpus callosum (cc), but also to reduced hippocampal neurogenesis. The current study sought to determine whether lacking the cc affected the recovery of forelimb function and hippocampal plasticity following training of the affected limb in mice with unilateral traumatic brain injuries (TBI). One week after TBI, produced by a controlled cortical impact to impair the preferred limb, *Emx1* wild type (WT) and knock out (KO) mice were subjected to the single-pellet reaching task with the affected limb for 4 weeks. Both TBI and *Emx1* deletion had overall adverse effects on the successful rate of reaching. However, TBI significantly affected reaching performance only in the WT mice and not in the KO mice. Both TBI and *Emx1* gene deletion also negatively affected hippocampal neurogenesis, demonstrated by a reduction in doublecortin (DCX)-expressing immature neurons, while limb training enhanced DCX expression. However, limb training increased DCX cells in KO mice only in the TBI-treated group, whereas it induced neurogenesis in both WT mice groups regardless of the treatment. Our finding also suggests that limb training enhances neuroplasticity after brain injury at functionally remote regions including the hippocampus, which may have implications for promoting overall recovery of function after TBI.
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1. Introduction

The two cerebral hemispheres are functionally balanced; however, unilateral dysfunction resulting from injury often disrupts this balance [28,18]. In humans, cortical excitability in the unaffected motor cortex can increase after a unilateral brain injury like stroke or after a transient suppression of cortical excitability such as hemispherectomy or transection of the corpus callosum [36], the

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principal fiber tract that connects the left and right hemispheres. The exact role of contralesional cortical activity in mediating functional recovery is not completely understood, although in healthy human subjects, suppression of excitability of one motor cortex via repetitive transcranial magnetic stimulation can enhance motor performance of the ipsilateral hand [17,18]. Hyperexcitability in the contralesional hemisphere after stroke is thought to occur as a consequence of disinhibition due to loss of interhemispheric connections from the affected cortex, an action largely mediated by transcallosal fibers [24,22]. Indeed in some stroke patients, an abnormally high interhemispheric inhibitory drive from M1 (intact hemisphere) to M1 (lesioned hemisphere) is observed during the process of generation of a voluntary movement by the paretic hand [25]. In rats, acute cortical lesions lead to an increase in excitability of homotopic areas of the contralateral hemisphere and facilitation of motor skill learning with the unaffected forelimb [1], further supporting the concept of interhemispheric rivalry. In suppressing the contralesional activity, we found that combining physical therapy and limb restraint with botulinum toxin significantly improved function in rats with TBI [21].

The mammalian homeobox *Emx1* gene family is involved in the development of the rostral brain. We previously found that germ line deletion of the *Emx1* gene reduced the size of the dentate gyrus (DG) and decreased the number of proliferating cells and immature neurons found within, but it did not affect baseline neurogenesis in the subventricular zone during adulthood [16]. Despite the acallosal phenotype, the adult naïve *Emx1* mutant mice displayed normal basic motor coordination and spatial memory function compared to wild type mice. However, *Emx1* gene deletion impaired performance in a skilled reaching task and attenuated training-induced hippocampal neurogenesis. Because brain injury recapitulates gene expression patterns that are observed during development or regeneration [10], the current study sought to determine whether unilateral TBI modifies functional impairment in the *Emx1* mutant mice under conditions of acallosal phenotype and reduced neurogenesis. We found that TBI appeared to impair limb reaching function less in mice lacking an intact interhemispheric connection compared to wild type mice. Enhanced neuroplasticity in response to limb training occurred in the acallosal mice only in the TBI group, in contrast to the wild type mice, which displayed this effect with or without brain injury.

2. Materials and methods

2.1. Animals, housing and general considerations

This study was conducted in accordance with the animal care guidelines issued by the National Institutes of Health and by the San Francisco Veterans Affairs Medical Center Animal Care and Use Committee. Adult male mice 2.5 months of age, weighing 24 to 30 g, derived from cryopreserved embryos at Jackson Laboratory were bred and maintained in house in the institutional standard cages (4 mice per cage) on a 12-h light/12-h dark cycle with ad libitum access to water and food before and during experimental procedures. All procedures including surgery, behavioral assessment and histological quantification were conducted by examiners blinded to experimental conditions.

2.2. Induction of traumatic brain injury

Emx1 wild type (WT) and *Emx1* knock out (KO) mice were randomly assigned to either a TBI group or a sham group. Animals were anesthetized with isoflurane/O_2/N_2O (1.5/30/68.5%) during surgery and their core temperature was maintained within $37 \pm 0.5\,^{\circ}C$ with a heating blanket and rectal thermistor servo-loop during both the surgical and the postoperative recovery period.

Controlled cortical impact (CCI) was conducted as described [26,41]. Secured in a stereotaxic frame (Kopf instrument, Tujunga, CA) followed by a midline skin incision, a 3 mm diameter circular craniotomy was performed with a dental drill, lateral (right side) to the mid-sagittal suture centering at [AP: −2.0 mm; ML: 2.0 mm] relative to Bregma. The mouse was then subjected to a CCI using an impactor of 2.0 mm in diameter, operated by a linear motor and microprocessor controller (Linmot, Zurich, Switzerland). The impactor tip was first centered over the craniotomy and was slowly lowered till the tip just contacted the dura (confirmed by an operating microscope). The impact injury was generated using the following parameters: 1.5 m/s strike velocity, 1.00 mm depth of penetration, and a 155 ms contact time. The scalp was then closed with sutures and each animal was given 1.0 ml of isotonic saline subcutaneously to prevent dehydration. Sham animals received craniotomy but no impact.

2.3. Single pellet reaching task

The skill reaching training was performed as described [42,1,16]. Mice were reduced to 90% of their starting body weight and maintained at this level throughout the experiment. To determine limb preference, mice were placed on a restricted diet one day prior to training, followed by a brief shaping period for 3 days during which the frequency of using left versus right limb was recorded. Shaping and training required the mouse to reach a single banana-flavored food pellet placed into a well in front of the clear Plexiglas chamber (13.3 cm long × 21.4 cm high × 9.4 cm wide) where the animal resided. To prevent possible interaction between handedness and the performance of reaching, training was conducted on the preferred limb. To reinforce training of the preferred limb, an aluminum wall was placed ipsilateral to the reaching limb approximately 2 cm from the reaching window, preventing reaches with the non-preferred forelimb. For each reaching trial, mice were permitted up to five reach attempts until the pellet was grabbed successfully, dropped, knocked from its well or not touched at all. Performance was measured as the percentage of successful reaches divided by the total number of reaching trials (successful reaches + missed reaches + dropped pellets). A successful reach was one in which the mouse grabbed the pellet from the well and either brought it to its mouth and ate it, or put it to the floor for later consumption after properly grabbing. Missed reaches include reaches that did not contact the pellet at all or in which the mice knocked the pellet to the outside of the chamber. Drops were considered to be reaches in which the mouse grabbed the pellet but could not properly hold on to it so that it fell down. Training periods consisted of two daily sessions of 30-min or 40 single-pellet trials, whichever came first, for 28 days beginning at one week after CCI or sham surgery. Mice that did not reach for at least 30 pellets per session after 2 weeks of training were excluded from the experiment. Mice were euthanized at the end of the 28-day training.

2.4. BrdU labeling

To determine the effect of motor learning on the survival of newborn hippocampal cells, mice received a single daily intraperitoneal injection of thymidine analog 5-bromo-2′-deoxyuridine-5′-monophosphate (BrdU) (Sigma, St. Louis, MO) at 50 mg/kg for 14 consecutive days, beginning on the first day of skill reaching training.

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