with PMA and ionomycin,  $CD4^+$  T cells from LATY136F<sup>m/m</sup> mice produced large amounts of IL-4 relative to  $CD4^+$  T cells from heterozygous knock-in or LAT<sup>+/+</sup> mice (Fig. 2B). Consistent with the T<sub>H</sub>2 cytokine profile, serum concentrations of immunoglobulins G1, E, and M were elevated (fig. S6) and the multiorgan infiltrates in LATY136F<sup>m/m</sup> mice included large numbers of eosinophils (9). Lastly, LATY136F<sup>m/m</sup> mice also had elevated serum levels of DNA and nuclear antigen autoantibodies (fig. S6).

The in vivo effects of a defined point mutation in LAT reveal a role for the PLCy1calcium signaling pathway in early T cell development. Calcium signaling is important for setting the threshold for negative selection in the thymus (19, 20). Thus, in LATY136F<sup>m/m</sup> mice, thymocytes that normally would be eliminated by negative selection may survive and populate the periphery. These cells may differ from normal T cells in their requirement for PLC- $\gamma 1$  for Ras-Erk activation. In this regard, it is notable that PLC- $\gamma$ 1–DAG independent activation of Ras has been described in peripheral blood-derived T cells (21) and that the LAT132YF mutant protein retains the ability to recruit Grb2 together with the Ras guanine nucleotide exchange factor Sos after TCR stimulation (3-5). Alternatively, the low levels of catalytically active PLC-y1 generated in LAT136YF<sup>m/m</sup> T cells may be sufficient to activate Ras-GRP or protein kinase C, resulting in Ras-Erk activation. The profound disturbance in T cell homeostasis caused by the selective loss of PLC- $\gamma$ 1-mediated signaling but not Erk signaling in LATY136F<sup>m/m</sup> mice further demonstrates a critical role for LAT in integrating signaling downstream of the TCR.

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Materials and Methods Figs. S1 to S6

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## Cerebellum Activation Associated with Performance Change but Not Motor Learning

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The issue of whether the cerebellum contributes to motor skill learning is controversial, principally because of the difficulty of separating the effects of motor learning from changes in performance. We performed a functional magnetic resonance imaging investigation during an implicit, motor sequencelearning task that was designed to separate these two processes. During the sequence-encoding phase, human participants performed a concurrent distractor task that served to suppress the performance changes associated with learning. Upon removal of the distractor, participants showed evidence of having learned. No cerebellar activation was associated with the learning phase, despite extensive involvement of other cortical and subcortical regions. There was, however, significant cerebellar activation during the expression of learning; thus, the cerebellum does not contribute to learning of the motor skill itself but is engaged primarily in the modification of performance.

Despite extensive research, the role of the cerebellum in learning motor skills remains controversial (1, 2). The concept of the cerebellum as a learning machine comes from the theoretical work of Marr (3) and Albus (4) and has been supported by data showing that it is essential for adaptive modification of reflex behavior (5, 6) and is activated during motor learning (7-9). However, learning invariably leads to changes in motor performance, which in itself can activate the cerebellum (10, 11). Previous efforts to deal with the issue of learning versus performance have required complex behavioral manipulations, such as subtracting an estimate of the performance effect (9). Here, we present a learning paradigm in which learning and performance change are effectively dissociated, using a modification of the serial reaction time task

<sup>1</sup>Brain Sciences Center (11B), Veterans Affairs Medical Center, Minneapolis, MN 55417, USA. <sup>2</sup>Department of Neuroscience, University of Minnesota, 321 Church Street, Minneapolis, MN 55455, USA. <sup>3</sup>Center for Magnetic Resonance Research, University of Minnesota, 2021 Sixth Street, Minneapolis, MN 55455, USA. <sup>4</sup>Department of Psychology, University of Virginia, Gilmer 102, Post Office Box 400400, Charlottesville, VA 22904, USA. (12, 13). Typically, participants learn the sequence embedded in the serial reaction time task within a few hundred trials. However, when asked to perform the task concomitantly with certain distractor tasks, they show no evidence of sequence learning (14). When retested upon removal of this distractor, it is evident that participants did actually learn the sequence during the initial training. Therefore, the distractor task served only to suppress performance change but did not prevent learning, allowing the determination of the underlying neural substrates for sequence learning separately from performance.

We obtained high-field (4 T) functional magnetic resonance imaging (fMRI) images of the cerebellum (15, 16) in participants performing the modified serial reaction time task. Participants (n = 6) pressed one of four buttons with the right hand when instructed by a visual display. Trials were presented in blocks in which the lights were illuminated either randomly or on the basis of a 12element repeating sequence. During the encoding phase, participants performed the sequence task concurrently with a distractor task, whereas for the expression phase the distractor was absent. Finally, participants were probed for their awareness of the existence of the sequence with a questionnaire and then asked to perform a free recall task.

During the encoding phase, there were no

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changes in performance either on the basis of response time (RT) or in the number of errors across the four blocks with the repeating sequence (Fig. 1, P > 0.1). Despite the absence of performance changes, however, participants did learn the sequence, as shown by the savings in response time and errors during the sequence as compared to the random presentation observed during the expression phase (P < 0.01 for response time, P < 0.05for errors). In addition, there was a savings in performance during block 8 (sequence performance upon expression) compared to the average of blocks 2 through 5 (sequence performance during encoding, P < 0.01 for RT, P < 0.05 for errors). This comparison vali-

Fig. 1. Temporal separation of learning and expression. (A) Response time data (mean and SD values). It can be seen that there was no change in response time for repeated sequence presentations during concurrent performance of the distractor task. There was a response time advantage for the sequence block once the distractor had been removed. (B) Number of errors made for each block. The pattern of results is the same as in (A). dated the performance-inhibiting effects of the distractor. Sequence learning was implicit, because the participants could only recall, on average, 3.6 of the 12 sequence elements (17). One might argue that the participants learned the sequence during the one block of trials to which they were exposed without the distractor; however, it has already been shown that this does not occur in this paradigm (17).

Functional imaging data were analyzed separately (i) for the encoding phase and (ii) for the expression phase (18-21). We tested two separate models of learning-related activity across the sequence-encoding blocks, based on the assumption that learning-related



activity could be either early or late (22, 23). The first model assumed that activity was initially high for the sequence scans as compared to the control period and then diminished across the remaining sequence blocks. The second model assumed that there was an increasing difference between the task and control periods across sequence blocks. Both models were tested with linear and exponential functions, because learning processes may conform to either function (24). During the encoding phase, learning-related activity was documented in the parietal, prefrontal, and motor cortex, as well as in the thalamus (25, 26) (Table 1). We detected no activation in the cerebellum during the encoding phase with any of the models (27). By contrast, we found prominent activation in the cerebellum during the expression of learning, as assessed by comparing the difference between task and control periods in the sequence block with that of the two random blocks during the expression phase (28). The active sites were in bilateral lobule VI (Fig. 2A and Table 2). The time course of activation during the expression of learning (Fig. 2B) was the inverse of the performance data shown in Fig. 1. Correlations between individual differences in response time savings and the intensity of activation were significant for sites within the cerebellum that overlapped with those observed from the expression contrast. This correlation analysis further validated the idea that the activation of the cerebellum during the expression of learning was related to an individual's behavioral performance.

In the current experiment, the use of a visual distractor task effectively separated the process of motor skill acquisition from its expression. Thus far, the only work to successfully separate learning from expression, in investigations of cerebellar function, is that examining the acquisition and expression of the conditioned eyeblink response in animals (29). Therefore, although it appears that the cerebellum may mediate some types of learning such as eyeblink



**Fig. 2.** Cerebellar activation during expression of learning. (A) Areas of activation for the expression of learning contrast (left) and for the regression with individual participants' response time savings (right). The peak of activation was in bilateral lobule VI for both contrasts. (B) Changes in activation averaged across these areas for the encoding and

expression blocks. The activation was computed as the average amplitude during the control period subtracted from that during the task period of each block, for the clusters activated in (A) (bars reflect the standard error). There was no change in activation until the expression blocks.

**Table 1.** Coordinates of local maxima for the areas showing learning-related activation during sequence encoding. R is right hemisphere, L is left hemisphere; PM refers to premotor; BA, Brodmann area.

Area activated	Coordinates of peak activation	Z score of peak activation
Premotor cortex		
L BA 6 (ventral PM cortex)	-58, 6, 18	3.47
Motor cortex		
L BA 4	-38, -16, 50	2.10
Prefrontal cortex		
R BA 9	46, 8, 36	3.40
Cingulate cortex		
R BA 24/32	8, 30, 24	2.28
Parietal cortex		
LBA7	-20, -48, 44	4.14
L BA 40	-32, -36, 56	2.04
R BA 40	48, -38, 54	2.39
Subcortical activation		
R thalamus	28, -32, 2	2.16
R thalamus	20, –26, 12	3.27

**Table 2.** Coordinates of local maxima for the cerebellar areas activated at expression of learning and for those correlated with individual differences in response time savings.

	Coordinates of peak activation	Z score of peak activation
	Areas activated at expression of learning	
Cerebellar cortex	1 , 5	
L lobule VI	-28, -76, -22	3.46
R lobule Vi	20, -74, -26	3.09
	Areas correlated with response time savings across participants	
Cerebellar cortex		
L lobule VI	-30, -76, -22	2.50
R lobule VI	16, -76, -24	3.27

conditioning (30), our data indicate that, during the acquisition of a motor skill, the cerebellum does not contribute to sequence learning per se but rather to its expression.

It is not entirely clear how the cerebellum contributes to improvement in performance, and the current experiment did not address this issue. There are, however, a number of possible explanations, including improved coordination of movement timing (31) and enhanced motor planning (32). Ample evidence underscores the importance of the cerebellum for the modulation of response time. Both lesions of the cerebellum in patients (33, 34) and reversible inactivation of the dentate nucleus by cooling in experimental animals (35, 36) lead to delays in response time and in movement initiation. Other imaging studies have found a negative association between blood flow in the cerebellum and response time (32). Collectively, these results suggest that the cerebellum plays a critical role in motor response facilitation, which is manifested in the present experiment by the improvement in performance seen during the expression phase.

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- 14. Sequence acquisition blocks were performed concurrently with a distractor task requiring participants to monitor the number of times a specific color appeared in a square (each side, 1.8 cm) that changed color at a rate of 3 Hz and to report the number at the end of each block. This square was centered 0.5

cm above the stimulus boxes. Three to seven targets appeared per block. Blocks 1 through 6 were performed concurrently with the distractor in the following order: random, sequence, sequence, sequence, random. Blocks 7 through 9 were performed without the distractor task with random, sequence, random presentations. Participants were 94 to 100% accurate at performing the distractor task. Each block consisted of 94 trials with an 800-ms interstimulus interval. Median reaction times were computed for each participant and averaged across participants.

- 15. The experiment was conducted in a 4-T magnet using an open-face volume coil (16). Functional images were acquired using single-shot gradient-echo echo plan imaging. The field of view was 220 mm by 220 mm; voxel size was 3.4 mm by 3.4 mm by 5 mm, repeat time = 3.38 s, echo time (TE) = 35 ms. The entire brain, including the cerebellum, was imaged for a total of 26 slices. A total of 31 volumes was collected for each block: 3 dummy scans, 7 control scans, and 21 task scans. Participants simply viewed the unlit light-emitting diodes (LEDs) during the control period. Structural images were acquired with a fast low-angled shot. The field of view was 240 mm by 240 mm; voxel size was 0.86 mm by 0.86 mm by 5 mm, TE = 4.3 ms, 26 slices.
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- 18. Statistical Parametric Mapping 99 was used for image analysis [Wellcome Department of Cognitive Neurology, London, UK (19)] in the Matlab environment. The functional images were realigned to the first one within the set. The data were examined to ensure that participants did not move more than 2 mm during the experiment. A mean functional image for each participant was also computed at this time. The structural image was then coregistered to this mean and spatially normalized to the Montreal Neurological Institute (MNI) template (20). The resulting parameters were applied to each participant's functional images, which were then smoothed with a Gaussian smoothing kernel with a full width at half maximum of 6 mm.
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- 21. Data were analyzed for the whole brain. In this report, we concentrate on the cerebellum as the region of interest. One analysis was performed over the first four sequence blocks (blocks 2 through 5). The data were modeled as boxcar functions timelocked to the effect of interest and were convolved with an estimate of the hemodynamic response function. To assess sequence encoding, an activation mask with P < 0.05 was first computed to reveal the voxels that showed significant activation during the task as compared to the control. Then we performed two different contrasts (P < 0.001) at the individual participant level, created to capture changes occurring across the four blocks, masked by the activation map. One contrast searched for activation that was initially high for the task period as compared to the control (such as strong activation in block 2) that then progressively decreased across the remaining sequence blocks (blocks 3 through 5), reflecting early learning processes. The second contrast searched for longitudinally increasing activation across the four sequence blocks, reflecting later learning processes. Individual participant data were then combined in a random effects analysis. The results of the analysis at the group level were evident in the analysis of each of the individual participants, both for the encoding and expression models.
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- 28. In order to assess expression of learning, sequence block 8 was compared to the average of random blocks 7 and 9, masked with an activation map for the three blocks. Single-participant results were combined in a random effects analysis. An additional random effects analysis was performed to determine

how activation predicted individual performance differences. For this analysis, RT savings at expression, for individual participants, were entered as a regression vector with the expression of learning activation. Thus, the expression contrast searched for areas that showed greater activation during the sequence block than during the two random blocks at expression, and the RT contrast searched for areas that showed greater sequence activation by an amount that was scaled to the individual participants' response time savings. Both contrasts were evaluated at a P < 0.05 level. Furthermore, all fMRI data were examined at the group and single-participant level. The results presented at the group level were evident in the analysis of each of the single participants.

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# A Heat-Sensitive TRP Channel Expressed in Keratinocytes

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Mechanical and thermal cues stimulate a specialized group of sensory neurons that terminate in the skin. Three members of the transient receptor potential (TRP) family of channels are expressed in subsets of these neurons and are activated at distinct physiological temperatures. Here, we describe the cloning and characterization of a novel thermosensitive TRP channel. TRPV3 has a unique threshold: It is activated at innocuous (warm) temperatures and shows an increased response at noxious temperatures. TRPV3 is specifically expressed in keratinocytes; hence, skin cells are capable of detecting heat via molecules similar to those in heat-sensing neurons.

TRPs belong to a large family of nonselective cation channels that function in a variety of processes, including temperature sensation (1, 2). Vanilloid receptor 1 (TRPV1, also called VR-1) is activated by noxious heat (>42°C) (3). TRPV2 (VRL-1) is also activated by heat, but with a higher threshold (>50°C), whereas TRPM8 (CMR1) is induced by cool/cold temperatures (<25°C) (4-6). A receptor for innocuous warm temperatures has not been identified. Also, the persistent sensitivity of VR-1 knockout animals to moderately noxious heat stimuli implies the presence of another heat receptor (7, 8).

We searched DNA databases for TRPV1related TRP channels by constructing a hidden Markov model (HMM) of the TRPV1 and TRPV2 protein sequences from different mammalian species. With this model, we queried the six-frame translation of human genomic sequences and identified multiple putative exons with a great degree of sequence similarity to the ankyrin and transmembrane domains of TRPV1. We mapped these exons to two genes, one of which is now known as TRPV4 (9, 10). The other gene we have named TRPV3. Full-length sequence of mouse TRPV3 (GenBank accession number AF510316) was derived from a combination of exon prediction software, polymerase chain reaction, and rapid amplification of cDNA ends (RACE) from newborn skin cDNA (11). Several murine expressed sequence tags from skin tissues contain 3'-untranslated region TRPV3 sequence (e.g., BB148735, BB148088), and recently the human TRPV3 sequence has been annotated in GenBank (accession numbers GI 185877 and GI 18587705) (12).

The predicted TRPV3 protein is composed of 791 amino acid residues. The overall

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sequence of mouse TRPV3 has 43% identity to TRPV1 and TRPV4, 41% to TRPV2, and 20% to TRPV5 and TRPV6 (fig. S1). TRPV3 has six putative transmembrane domains and four predicted NH<sub>2</sub>-terminal ankyrin domains that are thought to be involved in proteinprotein interactions. We also identified two coiled-coil domains NH2-terminal to the ankyrin domains in TRPV3 (fig. S1). Coiledcoil domains have been previously reported to be present in some TRP channels, but not in TRPVs (6, 13). Further examination showed that TRPV1, but not the other members of the TRPV family, also has putative coiled-coil domains in the same location. Interestingly, both TRPV1 and TRPV3 were predicted to reside on human chromosome 17p13 and mouse chromosome 11B4. Our mapping analysis of bacterial artificial chromosome clones, and later the assembled human and mouse genome sequences, revealed the distance between the two genes to be about 10 kb (Fig. 1B) (14).

Given the high degree of homology of TRPV3 to TRPV family members, we tested whether TRPV3 responds to stimuli that activate other closely related family members. The murine full-length TRPV3 was stably expressed in Chinese hamster ovary (CHO) cells, which do not express an endogenous TRPV3 isoform (11, 14). Transfected cells were assayed electrophysiologically by whole-cell voltage-clamp techniques (11). Capsaicin (1 µM), an activator of TRPV1, did not evoke a response in TRPV3-expressing cells (14). Similarly, no current responses were seen when TRPV3-expressing cells were challenged with a hypo-osmotic solution containing 70 mM NaCl or with low pH (pH 5.4) (14). However, raising the temperature of superperfused external solution from room temperature to 45°C evoked currents in TRPV3-expressing cells. Analysis of currents evoked by temperature ramps from  $\sim 15^{\circ}$  to  $\sim$ 48°C (Fig. 2A) showed that little current was elicited until temperatures rose above  $\sim$ 33°C and that the current continued to increase in the noxious temperature range

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