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Cerebro-cerebellar functional connectivity revealed by the laterality index in tool-use learning

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THE function of the lateral part of the human cerebellum was investigated through cerebro-cerebellar functional connectivity. We propose a laterality index method to reveal a functional and possibly anatomical pathway between the cerebral cortex and the cerebellum. The brain activity involved in learning a visually-guided tracking skill using a novel computer mouse was measured by functional magnetic resonance imaging. The imaging data analyzed using the method suggest that the simple lobule and semilunar lobule of the lateral cerebellum have connections with the pars opercularis and pars triangularis in the inferior frontal gyrus. A possible function of this cerebro-cerebellar communication loop is tool usage, which is in-between the cognitive and motor functions of the human cerebellum. *NeuroReport* 10:325-331 © 1999 Lippincott Williams & Wilkins.

Key words: Cerebro-cerebellar functional connectivity; Functional magnetic resonance imaging; Higher brain function; Laterality index; Learning

Introduction

Recent neuroimaging and lesion studies have shown that the most evolved, lateral part of the cerebellum is involved in higher brain functions than motor control; such higher functions include visual attention, motor imagery, and cognitive planning [1-4]. However, the topography, i.e. which function is assigned to which region, in this part of the human cerebellum is still unknown although it has been well investigated in the phylogenetically older part of the cerebellum consisting of the vermis and intermediate hemisphere. Often called the cerebro-cerebellum, the lateral part of the cerebellum receives inputs from and sends outputs to the cerebral cortex. Its mossy fibre inputs originate in pontine nuclei that relay information from the cerebral cortex, and its outputs are conveyed by the dentate nucleus to the thalamus and from there to the cerebral cortex. The cerebellar output to individual cerebral cortical areas originates from distinct clusters of neurons in the output nuclei, and individual clusters show little or no overlap [5]. Therefore, the function of this cerebellar region could be revealed by the function of the cerebral cortical region with which a given cerebellar region connects because more is known about functional localization in the cerebral cortex.

In monkey brains, the details of pathways between the cerebral cortex and the cerebellum have been investigated in electrophysiological and morphological experiments [5-9]. However, the methodologies used in monkey studies are not applicable to the living human brain. Instead, we have functional neuroimaging methods. In typical neuroimaging experiments conducted by positron emission tomography (PET) or functional magnetic resonance imaging (fMRI), the functional connectivity between two given regions is investigated through temporal correlation between activities of both regions elicited by a certain task. Showing functional connectivity is, however, not sufficient. It is necessary to show that there is underlying anatomical connectivity. Although some methods to find functional connectivity between cerebral cortical regions under anatomical constraints have been developed [10,11], it is impossible to apply them to unknown connectivity, and the anatomical knowledge on which imposed constraints are based is often infirm. One of the most successful techniques to overcome these drawbacks is transcranial magnetic stimulation (TMS) during PET. Paus *et al.* stimulated a given cortical region by TMS and using PET identified the precise location stimulated and the regions in which secondary activities are elicited [12]. Although this technique is reliable, there are severe limitations in areas that can be stimulated by TMS, especially the

subcortical areas. Here we propose a new method to find functional connectivity under a valid and loose anatomical constraint that is applicable to unknown cerebro-cerebellar connectivity. This method thus has both functional and anatomical constraints. The functional constraint is that the task related to a given function elicits coactivation in cerebral cortical and cerebellar regions if both regions are involved in the function. The anatomical constraint is that the anatomical connection between the cerebral cortex and the cerebellum is contralateral. This constraint is based on the fact that most corticopontine projections and almost every cerebellar projection to the cerebral cortex are contralateral [5-9,13-15]. Thus, the output signal of a given cerebral cortical region is conveyed to a contralateral cerebellar region and vice versa if there is a pathway between these regions. It follows that *laterality* of neural activation in the cerebral cortical region is the opposite to that in the cerebellar region. To quantify such laterality, we defined a *laterality index* (LI) as LI = (L - R) / (L + R), where, L denotes the activation of a given region in the left hemisphere and R denotes that of the corresponding region in the right hemisphere. It is predicted that, if there is an anatomical pathway between cerebellar region A and cerebral cortical region B and if both regions are activated by a task, there is *negative* correlation between LI in A and that in B. LI could serve as an index of cerebro-cerebellar connectivity that combines functional and anatomical constraints. Thus, the LI method we propose has three improvements over existing methods to investigate functional connectivity. First, the possibility that anatomical connectivity underlies an observed functional connectivity is much higher than with conventional temporal correlation methods that impose no anatomical constraint. Second, it is applicable to unknown connectivity that cannot be investigated by some methods that impose anatomical constraints. Third, it can reveal cerebro-cerebellar connectivity while the technique of TMS during PET cannot.

The purpose of the present study is to investigate the function of the *simple lobule and superior semilunar lobule* in the lateral part of the cerebellum by investigating a cerebral cortical region with which this cerebellar region connects. Our previous studies showed that the neural activity in this region is modulated by learning a visually-guided tracking task with a novel computer mouse; vigorous activity was observed in the early stage of learning, while the activity area diminished in the late stage of learning [16,17]. We can predict therefore, after this cerebellar region begins to play a functional role in the feedforward control and the anatomical pathway between this cerebellar region and the cerebral cortex becomes *functionally effective* as a result of learning, the pathway will be revealed with the proposed method. Thus, it is expected that a negative correlation between LI of the cerebellar region and LI of the cerebral cortical region that is connected with it would be observed only after learning.

Materials and Methods

Subjects: Three female and two male volunteers (20-23 years old) participated in this study after giving their informed consent. All of them were right-handed. The protocol of the present study was in accordance with the ethical guidelines of the Communications Research Laboratory.

Task and procedure: All of the tasks and procedures were the same as in previous studies [16,17]. Subjects lay in a supine position with their heads inside a head coil. A tilted mirror attached to the coil provided them with a view of the screen. The task was visually-guided tracking with a computer mouse. The subjects were asked to manipulate a computer mouse with their right hands, tracking a small randomly moving target on the screen by using a cursor. There were two experimental conditions: *test* and *control*. Under the test condition, the subjects used a novel computer mouse in which the relationship between the mouse position and the corresponding cursor position was rotated in the following fashion:

$$\begin{pmatrix} x \\ y \end{pmatrix} = \begin{pmatrix} \cos 120^\circ & \sin 120^\circ \\ -\sin 120^\circ & \cos 120^\circ \end{pmatrix} \begin{pmatrix} p \\ q \end{pmatrix},$$

where, (x, y) and (p, q) denote the position of the cursor and the mouse, respectively. Under the control condition, the subjects used a normal computer mouse (i.e., $(x, y) = (p, q)$).

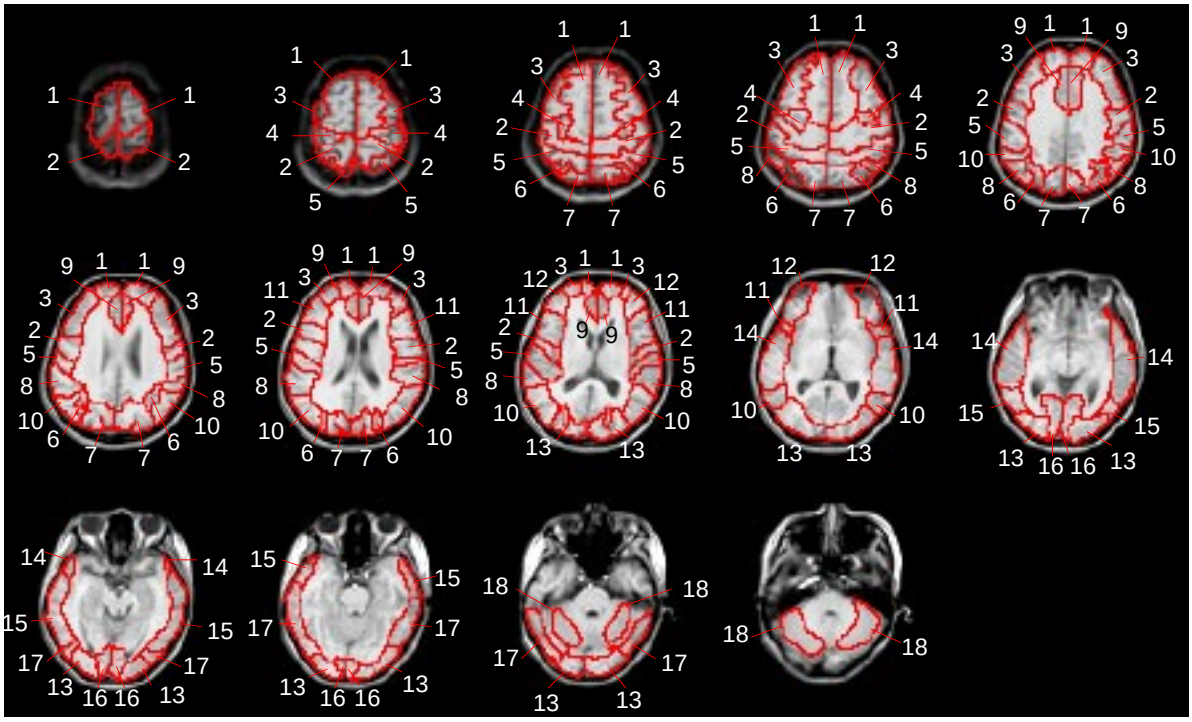
A session consisted of eight blocks; each block continued a 35.2 s test period and a 35.2 s control period. The session thus lasted about 9.5 min. Subjects participated in 18 sessions over 2–4 days. The first three sessions were defined as *pre-learning* sessions, the last three sessions as *post-learning* sessions, and the other sessions as training sessions.

We recorded tracking errors to assess the degree of learning. The error was defined as averaged visual angles subtended by the distance between the target and the cursor over a session. The sampling rate of the errors was about 17 ms.

The previous experiment suggested that the activity in the lateral cerebellum in the late stage of learning is combined with feedforward control process rather than error detection and correction processes [17]. Therefore, we used different experi-

mental situations in the pre-learning and post-learning sessions by changing a task load in the control periods. In the pre-learning session, we used the same target velocity in the test and control periods. In the post-learning sessions (16th to 18th sessions) and two training sessions (ninth and tenth sessions), we controlled the target velocity in the control periods so that the amount of tracking errors in the test periods would be equivalent to those in the control periods. This procedure was designed to verify that the observed activation differences between test and control conditions after learning were not caused by error detection and correction but by feedforward control acquired through learning. Thus, brain activity in the pre-learning sessions reflected mainly error detection and correction processes, while that in the post-learning sessions reflected feedforward control processes.

Magnetic resonance imaging: MRI data acquisitions were carried out with a 1.5 T Siemens MRI scanner (MAGNETOM Vision). During one of the three pre-learning and one of the three post-learning sessions, we acquired echo planar functional MRI images in 16 transverse sections (typical parameters: TR = 4.4 s, TE = 66 ms, flip angle = 90, matrix size = 128**x**128, FOV = 280 mm, slice thickness = 6 mm, slice gap = 0.3 mm). We obtained eight sets of images for test and eight sets of images for control conditions in individual blocks. A session consisted of eight repetitions of blocks. Thus, the number of total scans in a session was 128: 64 for test and 64 for control conditions. After finishing each experimental session, T1-weighted structural images at the same slice positions were acquired by a gradient echo sequence (typical parameters: TR = 560 ms, TE = 6 ms, flip angle = 90, matrix size = 256**x**256). We also took a set of high-resolution T1-weighted whole brain images of individual subjects, either before or after the entire set of experimental sessions. This series of images consisted of 180–200 sagital images obtained by FLASH (parameters:



1. Superior frontal gyrus
2. Precentral gyrus
3. Middle frontal gyrus
4. Depth of precentral sulcus
5. Postcentral gyrus
6. Intraparietal sulcus
7. Superior parietal lobule
8. Supramarginal gyrus
9. Cingulate sulcus
10. Angular gyrus
11. Pars opercularis and pars triangularis
12. Orbital gyri
13. Extrastriate cortex
14. Superior temporal gyrus
15. Middle temporal gyrus
16. Striate cortex
17. Inferior temporal gyrus
18. Simple lobule and semilunar lobule

FIG. 1. Example ROI-parcellation in one subject.

TR = 9.7 ms, TE = 4 ms, flip angle = 15, matrix size = 256 **x** 256, FOV = 256 mm, slice thickness = 1 mm, slice gap = 0 mm). These two types of T1-weighted structural images enabled identifying anatomical regions of the brain structure.

Analyses: All of the functional images were motion-corrected by AIR 3.0 [18] and then spatially filtered with an isotropic low-pass Gaussian filter (FWHM = 4 mm). We then performed a two-step analysis: (1) we identified the regions showing the effect of learning, (2) for individual cerebral cortical regions identified in (1) , we examined the correlation between its LI and the LI in the simple lobule and the superior semilunar lobule in the lateral cerebellum.

First, for each pre- and post-learning *session* of individual subjects, we performed a cross-correlation [19] to examine the linear correlation pixel-by-pixel between an actual time course of signal values and an ideal box-car reference function representing the condition protocol shifted by one data point to account for the delay of the rise and fall time of the blood oxygenation level. An *active pixel* was determined as one showing significantly higher signal intensity under the test conditions than under the control conditions ($r > 0.29$, $p < 0.001$). We superimposed the results of the cross-correlation on the corresponding T1-weighted structural images. On each T1-weighted structural image obtained at the same slice position as the functional slice position, we segmented the cerebral cortical and cerebellar cortices into anatomically identifiable regions of interest (ROIs) based on anatomical landmarks (Fig. 1). Sulci were examined through high-resolution T1-weighted whole brain images with reference to the brain atlases of Hirayama and Kawamura [20]. We then calculated the number of active pixels in each ROI and performed a one-way-within-subject ANOVA (learning; pre-learning and post-learning) on the number of active pixels in each ROI to investigate the cerebral cortical ROIs in which neural activities were modulated by learning.

Second, for each *block* of each pre- and post-learning session of individual subjects, we performed a cross-correlation and calculated the number of active pixels in the cerebral cortical ROIs that show the effect of learning and in the cerebellar ROI of the region of the simple lobule and the superior semilunar lobule reported as being activated in the same experimental paradigm [16,17]. For each of these ROIs, we obtained 40 LI data (from eight blocks **x** five subjects) in each of the pre-learning and post-learning sessions. We then plotted LI in each cerebral cortical ROI as a function of LI in the cerebellar ROI to investigate whether there was negative correlation between LI in a cerebral cortical ROI and LI in the cerebellar ROI.

Results

As shown in Fig 2, tracking errors under the test condition decreased as the sessions proceeded, and no change was observed in the amount of errors under the test condition in the final few successive sessions. This indicates that subjects learned to use the novel computer mouse in the sessions. Furthermore, the error levels in the test sessions were equivalent to those under the control conditions in the five sessions (ninth and tenth and 15th to 17th sessions) in which we controlled the target velocity that confirmed the success of the experimental manipulation of the target velocity.

Figure 3 depicts typical slices of the results of the cross-correlation for pre- and post-learning sessions of one subject. Table 1 summarizes the number of active pixels in each ROI and the results of the ANOVA. We found a main effect of learning in four cerebral cortical ROIs: the *extrastriate cortex*, *the depth of the precentral sulcus*, *the middle frontal gyrus*, and *the pars opercularis and pars triangularis*, as well as in the cerebellar ROI of the simple lobule and superior semilunar lobule ($p < 0.05$). In all these ROIs, the number of active pixels decreased as learning proceeded. By further analyses of LI, we found that no significant negative correlation be-

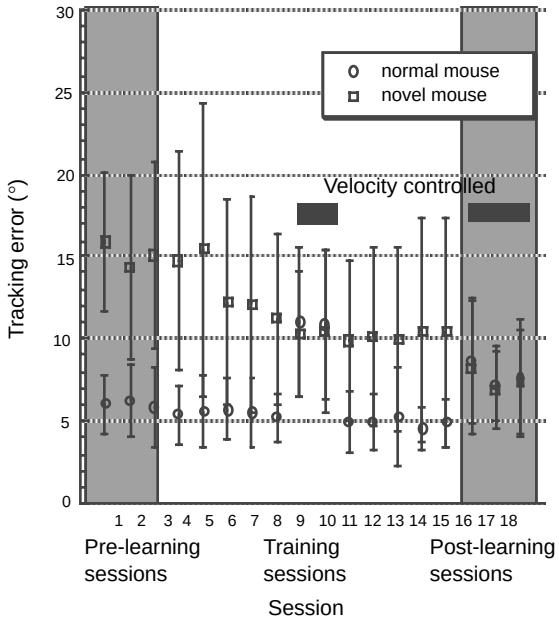


FIG. 2. Averaged tracking errors among all subjects. Error bars show s.d. fMRI data acquisitions were carried out in one of the first three successive sessions (pre-learning) and one of the last three successive sessions (post-learning). Velocity of the target was controlled in the ninth, tenth, and 16th to 18th sessions so that the amount of tracking errors under each condition was the same.

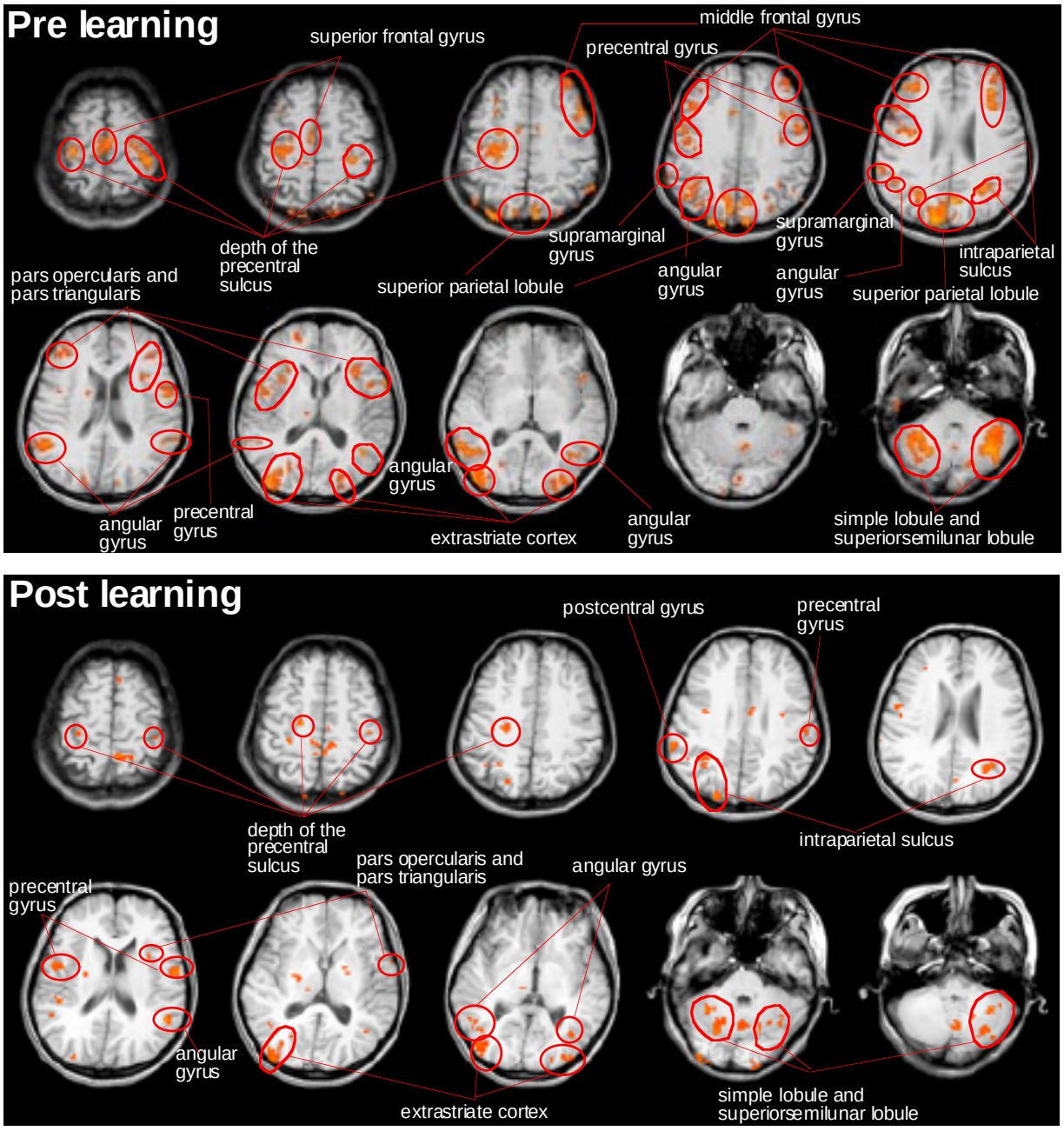


FIG. 3. Typical slices showing results of cross-correlation in pre- and post-learning sessions of one subject.

tween LI in the cerebellar ROI and LI in the cerebral cortical ROI existed for any ROI in pre-learning sessions. In post-learning sessions, however, we found such negative correlations only for the pars opercularis and pars triangularis ($r^2 = 0.167$, $p < 0.01$; Fig. 4).

Discussion

First, it should be emphasized that we found a negative correlation only in the post-learning session. This result could verify that, once feedforward control has been enabled through learning, the simple lobule and superior semilunar lobule in the lateral part of the cerebellum play a specific role in performing the task and that the existing anatomical pathway between this cerebellar region and the cerebral cortex becomes functionally effective.

Anatomical studies with monkeys showed that the lateral portion of the dentate nucleus sends its outputs via the area X in the thalamus to the PMv [5,7,9]. PMv in monkey brains largely overlaps with

Table 1. Summary of the number of active pixels averaged among all subjects and the results of ANOVA. Numbers in parentheses are s.d.

Cerebral ROI	Pre-learning		Post-learning		Effect of learning
	Left	Right	Left	Right	
Extrastriate cortex	7.2 (7.3)	15.4 (10.2)	3.0 (4.2)	3.8 (3.5)	$p < 0.05$
Superior parietal lobule	50.2 (64.0)	67.0 (65.3)	9.0 (16.3)	18.8 (18.0)	ns
Intraparietal sulcus	89.2 (115.3)	76.0 (90.8)	25.0 (35.8)	18.6 (23.1)	ns
Supramarginal gyrus	8.8 (8.7)	9.8 (16.8)	4.6 (10.3)	14.2 (18.8)	ns
Angular gyrus	12.2 (11.8)	35.4 (40.3)	3.8 (8.5)	16.0 (23.1)	ns
Middle temporal gyrus	9.6 (18.3)	16.2 (18.4)	2.4 (5.4)	1.6 (2.6)	ns
Postcentral gyrus	12.8 (12.1)	12.4 (20.2)	8.6 (15.6)	19.6 (35.2)	ns
Precentral gyrus	37.0 (36.6)	37.4 (28.8)	8.6 (12.7)	16.8 (17.6)	ns
Depth of the precentral gyrus	29.2 (25.5)	57.6 (36.8)	12.6 (22.4)	30.4 (45.8)	$p < 0.05$
Superior frontal gyrus	16.6 (9.7)	19.8 (20.1)	19.2 (30.8)	19.4 (28.2)	ns
Middle frontal gyrus	75.2 (79.1)	47.2 (9.6)	3.4 (4.7)	4.6 (4.6)	$p < 0.0$
Pars opercularis and pars triangularis (including sulcus of insula)	25.2 (30.7)	29.0 (23.9)	7.0 (13.5)	10.0 (12.2)	$p < 0.05$

ns = not significant

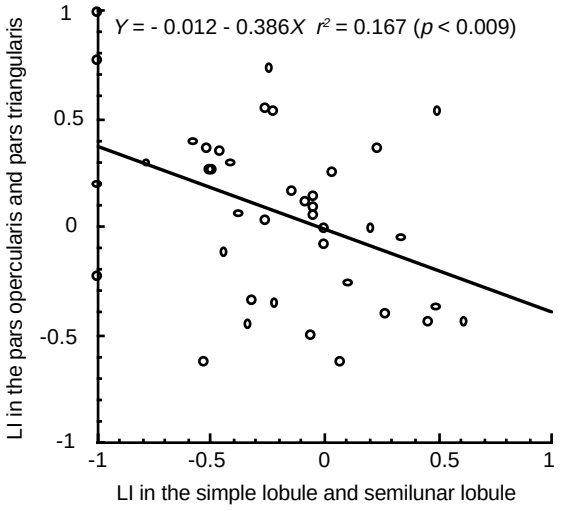


FIG. 4. Linear regression between LI in the pars opercularis and the pars triangularis and that in the simple lobule and superior semilunar lobule in the cerebellum.

the pars opercularis and pars triangularis in the inferior frontal gyrus in human brains [21]. Taking these anatomical findings into account, we concluded that the pars opercularis and pars triangularis might be the target of output from the simple lobule and superior semilunar lobule in the lateral part of the cerebellum. Physiological studies have suggested that PMv in monkey brains is related to visual guidance of hand and arm movements [22]. The task in the present study also required visual guidance of arm movements. It therefore seems reasonable to conclude that the simple lobule and superior semilunar lobule subserves visual guidance of movements. It is possible, however, that another function of this cerebellar region is more cognitive than visual guidance of movements. First, the previous study [17] showed that the activation in this cerebellar region was not elicited by visually-guided tracking with a normal mouse but by the novel mouse. Second, the pars opercularis and pars triangularis in the inferior frontal gyrus in the left hemisphere is known as the Broca's area. There is general agreement that the Broca's area plays an important role in language production. These facts suggest that the functions of this cerebellar region can be understood in more cognitive terms such as *tool usage*. We need further studies with carefully controlled task design and further analyses to investigate whether there are functional subdivisions in this cerebellar region.

The method of revealing cerebro-cerebellar connectivity that we propose here overcomes the drawbacks of conventional temporal correlational methods, that is, its inapplicability to unknown connectivity and infirmness of its anatomical constraint. Unknown connectivity can be revealed by this method and the anatomical constraint we imposed is valid because the underlying anatomical fact that cerebro-cerebellar anatomical connectivity is contralateral is well established. This method also supplements the combined method of TMS and PET in the sense in its applicability to cerebro-cerebellar connectivity. Thus, the LI method can reveal unknown cerebro-cerebellar functional connectivity under a firmer anatomical constraint. However, it also has some problems. There is a slight possibility that such correlation may be caused by a reason other than the underlying anatomical connectivity; both regions may be simply coactivated contralaterally. Furthermore, it is possible that an observed negative correlation between LI in a cerebral cortical region A and that in a cerebellar region B does not indicate the direct pathway between them if both regions connect with each other via another hidden region C that is too small to identify activities by

any functional imaging method such as fMRI and PET and if the region C is activated ipsilaterally to A or B. We therefore must consider many other functional and anatomical findings in order to draw firm conclusions from the results we obtained.

Conclusion

We proposed a LI method to investigate cerebro-cerebellar functional connectivity in humans. This method is unique in that it imposes both functional and anatomical constraints. We investigated changes in neural activities involved in visually-guided tracking with a novel computer mouse and found a negative correlation between the LI in the pars opercularis and pars triangularis in the inferior frontal gyrus and that in the simple lobule and superior semilunar lobule in the lateral part of the cerebellum. The results suggest that these two regions have functional and possibly anatomical connectivity and that the function of this cerebellar region might be rather cognitive. A possible function of this cerebro-cerebellar communication loop is tool usage, which is in-between the cognitive and motor functions of the human cerebellum.

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