Eco RI–Nco I fragment. The 2C GPI-TCR α and β and the D10 GPI-TCR α and β constructs (50 μg of each plasmid) were electroporated into BW5147 cells, and cells were initially selected in methotrexate (MTX) at a concentration of 50 nM. After amplification to 500 μM MTX, the amount of both TCRs on the cell surface was 5×10^6 molecules per cell for both 2C16 cells (2C GPI-TCR) and Ak19 cells (D10 GPI-TCR) as determined by Scatchard analysis.

32. Soluble TCRs were purified after cleavage from the cells by phosphatidyl inositol–phospholipase C (PI-PLC) by immunoadsorbent chromatography on columns of clonotypic mAbs 1B2 (*28*) for 2C and 3D3 for D10 (*9*) or with the C_β mAb H57-597 (*29*). Approximately 2×10^9 cells, grown either in Dulbecco's modified essential medium [supplemented with 500 μM MTX, 10% heat-inactivated fetal calf serum, 2 mM l-glutamine, nonessential amino acids, and gentamicin (5 μg/ml)] or in α-minimal essential medium (BioWhittaker, Gaithersburg, MD) supplemented with the same additives but with 1.3 μM MTX, were harvested by centrifugation, washed once in phosphate-buffered saline (PBS), and resuspended in 12 ml of RPMI medium (BioWhittaker) containing 1 mM Hepes (pH 7.4), 1 μM sodium pyruvate, and 2 units of PI-PLC (Sigma) for about 90 min at 37°C with frequent adjustment of the pH with 1 M $NaHCO_3$. The supernatant was clarified by centrifugation (3500 rpm for 10 min in the Sorvall HB1000 rotor) then passed through a 0.22-μm filter and applied to immunoadsorbent columns prepared from protein-A–purified mAbs coupled to Affiprep 10 (Bio-Rad). After washing with PBS, bound TCR was eluted with either glycine HCl (0.1 M, pH 2.7) or potassium phosphate buffer (0.01 M, pH 11.5). Eluted protein was immediately neutralized, dialyzed against PBS, and concentrated by ultracentrifugation (Centricon 10) to >1 mg/ml.
33. Protein (either the purified 2C or D10 TCR) was diluted to a concentration of 100 μg/ml in 10 mM sodium acetate at pH 4.6 and was coupled to the dextran-modified gold surface of a Sensor Chip CM5 (Pharmacia) with standard coupling chemistry with *N*-hydroxysuccinimide and *N*-ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide hydrochloride in the Pharmacia BIAcore as described (*12, 30*). Purified MHC class I molecules sH-2L^d (consisting of the α1 and α2 domains of H-2L^d linked to the α3 and COOH-terminus of Q10^b), sH-2K^b (α1 and α2 domains of H-2K^b linked to the α3 domain of H-2D^d and the COOH-terminus of Q10^b), and sH-2D^d (α1, α2, and α3 domains of H-2D^d and the COOH-terminus of Q10^b) were mixed with the indicated peptides at the indicated concentrations (Fig. 1), diluted into HBST [10 mM Hepes (pH 7.5), 150 mM NaCl, 3.4 mM EDTA, and 0.05% Tween-20], and injected over the 2C-coupled surfaces. Flow rates for the binding and dissociation phases were 5 μl/min. Binary data files were transferred from the BIAcore to a Macintosh computer and analyzed and plotted with Igor (WaveMetrics, Lake Oswego, OR). All sensorgrams were normalized to a base line of 0 resonance units (RU). For the sensorgrams (Fig. 1, D and E), refractive index artifacts were corrected by subtraction of the sH-2D^d–p2CL background sensorgram.
34. A biosensor surface coupled with 2C TCR (Fig. 1) was exposed to complexes of sH-2L^d–p2CL at the indicated concentrations of p2CL or sH-2L^d. Equilibrium values (taken as resonance units at t = 360 s) of the concentration-dependent resonance units were fit to the four-parameter equation

$$y = \{(a - d)/[1 + (x/c)^b]\} + d$$

where a is the minimal RU; b is the slope factor; c is the value of x with half-maximal binding; and d is the maximal RU with the use of Kaleidagraph (Synergy Software, Reading, PA). For Fig. 2D, a = 15.5 ± 44.8; b = 1.2 ± 0.15; c = 0.047 ± 0.0053; d = 1857.2 ± 73.4; χ^2 = 6089.7; and the correlation coefficient, R, is 0.99891. Values for the concentration of preparations of sH-2L^d are based on the protein concentration corrected for the proportion of molecules capable of binding peptide, which was estimated to be 40% in an assay of peptide-dependent epitope induction (*12, 16*). For the competition curve (Fig. 2E), sH-2L^d (500 μg/ml, saturated with p2CL) was mixed with graded amounts of the 2C TCR and injected over the biosensor surface coupled with the 2C TCR.
35. Data points for the experiment of Fig. 2C were fit for the 401 data points of the first 200 s for the association phase with use of the program Igor to the single exponential equation

$$B_t = B_\infty - B_{max} \exp(-k_{obs}\, t)$$

where B_t was the amount bound in RU at time t, B_∞ was the amount bound at equilibrium, and B_{max} was the maximum bound (B_{max} always was ≥0.9 B_∞). For fitting to a double exponential equation (descriptive of two simultaneous independent processes), we used the equation

$$B_t = B_\infty - [B_{max}\text{fast} \exp(-k_{obs}\text{fast}\, t)] - [B_{max}\text{slow} \exp(-k_{obs}\text{slow}\, t)]$$

where fast and slow components were considered. The dissociation phase was fit to the single exponential expression

$$B_t = B_0 \exp(-k_{off}\, t)$$

or to the double exponential expression

$$B_t = B_{fast} \exp(-k_{off}\text{fast}\, t) + B_{slow} \exp(-k_{off}\text{slow}\, t)$$

Residuals, the differences between the fit curve and the experimental data, are plotted. The larger residuals were characteristic of the curves with higher concentrations of sH-2L^d.
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Activation of a Cerebellar Output Nucleus During Cognitive Processing

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Magnetic resonance imaging was used to examine the involvement of the dentate nucleus of the cerebellum in cognitive operations. All seven people examined displayed a large bilateral activation in the dentate during their attempts to solve a pegboard puzzle. The area activated was three to four times greater than that activated during simple movements of the pegs. These results provide support for the concept that the computational power of the cerebellum is applied not only to the control of movement but also to cognitive functions.

The cerebellum is a part of the central nervous system and is essential for the proper control of limb and eye movements (*1, 2*). This brain structure consists of a large cortex, which processes input, and several deep nuclei, which process output. The lateral cerebellar cortex and the lateral deep nucleus, the dentate, underwent a marked expansion in the course of hominid evolution (*3*).

Evidence for the involvement of the cerebellum in aspects of motor learning and in the adaptive modification of motor output is accumulating (*2, 4*). On the other hand, there has been controversy about the participation of the cerebellum in nonmotor, cognitive operations (*5–7*). Leiner *et al.* (*5, 6*) have argued that the increase in the size of the dentate nucleus is paralleled by an increase in the size of the cortical areas influenced by cerebellar output and, consequently, by an expansion of cerebellar function to include involvement in some language and cognitive tasks. Perhaps some of the best support for this view comes from the results of a positron emission tomography (PET) study that showed activation in an inferior and lateral part of the cerebellum during a rule-based word generation task (*8, 9*). This activation was spatially separate from that found during motor tasks, including speech. Other imaging studies have reported activation in the inferior lateral cerebellum during silent counting and mental imagery (*10*).

The activation of the cerebellum observed in earlier studies reflects changes occurring largely, if not exclusively, in the cerebellar cortex. Large modulations in the input to the cerebellar cortex can take place without leading to observable changes in motor behavior (*11*). Thus, evidence that cerebellar output from the deep nuclei participates in cognitive processing has been lacking. To examine this issue, we used magnetic resonance imaging (MRI) to study functional activation of the dentate nucleus during a task that included a cognitive component. The greater spatial resolution of MRI (*12*) relative to other imaging methods allowed us to visualize activity changes in the dentate.

Seven healthy human volunteers participated in these experiments (*13*). Par-

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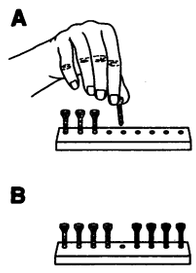

Fig. 1. The pegboard, pegs, and tasks used during imaging. For the visually guided task (**A**), participants were instructed to move each peg, one hole at a time, to the holes at the opposite end of the board. For the Insanity task (**B**), participants were instructed to move the four pegs of each color from one end to the other. See text for detailed description of tasks.

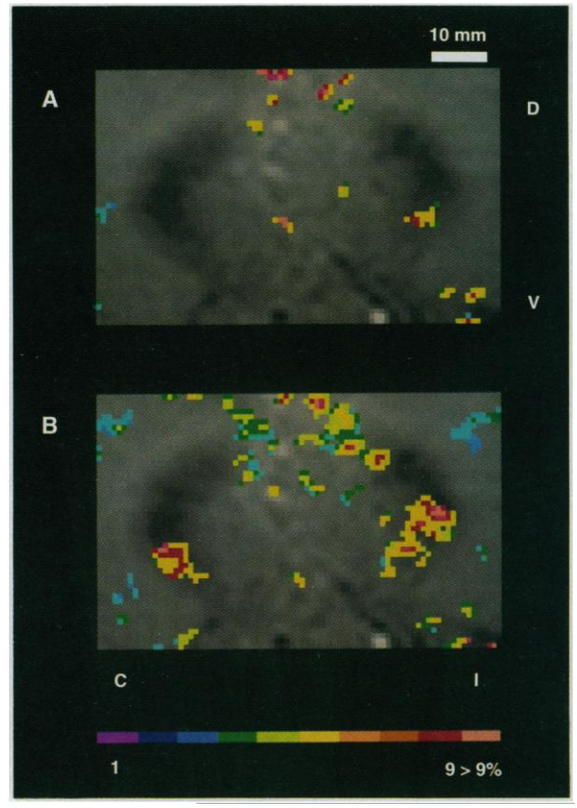

Fig. 2. Functional maps of dentate activation for one participant during the visually guided task (**A**) and during the Insanity task (**B**) (*15*). The dentate nuclei are the dark crescent-shaped regions with low background signal intensity. Some activation sites are located outside of the dentate in regions of adjacent deep cerebellar nuclei and cerebellar cortex. C, dentate contralateral to the moving arm; I, dentate ipsilateral to the moving arm; D, dorsal; V, ventral. Scale at bottom shows colors corresponding to percentages of activation.

ticipants lay supine in a 4-T MRI instrument (*14*). Images were acquired (*15*) while participants used their preferred hand to perform two different tasks. A small lightweight pegboard with nine holes was securely positioned over each person's chest (Fig. 1A). The pegboard holes were approximately 4.5 mm in diameter and were spaced 12.5 mm apart. For the first task, four red pegs were placed in the holes at the right end of the board, leaving the five holes at the left end empty. The task was to move each peg, one hole at a time, to the holes at the opposite end of the board. When all four pegs had been transferred to one end of the board, they were to be returned, one hole at a time, to the other end. We called this the visually guided task. Participants wore glasses with prisms so that they could see their hand, the pegs, and the pegboard holes while in the bore of the magnet. For the second task, four red pegs were placed in holes at the right end of the board, and four blue pegs were placed in holes at the left end of the board (Fig. 1B). The center hole was left open. Participants were instructed to move the four pegs of each color from one end to the other using three rules: (i) Only one peg may be moved at a time. (ii) A peg may be moved to an adjacent open space or may jump an adjacent peg (of a different color). (iii) A peg may be moved forward, never backward. This was called the Insanity task (*16*). Participants were instructed to make all task movements at a comfortable speed. During imaging, each person was asked to do the visually guided task and then to do the Insanity task.

In comparison with surrounding tissue, the dentate has a decreased signal intensity in images weighted with apparent transverse relaxation time (T_2*) because of its propensity to accumulate iron (*17*). Consequently, the outline of the nucleus was clearly visible in images that were made through the appropriate level of the cerebellum (Fig. 2). Six of the seven participants showed a small region of activation in the dentate during the visually guided task of moving pegs from one hole to another (Figs. 2A and 3A) (*18*). In most participants (four out of six), dentate activation was found both ipsilateral and contralateral to the moving arm. The presence of activation in the dentate during the visually guided task is consistent with the results of single-neuron recording studies in primates, which have reported changes in dentate activity related specifically to movements of single joints (*19*), to visually guided arm movements (*20*), and to saccadic eye movements (*21*). It is likely that the bilateral activation in the dentate is due partly to the bilateral nature of the control of eye movements. It is also worth noting that even simple repetitive movements of the fingers lead to bilateral activation of the primary motor cortex (*22*), a major source of input to the cerebellum.

All seven of the participants showed a large bilateral activation in the dentate during attempts to solve the Insanity task (Figs. 2B and 3B). Furthermore, in every participant, the size of this activation was larger than that found during the visually guided task. In fact, the mean number of pixels activated in the dentate during the Insanity task was three to four times greater than that activated during the visually guided task (*23*).

The main difference between the two tasks lies in the need to solve the puzzle of the Insanity task (*24*). Although some subjects figured out the solution to the puzzle, none did so during the period of scanning. Thus, our results suggest that the cognitive processing associated with attempts to solve the Insanity task leads to an activation of the dentate nucleus (*25*). Moreover, the dramatic increase in the size of dentate activation during the Insanity task as compared with that during the visually guided task implies that the regions of the dentate involved in cognitive processing are distinct from those involved in the control of eye and limb movement.

It is possible that the actual amount of the dentate involved in cognitive processing is even larger than that revealed in the present study. Our images of the dentate were confined to the central region of this nucleus and did not examine the rostral or caudal poles of the dentate. Also, the thickness of our images (8 mm) may lead to an underestimation of the size of activation because of partial volume effects. Furthermore, the Insanity task probes only a relatively small aspect of cognitive function. It does not examine other components of behavior proposed to be under cerebellar control, such as language, the voluntary shift of attention between sensory modalities, and the detection of timing differences (*5–7*).

The dentate nucleus exerts its influ-

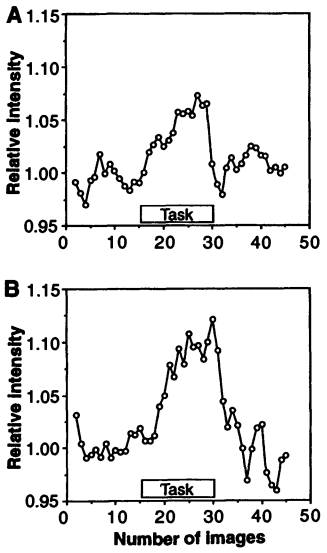

Fig. 3. Time course of image intensity for activated pixels in the dentate of one participant before, during, and after performance of the visually guided task (**A**) and of the Insanity task (**B**). Data points have been smoothed with a moving average of three points. "Task" is the period of task performance. The total time to acquire the 45 images was approximately 360 s.

ence by projecting, via the thalamus, to the cerebral cortex. The regions of the dentate that are concerned with the generation and control of limb and eye movements are connected with primary motor and premotor areas of the cerebral cortex (*26*). The cortical target of dentate regions involved in cognitive processing remains to be determined.

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13. Participants were recruited from the academic environment of the University of Minnesota Medical School. All of them were male (mean age, 30 years; range, 20 to 44 years). Five of the participants were right-handed and two were left-handed. Handedness was quantitatively assessed by means of the Edinburgh Handedness Inventory [R. C. Oldfield, *Neuropsychologia* **9**, 97 (1971)].
14. The MRI studies were done with a 4-T whole body system (SIS, Sunnyvale, CA, and Siemens, Erlangen, Germany) with actively shielded gradient coils and an anatomically fitted surface coil. These experiments were approved by the institutional review board of the University of Minnesota Medical School.
15. High-resolution images of the dentate, weighted with apparent transverse relaxation time (T_2*), were acquired in an oblique plane that was orthogonal to the brainstem (between the axial and coronal planes). Typically, two slices through the dentate, 8 mm thick and with centers separated by 5 mm, were selected for functional studies. The T_2*-weighted images were collected with a conventional gradient echo sequence during three consecutive periods: resting (no stimulation), task (stimulation), and recovery (no stimulation). The beginning and end of each period were indicated to participants over an intercom. Fifteen images were acquired within each period (8.06 s per image). Typical imaging parameters were: echo time, 26 ms; repetition time, 47 ms; and interscan delay, 2 s. Images were acquired with 128 complex pairs in the readout direction and 128 phase-encoding steps in a field of view 16 by 16 cm^2, with an in-plane resolution of 1.25 by 1.25 mm^2. To reduce physiological fluctuations in images, a complex data point was collected before application of phase-encoding and readout gradients; then the phase and magnitude of subsequently acquired *k*-space lines were corrected [X. Hu and S.-G. Kim, *Magn. Reson. Med.* **31**, 495 (1994)]. The acquired data were zero-filled to 256 by 256, then Fourier-transformed. Maps of functional activation were calculated by comparison of T_2*-weighted images acquired during the nonstimulated periods with those acquired during task periods. The first images acquired after the onset and cessation of each collection period were not included in this analysis because of delays in the onset and offset of the hemodynamic response that are related to functional activation (*12*). Activation was defined by the following two criteria: (i) Student's *t* tests were done to compare the resting-recovery baseline with activation during a task period; only pixels with a statistically significant activation ($P < 0.05$) were included in the functional map. (ii) Regions with less than four contiguous activated pixels were not included in the functional map, because a single pixel of the raw data (128 by 128) approximately corresponded to four pixels in the zero-filled image (256 by 256). Then the functional map was overlaid on a T_2*-weighted image to determine activation sites. The area of activation used for comparisons was the number of activated pixels within the dentate nuclei.
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23. Activation was measured in two MRI slices through the dentate of each participant. During the visually guided task, the number of activated pixels (mean ± SEM) in the dentate ipsilateral (I) and contralateral (C) to the moving arm was: I, 17.3 ± 6.1; C, 17.4 ± 8.3. In contrast, the number of activated pixels in the dentate during the Insanity task was: I, 58.7 ± 20; C, 70.8 ± 6.4. The difference between the two tasks in the number of activated pixels was statistically significant (repeated analysis of variance measures: I, $P < 0.05$; C, $P < 0.01$).
24. The two tasks inherently required the same eye and hand movements because of the small pegs and the narrow spacing between the pegboard holes. Videotape recordings of hand and eye movements showed that when participants moved the pegs, they moved them in the same manner for both tasks. However, participants were unaware of the solution of the Insanity task. This uncertainty led them to make fewer movements during the Insanity task than during the visually guided task. In brain regions solely involved in controlling movement, a decrease in the number of movements could result in less activation. Instead, we found more activation in the dentate during the Insanity task than during the visually guided task. Thus, the difference in the number of movements is unlikely to account for our results.
25. An important issue for this and other functional imaging studies is the extent to which activation reflects changes in cellular activity (and the output of an area) versus synaptic activity (and the input to an area). This issue has not been resolved in previous studies [such as G. F. Wooten and R. C. Collins, *J. Neurosci.* **1**, 285 (1981)]. In relation to the present experiment, marked changes in neuron activity have been seen in the dentate during tasks similar to our visually guided task (*19–21*). Hence, the MRI activation we observed during this task reflected, in part, cellular activity and output from the nucleus. Given this fact, there is every reason to believe that the even larger activation of the dentate that occurred during the Insanity task was accompanied by even larger changes in the activity of neurons in the dentate nucleus. This supposition will need to be tested with other techniques that directly examine cellular activity.
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