Behavioural Brain Research 303 (2016) 103–108

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Long-term deficits of the paretic limb follow post-stroke compensatory limb use in C57BL/6 mice

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HIGHLIGHTS

- Nonparetic limb training in the mouse functions as a model of compensatory limb use.
- Fifteen days of early nonparetic limb training in the mouse results in long-term deficits of the paretic limb.
- Twenty-eight days of rehabilitative training after early nonparetic limb training does not improve skilled reaching.

ARTICLE INFO

Article history:
Received 14 December 2015
Received in revised form 20 January 2016
Accepted 22 January 2016
Available online 26 January 2016

Keywords:
Ischemia
Rehabilitation
Recovery
Behavioral flexibility

ABSTRACT

Stroke is a leading cause of long-term disability that most often results in impairment of a single limb, contralateral to the injury (paretic limb). While stroke survivors often receive some type of rehabilitative training, chronic deficits persist. It has been suggested that compensatory use of the nonparetic limb immediately after injury may underlie these long-term consequences. The current study investigated the behavioral effects of early compensatory limb use on behavioral outcome of the paretic limb in a mouse model of stroke. Mice received unilateral stroke after acquiring skilled motor performance on a reaching task. Following injury, mice received either delayed rehabilitation of the paretic limb or compensatory limb training prior to delayed rehabilitative training. After 28 days of focused rehabilitative training of the paretic limb, mice that had previously received compensatory limb training exhibited performance that was similar to their initial deficit after stroke while mice that received delayed rehabilitative training improved to pre-operative performance levels. Our results indicate that even with extensive focused training of the paretic limb, early compensatory limb use has a lasting impact on the behavioral flexibility and ultimate functional outcome of the paretic limb.

1. Introduction

Stroke is a leading cause of disability, with upper limb impairment among the most common, chronic deficits reported in stroke survivors [1]. This deficit is often unilateral, resulting in a loss of function in the body side contralateral to the damaged hemisphere (i.e., paretic body side). The body side that is ipsilateral to the lesion locus is largely unaffected by the stroke and is therefore termed the nonparetic body side. A common coping strategy following stroke is a compensatory reliance on the nonparetic limb for completion of daily tasks involving skilled hand use (e.g., grasping or manipulating objects). This compensatory use of the nonparetic limb may present as a learned non-use of the paretic limb [2,3], or as a result of rehabilitative therapy focusing on improving functional use of the nonparetic limb to circumvent the paretic limb deficit [4]. While compensatory limb use may promote efficient daily task completion, it also may limit long-term functional outcome following stroke. Research conducted in rodent models of stroke suggests that, at least in the short term, reliance on the nonparetic limb has a negative impact on functional outcome of the paretic limb [5–7]. However, the long-term impact of compensatory limb use on functional outcome of the paretic limb is unclear.

"Learned non-use" refers to a reliance on the nonparetic limb despite appropriate, functional anatomical connections to promote successful use of the paretic limb [8,9]. It is believed that the preference for the nonparetic limb persists because of early, unsuccessful or painful attempts to engage the paretic limb. Over time, individuals learn to neglect the paretic limb because they have received little to no reinforcement from its use since injury. Such a phenomenon has also been observed in rodent models of stroke [7,10–14].

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http://dx.doi.org/10.1016/j.bbr.2016.01.055

Current data indicate that compensatory limb training following ischemic stroke results in impaired functional outcome of the paretic limb. In rat models, nonparetic limb training appears to retard functional improvement of the paretic limb [5,6,10], though focused rehabilitative training after compensatory training of the nonparetic limb ultimately improves functional outcome to pre-stroke performance levels. The C57BL/6 mouse model suggests a more long-term paretic limb deficit following compensatory limb training, with seven days of focused rehabilitative training of the paretic limb having a null effect on functional outcome [7]. The current study was conducted to investigate the long-term impact of early compensatory limb use by assessing functional outcome of the paretic limb following one-month of focused rehabilitative training of the paretic limb after initial compensatory limb use. The findings of this study suggest long-term consequences of reliance on the nonparetic limb to ultimate functional outcome following stroke. That is, one month of focused rehabilitative training does not improve functional outcome following compensatory limb use. These results suggest that the C57BL/6 mouse strain provides a highly-clinically relevant model of stroke recovery wherein the mechanisms of the long-term deficits associated with compensatory limb use can be studied.

2. Experimental procedures

2.1. Subjects

A total of 24 male C57BL/6 mice were housed in groups of four with standard housing supplementation (PVC pipe, nesting materials, cardboard rolls). Mice were maintained on a 12:12 light/dark cycle. The mice were 2-months of age upon arrival and were handled for three weeks prior to the onset of behavioral training. Mice were then placed on a restricted feeding schedule (2.5–3 g/mouse) for the duration of the study. All mice were weighed daily and daily food allotment was adjusted such that no mouse lost more than 10% of its pre-experimental free-feeding weight. Two mice were used to optimize training and surgical procedures. Their data were not used in the current study. Two additional mice did not adequately acquire the skilled reaching task (criterion described in Section 2.3.1) and were excluded from the study prior to the surgical procedure. Animal use was in accordance with a protocol approved by the Illinois Wesleyan University Animal Care and Use Committee.

2.2. Surgical procedure

After the mice completed pre-operative motor skill learning (described in Section 2.3 below), a total of 20 mice received an intracortical infusion of the vasoconstricting peptide, endothelin-1 (ET-1). Surgical procedures have been described in detail previously [14]. Briefly, mice were anesthetized with isoflurane in oxygen. The animals were then secured in a stereotaxic frame (Stoelting, Wood Dale, IL). A midline incision was made to expose the skull and a small burr hole was drilled over the region of the motor cortex contralateral to the preferred forelimb (0.3 mm anterior to Bregma; 1.5 mm from midline). Ischemic stroke was induced with 3 μl of ET-1 (American Peptide; 320 pmol) injected 800 μm below the surface of the motor cortex over a 10 min period. The burr hole was filled with Ethicon Bone Wax and the wound was sutured and covered with an antibiotic ointment. The mice awoke from the procedure in heated chambers and were monitored for a minimum of 2 h before they were returned to their home cages. All mice were allowed a recovery period of 4 days during which the restricted feeding schedule was maintained.

In order to evaluate the validity of the surgical procedure, five mice received sham operations that were identical to the ischemic strokes listed above with the exception that saline was injected into the motor cortex instead of ET-1. These mice were sacrificed and perfused 30 days after the sham procedure. No notable lesions were present in this group.

2.3. Behavioral methods: Pasta Matrix Reaching Task

Mice were trained on the Pasta Matrix Reaching Task (PMRT; described previously [14,15]) both pre- and post-operatively. Mice were placed in a Plexiglas chamber and were trained to reach through a slit in the center wall to break pieces of vertically arranged pasta (De Cecco brand, Fratelli De Cecco di Filippo Fara San Martino S.P.A., Italy; 3.2 cm in height, 1 mm in diameter) that were inserted into a heavy-duty plastic block outside the reaching chamber. All pasta pieces were 2 mm apart.

2.3.1. Preoperative training

Prior to training, 22 mice underwent shaping procedures. The shaping procedures determined limb preference and lasted a maximum of 7 days. Mice were placed individually in the reaching chamber with a full pasta matrix placed outside of the chamber. Mice were allowed to reach for pasta pieces using either limb. A daily trial consisted of a maximum of 10 reaches (combined across both limbs) or 10 min, whichever occurred first. A reach was defined as extending a single limb through the reaching aperture such that the entire forepaw was outside of the chamber walls. Limb preference was determined when a minimum of 70% of a mouse's daily reaches were made with a single limb. Shaping continued daily until each mouse's limb preference was determined.

Mice were trained on the PMRT following shaping to establish the motor skill. Mice were placed in the chamber with half of the matrix (contralateral to the preferred limb) filled with pasta. As mice must reach across their body to successfully retrieve pasta, this procedure forces the use of the preferred limb during pre-operative training. Each daily trial consisted of 15 min or 100 reaches, whichever occurred first. Mice were trained five days a week for a total of 16–19 days, until performance was considered stable (determined as asymptotic performance visualized on a line graph). Two mice were excluded from the study following pre-operative training for failing to acquire the skill, defined as breaking a minimum average of 9 pasta pieces on the final three days of training.

After pre-operative training, 20 mice underwent the surgical procedures described in Section 2.2 above to selectively impair function of the preferred limb. After four days of recovery, all mice received a single PMRT session with their paretic limb to assess initial post-stroke performance.

2.3.2. Post-operative training

Mice were split into two groups post-operatively; 10 received nonparetic limb training (NPT) while the other 10 received control procedures (Control). The post-operative training procedure was similar to the pre-operative training procedure. The only exception was that NPT mice reached for pasta pieces using their intact limb (i.e., ipsilesional, nonparetic limb). This was achieved by placing a half-filled matrix in front of the chamber as in Section 2.3.1, but the half of the matrix that was filled was opposite the animal's nonparetic limb as described previously [15]. Mice receiving control procedures were placed in the training chambers but did not reach for pasta. Instead, the control mice received an equivalent amount of pasta that was broken into small pieces placed on the floor of the training chamber. This prevented control mice from engaging either limb during post-operative training. Post-operative training lasted a total of 14 days (7 days/week for 2 weeks).