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Concurrent silent strokes impair motor function by limiting behavioral compensation

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ABSTRACT

Silent strokes occur more frequently than classic strokes; however, symptoms may go unreported in spite of lasting tissue damage. A silent stroke may indicate elevated susceptibility to recurrent stroke, which may eventually result in apparent and lasting impairments. Here we investigated if multiple silent strokes to the motor system challenge the compensatory capacity of the brain to cumulatively result in permanent functional deficits. Adult male rats with focal ischemia received single focal ischemic mini-lesions in the sensorimotor cortex (SMC) or the dorsolateral striatum (DLS), or multiple lesions affecting both SMC and DLS. The time course and outcome of motor compensation and recovery were determined by quantitative and qualitative assessment of skilled reaching and skilled walking. Rats with SMC or DLS lesion alone did not show behavioral deficits in either task. However, the combination of focal ischemic lesions in SMC and DLS perturbed skilled reaching accuracy and disrupted forelimb placement in the ladder rung walking task. These observations suggest that multiple focal infarcts, each resembling a silent stroke, gradually compromise the plastic capacity of the motor system to cause permanent motor deficits. Moreover, these findings support the notion that cortical and subcortical motor systems cooperate when adopting beneficial compensatory movement strategies.

Introduction

About 12–15% of major strokes are preceded by a transient ischemic attack or minor stroke (Hankey, 2003). Minor strokes represent small brain infarctions followed by rapid clinical recovery. In the case of a "silent stroke" neurological symptoms may not be noticeable and go unreported in spite of lasting tissue damage (Herderschee et al., 1992; Kang et al., 2006). Silent or subclinical strokes in humans are associated with small single or multiple lesions in the non-eloquent (either cortical or subcortical) areas of the brain (Masuda et al., 2001). Notably, silent strokes occur 15 times more often than classic strokes (Brown et al., 2005; Das et al., 2008). A silent stroke indicates a high risk for recurrent stroke, which then may become a major event with severe and lasting impairments (Bernick et al., 2001; de Lau et al., 2009; Kobayashi et al., 1997; Olsson, 1990; Yatsu and Shaltoni, 2004). Cumulatively, silent strokes may also accelerate age-associated neurological decline and contribute to dementia in the elderly (Davalos and Castillo, 1999; Nascimbeni et al., 2006; Prabhakaran et al., 2008). Once a silent stroke has been diagnosed, however, treatments are available to prevent recurrent stroke (Hakim, 1994). This emphasizes the pressing need to identify predictors for silent and recurrent stroke (Gállego et al., 2009).

Silent strokes frequently involve cortical and subcortical motor systems (Vermeer et al., 2003). Both sensorimotor cortex (SMC) and striatum are widely implicated in skilled limb function, as shown in rats when reaching for food or walking across challenging territory (Bures and Bracha, 1990; Döbrössy and Dunnett, 2003; Faraji and Metz, 2007; Klein et al., 2011; Teskey et al., 2003; Metz and Whishaw, 2002; Whishaw et al., 1986;). In particular, skilled reaching movements in rats are mainly affected by lesions to the motor cortex (Moon et al., 2009) or its main efferent pathway, the corticospinal tract (Thallmair et al., 1998; Whishaw et al., 1993). Extrapyramidal components, such as the striatum may play a central role in recovery and compensation of skilled motor functions. For example, it has been suggested that the dorsolateral striatum (DLS) is crucially involved in the control and learning of sensorimotor tasks (Alloway et al., 2006; Dunnett and Iversen, 1982; Reading et al., 1991). Depending on the lesion size and location, the resulting deficits can be largely attenuated by compensatory adjustments (Alaverdashvili and Whishaw, 2008; Metz et al., 2005).

The purpose of the present study was to investigate the plastic capacity of the motor system to effectively compensate for functional loss caused by single versus multiple focal ischemic lesions. We hypothesized that repeated focal infarcts resembling silent strokes in the cortical or subcortical brain regions challenge the compensatory capacity of the motor system to cumulatively result in permanent motor deficits. Using a new rat model of recurrent silent stroke, we show that compensation for a single focal cortico-striatal infarct may

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be sufficient to overcome the primary deficits, while multiple lesions exhaust the compensatory capacity of the motor system to reveal cumulative motor deficits.

Material and methods

Subjects

Twenty-nine adult male Long-Evans rats, weighing 330–375 g at the beginning of the experiment, were used. To enhance motivation in the reaching task, animals received a restricted diet to maintain 90% of their free feeding body weight. All procedures were approved by the Animal Care Committee of the University of Lethbridge in compliance with the guidelines of the Canadian Council on Animal Care.

Experimental design

Rats were trained in skilled forelimb reaching (19 days) and ladder rung walking tasks (1 day). Reaching and walking performances were video recorded on the day prior to lesion (baseline) for qualitative movement analysis. The animals were then matched for their reaching success rates and randomly assigned to the following groups: sham ($n=7$), sensorimotor cortex (SMC) lesion-only ($n=8$), dorsolateral striatum (DLS) lesion-only ($n=7$) and combined (SMC + DLS) lesion ($n=7$). Animals assigned to the lesion groups received endothelin-1 (ET-1) injections into the SMC, DLS or SMC + DLS on the side contralateral to the paw preferred for reaching. Animals were allowed to rest for 3–4 days. All groups were tested daily in skilled forelimb reaching and weekly in ladder rung walking for post-lesion behavioral assessment up to 14 days post-ischemia. Performance in both tasks was also video recorded on post-lesion days 4 (acute time) and 14 (chronic time). It should be pointed out that the 14-day chronic time point in the present study was selected based on previous reports in rodent models (Hilger et al., 2004; Karl et al., 2010; Soltanian-Zadeh et al., 2003). After behavioral assessments the animals were euthanized for histological analysis.

Skilled forelimb reaching task

Assessments of skilled forelimb reaching were based on earlier descriptions (Metz and Whishaw, 2000; Whishaw et al., 1986). Each training and test session required the rats to reach for 20 food pellets. Baseline training was considered complete once success rates reached asymptotic levels. On the last day of each testing period, reaching performance was video recorded from a frontal view using a digital camcorder (Canon ZR70 MC) at 25 frames/s with a shutter speed of 1/500. The tapes were analyzed frame-by-frame on a Sony DV player. Quantitative analysis included success percent and number of reaching attempts.

A successful reach was defined as obtaining the pellet on the first attempt, withdrawing the paw with the pellet through the slit and releasing the pellet into the mouth. Percent reaching success was calculated by counting the number of successful reaches divided by the number of pellets (20) given in each session multiplied by 100. Moreover, an attempt was defined as a repeated forelimb movement towards the pellet and obtaining the pellet after more than one reach (Metz and Whishaw, 2000).

Qualitative movement analysis of limb movements was performed for the first three successful reaches for each limb. Eleven reaching movement components [(1) Orient, (2) Limb lift, (3) Digits close, (4) Aim, (5) Advance, (6) Digits open, (7) Pronation, (8) Grasp, (9) Supination 1, (10) Supination 2, and (11) Release] and 35 subcomponents were scored according to earlier descriptions (Metz and Whishaw, 2000). Each movement component was rated on a three-point scale: 0 point, movement absent; 0.5 point, the movement was present but abnormal; 1 point, the movement was normal.

Ladder rung walking task

Animals were trained to cross a 1-m long horizontal ladder rung walking task with irregularly spaced round metal rungs (Metz et al., 2000; Metz and Whishaw, 2002, 2009). The rungs were arranged at random distances ranging from 0.5 to 5 cm. The pattern was maintained for all training and test sessions. Animals were trained in five trials to cross the ladder. Each test session consisted of three trials per animal, during which the animals' performance was video recorded from a lateral perspective for further movement analysis. A 7-category rating system according to Metz and Whishaw (2002) was used to determine the type of foot placement on the rung. A foot fault score of 0 was given for a total miss, a score of 1 for a deep slip, and a score of 2 for a slight slip. A score of 3 indicated a replacement of the limb on the rung, a score of 4 a correction of limb position, a score of 5 a partial placement of the distal limb on the rung, and a score of 6 indicated a correct limb placement with full weight support. Scores for both ipsilateral and both contralateral limbs were averaged.

The number of errors in each crossing was also counted. Based on the 7-category rating system (Metz and Whishaw, 2002), an error was defined as a limb placement that received a score of 0, 1 or 2 points. Thus, an error represents any kind of foot slip or total miss. The number of errors and the number of steps were recorded for each limb separately. In the present study, the mean number of errors per step was calculated and averaged for three trials.

Both measures of skilled walking performance (foot fault score and number of errors) were previously shown to represent valid indicators of skilled motor impairments (Metz and Whishaw, 2002).

Cortical and subcortical focal ischemia induced by endothelin-1

Procedures for inducing focal ischemia were similar to those reported by Faraji et al. (2009), except the coordinates, injection volume and rate. Briefly, the lesion groups received unilateral ET-1 infusion into different points of cortical (SMC) and subcortical (DLS) regions. Injections of ET-1 were made into the SMC (*AP*: +0.70, +2, +2, +2, +3; *ML*: ±3, ±3, ±3.5, ±2.40, ±3.5; *DV*: −1.5, −1.5, −2, −2, −3; 175 pmol; 0.15 μl; 0.1 μl/min), DLS (*AP*: +0.70, +0.70, +0.70, −0.40, −0.40, −0.40; *ML*: ±2, ±3, ±4, ±2, ±3, ±4; *DV*: all −4.60; 175 pmol; 0.18 μl; 0.1 μl/min), and SMC + DLS (combined coordinates; 175 pmol; 0.33 μl; 0.1 μl/min) on the side contralateral to the paw preferred for reaching. Sham-operated animals received all surgical procedures except skull trephination to prevent potential behavioral and neurochemical asymmetries (Adams et al., 1994).

Histological confirmation of lesion extent and location

After behavioral testing was completed, animals were sacrificed by an overdose of sodium pentobarbital (300 mg/kg i.p.) and perfused transcardially with 0.9% phosphate buffered saline followed by 4% paraformaldehyde (PFA). Brains were removed, post-fixed for 24 h in 4% PFA, and stored in 30% sucrose–formalin solution for cryoprotection until they were sectioned on a cryostat microtome at a thickness of 40 μm. Every fourth section was mounted on glass slides and stained with cresyl violet. The stained sections were examined under a microscope (Zeiss, Germany) and images were captured using an AxioCam camera (Zeiss, Germany) to quantify lesion extent (Faraji et al., 2009). The extent of cortical and subcortical damage in each lesion rat was calculated. Four to five images were captured under 1× magnification, corresponding approximately to 3.20, 1.70, 1.00, and 0.20 mm (SMC lesion-only) and 1.00, 0.48, −0.26, −0.40, and −0.90 (DLS lesion-only) relative to bregma. A systematic sampling grid with an area per point of 2000 pixels^2 was randomly projected on each image and the number of points hitting intact cortical and subcortical tissues was counted. Grids were generated using ImageJ