Quantitative and Qualitative Impairments in Skilled Reaching in the Mouse (Mus musculus) After a Focal Motor Cortex Stroke

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Background and Purpose—Skilled reaching movements are an important aspect of human motor behavior but are impaired after motor system stroke. The purpose of this study was to document skilled movements in mice before and after a focal motor cortex stroke for the purpose of developing a mouse model of human stroke.

Methods—Male C57/BL6 mice were trained to reach with a forelimb for food pellets and then given a motor cortex stroke, induced by pial stripping, contralateral to their preferred reaching limb. Reaching success and the movements used in reaching were analyzed by frame-by-frame inspection of presurgical and postsurgical video records.

Results—Reaching success was severely impaired after the stroke. Improvement in success over 2 postsurgical weeks was moderate. Analysis of 10 movement components comprising reaches pre- and postsurgically indicated that most of the rotatory movements of the limb used for aiming, advancing, pronating, and supinating the paw were impaired. When successful reaches did occur, body movements that compensated for the impairments in limb rotatory movements aided them.

Conclusions—The results indicate that skilled reaching in the mouse is impaired by focal motor cortex stroke and they suggest that the mouse, and the skilled reaching task, provides an excellent model for studying impairments, compensation, and recovery after motor system stroke. (Stroke. 2002;33:1869-1875.)

Key Words: behavior ■ motor cortex ■ stroke ■ mice

Comparative analyses of food handling and skilled reaching by laboratory rats (Rattus norvegicus) and mice (Mus musculus) show that the forelimb movements used by the 2 species are similar.1–5 To eat, they use 5 movements in sequence. They identify the food via olfaction, pick up the food using the mouth, sit back on their haunches, grasp the food with the forepaws by bringing the elbows inward, and manipulate the food with the digit tips.

Similarly, while performing in a formal laboratory skilled reaching task, requiring extension of a forelimb through an opening in a cage to obtain a food pellet, rat and mouse movements are similar.1,6 The paw is lifted so that the digits, semiflexed, are aligned with the midline of the body, and then the elbow is also brought to the midline, to an “aiming” position. As the limb is advanced through the slot, the digits are extended. As the limb is pronated, the digits are opened, thus placing the paw over the food. The food item is grasped by digit closing, the wrist is extended to lift the paw, and the paw is withdrawn through the slot. The paw is then supinated further so that the food can be presented to the mouth. Aiming, pronation, and supination are performed mainly by upper arm movements.7,8 Pronation consists of rotating the paw so that digits 5 (outermost) through 2 (innermost) contact the surface on which the food pellet is placed, and this “arpeggio” movement is assisted by movements around the wrist.9

Studies using rats show both spontaneous and skilled limb use are impaired after damage to the motor system.3,4,6,10–21 Impairments produced by damage to different motor regions are also distinctive. For example, after a motor cortex injury, a rat still retrieves food by reaching with the affected forelimb, but success is reduced.4,14,22 In addition, most of the movement components of the reach are abnormal. Compensatory body movements provide the rotatory movements to assist pronation and supination.4 Thus, the species has provided a primate substitute for studying the neural basis of skilled movements.

Given recent interest in using transgenic mice for stroke research,23–29 the present study investigated the effects of motor cortex stroke on skilled reaching in mice. The mice were trained to reach through a slot for food, and their performance was video recorded before and after a cortex stroke produced by pial stripping.

Materials and Methods

Subjects

Eight 5-month-old male C57/BL6 mice (Mus musculus) weighing between 25 and 30 g were raised in the University of Lethbridge.
animal colony from animals originally obtained at The Jackson Laboratories (Bar Harbor, Maine). Animals were housed individually in standard plastic mouse cages (lights on a 12:12 hour cycle beginning at 8:00 AM; room temperature 22 °C) and given 1 piece of laboratory chow (4 g) after the testing period each day. Two of the 8 animals consistently had less than half of a gram of food remaining in the morning, which was removed at 8:00 AM. Because testing began at 1:00 PM, even the mice with remaining food had to wait 5 hours before regaining access to food. Each mouse was thus sufficiently restricted and motivated to perform the task. The experiment was conducted according to the Canadian Council on Animal Care code.

Surgery
After adaptation to reaching and 6 days of training, the animals were anesthetized using isoflurane (BIMEDA-MTC Animal Health Inc.). Eight mice, 5 with left and 3 with right paw preference, received a pial strip stroke to the motor cortex contralateral to the preferred forelimb. Using stereotactic surgery, a 3 mm × 3 mm region of the skull was removed, 0.5 mm behind bregma to 3 mm anterior to bregma, and from 0.5 mm lateral to the midline to 3.5 mm lateral to the midline. The dura was removed within the trephination, and the underlying pia, arachnoid, and vasculature was wiped with a cotton swab until no vasculature was visible. Shams were not performed because the removal of the skull and dura did not result in any motor deficits in similar experiments in rats. Vetroprolycin (Janssen) gel was applied to the eyes, and the incisions were closed. The animals were returned to their home cages, and postsurgical testing began the following day.

Reaching Task
The Plexiglas reaching box1 was 19.5 cm long, 8 cm wide, and 20 cm high (Figure 1). A 1-cm wide vertical slit ran up the front of the box. A 0.2-cm thick plastic shelf (8.3 cm long and 3.8 cm wide) was mounted 1.1 cm from the floor on the front of the box. Twenty-milligram food pellets (Bioserve Inc) were placed in indentations, allowing the mice to display which paw they preferred to use, after which the food was placed in the indentation contralateral to the preferred paw. As the animal pronates the paw medially, this placement allows the mouse to obtain the pellet with a paw and not with the tongue.20

Video Recording
Filming was done on the last 2 pre- and postsurgical days of training with a Sony DSRPD100 digital camcorder (30 frames/s; shutter speed of 1000). Illumination was provided with a 2-arm Nikon MKII 150W fiber optic light. The animals were filmed from a frontal and ventral view (by placing the apparatus over an inclined mirror table). The tapes were viewed on a Sony DV cam DSR-20 player and Trinitron monitor. Representative movements were captured using Final Cut Pro frame grabbing software on a Macintosh G3 computer.

Reaching Success
Each animal reached for 20 pellets each day during the testing period. All animals were more than sufficiently motivated to attempt to obtain 20 pellets. If an animal reached through the slot and obtained a food pellet, the reach was scored as a success. If an animal knocked the food away or dropped the food after grasping it, the reach was scored as a miss. Performance was defined by: Percent Success = (number of successful retrievals/20) × 100

Qualitative Analysis
Qualitative movement scoring was derived from a conceptual framework adapted from Eshkol-Wachmann Movement Notation (EWMN). EWMN is designed to express relations and changes of relation between the parts of the body. The body is treated as a system of articulated axes (ie, body and limb segments). A limb is any part of the body that either lies between 2 joints or has a joint and a free extremity. These are imagined as straight lines (axes), of a constant length, which move with one end fixed to the center of a sphere. On the basis of descriptions obtained from EWMN, rating scales of movements were derived.7

Five presurgical and 5 postsurgical reaches from each mouse were rated for qualitative features of the movement.20 Ten components of a reach were rated: (1) Digits to the midline: Using mainly the upper arm, the reaching limb is lifted from the floor so that the tips of the digits are aligned with the midline of the body. (2) Digits semiflexed: As the limb is lifted, the digits are flexed and the paw is supinated so that the palm of the paw is aligned almost vertically. (3) Aim: Using an upper arm movement, the elbow is adducted to the midline while the tips of the digits remain aligned with the midline. (4) Advance: The limb is advanced directly through the slot toward the food target using an upper arm movement, and during advancement the snout is raised to allow passage of the paw into the slot. (5) Digits extend: The digits extend during the advance. (6) Pronation: When the paw is over the target, the paw pronates and digit 5 (the outer digit) through to digit 2 touches the surface in succession, mainly by abdution of the elbow and also by a rotational movement around the wrist. During pronation, the digits open. (7) Grasp: The digits flex over the food and close around it. The paw remains in place and the wrist is slightly extended to lift the food. (8) Supination I: As the paw is withdrawn, it supinates by almost 90°. (9) Supination II: Once the paw is withdrawn from the slot the paw further supinates by 45° to present the food to the mouth. (10) Release: The mouth contacts the paw, and the digits open to release the food.

Each movement was rated on a 3-point scale. If the movement was normal, a score of 0 was given. In cases where there was some ambiguity concerning the occurrence of a movement, or the movement was present but incomplete, a score of 1 was given. If the movement was absent, a score of 2 was given.

Histology
The mice were deeply anesthetized and perfused through the heart with 0.9% saline followed by 4% para-formaldehyde/0.9% saline. Brains were removed and cryo-protected in 30% sucrose/4% para-formaldehyde for 1 week. The brains were sectioned (50 μm) on a 2800 Frigocut E cryostat (Reichert-Jung), mounted on 1% gelatin
and 0.2% Chromalum-dipped slides, stained with Cresyl violet, and coverslipped using Permount (Fisher Scientific).

Results

Histology
All of the brains had damage in the motor cortex. Figure 2 indicates representative dorsal (Figure 2A and 2B) and coronal views (Figure 2C through 2E) of the lesion. Pial stripping produced a conical cavity beneath the area of pial removal, and the cortex adjacent to the lesion evaginated and filled in the cavity produced by the lesion, making quantification of the actual lesion size difficult. Nevertheless, based on a comparison of the lesion location relative to the large layer V cells in the contralateral hemisphere, the lesions appeared restricted to the caudal and rostral forelimb areas as defined by electrophysiological stimulation studies in the rat.31

Quantitative Changes in Reaching After Stroke

Reaching Success
The ANOVA on success revealed a significant treatment effect [F(1, 15) = 38.962, P < 0.001], indicating that the mice achieved higher reaching scores before the motor cortex lesion. There was a significant effect of days [F(5, 75) = 3.48, P < 0.007], but there was no significant group by days interaction [F(5, 75) = 2.114, P = 0.0729]. The mice achieved an average percent success of 40 ± 10 before the lesion, and the average percent success dropped to 10 ± 10 after the lesion (Figure 3). An ANOVA on postsurgical performance was not significant, although the animals displayed a trend toward improving.

Movement Components
The ANOVA of 5 presurgical and postsurgical reaches revealed a significant main effect of group [F(1, 16) = 65.01, P < 0.001], indicating that the mice accumulated higher scores for the 10 movement components after cortical injury. There was also a main effect of movement [F(9, 144) = 14.68, P < 0.001], indicating that some movements were more impaired than others. More importantly, there was a significant group by movement interaction for the postsurgery stroke group [F(9, 144) = 8.232, P < 0.001], because some movements in the stroke group were more impaired relative to control scores. Follow-up Student’s unpaired t tests indicated significant group differences for the aim, advance, pronation, supination II, and release components of the reach, but not for the digits to the midline, digits semiflexed, digits extend, grasp and supination I (Figure 4).

Qualitative Changes in Movement After Stroke
The following descriptions and the ratings were all taken from reaches that were successful. Nevertheless, it is likely that the abnormalities displayed by the stroke mice on successful reaches did contribute to the lower incidence of successful reaches.

Digits to the Midline and Aim
Control mice lifted the reaching limb from the surface, semiflexed the digits, and aligned the digit tips with the midline of the body with a single movement (Figure 5A).
They then adducted the elbow to the midline of the body so that the forelimb was aligned with the body midline in an “aiming” position (Figure 5B). Stroke mice also lifted the limb and semiflexed the digits, but the digit tips were displaced more laterally. Then, rather than adducting the elbow to the midline, to bring the paw to a position from which it could enter the slot, the animals made an ipsiversive rotatory movement of the body to bring the limb to an aiming position. Thus, the comparison in Figure 5A (control) and Figure 5C (stroke) indicates that although the digits seem aligned with the body in both animals, the stroke animal has achieved alignment in part by using body rotation. The end result was that the control mice were able to advance the paw from an “aiming” position directly through the slot toward the food, whereas the stroke mice directed the paw through the slot diagonally.

**Advance and Digits Extend**

The control mice advanced the paw directly forward over the food item, while at the same time extending the digits (Figure 6A), leaving the paw in a position to pronate over the food pellet (Figure 6B). The stroke mice also opened the digits as the paw advanced, but the paw entered the slot diagonally so that it was located dorsally and medially relative to the food pellet (Figure 6C and 6D).
Pronation

Once the limb is advanced and the digits are extended, the paw is pronated over the food pellets as the digits are opened from digit 5 through digit 2. The digits also contact the shelf in the sequence digit 5 through digit 2. This arpeggio movement is illustrated for a control mouse reaching in an instance in which no food pellet was located in the slot (Figure 7A through 7D, top). Typically, when the paw and digits contact a food pellet, grasping occurs. If the paw fails to contact a food pellet, grasping does not occur. The stroke mice slapped the paw laterally, instead of using a rotatory wrist movement, and grasped whether or not a food pellet was contacted (Figure 7A through 7D, bottom).

Grasp and Supination I

Once the food was contacted, the control mice closed the digits around the pellet and supinated the closed paw so that the palm of the paw was oriented vertically (Figure 8A), from which position the paw was withdrawn through the slot (Figure 8B). Stroke animals displayed little supination after grasping the food (Figure 8C), and whatever supination they did achieve occurred by leaning on the pellet with the forelimb as they withdrew the paw from the slot (Figure 8D).

Supination II and Release

Once the paw with food was withdrawn, the control mice further supinated the paw, so that the palm faced upward to present the food to the mouth (Figure 9A). Supination II and release of the food was frequently accomplished without assistance from the nonreaching paw (Figure 9B). Stroke mice typically used the nonreaching forepaw to grasp the reaching forepaw and assist in supination II and release (Figure 9C and 9D).

Discussion

This study examined the effect of motor cortex stroke on quantitative and qualitative measures of skilled reaching in mice. After a focal motor cortex stroke, reaching success by the paw contralateral to the lesion was impaired and did not recover during 2 postsurgical weeks of testing. At this time, many component movements used for reaching remained abnormal, especially the rotatory movements of the limb associated with aiming, pronating, and supinating the paw. When reaches were successful, they were achieved using compensatory body movements. This is the first study to...
describe qualitative impairments in skilled reaching in the mouse, and the results suggest that this species provides a good model for analysis of motor skills, plasticity, and recovery processes.

The mice were trained and tested pre- and postsurgically in an apparatus in which they reached through a slot in a test box for single pieces of food located on a shelf just outside the slot. Before the lesion, the animals were able to orient to the slot, advance a paw through the slot, and grasp and retrieve the food pellets for eating. Performance presurgically was reasonably accurate, but after the lesion the mice were extremely impaired; this is consistent with findings on another motor task in mice subjected to prefrontal damage.\textsuperscript{32}

Before surgery, the mice used a distinctive pattern of movement when reaching for food. They located the food by sniffing for the food pellet, oriented their body, and then made a characteristic series of limb movements to retrieve a food pellet. The paw was lifted so that the slightly flexed digits were aligned with the tips to the midline of the body. The elbow was then adducted to the midline of the body so that the limb was aimed to enter the slot. As the paw was advanced through the slot to the food, the digits were extended, and then the paw was pronated while opening the digits to grasp the food. The paw was then supinated to withdraw the food through the slot, and then once through, was supinated further to place the food in the mouth.

After the stroke reaching movements were altered, even when the animals successfully obtained food. The paw and elbow were not aligned to the aiming position along the midline of the body, but rather proceeded toward the food diagonally. Some aiming was obtained by an ipsiversive rotation of the foreportion of the trunk. Pronation was incomplete as the animals swept at the food pellet with a sideways motion of the paw. Finally, the movements of supination during withdrawal and release were abnormal as the paw was incompletely supinated. Again, food retrieval was assisted by rotatory movements of the trunk and through the assistance of the nonreaching paw.

Some of the stroke animals’ movements were not impaired. Stroke animals remained able to semiflex and extend the digits as well as grasp the food. Again, these findings are consistent with the impairments described for the rat after a focal motor cortex injury.\textsuperscript{4,6,11,19,20}

These changes in movements after stroke suggest that the function of the motor cortex is in part to produce rotatory movements of the limb.\textsuperscript{33} This conclusion is consistent with other findings of impaired limb use,\textsuperscript{4,22,34–37} with recovery likely a result of compensatory movements.

In conclusion, this is the first study to document a detailed qualitative impairment in skilled movements after a motor cortex stroke in mice. The main findings are that mice perform skilled reaching movements quite well, and the skilled reaching test is sensitive to damage to the motor cortex. A strength of the model is that it is able to provide a quantitative score and a qualitative assessment of deficits, compensation, and recovery.

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