Lesion in the lateral cerebellum specifically produces overshooting of the toe trajectory in leading forelimb during obstacle avoidance in the rat

Sho Aoki,1,2 Yamato Sato,1 and Dai Yanagihara1,3
1Graduate School of Arts and Sciences, University of Tokyo, Tokyo, Japan; 2Japan Society for the Promotion of Science, Tokyo, Japan; and 3Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Tokyo, Japan

Submitted 3 December 2012; accepted in final form 18 April 2013

Aoki S, Sato Y, Yanagihara D. Lesion in the lateral cerebellum specifically produces overshooting of the toe trajectory in leading forelimb during obstacle avoidance in the rat. J Neurophysiol 110: 1511–1524, 2013. First published April 24, 2013; doi:10.1152/jn.01048.2012.—During locomotion, stepping over an obstacle under visual guidance is crucial to continuous safe walking. Studies of the role of the central nervous system in stepping movements have focused on cerebral cortical areas such as the primary motor cortex and posterior parietal cortex. There is speculation that the lateral cerebellum, which has strong anatomical connections with the cerebral cortex, also plays a key role in stepping movements over an obstacle, although this function of the lateral cerebellum has not yet been elucidated. Here we investigated the role of the lateral cerebellum during obstacle avoidance locomotion in rats with a lateral cerebellar lesion. A unilateral lesion in the lateral cerebellum did not affect limb movements during overground locomotion. Importantly, however, the lesioned animals showed overshooting of the toe trajectory specific to the leading forelimb ipsilateral to the lesion when stepping over an obstacle, and the peak toe position, in which the toe is maximally raised during stepping, shifted away from the upper edge of the obstacle. Recordings of EMG activity from elbow flexor and extensor muscles suggested that the overshooting toe trajectory in the ipsilateral leading forelimb possibly resulted from sustained elbow flexion and delayed elbow extension following prolonged activity of the biceps brachii. These results suggest that the lateral cerebellum specifically contributes to generating appropriate toe trajectories in the ipsilateral leading forelimb and to controlling related muscle activities in stepping over an obstacle, especially when accurate control of the distal extremity is achieved under visual guidance.

lateral cerebellum; obstacle avoidance; leading forelimb; toe trajectory; rat

STEPPING OVER OBSTACLES is crucial to achieving safe and smooth locomotion. Visually guided locomotion is an essential component of many aspects of locomotor behavior; the function of the brain in this type of locomotion has been tested by ladder walking, narrow walking, and stepping over obstacles (Beloozerova and Sirota 2003; Drew et al. 2008; McVea and Pearson 2009). Several regions in the central nervous system of humans and quadrupedal animals contribute to the ability to step over an obstacle (Beloozerova and Sirota 1993; Drew 1988, Drew 1993; Friel et al. 2007; Galna et al. 2010; Lavoie and Drew 2002; Morton et al. 2004). In animal studies, the main focus has been on particular cerebral cortical areas, such as the primary motor cortex (M1; Beloozerova and Sirota 1993; Drew 1988, Drew 1993) and the posterior parietal cortex (PPC; Andujar et al. 2010; Beloozerova and Sirota 2003; Marigold et al. 2011); these findings have been extensively reviewed (Drew et al. 2008; McVea and Pearson 2009). In addition to the M1 and the PPC, the lateral cerebellum is a candidate for involvement in the control of stepping over an obstacle during locomotion since it has anatomical connections with cerebral cortical areas including the M1 and the PPC (Giannetti and Molinari 2002; Glickstein 2000; Kakei et al. 1995; Lu et al. 2007; Massion and Rispal-Padel 1972; Ramnani 2006; Sasaki et al. 1975; Strick et al. 2009; Suzuki et al. 2012). It has been proposed that a cerebro-cerebellar loop via the lateral cerebellum may contribute to visually guided locomotion (see for reviews Drew et al. 2008; McVea and Pearson 2009). To date, however, the function of the lateral cerebellum in obstacle avoidance during locomotion remains uncertain.

Cerebellar patients with a lesion of the lateral cerebellum demonstrate hypermetric limb movements and overshooting trajectories of distal extremities in reaching (Hore et al. 1991; Manto 2009; Manto and Bosse 2003); these patients also display delayed onset and/or offset activities of related agonistic and antagonistic muscles. Likewise, monkeys with an inactivated dentate nucleus, which receives projections from the lateral cerebellum, show a lack of dexterity in visually guided reaching (Hore and Flamant 1986; Stein and Glickstein 1992; Thach et al. 1992). These reports indicate that the lateral cerebellum contributes to controlling the position and/or trajectory of the distal limb and to directing activities of the appropriate muscle groups during visually guided movements that require accuracy.

Electrophysiological studies have shown that the Purkinje cells (PCs) of the lateral cerebellum and the cerebellar output neurons, the dentate nucleus, are modulated to visual stimuli during visually guided reaching. These responses are evoked by visual stimuli related to the ongoing movements but not by irrelevant stimuli such as flash and light (Cerminara and Apps 2011; Cerminara et al. 2005; Cerminara et al. 2009). Additionally, visually dependent modification of limb movements during ladder walking is associated with modulation of the PCs in the lateral cerebellum and of the dentate neurons (Marple-Horvat and Criado 1999; Marple-Horvat et al. 1998). These observations show that the lateral cerebellum may be involved in visually guided locomotion, suggesting the possibility that the lateral cerebellum plays a key role in the control of stepping movements during obstacle avoidance locomotion under visual guidance.

In our previous study, we described the kinematic and electromyographic characteristics of forelimb movements in
Materials and Methods

Ethical approval. The present study was approved by the Ethical Committee for Animal Experiments at the University of Tokyo and was carried out in accordance with the Guidelines for Research with Experimental Animals of the University of Tokyo and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (revised 1996). All efforts were made to minimize the number of animals used and any discomfort or suffering throughout the course of the experiments.

Animals. Experiments were performed on 10 male Wistar rats (270–320 g; CLEA Japan). All animals were provided with food (CE-2, CLEA Japan) and water ad libitum and housed under standard conditions (12:12-h light-dark cycle, temperature ~22°C).

Apparatus. A runway box (length 140 cm, width 14 cm) was used for the analyses of behavior, overground locomotion, and stepping over an obstacle during locomotion. The obstacle was 2, 3, or 4 cm in height with a depth of 2 mm. The obstacle was attached at the midpoint of the runway. Detailed information on the runway box is provided in our previous reports (Aoki et al. 2012; Sato et al. 2012).

Training procedure. All animals were habituated to the runway for a week and were trained to walk from the starting position to the opposite end. They were also trained to walk forward and to step smoothly over an obstacle without stopping or touching the obstacle. Once the animals were trained, limb movements during the stepping phase were found to be highly reproducible.

General procedure. When the surgery implanting the EMG electrodes was completed, analyses of behavior and monitoring of limb movements during overground locomotion and stepping over an obstacle were carried out before the animals were subjected to surgery for the cerebellar lesion. These observations served as the prelesion control. The characteristics of forelimb movements in prelesion control rats have been fully described in our earlier report (Aoki et al. 2012). The unilateral lesion to remove a part of the lateral cerebellum was performed under anesthesia. Within 2–3 days after the surgery, we repeated the behavioral analyses to identify the effects of the cerebellar lesion on the limb movements during overground and obstacle avoidance locomotion. After completion of these analyses, the rats were euthanized, the brains were removed, and histological sections were prepared to verify the lesion in the cerebellum.

Surgery. The EMG electrodes were implanted 3 days before beginning the behavioral experiments. Animals were anesthetized with isoflurane (3.5% initial, ~2.5–3.0% maintenance in air) and were placed on a stereotaxic frame. The level of anesthesia was monitored during surgery by the absence of dynamic whisker movements and the pinch withdrawal reflex. Small incisions were made on the skin overlying the bilateral biceps and triceps muscles in order that bipolar electrodes could be implanted. The electrode wire implanted in the muscles was led subcutaneously to the head of the animals and attached to a connector mounted on the skull with dental cement and screws. After this process was completed the skin incisions were sutured, and the animals were allowed to recover.

For a unilateral lesion in the lateral cerebellum, animals were anesthetized with isoflurane as described above. The level of anesthesia was also monitored similarly. All surgical procedures were performed with the aid of a microscope. The animal was placed on a stereotaxic frame, and the right side of the cerebellar hemisphere was accessed by a small craniotomy of the right occipital bone with a surgical drill. The dura mater was carefully incised, and tissue of the lateral cerebellum was removed by suction with a custom-made glass micropipette. When aspirating the brain tissue, we aimed to suction lobule V to crus I under visual inspection and sought to avoid removing any tissue adjacent to the paravermal vein in order to leave the intermediate cerebellum intact. Subsequently, an antibiotic solution was applied. Finally, all skin incisions were carefully sutured, and the animals were allowed to recover. Animals were monitored for signs of stress or discomfort after the surgery, but all recovered uneventfully.

Kinematics. As stated above, the animals were encouraged to walk forward on the runway. Overground locomotion on the runway in the absence of an obstacle and stepping movements over an obstacle were captured bilaterally with a HAS-220 high-speed digital image camera system (DITECT) at 200 frames/s. The captured images were directly stored on a personal computer for later observation and analyses. Overground locomotion was recorded in five successful trials for each animal. Stepping over an obstacle was recorded over three successful trials for both leading limb and trailing limb at each of the three obstacle heights. In our previous study, we divided stepping over an obstacle into the approach phase and the stepping phase; the latter was defined as the swing phase of the stepping movement from lift-off to land-on of the toe (Aoki et al. 2012). In the present study, the analysis of movements focused on the stepping phase. In addition, the analysis was limited to the sagittal plane parallel to the direction of walking and stepping over the obstacle and was also limited to the standard strategy in which the leading hindlimb stepping over the obstacle was ipsilateral to the leading forelimb (Lajoie and Drew 2007). Successful stepping movements fulfilled the following criteria: 1) the trailing limb did not lift off the ground until the leading limb reached just above the obstacle, and 2) neither the forelimb nor the hindlimb came into contact with the obstacle (Aoki et al. 2012; Sato et al. 2012). Indeed, the occurrence frequency of trials that did not fulfill the first criterion was normally <10%, and it was not different before or after the lateral cerebellar lesion. Also, the hitting behavior mentioned as the second criterion was rarely observed (<2%), irrespective of whether before or after the lesion. All the analyzed steps were selected from first and prior trials that fulfilled the criteria for each obstacle condition in each animal.

Colored markers were placed on the skin at various anatomical landmarks of the ipsilateral and contralateral forelimbs, namely, the shoulder, wrist, and toe of the forelimbs. Since the skin overlying the elbow joints showed excessive slippage, the coordinates of the elbow were trigonometrically calculated. Reconstruction of the trajectories of the forelimb was made by digitizing the two-dimensional coordinates of the anatomical positions of the measured or estimated landmarks. The shoulder and wrist joint angles could not be obtained because of excessive slippage of the skin in the proximal part of the scapula; in addition, the position of the fifth metacarpus, which is crucial to calculating a wrist angle, was difficult to determine because of the flexible range of motions of the paw and digits in the rat.
Therefore, as reported previously, we could only measure the angular displacement for the elbow joint in the forelimb (Aoki et al. 2012). It must be stressed that although the angle composed of the elbow, wrist, and toe superficially resembles a “wrist joint angle” in Fig. 2 and Fig. 8, B and C, it is not the actual joint angle. Kinematic markers were also placed on hindlimb landmarks, and their motion was fully analyzed; however, we did not observe any movement deficit in the hindlimb after lateral cerebellar lesions. For this reason, the methodology for motion analysis of hindlimb movements is not described here.

For overground locomotion, we analyzed maximal toe heights in the swing phase. Mirror images below the runway were used to determine the time of lift-off and land-on of the toe. In stepping over an obstacle, we analyzed the toe trajectory, toe-obstacle distances at lift-off and land-on, and the toe height when the toe was just above the obstacle. A peak toe position, when the toe was maximally elevated in the stepping phase (Aoki et al. 2012), was also analyzed. The peak toe position was expressed as relative coordinates from the upper edge of the obstacle. Angular displacement of the elbow joint in Fig. 7, A and B, was normalized as a percentage of the swing phase by using cubic spline data interpolation without filtering. In an additional analysis, angular displacement and angular velocity of the leading forelimb were calculated within a particular time window 30 ms before and after the moment that the toe passed just above the obstacle during stepping over (see Fig. 7, C and D). These data were averaged but not filtered or normalized. The analyses were performed with custom-designed motion analysis software (DIPP-Motion Pro 2D, DITECT) and MATLAB software (The MathWorks).

**Electromyographic recording and analysis.** Implantation of the EMG electrodes was performed as described above, and the positions of the implanted electrodes in the muscles were visually inspected for each animal after completion of the behavioral experiments. EMG activities were amplified, band-pass filtered from 150 Hz to 10 kHz (MEG-6108, Nihon-Kohden), and sampled at 10 kHz (Powerlab/4SP, AD Instruments). The EMG data were synchronized with the kinematic analysis of forelimb movements, triggered via a computer-controlled TTL signal. For calculation of onset and offset of muscle activities, EMG data were full-wave rectified and filtered with a second-order Butterworth filter (cutoff frequency of 20 Hz). The threshold of onset and offset was arbitrarily determined in 6 SD from muscle activities in a resting state. Latency of onset and offset was calculated from the time that the forelimb toe ipsilateral to the EMG recording side reached just above the obstacle. This analysis was performed similarly to our previous study (Aoki et al. 2012).

---

**Fig. 1.** Histological verification of the extent of the lesion. A: representative image of the coronal section of the rat cerebellum in which the right side of the lateral cerebellum was unilaterally removed by surgical suction. B: 3 types of the lateral cerebellar lesion are superimposed on a diagrammatic series of normal cerebellar sections derived with permission from the standard rat brain atlas (Paxinos and Watson 2004; copyright Elsevier 2004). Numbers on left indicate the rostrocaudal level from the interaural line. The illustrated lesions show the smallest, the average, and the largest examples in the present study; these 3 lesions are indicated in black, dark gray, and light gray, respectively. Cerebellar regions are indicated as follows: V, lobule V; VI, lobule VI; simple, lobule simplex; PF, paraflocculus; PMD, paramedian lobule; FN, fastigial nucleus; AIN, anterior interpositus nucleus; PIN, posterior interpositus nucleus; DN, dentate nucleus. Scale bar, 2 mm.
Histology. On completion of the behavioral experiments, animals were given a lethal dose of urethane (2 g/kg) and then transcardially perfused with 150 ml of 0.9% saline and 300 ml of fixative, made up of freshly prepared 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB). After perfusion, the extracted brains were postfixed overnight in the same fixative and later were stored in 10% PFA in 0.1 M PB for a week and then in 10% sucrose in 0.1 M PB for a second week. After this postfixation period, serial coronal sections (40 μm) of the cerebellum and brain stem were prepared with a freezing microtome (REM-700, Yamato Kohki), collected in vials containing 0.1 M PB, and then mounted on glass microscope slides and fully dried. The sections were Nissl counterstained with cresyl violet. A representative section in Fig. 1A was photographed by a digital microscope (BZ-9000, Keyence). Determination of the extent of the cerebellar lesion and superimposition of the lesion cases onto representative drawings of the normal rat cerebellum in Fig. 1B were performed with a conventional light microscope (CX41, Olympus).

Statistical analysis. Data were analyzed by a one-way analysis of variance (ANOVA) with standard statistical software (SPSS Japan). In post hoc tests, Tukey’s honestly significant difference test was applied to data with equal variance and the Games-Howell test was used for data with unequal variance. The level of statistical significance for variables was set at $P < 0.05$. For the analyses that examine statistical significance, we applied the average of averages from each animal and the data are shown as means ± SE. The reason for this is that it is better to use the average of averages from each animal than the average of all the data points in order to test the statistical significance strictly. This is because in the case of using the average of all the data points the number of points increases, the significance could be easily detected, and it could lead to overestimation. By contrast, the exceptions are Fig. 5, C and D, and Fig. 6B, in which we did not test the statistical significance and we applied the average of all the individual points. Then the variability of the peak toe positions (Fig. 5, C and D) and the paw placement (Fig. 6B) was compared as means ± SD since the comparisons of the variability between different steps were performed for each condition.

RESULTS

As described above, we carried out histological verification of the lesion in the lateral cerebellum after the behavioral analyses were completed (Fig. 1). As shown in Fig. 1B, the suction surgery achieved unilateral removal of the lateral cerebellum including lobule V to crus I (in some cases to crus II). Additionally, the cerebellar nuclei were intact, except for three animals in which the dorsal dentate nucleus was slightly damaged. The effects of the lesion on the stepping behavior in these three animals were similar to the other cases, and thus the data obtained from these animals were also included in the results. Overall, the extent of the lesion in the cerebellar cortex showed relatively little variation and the effects of the lesion were similar among the animals. Therefore, the following comparisons of the kinematic and electromyographic data were performed with the data from all 10 rats.

In the following description of the kinematic analysis, the forelimb contralateral to the lesioned side is termed the “contralateral forelimb” in the text and the “Post-contra” in the figures and tables and the forelimb ipsilateral to the lesioned side is the “ipsilateral forelimb” in the text and the “Post-ipsi” in the figures. As stated above, we also fully analyzed hindlimb movements but did not observe any movement deficit of the hindlimb in overground or obstacle avoidance locomotion. Thus we concluded that the lesion in the lateral cerebellum did not influence hindlimb movements during stepping over an obstacle in the rat. For this reason, we do not include the data from the hindlimb movements in this study.

Forelimb movements and toe trajectories are illustrated as stick figures in Fig. 2 and the anatomical landmarks used in this analysis are illustrated in Fig. 2, left. A unilateral lesion of the lateral cerebellum had no effect on either the contralateral or ipsilateral forelimb during overground locomotion (Fig. 2, A–C). However, in stepping over an obstacle, there was clear overshooting of the toe trajectory of the ipsilateral forelimb when the limb led (arrowhead, Fig. 2F). In particular, the toe of the forelimb overshot the upper edge of the obstacle. By contrast, when the contralateral forelimb led, the toe trajectory was similar to that seen in prelesion animals, and the peak toe position of the trajectory was observed just above the obstacle (Fig. 2E). Thus the overshooting of toe trajectory was specific to the ipsilateral leading forelimb; no deficit in a trajectory of the ipsilateral forelimb was detected when it was utilized as the trailing forelimb. To illustrate and compare the overshoot of toe trajectories in the leading forelimb, representative tra-

![Fig. 2. Representative stick figures of forelimb movements: forelimb movements during the stepping (swing) phase from lift-off to land-on are represented. Left: anatomical landmarks measured in this study. A–C: forelimb movements during overground locomotion (Ground). D–F: leading forelimb movements over an obstacle. G–I: trailing forelimb movements over an obstacle. The lesion was induced in the right side of the brain; therefore, the contralateral forelimb was on the left side of the body and the ipsilateral side (Post-ipsi) was on the right side. Thus the stick figures for the postlesion contralateral forelimb (Post-contra) are shown as inverted mirror images to make visual comparison easier (B, E, H). Toe trajectories are represented as gray lines. Arrowhead indicates overshoot of the toe trajectory and the peak toe position (F). Arrows show the direction of movements. Vertical bars in D–I indicate the obstacle (3 cm). Stick figures are illustrated at 100 frames/s.](https://www.jn.org/content/jn/108/5/1514/F1.large.jpg)
The maximal heights of the forelimb toe in overground locomotion and toe heights just above the obstacle in the stepping phase are shown in Fig. 4, A–C. The lesion in the lateral cerebellum did not affect maximal toe heights during overground locomotion (Fig. 4A). Likewise, toe heights just above the obstacle were unaffected in either leading or trailing forelimb during obstacle avoidance locomotion (Fig. 4, B and C). In addition, toe-obstacle distances in either lift-off or land-on are indicated in Fig. 4, D and E. These results show that the lesion in the lateral cerebellum did not influence toe-obstacle distances of the leading or trailing forelimb in either lift-off or land-on (Fig. 4, D and E). The data shown in Fig. 4 and the statistical analysis are shown in Table 1.

Notably, peak toe positions during the stepping phase were clearly altered in the ipsilateral forelimb when this limb led (Fig. 5A). In this case, the peak toe positions shifted forward away from the upper edge of the obstacle in horizontal and vertical directions (Fig. 5A); this reflects the overshooting of the toe trajectory of the ipsilateral leading forelimb shown in Fig. 2F. The mean peak toe position in the ipsilateral leading forelimb was away from the upper edge of the obstacle and was more variable at the different obstacle heights (Fig. 5C). In contrast, prelesion peak toe positions and those of the contralateral leading forelimb were nearer to the upper edge of the obstacle and showed less variability (Fig. 5C).

These observations are similar to those in our previous study, in which we found that the peak toe positions of the leading forelimb were closer to the upper edge of the obstacle and were more accurately controlled than the trailing forelimb (Aoki et al. 2012). Here, the peak toe position of the trailing forelimb was not different among pre- or postlesion conditions (Fig. 5, B and D). Given that the peak toe positions of the leading forelimb are accurately and consistently controlled just above the upper edge of the obstacle in prelesion and contralateral forelimbs, it is likely that the peak toe position of the ipsilateral leading forelimb might lack consistency and accuracy. Addi-

Fig. 3. Superimposed trajectories of the toe in the leading forelimb: toe trajectories of the leading forelimb of each animal over a 3-cm obstacle for prelesion (Pre, A), Post-contra (B), and Post-ipsi (C). Traces are synchronized to the location of the obstacle with the reference of the coordinates of the obstacle. Arrow indicates movement direction. Arrowhead in C emphasizes that overshoot of the toe trajectory can be observed in all the treated animals.

Fig. 4. Toe heights and toe-obstacle distances in overground locomotion and in stepping over an obstacle. A: maximum heights of the forelimb toe during overground locomotion. B and C: toe heights just above the obstacle in the leading forelimb (B) and trailing forelimb (C). D and E: toe-obstacle distances at lift-off (D) and land-on (E). There was no statistical difference among the 3 groups in the data illustrated in A–E. Values are means ± SE. All averages are from 8–10 animals, and the number of analyzed animals is shown in Table 1.
tionally, the linear distance from the peak toe position to the upper edge of the obstacle was significantly larger in the ipsilateral leading forelimb compared with the prelesion and contralateral leading forelimbs (Fig. 5E); in contrast, those of the trailing forelimb did not differ significantly among the groups (Fig. 5F). The data and the results of the statistical analysis are shown in Table 1. Our observations indicate that the unilateral lesion in the lateral cerebellum significantly altered the toe trajectory of the ipsilateral leading forelimb, producing overshoot of the toe trajectory and inaccurate peak toe positions. With regard to the variability of end point control among steps, we subsequently analyzed toe-obstacle distances (i.e., the positions of paw placement before or after stepping over the obstacle) in the same manner as in Fig. 5, A–D. The data for this analysis are shown in Fig. 6. Toe-obstacle distances were normally more variable than the peak toe positions of the leading forelimb (Fig. 6 and see prelesion control in Fig. 5, A and C), and the variability seemed unaffected by the lesion (Fig. 6B). Although the trailing forelimb showed less variability of the paw placement at lift-off, its variability was also not influenced by the lesion. Thus the lesion of the lateral cerebellum did not affect the overall positions of the paw placement (Fig. 4, D and E) or its variability (Fig. 6). This conclusion is applicable to all types of paw placement (leading and trailing forelimb at either lift-off or land-on).

The angular displacement of the elbow joint was normalized in the swing phase during the stepping phase (Fig. 7, A and B). Flexion of the elbow joint in prelesion and contralateral leading forelimbs was coincident with the moment that the forelimb toe reached just above the obstacle (indicated by vertical lines in Fig. 7, A and B), and thereafter the elbow joint was rapidly extended toward land-on (Fig. 7A, left and center). By contrast, flexion of the elbow joint in the ipsilateral leading forelimb was sustained even after the toe passed over the obstacle (Fig. 7A, right); thus elbow extension in the ipsilateral leading forelimb was delayed compared with prelesion and contralateral leading forelimbs. This observation is emphasized by the red line in Fig. 7A, right. In contrast to the leading forelimb, the displacement of the elbow in the trailing forelimb was not different among the three groups (Fig. 7B). Next, we examined the angular displacement and angular velocity of the elbow joint in the leading forelimb within a particular time window (Fig. 7, C...
Angular displacement and velocity were averaged over the time window from -30 ms to +30 ms, with time zero representing the point at which the toe of the leading forelimb passed just above the obstacle. Normalization of angular displacement and velocity was not applied to this analysis. Both Fig. 7C and D indicate delayed elbow extension after time zero, but it is more emphasized in Fig. 7D, showing that the time when the angular velocity switched from negative to positive numbers, which corresponds to the switching point of elbow flexion to extension, occurred at the moment that the toe passed just above the obstacle in the prelesion and contralateral leading forelimbs (black arrows in Fig. 7D). By contrast, this switch was delayed in the ipsilateral leading forelimb (red arrows in Fig. 7D). These observations imply that sustained elbow flexion and delayed elbow extension are specifically induced in the ipsilateral leading forelimb.

Next, we sought to determine whether the girdle (shoulder) position might also contribute to overshooting of the toe trajectory in the ipsilateral leading forelimb. Previous studies reported that elevation of the girdle position was important for achieving successful obstacle avoidance (Aoki et al. 2012; Aoki et al. 2012). Results for the leading (C) and trailing (D) forelimbs are shown separately. The number of data points in A–D are as follows: leading forelimb: 2-cm obstacle: Pre, n = 44, Post-contralateral, n = 30, Post-ipsilateral, n = 34; 3-cm obstacle: Pre, n = 37, Post-contralateral, n = 35, Post-ipsilateral, n = 41; 4-cm obstacle: Pre, n = 40, Post-contralateral, n = 27, Post-ipsilateral, n = 34; trailing forelimb: 2-cm obstacle: Pre, n = 45, Post-contralateral, n = 34, Post-ipsilateral, n = 30; 3-cm obstacle: Pre, n = 39, Post-contralateral, n = 41, Post-ipsilateral, n = 35; 4-cm obstacle: Pre, n = 41, Post-contralateral, n = 34, Post-ipsilateral, n = 27. E and F: linear distances from peak toe positions to the upper edge of the obstacle for the leading (E) and trailing (F) forelimbs. Values are means ± SE. *P < 0.05, **P < 0.01, ***P < 0.001. All averages are from 8–10 animals, and the number of analyzed animals in E and F is shown in Table 1.
The displacement of the shoulder positions of the leading forelimb during the stepping phase for each treatment group is illustrated in Fig. 8A. We found that the extent of elevation of the shoulder positions was gradually altered in relation to the height of the obstacle, and the displacement of shoulder positions in the ipsilateral leading forelimb was similar to those of the prelesion and contralateral leading forelimbs (Fig. 8A). Since girdle position itself did not overshoot during the stepping phase, it is unlikely that the girdle position directly contributes to the overshooting toe trajectory in the ipsilateral leading forelimb. It was also found that the shoulder positions were continuously elevated even after the toe passed just above the obstacle (indicated by cross in Fig. 8A). Because elbow flexion was sustained and elbow extension was delayed in the ipsilateral leading forelimb after this point, a possible explanation for the overshooting toe trajectory in the ipsilateral leading forelimb might be that it was caused by delayed elbow extension in combination with the normal elevation of the shoulder positions (Fig. 8C). In the prelesion and contralateral leading forelimbs, the shoulder position remained elevated after the toe passed just above the obstacle and the elbow joint was properly extended at that point; this enabled a downward toe trajectory (Fig. 8B).

To investigate the behavior of the agonist and antagonist muscles involved in control of the elbow joint, we recorded the EMG activities of the biceps and triceps brachii (Fig. 9). Offset of the biceps brachii and onset of the triceps brachii in the ipsilateral leading forelimb were delayed to the time that the toe reached just above the obstacle (see arrows in Fig. 9E) compared with the prelesion leading forelimb (Fig. 9A) and
contralateral leading forelimb of postlesion rats (Fig. 9C). In contrast to the leading forelimb, the EMG activities of the trailing forelimb were similar among the three groups (Fig. 9, B, D, and F). The offset latency of the biceps brachii and the onset latency of the triceps brachii from the time that the toe of the leading forelimb reached just above the obstacle are shown in Fig. 10. Unfortunately, the results from the 4-cm obstacle condition could not be included in the analysis because sufficient EMG data were not obtained for a statistically reliable comparison. Detection of the offset of biceps activity and the onset of triceps activity with Butterworth-filtered signals with a threshold of 6 SD is illustrated in Fig. 10A. We found that the offset latency of the biceps brachii in the ipsilateral leading forelimb was significantly delayed compared with the prelesion control and contralateral leading forelimbs (Fig. 10B). By contrast, the offset latency of prelesion and contralateral leading forelimbs was synchronized with the toe reaching just above the obstacle. All the statistical data are shown in Table 2. The lesion in the lateral cerebellum therefore induced a clear prolongation of the activity of the biceps brachii. We postulate that the prolonged activity of biceps brachii might result in the sustained elbow flexion and delayed elbow extension seen in the ipsilateral leading
the cerebellar cortex were mainly located in lobule V– crus I during locomotion. In the present study, the lesioned areas of the limb movements involved in stepping over an obstacle was to elucidate the effects of a lesion in the lateral cerebellum (Voogd 2004). Two aspects of the methodology need to be taken into consideration when interpreting the data obtained in the present study. First, we could not completely exclude the possibility that the lesion in the cerebellar cortex extended beyond the boundary between the intermediate and lateral zones since electrophysiological and/or anatomical identification of the lateral zone was not performed. It was previously reported that inactivation of lobule V in the intermediate cerebellum results in excessive toe elevation and hyperflexion of the elbow joint during overground locomotion in cats (Udo et al. 1979, Udo 1980). In agreement with this, we observed that unilateral cerebellar damage that included more medial areas of the cerebellar cortex from lobule IV–VI (which could be equivalent to the intermediate cerebellum) or pharmacological inactivation of the intermediate cerebellum had severe effects on ipsilateral limb movements in overground walking in rats (Aoki et al., unpublished observations). However, in the present study, the lesion had no apparent effect on limb movements in overground locomotion. In light of these findings, we conclude that the lesion in the present study was probably confined to the lateral cerebellum.

The second methodological consideration is compensatory functions of other brain areas beyond the cerebellar cortex. It is possible that compensation might have occurred during the 2-day recovery period after surgery because compensatory processes are expected to start immediately after the occurrence of the lesion. However, a previous study in the cat showed that evidence of compensation for a lesion in the primary motor cortex was not observed until 3–4 days after surgery (Beloozerova and Sirota 1993). Thus, although we cannot completely exclude the possibility, it is likely that after 2 days compensatory effects on locomotor movements would be small and have little or no influence on the results obtained here. It is possible that in a future study acute inactivation of the lateral cerebellum could offer an alternative to suction surgery to exclude compensatory effects.

Comparison with previous studies. As stated above, the lesion in the lateral cerebellum did not influence limb movements during overground locomotion. This result is consistent with the previous description of locomotor control by the cerebellum (see for review Morton and Bastian 2007). In contrast, we found that the lesion in the lateral cerebellum resulted in the overshooting of the toe trajectory when the animal stepped over an obstacle. The overshooting toe trajectory can be classified as a hypermetric movement. From a clinical standpoint, hypermetria is a major symptom in cerebellar patients who have a lesion in the lateral cerebellum and/or dentate nucleus and is assumed to be caused by delayed onset and/or offset of muscle activities during a reaching task (Hore et al. 1991; Manto 2009). In the monkey, cooling of the dentate nucleus results in hypermetric limb movements following delayed muscle activity in reaching (Diener and Dichgans 1992; Flamant and Hore 1986). Furthermore, a recent clinical study using transcranial magnetic stimulation reported that interruption of the lateral cerebellum caused augmented errors in the final limb positions in a reaching task (Miall et al. 2007).

In the present study, we obtained similar findings that delayed onset in the final limb positions in a reaching task (Miall et al. 2007). In the monkey, cooling of the dentate nucleus results in hypermetric limb movements following delayed muscle activity in reaching (Diener and Dichgans 1992; Flamant and Hore 1986). Furthermore, a recent clinical study using transcranial magnetic stimulation reported that interruption of the lateral cerebellum caused augmented errors in the final limb positions in a reaching task (Miall et al. 2007).

In the present study, we obtained similar findings that delayed onset in the final limb positions in a reaching task (Miall et al. 2007). In the monkey, cooling of the dentate nucleus results in hypermetric limb movements following delayed muscle activity in reaching (Diener and Dichgans 1992; Flamant and Hore 1986). Furthermore, a recent clinical study using transcranial magnetic stimulation reported that interruption of the lateral cerebellum caused augmented errors in the final limb positions in a reaching task (Miall et al. 2007).
In light of these observations, we conclude that impairment of the lateral cerebellum can result in hypermetric movements and an increased rate of errors in distal limb positions that arise from delayed muscle activities.

Cerebellar patients with a lesion in the lateral cerebellum show both hypermetric and hypometric movements; interestingly, cerebellar hypermetria can often be observed in fast movements while hypometria occurs in slow movements (Dieiner and Dichgans 1992; Hore et al. 1991; Manto 2009). Hypermetric movements are frequently associated with more prolonged activities of agonistic muscles (Hore et al. 1991); this type of behavior was detected in the present study. In contrast, we did not observe hypometric movements, such as undershooting of the toe trajectory. We propose that the lesion in the lateral cerebellum induced dysmetria with regard to toe trajectory, particularly in the hypermetric direction, when stepping over an obstacle; we further suggest that a possible cause of the hypermetria is comparatively faster limb movements when stepping over an obstacle.

A previous study in the cat reported that a lesion in the PPC induced abnormal paw placement prior to stepping over an obstacle during treadmill locomotion in both the leading and trailing limbs; the impaired paw placement resulted in the paw frequently hitting the obstacle (Lajoie and Drew 2007). When the M1 of cats was inactivated, the animals showed a normal limb trajectory when stepping over an obstacle; however, they placed their paws farther away from the obstacle after stepping over it (Friel et al. 2007). In the present study, we found that even in prelesion animals the toe-obstacle distances at lift-off and land-on (defined as paw placement) showed a relatively wider dispersion than the peak toe positions of the leading forelimb (Fig. 5, A and C, Fig. 6), suggesting that accurate and consistent control of the distal extremity is unnecessary for paw placement control. Indeed, the toe-obstacle distances and their variability were unaffected by the lesion in the lateral cerebellum. Thus we suggest that the lateral cerebellum is unlikely to be involved in the end point control of paw placement when accurate control of limb movement is not required. In contrast, the toe trajectory and the peak toe position were affected by the lesion, probably because accurate and consistent control of the distal limb position is achieved in this situation. Another possibility is that the lateral cerebellum might contribute to the control of a transition point from upward to downward in the trajectory of the distal extremity rather than simple end point control such as paw placement. This possibility is supported by the observation that the paw placement in the trailing forelimb at lift-off was also unaffected by the lesion despite less variability (Fig. 6). In summary, we conclude that the lateral cerebellum is not involved in control of paw placement but is required for the accurate control of the toe trajectory.

Overshooting toe trajectory is specific to the ipsilateral leading forelimb. A principal finding of the present study was that a unilateral lesion in the lateral cerebellum resulted in overshooting of the toe trajectory during obstacle avoidance. Importantly, this behavior was specifically seen in the leading forelimb and was not observed in the other types of ipsilateral limb movements (trailing forelimb, leading or trailing hindlimb).
The lateral cerebellum is thought to receive massive visual inputs that are used for the guidance of movements (Glickstein et al. 1998). Thus we suggest that the lateral cerebellum is likely to be specifically involved in the control of limb movements that require visually dependent modification. Second, some neurons in the PPC show preferential modulations to the leading forelimb, irrespective of which ipsilateral or contralateral forelimb actually leads (Andujar et al. 2010). These neurons are located in layer 5 of the PPC in which the neurons project contralaterally to the lateral cerebellum via pontine nuclei (Drew et al. 2008). In turn, the ascending projections from the cerebellum are predominantly to the contralateral cerebral cortex. Therefore, although the lateral cerebellum could obtain information on both the ipsilateral and the contralateral leading forelimbs from the PPC, it has a limited influence on the contralateral cerebral cortex, especially on the M1. This may also explain why a unilateral lesion in the lateral cerebellum specifically affects the ipsilateral leading forelimb.

Another question to be considered is why the lesion had a selective influence on forelimb but not hindlimb movements. The lateral cerebellum is thought to receive massive visual inputs that are used for the guidance of movements (Glickstein 2000; Kakei et al. 1995; Sasaki et al. 1975). It is likely that the forelimb can be controlled under vision and may require much more visual information than the hindlimb for skillful movement control. Thus we suggest that the lateral cerebellum preferentially communicates with the forelimb areas of the cerebral cortex rather than the hindlimb areas.

With regard to cerebellar somatotopy in which the distal extremity is more laterally located than the proximal segments (Manni and Petrosini 2004), it is possible that wrist joint and/or digit movements may be partly involved in the overshooting toe trajectory. Since we were unable to carry out kinematic analyses of the wrist joint and digit motions (see MATERIALS AND METHODS), we could not address the specific contributions of these joints. Our evidence indicates a significant role of the elbow joint and its related muscles in the overshooting toe trajectory in the ipsilateral leading forelimb, but it remains not account for the overshooting of toe trajectory specific to the leading forelimb and the absence of effects on the other types of ipsilateral limb movements. Two possible explanations can be suggested for this phenomenon. First, control of the leading forelimb movements may involve a greater visual input since it is the first limb to step over an obstacle and is the only limb under visual control during stepping over the obstacle. It has been reported that some neurons in the lateral cerebellum and the dentate nucleus are responsive to the visual stimuli required for performing visually guided reaching and locomotion but are not responsive to unrelated visual stimuli such as flash and light (Cerminara and Apps 2011; Cerminara et al. 2005, Cerminara 2009; Marple-Horvat and Criado 1999; Marple-Horvat et al. 1998). Thus we suggest that the lateral cerebellum is likely to be specifically involved in the control of limb movements that require visually dependent modification.

Table 2. Numerical and statistical parameters for the offset latency of biceps and the onset latency of triceps in the leading forelimb

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post-contralateral</th>
<th>Post-ipsilateral</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Offset latency of biceps (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 cm</td>
<td>4.7 ± 1.1 (8)</td>
<td>1.3 ± 3.1 (6)</td>
<td>28.2 ± 5.3 (7)</td>
<td>F(2,18) = 16.623</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>3 cm</td>
<td>1.1 ± 2.4 (9)</td>
<td>1.1 ± 2.4 (6)</td>
<td>25.1 ± 5.0 (9)</td>
<td>F(2,21) = 13.510</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>Onset latency of triceps (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 cm</td>
<td>59.7 ± 8.0 (8)</td>
<td>66.5 ± 10.8 (6)</td>
<td>118.4 ± 10.0 (7)</td>
<td>F(2,18) = 11.682</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>3 cm</td>
<td>69.3 ± 8.5 (9)</td>
<td>71.0 ± 8.0 (6)</td>
<td>120.6 ± 16.1 (9)</td>
<td>F(2,21) = 5.846</td>
<td>&lt;0.01**</td>
</tr>
</tbody>
</table>

Values (in ms) are means ± SE for n analyzed animals (in parentheses). Numerical and statistical parameters for the latency of EMG activities are shown. **P < 0.01, ***P < 0.001.

J Neurophysiol • doi:10.1152/jn.01048.2012 • www.jn.org
possible that the behavior of more distal parts of the limb, such as wrist and digits, are also affected by the lesion.

Relationships with other brain structures. Previous studies on the regions of the brain involved in the control of obstacle avoidance locomotion have focused mainly on the cerebral cortical areas, such as the M1 and PPC of cats (Beloozerova and Sirota 1993; Drew 1993; Drew et al. 2008; Lajoie and Drew 2007; Marigold et al. 2011; McVea et al. 2009). The lateral cerebellum has abundant anatomical connections with these cortical areas. It has been hypothesized that a cerebro-cerebellar loop via the lateral cerebellum may contribute to stepping over an obstacle during locomotion (Drew et al. 2008; McVea and Pearson 2009). As a result of testing the hypothesis, we found that a unilateral lesion in the lateral cerebellum resulted in the overshoot of the toe trajectory solely in the ipsilateral leading forelimb.

How does the lateral cerebellum act in concert with other areas of the brain to contribute to the limb movements required for stepping over an obstacle? Drew et al. (2008) suggested that the cerebro-cerebellar loop from the PPC to the M1 via the lateral cerebellum is important for the proper generation of stepping movements and limb trajectories in obstacle avoidance locomotion. This suggestion was based on accumulated anatomical evidence indicating that the lateral cerebellum receives afferents from the PPC and that PCs of the lateral cerebellum and output neurons of the lateral cerebellum, the dentate nucleus, project back to the M1 (Dum and Strick 2003; Glickstein 2000; Kakei et al. 1995; Kelly and Strick 2003; Sasaki et al. 1975; Stein and Glickstein 1992; Strick et al. 2009). It was also reported that the ventrolateral thalamic nuclei, which mainly project to the M1 and which receive inputs from the cerebellum and basal ganglia, contribute to rhythmic modulation of the pyramidal neurons of the M1 in response to the phase of stepping in cats (Marlinski et al. 2012). Given that a major source of the ventrolateral thalamus originates from the lateral cerebellum via the dentate nucleus, it is suggested that the lateral cerebellum as well as the dentate nucleus are probably involved in generating properly modulated activity in the pyramidal neurons of the M1 via the thalamus. Therefore, one possible explanation is that the lesion in the lateral cerebellum induced dysfunction of the PPC-lateral cerebellum-M1 loop believed to play an important role in obstacle avoidance, and that the lesion resulted in the overshooting toe trajectory in the ipsilateral leading forelimb due to inappropriate modulation of the pyramidal neurons of the M1. In support of this proposal, the lesion caused similar effects irrespective of whether the lesion contained crus II or not. Since the strongest connections of crus II are with the parietal and prefrontal cortex (Glickstein et al. 2011; Kelly and Strick 2003; Strick et al. 2009), the effects observed here are more likely mediated by the M1 rather than these association areas. However, the lateral cerebellum also communicates with other areas of the brain (Hashimoto et al. 2010; Stein and Glickstein 1992; Suzuki et al. 2012), and it is also possible that these structures were affected by the lesion in the lateral cerebellum.

In a future study, we propose to investigate the cerebellar contribution to modulation of neuronal activities in M1 pyramidal neurons during obstacle avoidance locomotion. Additionally, it will be necessary to clarify in detail the anatomical connections of the cerebellum with the cerebral cortex, particularly with the M1 and the PPC in the rat. This information should provide further insights into the neural substrates involved in obstacle avoidance behavior.

In conclusion, we have shown here that a unilateral lesion in the lateral cerebellum induced the overshooting toe trajectory that was specific to the leading forelimb ipsilateral to the lesion. Our analyses indicated that this effect on the toe trajectory was mediated by sustained elbow flexion and delayed elbow extension following delayed muscle activities. We suggest that the lateral cerebellum specifically plays a key role in the generation of the accurate toe trajectory in the leading forelimb and is involved in the appropriate control of muscle activity in stepping over an obstacle during locomotion. This role is particularly important for highly accurate control of limb movements under visual guidance.

GRANTS

This work was supported by Grants-in-Aid for Scientific Research (B) and Priority Areas “Emergence of Adaptive Motor Function through Interaction between Body, Brain and Environment” from the Ministry of Education, Culture, Sports, Science and Technology of Japan to D. Yanagihara, the research funds of Tateishi Science and Technology Foundation to D. Yanagihara, and Grant-in-Aid for JSPS Fellows to S. Aoki.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: S.A. and D.Y. conception and design of research; S.A. performed experiments; S.A. analyzed data; S.A., Y.S., and D.Y. interpreted results of experiments; S.A. prepared figures; S.A. drafted manuscript; S.A., Y.S., and D.Y. edited and revised manuscript; S.A., Y.S., and D.Y. approved final version of manuscript.

REFERENCES


