Attentional Activation of the Cerebellum Independent of Motor Involvement

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The cerebellum traditionally has been viewed as a neural device dedicated to motor control. Although recent evidence shows that it is involved in nonmotor operations as well, an important question is whether this involvement is independent of motor control and motor guidance. Functional magnetic resonance imaging was used to demonstrate that attention and motor performance independently activate distinct cerebellar regions. These findings support a broader concept of cerebellar function, in which the cerebellum is involved in diverse cognitive and noncognitive neurobehavioral systems, including the attention and motor systems, in order to anticipate imminent information acquisition, analysis, or action.

The human cerebellum has more neurons than the remainder of the brain combined (*1*). It is physiologically connected, by monosynaptic or multisynaptic pathways, with all major subdivisions of the central nervous system (CNS), including the cerebrum, basal ganglia, diencephalon, limbic system, brainstem, and spinal cord (*2–6*). It is, therefore, one of the busiest intersections in the human brain. Nonetheless, for more than a century, neurologists and neuroscientists alike have held the view that the singular function of the human cerebellum is to help coordinate movement (*6*).

Controversy over this long-established position has emerged because of evidence from recent functional neuroimaging and neurobehavioral studies (*2, 7–14*). These studies show that the cerebellum may be involved in a variety of nonmotor functions, including sensory discrimination (*7*), attention (*2, 8–10*), working memory (*11*), semantic association (*12*), verbal learning and memory (*13*), and complex problem solving (*14*). However, in almost all of these studies, movement or motor planning were necessary components of the sensory or cognitive experimental task (*15*). In the face of such critical confounding factors, the traditional concept remains largely unmoved. One recent advance over the more traditional view suggests that the cerebellum modulates the motor control system in the service of acquiring high-quality sensory information (*7, 16*). Although this model incorporates a sensory role for the cerebellum, it still construes the cerebellum as a device whose function is motor control.

Missing from experiments to date is a single design that addresses two crucial questions. First, is the cerebellum involved in cognitive operations that do not involve the motor system for learning, planning, or guiding movements? Second, if there is such cognitive cerebellar involvement, is it colocalized to the same region (or regions) involved in movement when movement is required, or is it localized to a separate region within the cerebellum? We used functional magnetic resonance imaging (fMRI) to examine the differential involvement of the human cerebellum in three tasks: (i) a visual attention task that neither required motor learning nor made use of or guided motor operations, (ii) a motor task, and (iii) a task combining these two. We found evidence of a classic double dissociation in structure and function between areas of the cerebellum: Visual attention activates one anatomic location within the cerebellar cortex, whereas motor performance activates a distinctly different location. Moreover, attention activation can occur independently of motor involvement.

Six right-handed, healthy, normal volunteers (three male, three female) ranging in age from 23 to 29 years (mean ± SD, 25.8 ± 2.1 years) participated after informed consent. During the Attention task, circles, squares, or triangles in red, green, or blue were presented one at a time at a single spatial location in the center of foveal vision (*17*). This task tested the ability to attend selectively to targets (squares or red shapes) within a visual dimension (form or color). Subjects were instructed to silently count each target stimulus, which required attention to visual stimuli in the absence of a motor response. In the Motor task, subjects were instructed to execute repeatedly a self-paced movement of the right hand in the absence of visual stimuli. This movement was then used in the Attention-with-Motor task, which was identical to the Attention task, with one exception: Rather than silently counting target stimuli, subjects were instructed to respond to each target using movement of the right hand. Within each of the three tasks, a task activation condition was alternated with a baseline control condition. As a control for visual sensory stimulation, both the Attention task and the Attention-with-Motor task were alternated with passive visual stimulation, during which the subjects were instructed to observe the same set of visual stimuli but not selectively attend or respond to targets. The Motor task was alternated with rest (*18*).

During all three tasks, a time series of 128 gradient-echo echo-planar (EPI) images per slice was acquired (*19*) at five coronal slice locations through the cerebellum. Three slices at comparable locations within the cerebellum for all six subjects were analyzed (Fig. 1A). For each slice in each subject, the number of significantly activated voxels (*20*) during the three tasks was calculated within two regions of interest (ROIs) (Fig. 1B) defined a priori (*21*). The location of both ROIs was determined and drawn using standard cerebellar landmarks (*22*) on a single EPI image of each slice for each subject before the calculation of activations. The two ROIs were collapsed

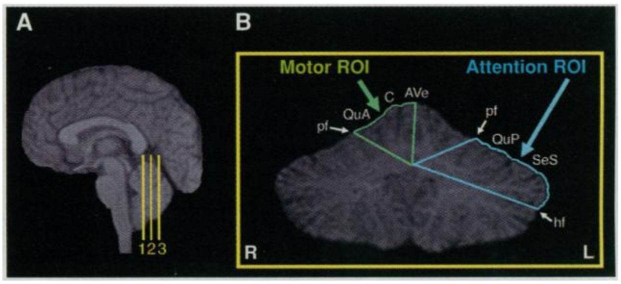

Fig. 1. (**A**) Approximate positions of slices 1 to 3 used for data analysis, shown on a midsagittal anatomical magnetic resonance (MR) image from a single subject. Slice 1 is the most anterior slice. Intracerebellar landmarks were used to guide the choice of slice positions in order to obtain images from comparable anatomical locations within the cerebellum across subjects. (**B**) Locations of ROIs (*21, 22*) shown on an anatomical MR image of slice 1 from a single subject (pf, primary fissure; hf, horizontal fissure). The Attention ROI included the left posterior quadrangular lobule (QuP) and the left superior semilunar lobule (SeS). The Motor ROI included the right anterior vermis (AVe), the right central lobule (C), and the right anterior quadrangular lobule (QuA).

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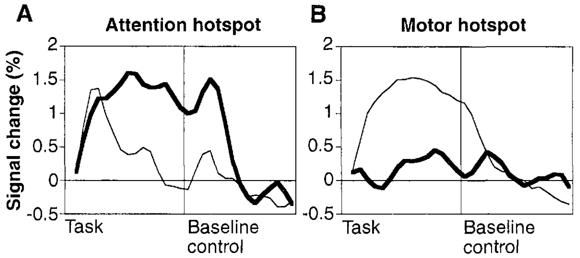

Fig. 2. Intertask comparisons within the Attention (**A**) and Motor (**B**) hotspots. For each hotspot, the time-course signal data for each subject were averaged, collapsed across the four cycles between task activation and baseline control conditions, and plotted in terms of percent change in MR signal (thick line, Attention task; thin line, Motor task).

across the three slices to create two volumes of interest (VOIs), an Attention VOI and a Motor VOI. The percent volume active during each task was then calculated for the two VOIs.

In all subjects, the cerebellum was active during the Attention task, which was performed in the absence of movement or motor planning (*23*). It was also active during the Motor task, which involved movement but demanded no selective attention. Moreover, the two tasks differentially engaged the Attention and Motor VOIs. The Attention hotspot—the maximally activated voxel in the Attention VOI during the Attention task in each subject (mean $r = 0.56$; mean percent signal change = 1.58)—was not active during the Motor task [mean $r = 0.15$; mean percent signal change = 0.38; matched-pairs t_5 (comparing r values) = 4.9, $P < 0.01$]. In contrast, the Motor hotspot—the maximally activated voxel in the Motor VOI during the Motor task in each subject (mean $r = 0.58$; mean percent signal change = 1.37)—was not active during the Attention task [mean $r = 0.16$; mean percent signal change = 0.28; matched-pairs t_5 (comparing r values) = 7.29, $P < 0.01$].

A closer look at the time course of activation underlying the above differences reveals the sharp distinction between attention and motor activation in the cerebellum (Fig. 2). At the onset of the Motor task, which was performed in the absence of the visual sensory stimulation used in the Attention task, there was a transient increase in activation in the Attention hotspot (Fig. 2A). This suggests that the initiation of the required simple motor action involved some degree of attention, whereas sustaining the simple actions did not. In contrast, during the Attention task, which was performed without any motor planning or execution, there was no increase in activation in the Motor hotspot (Fig. 2B), suggesting that neither the initiation nor the sustained execution of the Attention task required the use of those cerebellar regions most involved in the Motor task. These results highlight the functional independence of cerebellar activation by attention: Motor activation required attention, but attention activated the cerebellum regardless of whether there was visual sensory input or motor output.

Repeated-measures analysis of variance (ANOVA) of the percent volume active in the two VOIs during the three tasks (Fig. 3A) resulted in a statistically significant task × VOI interaction ($F_{2,10} = 5.81$, $P < 0.05$). Follow-up comparisons demonstrated that there was significantly greater activation in the Attention VOI during the Attention task ($F_{1,5} = 10.35$, $P < 0.05$), and, conversely, significantly greater activation in the Motor VOI during the Motor task ($F_{1,5} = 6.95$, $P < 0.05$). Both VOIs were activated during the Attention-with-Motor task, and the difference between the two was not significant.

Together, these results reflect a double dissociation between these two areas of the cerebellum with respect to their involvement in visual selective attention and movement. This dissociation is emphasized by the differential extent of activation within the ROIs across slices, with motor activity greatest in the most anterior slice and attention activity greatest in the more posterior slices (Fig. 3, B to D). The dissociation is most clearly demonstrated by the functional maps (Fig. 4) showing the differential neuroanatomical localization of these two distinct types of activity. This double dissociation is of theoretical importance because it shows that the cerebellum is not designed to perform a single neurobehavioral function, such as motor control or attention, but instead is a system composed of different regions that influence distinctly different neurobehavioral functions.

Such cerebellar influences, though differentially localized, might serve comparable, if not also complementary, goals. For instance, the cerebellum may modulate attention and sensory responsiveness (*2*, *9*, *24*) as well as movements that reposition sensory receptors (*7*, *16*) or track the trajectories of sensory information (*25*). These are complementary preparatory actions that optimize the acquisition and analysis of relevant sensory information during a search for a known and expected target stimulus or during exploration of a novel environment.

Our results demonstrate that such cerebellar preparatory influences can occur independently of motor involvement. In the Attention task, attention to sensory information alone was sufficient to activate the cerebellum, and engagement of the motor system was not necessary to produce cerebellar activation. Cerebellar attention activation occurred even though no motor learning was required; no motor response selection, error detection, or error correction was required; no imagined motor action was required; and no guidance of motor systems was required. In sum, these findings are contrary to the expectation of traditional theories of the cerebellum as a motor control system (*6*).

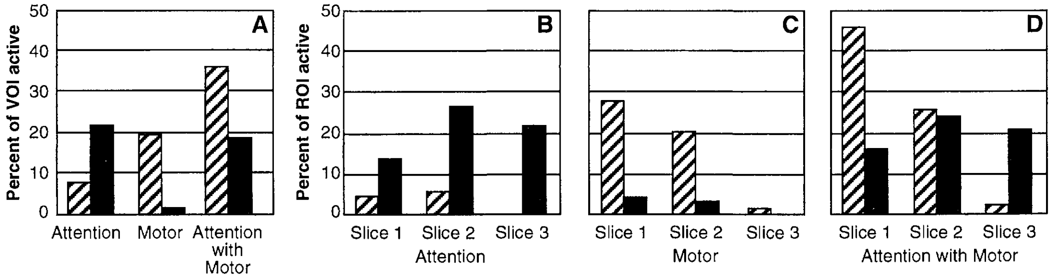

Fig. 3. (**A**) Percent (median of six subjects) of the two VOIs activated during each of the three tasks (Motor VOI, striped bars; Attention VOI, solid bars). (**B** to **D**) Percent of the two ROIs activated at each slice location during the three tasks. During the Attention task (B), the extent of activation in the Attention ROI (solid bars) was greatest in the posterior slices, falling off in the most anterior slice, whereas the Motor ROI (striped bars) was only 5% active in both slices 1 and 2, with no activation in slice 3. Conversely, during the Motor task (C), the extent of activation in the Attention ROI was minimal, while in the Motor ROI, the extent of activation was greatest in the most anterior slice, falling off in a gradient toward posterior slices. During the Attention-with-Motor task (D), the extent of activation in the two ROIs approximated a summation of results from the other two tasks (for example, the Motor ROI in slice 2 was 5.3% active during the Attention task, 20.2% active during the Motor task, and 25.8% active during the Attention-with-Motor task), with the exception of the Motor ROI in slice 1.

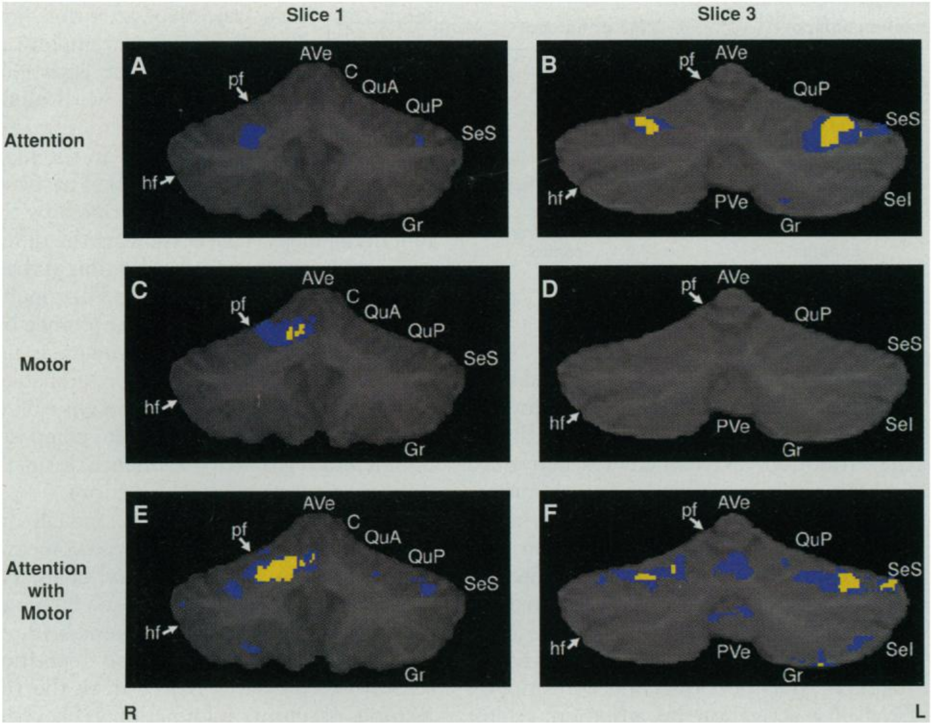

Fig. 4. Functional maps (*32*) demonstrating the most common sites of activation across subjects overlaid on an averaged coronal anatomical image of the cerebellum. Yellow, overlap of three or more subjects; blue, any two subjects. (**A** and **B**) During the Attention task, the most common site of activation was in the left superior posterior cerebellum [the posterior portion of the quadrangular lobule (QuP) and the superior portion of the semilunar lobule (SeS); approximate Talairach coordinates of center of mass, $x = -37$, $y = -63$, $z = -22$]. (**C** and **D**) During the Motor task, the most common site was in the right anterior cerebellum [the anterior portion of the quadrangular lobule (QuA), the central lobule (C), and the anterior vermis (AVe); approximate Talairach coordinates of center of mass, $x = 7$, $y = -51$, $z = -12$]. (**E** and **F**) During the Attention-with-Motor task, common sites of activation were in a combination of the areas from the other two tasks, with the addition of the posterior vermis (Pve). Sel, inferior portion of the semilunar lobule; Gr, gracile lobule; other abbreviations are as in Fig. 1.

These findings, in concert with other evidence about the cerebellum [for example, its homogeneous anatomical structure (*26*), its widespread connections with virtually all levels of the CNS (*2*–*6*), and its apparent involvement in a wide range of neurobehavioral functions (*2*, *7*–*14*, *16*, *21*, *23*–*25*, *27*, *28*)], highlight the need for a new conception of cerebellar function.

Such a conception, consistent with our findings, is provided by a recent hypothesis (*2*, *9*, *29*) that the cerebellum influences a variety of neurobehavioral systems—including sensory (*24*), motor (*7*, *16*, *25*), attention, and other cognitive and noncognitive systems—in order to accomplish its prime function, which is to learn to predict and prepare for imminent information acquisition, analysis, or action (*30*). As would be predicted by this hypothesis, cerebellar activation has been reported to be highest in the early stages of learning novel information, responses, or skills, or when nonmotor and motor sequences of information that are difficult to predict (such as the random sequences used in our experiment) must be processed (*27*, *28*). Successful anticipation of imminent real-time events improves the potential for the effective and timely directing of cognitive and noncognitive resources (*31*) to facilitate the learning of new information; it also improves the rapid, accurate, and effortless coordination of previously learned cognitive and noncognitive operations. We suggest that the human cerebellum may play a key role in the learning and smooth coordination of such anticipatory operations.

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15. The only exception is a recent paper showing dentate nucleus activation during tactile discrimination (*7*); all other studies cited above involved an overt motor response.
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17. Stimuli (duration 50 ms) appeared randomly at a rate of $\sim$3 per second (target rate, $\sim$1 per second; maximum and minimum intertarget intervals, 4630 ms and 240 ms, respectively).
18. Every 40 s over the course of 320 s, a one-word instruction cued the alternation between activation and control conditions. In the Attention tasks, "FORM" cued subjects to respond to each square, "COLOR" cued them to respond to every red shape, and "STOP" cued the control condition. For the Motor and Attention-with-Motor tasks, the movement was the squeezing of a bulb for two subjects and the pressing of a key for the other four. "GO" cued the onset of movement, and "STOP" cued rest.
19. Functional images were acquired at 1.5 T using conventional gradient hardware and a gradient-echo EPI pulse sequence [repetition time (TR) = 2500 ms; echo time (TE) = 40 ms; flip angle = 90°; matrix = 64 × 64; field of view (FOV) = 32 cm; slice thickness = 5 mm; slice gap = 1 mm]. Anatomical images [3D spoiled gradient-echo (SPGR) pulse sequence: TR = 30 ms; TE = 5 ms; flip angle = 45°; matrix = 256 × 192 × 60; FOV = 24 cm; slice thickness = 2 mm] were acquired at the same locations during the same scan session for each subject.
20. To locate significant activations, we used the correlational analysis described in P. A. Bandettini, A. Jesmanowicz, E. C. Wong, J. S. Hyde, *Magn. Reson. Med.* **30**, 161 (1993). The time-course signal data from each voxel were correlated with a model response function, a boxcar wave with sloped sides (duration 7.5 s), to approximate the delay between task onset and maximum signal change. Significantly activated voxels were those that exceeded a threshold r value equivalent to one-tailed $P < 0.05$ with Bonferroni correction for multiple comparisons, corresponding to $r = 0.35$ at $P < 0.00003$.
21. ROIs were based in part on our pilot fMRI studies of attention and motor activation and in part on our previous work showing cerebellar activity during self-paced right-hand movements localized primarily to the ipsilateral, anterior cerebellum [U. Sabatini *et al.*, *J. Cereb. Blood Flow Metab.* **13**, 639 (1993); H. Shibasaki *et al.*, *Brain* **116**, 1387 (1993)], whereas activity during tasks requiring attention to a stimulus guiding right-hand movements extended into the contralateral, posterior cerebellum (*28*) [M. Blinkenberg, C. Bonde, O. B. Paulson, C. Svarer, I. Law, *Hum. Brain Mapp.* (suppl. 1), 280 (1995); C. T. Bonde, M. Blinkenberg, I. Law, C. Svarer, O. B. Paulson, *ibid.*, p. 313; J. M. Ellermann *et al.*, *NMR Biomed.* **7**, 63 (1994)]. Together, these findings suggested a functional distinction between cerebellar

regions forming the basis for our ROIs with further support from cerebrocerebellar circuitry. For instance, the lateral neocerebellum sends output to the prefrontal cortex [F. A. Middleton and P. L. Strick, *Science* **266**, 458 (1994)]; the right prefrontal cortex is involved in attention tasks of the type we used [D. T. Stuss, T. Shallice, M. P. Alexander, T. W. Picton, *Ann. N.Y. Acad. Sci.* **769**, 191 (1995)]; and cerebellar input to the right cerebrum is from the left cerebellum [M. B. Carpenter, *Core Text of Neuroanatomy* (Williams & Wilkins, Baltimore, ed. 4, 1991)].

22. ROI drawing was guided by a human cerebellar atlas [G. A. Press, J. W. Murakami, E. Courchesne, M. Grafe, J. R. Hesselink, *Am. J. Neuroradiol.* **11**, 41 (1990)]. The Motor ROI was drawn from the surface location of the right primary fissure (pf) to the center of the band of white matter separating the anterior vermis (AVe) from the posterior vermis. From there, a line was drawn to the apex of the AVe. This ROI was completed by a line drawn along the surface of the cerebellum, back to the right pf. The Attention ROI was drawn from the surface location of the left pf to the center of the same white-matter band. A second line was drawn from this point to the surface location of the left horizontal fissure (hf). A line drawn along the surface of the cerebellum back to the left pf completed this ROI.
23. Because all stimuli were presented at a single spatial location in the center of foveal vision, eye movement activation was not predicted to occur. Moreover, previous work would predict that if eye movements had occurred, the resulting activation would occur in the cerebellar vermis [L. Petit *et al.*, *J. Neurosci.* **16**, 3714 (1996)], a region not activated during the Attention task. All areas that were active during the Attention task were also active during the Attention-with-Motor task, indicating that those behavioral requirements unique to the Attention task—namely, silent counting and encoding the number of targets—did not add to the results. Silent counting activation has been reported in the inferior cerebellum [E. Ryding, J. Decety, H. Sjoholm, G. Stenberg, D. H. Ingvar, *Brain Res. Cogn. Brain Res.* **1**, 94 (1993)] and in a "midline cerebellar region" [J. A. Fiez *et al.*, *J. Neurosci.* **16**, 808 (1996)]. In our study, the focus of attention activity was in the left cerebellar hemisphere, yet the right cerebellar hemisphere is consistently active during verbal tasks (*12*). Thus, nonverbal visual attention activated a side and region inconsistent with predicted silent counting effects. Still, to investigate whether silent counting might have contributed to these results, we instructed four subjects to silently count from 1 to 10 repeatedly in the absence of any visual stimuli. Examination of activation during this task revealed no cerebellar activation within the Attention VOI. Working memory activation of the cerebellum has also been reported (*11*), and the requirement to encode the number of targets during the Attention task placed minor demands on working memory. However, encoding the number targets was not required by the Attention-with-Motor task, and there were no regions of cerebellar activation unique to the Attention task as compared with the Attention-with-Motor task. Thus, like silent counting, working memory did not contribute to the activation effects observed during the Attention task.
24. In rats, when cerebellar stimulation occurs in advance of a sensory stimulus, neural responsiveness to that stimulus is altered at the brainstem, thalamic, hippocampal, and cortical levels (*4, 5*), and neural signal-to-noise enhancement can result (*4*); such effects are independent of the engagement of motor systems. For instance, when background luminance reduces to noise levels the colliculus response to a flash, stimulation of vermis lobules VI–VII causes the colliculus response to that flash to emerge above noise if stimulation occurs in advance of the visual stimulus (*4*).
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29. When sensory information is anticipated, attention is quickly and accurately redirected toward the predicted source of information. On the basis of neurobehavioral and neurophysiological evidence in patients with cerebellar lesions, it has been hypothesized that the cerebellum, through its connections with attention systems (*2*), influences the speed and accuracy of such attention changes (*2, 9*).
30. The cerebellum accomplishes this anticipatory function by encoding ("learning") sequences of multidimensional information about external and internal events. A large body of evidence shows that the cerebellum may be involved in such learning [J. L. Raymond, S. G. Lisberger, M. D. Mauk, *Science* **272**, 1126 (1996)]. Whenever an analogous sequence begins to unfold, the cerebellum predicts what is about to happen, reads out the rest of the sequence, and triggers changes in the neural responsiveness of systems expected to be needed in upcoming moments (*2, 9*).
31. Anticipation involves predicting the internal conditions needed for a particular motor or mental operation and setting those conditions in preparation for that operation. Complete knowledge of upcoming events is not necessary; simple exposure to aspects of a stimulus that may soon arrive will trigger anticipatory responding of the cerebellum. The anticipatory response is neither a sensory nor a motor activity, but rather a general response that prepares whichever neural systems may be necessary in upcoming moments. An example may be changes in the vestibulo-ocular reflex (VOR) in anticipation of changes in vergence observed in the monkey [L. H. Snyder, D. M. Lawrence, W. M. King, *Vision Res.* **32**, 569 (1992)]. A model of how the cerebellum might mediate such anticipatory modulation of the VOR has been proposed [O. Coenen and T. J. Sejnowski, in *Advances in Neural Information Processing 8,* D. Touretzky, M. Mozer, M. Hasselmo, Eds. (MIT Press, Cambridge, MA, 1996), pp. 89–95].
32. To create functional maps, we interpolated the correlation coefficient images to match the resolution of anatomical images and registered them to the anatomical images to reduce warping. Next, through rotation, translation, and scaling, each subject's cerebellum was transformed to a standard anatomical space by normalizing to a single subject chosen as the standard. All activated voxels were then superimposed across subjects for each task and slice.

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PTEN, a Putative Protein Tyrosine Phosphatase Gene Mutated in Human Brain, Breast, and Prostate Cancer

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Mapping of homozygous deletions on human chromosome 10q23 has led to the isolation of a candidate tumor suppressor gene, *PTEN*, that appears to be mutated at considerable frequency in human cancers. In preliminary screens, mutations of *PTEN* were detected in 31% (13/42) of glioblastoma cell lines and xenografts, 100% (4/4) of prostate cancer cell lines, 6% (4/65) of breast cancer cell lines and xenografts, and 17% (3/18) of primary glioblastomas. The predicted *PTEN* product has a protein tyrosine phosphatase domain and extensive homology to tensin, a protein that interacts with actin filaments at focal adhesions. These homologies suggest that *PTEN* may suppress tumor cell growth by antagonizing protein tyrosine kinases and may regulate tumor cell invasion and metastasis through interactions at focal adhesions.

As tumors progress to more advanced stages, they acquire an increasing number of genetic alterations. One alteration that occurs at high frequency in a variety of human tumors is loss of heterozygosity (LOH) at chromosome 10q23. This change appears to occur late in tumor development: although rarely seen in low-grade glial tumors and early-stage prostate cancers, LOH at 10q23 occurs in ~70% of glioblastomas (the most advanced form of glial tumor) and ~60% of advanced prostate cancers (*1, 2*). This pattern of LOH, and the recent finding that wild-type chromosome 10 suppresses the tumorigenicity of glioblastoma cells in mice, suggest that 10q23 encodes a tumor suppressor gene (*3*).

To identify this putative tumor suppressor gene, we performed representational difference analysis (RDA) on 12 primary breast tumors (*4*). A probe, CY17, derived from one of the tumors was mapped to chromosome 10q23, near markers WI-9217 and WI-4264, on the Whitehead-MIT radiation hybrid map (*5*). To map the location of CY17 more precisely, we isolated three yeast artificial chromosomes (YACs) containing CY17 that are present on the