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ENRICHED ENVIRONMENT PROMOTES EFFICIENCY OF COMPENSATORY MOVEMENTS AFTER CEREBRAL ISCHEMIA IN RATS

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Abstract—Rehabilitation therapy is known to drive motor improvement in stroke patients. However, the interplay of functional recovery and compensation in postischemic motor behavior is poorly understood. This study focused on the time course of functional recovery versus motor compensation in skilled forelimb movements after cerebral ischemia in rats. Young adult male rats underwent a focal cerebral ischemia by unilateral photothrombotic lesion of the motor cortex related to the preferred forelimb. In a first set of experiments animals were exposed to small cortical lesions comprising the forelimb motor cortex (n=8) or to larger lesions additionally extending into the hind limb motor area (n=8). In a second set of experiments animals with large lesion were either housed in standard (n=10) or enriched environment (n=14). Skilled reaching was assessed for 25 to 28 days postischemia. This task allows the distinction between recovery and compensation by parallel quantitative (reaching success) and qualitative (movement pattern) analysis. The results reveal that lesion size determines the initial magnitude of motor deficits, but not the degree of chronic impairments in movement pattern in all experimental groups. Compensatory movements represent the major mechanism of functional improvement and were accompanied by a partial functional restitution. Enriched environment facilitates effective compensation in skilled reaching, while it does not promote restitution of function. In particular, rotating movements of the forelimb during reaching were permanently impaired and required functional compensation through intensified use of the upper body. We conclude an activity dependent postischemic restoration of movement success. Enriched environment provides benefit by increased motor activity mainly due to compensation. Furthermore, these findings emphasize the power of comprehensive movement analysis to gain insight into recovery processes after stroke. © 2009 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: skilled reaching, recovery, compensation, functional improvement.

Loss of motor function is a common consequence of stroke. While partial functional restitution might occur spontaneously, the functional improvement in stroke patients is usually incomplete. Lesion size and lesion location determine the severity of initial functional deficits and time course of recovery (Calvanio et al., 1993; Chen et al., 2000), but compensatory mechanisms substantially contribute to functional improvements (Krakauer, 2006; Whishaw, 2000). Compensation reflects the use of alternate behavioral strategies in order to solve a specific task (Metz et al., 2005; Whishaw, 2000) accompanied by structural adaptation in the intact neural substrate (Nudo et al., 2001) and enhanced brain plasticity (Kunze et al., 2006; Witte et al., 2000).

In humans, many rehabilitation strategies achieve partial functional improvement through exercising specific movements in a repetitive fashion. In animal studies, enriched environment (EE) has been shown to improve functional outcome after middle cerebral artery occlusion in rats (Biernaskie et al., 2005; Risedal et al., 2002). EE-related functional improvement is associated with enhanced dendritic growth (Biernaskie and Corbett, 2001), changes in brain-derived neurotrophic factor levels (Gobbo and O'Mara, 2004; Zhao et al., 2001), gene expression (Keyvani et al., 2002; Kolb and Whishaw, 1998; Rampon et al., 2000) or neurogenesis (Komitova et al., 2005; Wurm et al., 2007). Although there is growing evidence for a beneficial effect of EE on post-ischemic motor outcome, it is not clear, whether EE influences functional restitution or compensation. Thus, EE may potentially rewire the neuronal circuit and promote restitution of the original function. However, EE seems likely to encourage the development of successful compensatory strategies that account for the functional improvements.

The purpose of the present study was (i) to compare the effects of lesion size on compensatory movements, and (ii) to determine if EE supports functional restitution or compensatory movements after experimental cerebral ischemia of the motor cortex in rats. Animals were trained and tested in a skilled reaching task, and post-ischemic movements were repeatedly analyzed up to 25 and 28 days. Furthermore, skilled walking and non-skilled motor functions were assessed at various time points. The time courses of quantitative and qualitative behavioral changes were compared.

EXPERIMENTAL PROCEDURES

Experimental subjects

The animal experiments were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All experiments were approved by the animal welfare commission of the Thuringian government. All efforts were

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Abbreviations: DPO, postoperative day; EE, enriched environment; LTP, long-term potentiation; PT-L, large photothrombosis; PT-S, small photothrombosis; SE, standard environment.

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made to minimize the number of animals used in the present study. Forty adult male Wistar rats (280–300 g), raised and housed at the Institute of Laboratory Animal Science of the Friedrich Schiller University of Jena, were subjected to this study (12-h light/dark cycle at 22 °C, water *ad libitum*).

Prior to and during training sessions rats were food deprived to enhance motivation for grasping. We fed the rats with a certain weight-adapted amount of food per day. However, body weight was maintained at 95% of the initial weight by additional feeding after daily behavioral training and testing.

Experimental design

Motor behavior was evaluated after unilateral motor cortex lesion in two sets of experiments. Set 1 aimed to evaluate the impact of lesion size on post-ischemic severity and time course of functional improvement after small photothrombosis [PT-S, $n=8$] vs. large photothrombosis [PT-L, $n=8$] in a skilled reaching (Metz and Whishaw, 2000), a skilled walking (Metz and Whishaw, 2002) and a forelimb asymmetry task (Schallert et al., 2000a). The motor performance in these tasks was video recorded at the last day prior to the lesion (baseline) and at postoperative days (DPO) 3, 7, 14, 21 and 25 for qualitative movement analysis. Reaching success was assessed at each DPO. In addition, set 2 of experiments was performed to elucidate the therapeutic potential of post-ischemic EE exposure to promote compensation-related functional improvement in reaching after PT-L [EE, $n=14$] in comparison to housing in standard cages [standard environment [SE], $n=10$]. Up to the experimental ischemia rats were housed in groups each of three to five animals in standard cages (55×18.5×34.7 cm) covered with mulch. Two hours after lesion 14 of these animals were transferred to an EE facility (67.0×36.0×83.0 cm) divided into two groups of seven animals each. The EE facility provided various toys, including plastic tubes, seesaws, and a running wheel in a variable combination that was changed daily. Test sessions including video recording were performed as mentioned for the experimental set 1. After behavioral tests were completed, brains were taken for histological examination.

Surgery

Focal cerebral ischemia was induced by photothrombosis (Buchkremer-Ratzmann et al., 1996; Watson et al., 1985) in two different sizes: a small lesion (PT-S) comprising the caudal and rostral forelimb area of motor cortex, and a large lesion (PT-L) comprising the motor cortex forelimb and hind limb areas according to Paxinos and Watson (1998), respectively contralateral to the forepaw individually preferred in reaching.

Animals were anesthetized using 2.5% enflurane in 20% O_2 and 80% N_2, and the skull was fixed in a stereotactic frame. After skin incision a fiberoptic light mounted on a cold light source (Schott KL 1500, Jena, Germany) was positioned over the skull at the appropriate coordinates, i.e. in set 1: at 0.3 mm anterior and 3.7 mm lateral to bregma with an aperture of 2.4 mm diameter for PT-S and, at 1.0 to 4.5 mm lateral and −2.5 to 4 mm relative to bregma with rectangle aperture of 3.5×6 mm for PT-L. To assess the capability of EE to improve motor impairment, the lesion in experimental set 2 was extended to 4×7 mm within the coordinates at 0.5 to 4.5 mm lateral and –2.5 to 4.5 mm relative to bregma for EE and SE. With the onset of illumination, 0.4 ml of a 10% solution of the photosensitive dye Rose Bengal (Sigma Aldrich Chemie GmbH, Steinheim, Germany) was administered through the tail vein via a butterfly catheter. After a 20 min illumination period the skin was sutured and animals were allowed to recover in their home cages.

Skilled reaching task

The reaching boxes were made out of Plexiglas (40×45×13 cm, custom made) with a 1 cm wide slot in the middle of the front wall to reach for foot pellets that were placed on a shelf attached to the outside of the front wall (4 cm above the floor) in two small indentations (5 mm diameter, 15 mm deep). Rats were individually habituated to the reaching boxes (Whishaw, 2000) and trained to reach for food pellets (45 mg, Bioserv-Inc., Frenchtown, USA). The pellets were placed contralateral to their preferred paw. Training lasted about 4 weeks until reaching success in individual rats reached asymptotic level. Then, success rates from five consecutive training days were averaged for baseline. Rats were trained to reach for the pellets using a strict protocol. After a reach attempt rats were required to walk back to rear the end of the reaching box. If rats reached when no pellet was presented, rats only received a pellet on the shelf after they began to insert their nose into the slit to sniff properly determining the presence of a food pellet. Twenty food pellets per rat were provided in each training and test session. The extensive training was necessary to prevent post-operative training effects.

Preconditions due to body position seem to considerably influence reaching success. In set 1 rats started a reaching movement partially out of the walking to the front wall. To optimize the qualitative rating procedure, we trained the animals of experimental set 2 to locate the pellet first before starting to grasp. Therefore, in early training sessions we observed, whether or not the rats located the pellet before initiating a reach attempt by intermittent pellet presentation.

Rats were video recorded from a frontal view with a Panasonic Video high 3-CCD portable camera (30 frames/s, shutter speed at 1/1000 s).

Reaching success (quantitative analysis)

A successful reach was defined as a reach in which the rat grasped a food pellet, transported it into the box, and placed it into its mouth. A failed reach occurred if the rat advanced the paw through the slot but missed the pellet, knocked it back into the box or dropped it. Reaching accuracy was scored as the percentage of successful reaches per total number of pellets given in each session.

Reaching movement rating score (qualitative analysis)

Scoring was achieved by frame-by-frame inspection of the video recordings (Panasonic video recorder) of the first three successful reaches, excluding the first successful reach in a session (Metz and Whishaw, 2000; Whishaw et al., 1991). The main movement components in reaching: orient, limb lift, digits close, aim, advance, digits open, pronation, grasp, supination I, supination II and release were divided in 35 subcategories in total. Each of the reaching movement subcategories was rated using the following scale. One point was given for normal motor performance, 0.5 points were given for altered performance, and 0 points were given for absent movements, resulting in maximal 35 points per reach (Metz and Whishaw, 2000; Whishaw et al., 1991). For presentational purposes, in modification to the original method (Metz and Whishaw, 2000; Whishaw et al., 1991), the reaching movement pattern was summed for three main parts: (1) reaching, summarizing movement components that cover the forward movement to the pellet (limb lift until pronation); (2) grasp; (3) withdrawal, summarizing movement components that cover the consumption of the reached pellet (supination I until release, Fig. 4).

Cylinder forelimb asymmetry test

Forelimb use during explorative activity was examined by placing rats in a transparent Plexiglas cylinder 20 cm in diameter and 30 cm high (Schallert et al., 2000b; Shanina et al., 2006). A mirror was placed underneath the cylinder to allow the investigator to videotape the animal's activity from a ventral view. Animals were