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36. Transient transfection of 293 cells by pRK5 itself resulted in sensitization to TRAIL-induced apoptosis relative to untransfected cells. The levels of background apoptosis in 293 cells as measured by morphology, directly in the culture dish (Fig. 4), were lower than the levels measured by fluorescence-activated cell sorting (FACS) analysis after harvest and staining of the cells with annexin V (*9*).
37. Treatment of 293 cell monolayers without removal from the culture dish led to a 33% reduction in ^{125}I-TRAIL binding (*25*), consistent with the 39% sensiti-

zation to TRAIL-induced apoptosis of cells treated similarly with PI-PLC (Fig. 4B).
38. We thank C. Clark for comments; D. Goeddel for CrmA and FADD-DN plasmids; V. Dixit for DR4 and DR4-IgG plasmids; A. Rosenthal for PI-PLC; P. Juhrani, P. Ng, and M. Vasser for DNA synthesis; J. Lee and A. Gray for cDNA libraries; C. Watanabe for

Neural Correlates of Motor Memory Consolidation

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Computational studies suggest that acquisition of a motor skill involves learning an internal model of the dynamics of the task, which enables the brain to predict and compensate for mechanical behavior. During the hours that follow completion of practice, representation of the internal model gradually changes, becoming less fragile with respect to behavioral interference. Here, functional imaging of the brain demonstrates that within 6 hours after completion of practice, while performance remains unchanged, the brain engages new regions to perform the task; there is a shift from prefrontal regions of the cortex to the premotor, posterior parietal, and cerebellar cortex structures. This shift is specific to recall of an established motor skill and suggests that with the passage of time, there is a change in the neural representation of the internal model and that this change may underlie its increased functional stability.

As one practices a motor task, stiffness of the limbs decreases (*1*), movements become smoother (*2*), and the muscle activations reflect a reliance of the motor output on an internal model (IM) that anticipates the force requirements of the task (*3, 4*). In a computational framework, the IM for arm movements may be characterized, in part (*5*), as a map from a desired trajectory for the hand to a set of muscle torques (*6*). Because we routinely use our hands to interact with a diverse variety of objects and systems, we rely on visual and haptic properties of the task to act as cues that facilitate recall of an appropriate IM from motor memory (*7*). Attempting to pick up an empty bottle of milk that has been painted white readily illustrates the consequences of visually cued recall of an inappropriate IM.

A single session of practice with a novel mechanical system may lead to long-term storage of an IM in the brain (*8*). However, when practice ends, a functional property of the IM continues to develop. Within 5 hours, the recently acquired IM gradually becomes resistant to behavioral interference (*8, 9*), that is, it consolidates. Although the mechanisms of motor memory

consolidation are unknown, examples from other memory systems of the brain show that a change in the neural representation of memory may contribute to consolidation (*10*). There is also evidence that neural representation of motor function is dynamic (*11*) and that motor areas of the primate brain are differentially associated with the performance of either a new or well-practiced motor task (*12*). Here we ask whether with the passage of time, as the IM becomes less fragile, there is a change in the neural representation of its motor memory.

We used positron emission tomography (PET) to monitor changes in regional cerebral blood flow (rCBF), an indirect marker of neural activity, mainly around the synapses (*13*), as participants ($n = 16$) learned an IM of a novel mechanical system (Fig. 1A). The dynamics of the novel system were represented as a force field and were produced by the torque motors of a robotic arm (*6*). The task was to make rapid reaching movements to a series of targets while holding the handle of the robot (*14*). Participants initially practiced the task with the robot motors turned off (300 targets, during which no rCBF measures were taken). They made accurate, straight movements, similar to that shown in Fig. 1B. In session 1, we acquired rCBF measures (*15*) as participants performed the task during two repetitions of four successive conditions: (i) during a null field condition in which the robot's motors were off (Fig. 1B); (ii) during a random field condition in which the robot produced a random, non-

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stationary, velocity-dependent force field representing an unlearnable mechanical system (Fig. 1C); (iii) during early learning of a force field (16) (Fig. 1D), in which the robot produced a stationary force field we labeled "A" and which represented a learnable mechanical system (Fig. 1E); and (iv) during late learning of force field A, in which participants performed the task skillfully (Fig. 1F) after further practice in field A. When participants were first exposed to the forces, movements deviated from the straight-line trajectories (Fig. 1G). In the random field, movements did not significantly improve with practice. However, rapid improvements occurred when the field was held stationary. With practice, the movements gradually converged to those recorded in the null field condition (Fig. 1H). Participants then returned 5.5 hours later for session 2, in which we acquired rCBF measures during two repetitions of only one of the following conditions: (i) recall of the IM of field A ($n = 9$ participants) or (ii) early learning of field B ($n = 7$ participants) (16).

We initially asked whether during session 1 there were brain regions where rCBF correlated with measures of total motor output. The average length of a movement was selected as an indicator of motor output (Fig. 1G) (17). Statistical parametric maps were generated (18), and we found three regions where activations significantly correlated with motor output: the left sensorimotor cortex (SMC) (–58, –32, 52; Z = +4.81; Fig. 2A), with the peak corresponding to Brodmann's area (BA) 4; the right SMC (48, –40, 52; Z = +3.41); and the right putamen (30, –6, –4; Z = +3.49). Changes in rCBF in the SMC have been shown to correlate with arm and finger force production in a task that precluded motor learning (19). Given the significant projections from the SMC to the putamen, it is likely that changes observed in these regions are associated with large-scale reductions in motor output from the random to the late learning condition rather than with acquisition of an IM.

Because learning of the IM has components associated with visual perception, force production, attention, and error-reduction processes, a comparison of the adaptation condition with a rest condition does not imply learning-related activity. To test for learning-specific changes, we compared the rCBFs during the random condition, where every component of the task but learning was present, with that of early learning of A. The only significant change was an increase in a region encompassing the dorsomedial and medial pulvinar thalamus (peak at 4, –24, 10; Z = +4.47). This increase was accompanied with increases in the medial occipital gyrus (–14, –94, –12; BA 18; Z = +3.84) and dorsolateral prefrontal cortex (42, 40, 10; BA 46; Z = +3.78; Fig. 2B). There were no significant differences in comparisons of the early and late learning conditions, other than the decreases observed in the SMC (Fig. 2A) and putamen. This suggests that the improvement in performance from the random to the learning condition during session 1 was at least in part due to an increase in activation of visuomotor association areas of BA 46 in the prefrontal cortex (20).

We found that with the passage of time, however, significant changes took place in the representation of the IM. Participants returned 5.5 hours after completion of session 1 and were presented with either field A or a novel field B. Motor performances during the late learning stage of A and the recall stage of A were not significantly different (Fig. 1, G and H). However, there
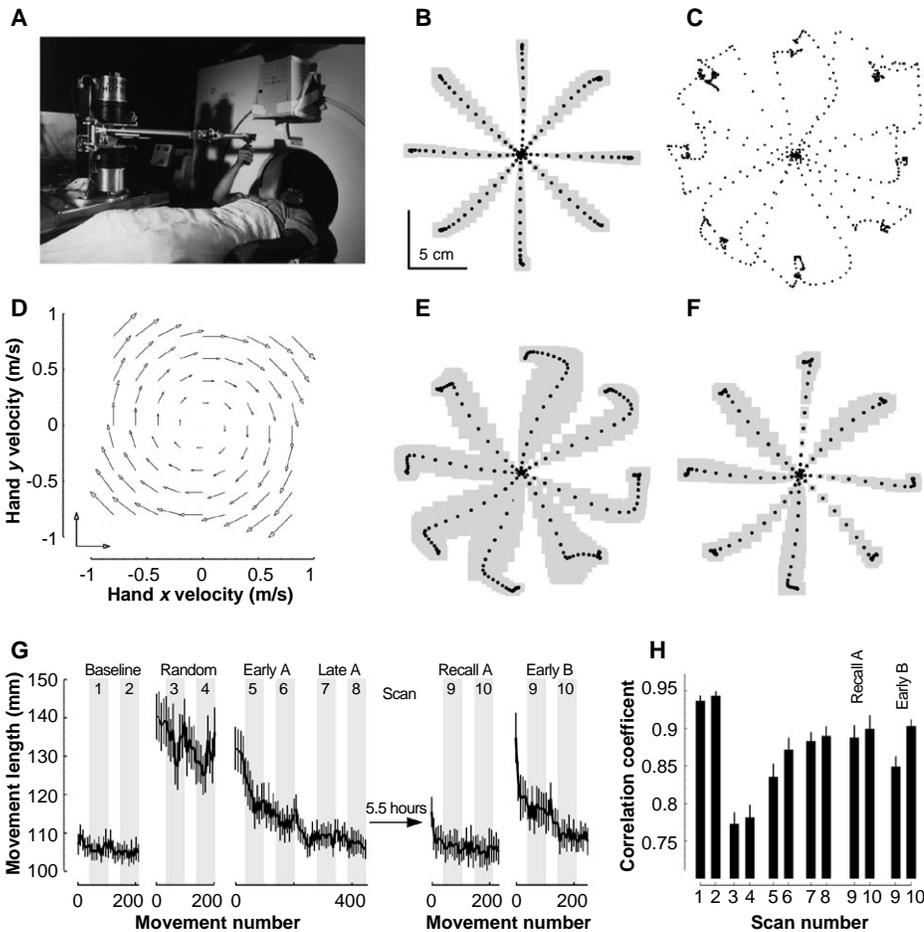

Fig. 1. The motor learning task. (**A**) Participants gripped the handle of a robot manipulandum and moved it to targets that appeared on the monitor in one of eight directions: 0 to 315° in 45° increments, randomly selected (8). Participants were provided with continous visual feedback. The task was to reach the target in a precise time (14). We acquired PET scans during five conditions: null field (robot motors not engaged); random force field (robot motors producing a nonstationary field); early learning of field A (16); late learning of field A; and, at +5.5 hours, recall of field A or early learning of field B. (**B**) Hand trajectories (mean ±SD) for a typical participant during the null field condition. (**C**) Typical hand trajectories during a random field condition. The robot's motors produced a velocity-dependent force field that randomly varied from target to target. This substantially disturbed the hand trajectories and required corrective movements while precluding the possiblity of learning an IM. (**D**) The force field A (16). (**E**) Trajectories (mean ± SD) during the early learning stage of field A (first 100 movements) for a typical participant. (**F**) Trajectories (mean ± SD) during the late learning stage of field A (last 100 movements) for a typical participant. (**G**) Length (mean ± SE) of reaching movements during task performance. Each point is an average of eight movements. Gray bars indicate periods of brain image acquisition. There was no significant improvement during the random field condition. However, participants were skillfully controlling their arms during the late stage of learning of A and were able to recall the appropriate IM at +5.5 hours. Performances were not significantly different at recall versus late learning. (**H**) Hand trajectories for each participant during each condition were correlated with that participant's typical trajectory during the null condition (6). Shown here are the population mean ± 95% confidence intervals (CI). With practice, movements converge to the trajectories recorded in the null field.

were significant increases in rCBF in three structures: in the left posterior parietal cortex (−30, −78, 54; BA 7; Z = +4.95; late A versus recall A; Fig. 3A); the left dorsal premotor cortex (PMC) (−50, −16, 60; BA 6; Z = +3.74; late A versus recall A; Fig. 3B); and the right anterior cerebellar cortex (18, −60, −20; Z = +4.93; early A versus recall A; Fig. 3C). These changes were specific to recall of the previously learned field. The group that was presented with field B during session 2 did not show similar changes, despite the fact that they had an increased motor output (Fig. 1G). We also found that recall of A involved significantly decreased levels of rCBF (with respect to late A) in the left (−46, 32, 28; BA 46; Z = −4.97) and right (42, 28, 20; BA 46; Z = −4.39) middle frontal gyri of the prefrontal cortex. The decreases in rCBF observed in these two regions were 7.0 ± 1.9% and 5.1 ± 2.0% (mean ± 95% confidence interval) for the left and right prefrontal regions, respectively. In comparison, no significant decreases were observed in the prefrontal cortex when participants were presented with field B.

It has been hypothesized that acquisition of a skilled movement is mediated principally through structures in the prefrontal cortex, and that with time or practice, as the task becomes "automatic," motor structures such as the cerebellum assume a greater role and possibly become the site of the motor memory (21). There is evidence that in humans, disruption of the prefrontal cortex prevents motor learning without disrupting motor execution (22). In our experiment, acquisition of the IM was associated with increased activation in the dorsolateral prefrontal cortex. Although the performance of our participants neared asymptotic levels during the late stage of learning (23), we did not observe an increased role for the anterior regions of the cerebellum or other motor structures with respect to random or early learning. This is in agreement with a number of other PET studies of motor learning (24, 25). However, we cannot rule out the influence of the cerebellum in initial acquisition of the IM, because posterior regions of the cerebellum were not sampled (18). When the participants were retested at +5.5 hours, there was no significant change in motor performance. However, comparison of rCBFs between recall and late learning stages of A revealed that there was a significant reorganization of the representation of the memory of the IM. With the passage of time, recall of the IM engaged areas of the contralateral dorsal premotor, contralateral posterior parietal, and ipsilateral anterior cerebellar cortex structures. This was coincident with a reduction in activations of the bilateral middle frontal gyri of the prefrontal cortex. The

decreased role of the prefrontal cortex has been observed in other studies in which a previously learned motor skill was recalled (25, 26).

A function of the prefrontal cortex is temporary storage of arbitrary sensorimotor information for use in the near term (27). Inherent in this faculty is the transient nature of the associations (28). Previous results on learning control of novel mechanical systems suggest that the representation of an IM in humans is most fragile soon after it has been acquired (8, 9). Within 5 hours after initial practice, the IM's representation becomes resistant to behavioral interference. We have shown here that this change in the functional stability of the acquired memory coincides with a reduced activation in the prefrontal structures and an increase in regions of the brain where long-term motor memory storage has been hypothesized (29).

Recordings of electromyographic activity from the arm during practice of this task suggest that participants gradually learn to recruit new arm muscles and precisely control the timing of activations of these muscles in order to compensate for the force field (30). Studies of similar tasks in highly trained monkeys suggest that the cerebellum is likely to play a

critical role in generating this response (3). In humans, cerebellar malfunction results in the loss of ability to anticipate and compensate for interaction torques that are generated in multijoint arm movements (31). Although the role of the cerebellar cortex in initial acquisition of the IM is unclear (32), it has been shown that within an hour after completion of motor learning, biochemical processes that are involved in the synaptic remodeling of Purkinje cells are initiated (33). Therefore, it seems likely that the cerebellum is part of the system that maintains long-term motor memories. On the other hand, lesion, inactivation, and recording studies of the PMC suggest that it is primarily involved in retrieval of a motor response as cued by a visual or auditory stimulus (34). Neuronal recordings show a phase lag between increased activity in some of the cells in the dorsal PMC and behavioral improvement (35). This has suggested that PMC cells function in the retrieval processes of an established visuomotor association, rather than in learning of the association (36). A major input to the dorsal PMC is from the posterior parietal cortex (37). The architecture of this network has been proposed to code reaching movements as the result of a combination
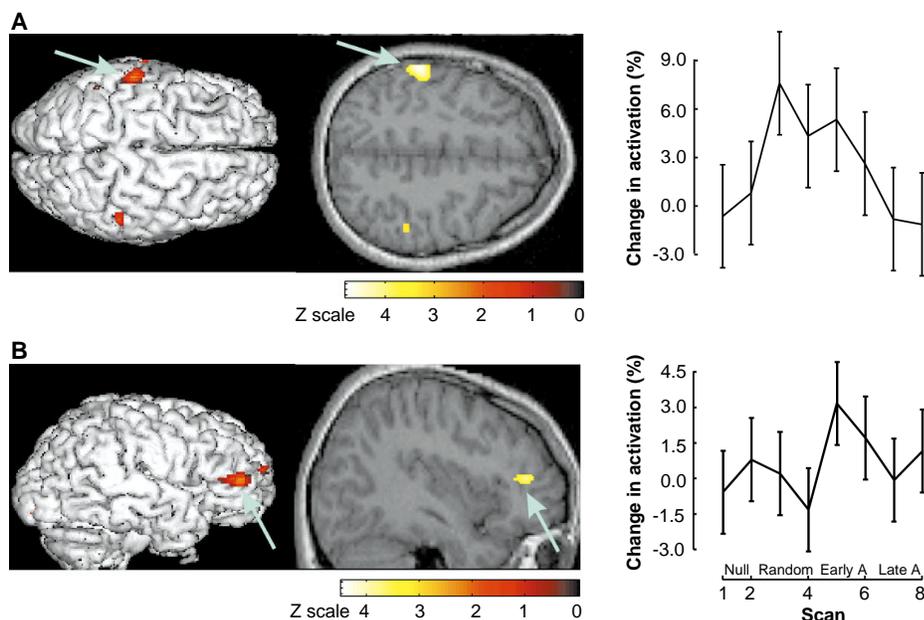

Fig. 2. Relative changes in rCBF (with respect to the null field condition) for two areas of the brain that showed increased activation during motor execution or learning. The regions are shown on the normalized MRI of a typical participant (18). Regions of activation are also localized on the rendered brain of the same participant. (**A**) We looked for regions of the brain where rCBF changes correlated with total motor output (18). The region with the highest correlation was located in the left sensorimotor cortex (peak location at −58, −32, 52; BA 4; Z = +4.81), shown here on a transverse view. The figure also shows the percentage change of the rCBF for all participants at the peak location (mean ± 95% CI). (**B**) Sagittal view of an area in the right prefrontal cortex (middle frontal gyrus) showing an increase in activity during early learning with respect to the random condition (peak location at 42, 40, 10; BA 46; Z = +3.78). Also shown are the rCBF changes at this location.
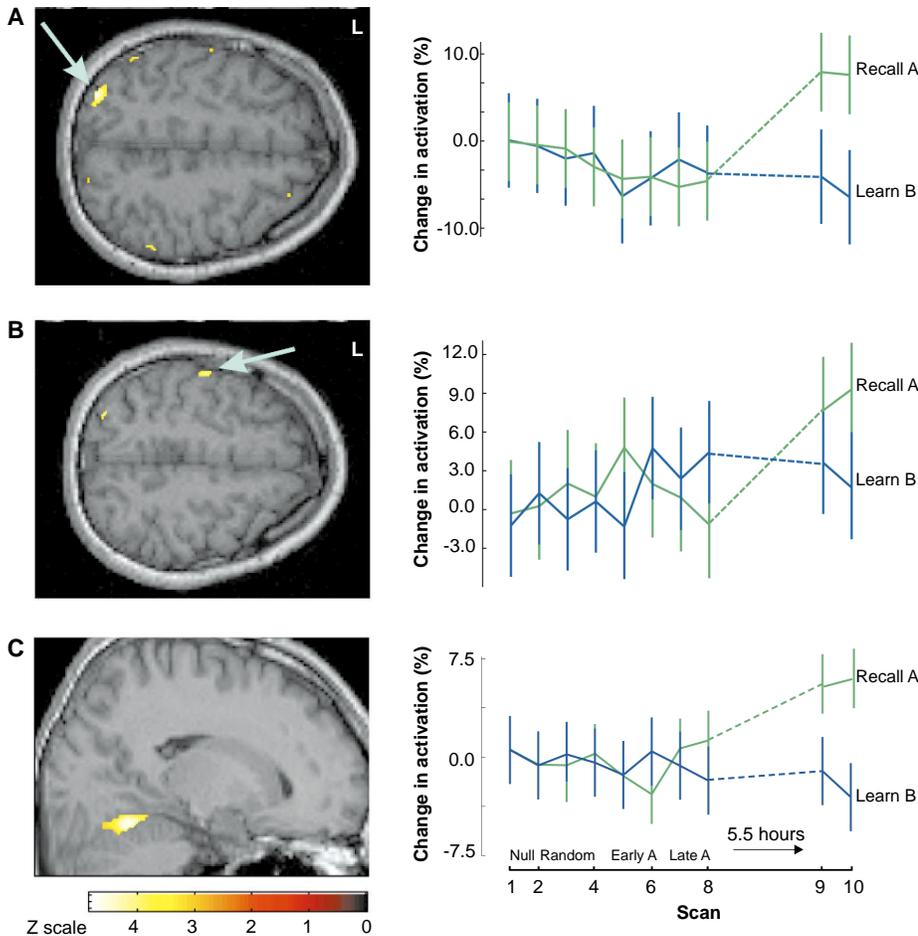
Fig. 3. Brain regions showing a significant increase in rCBF from the learning stage of field A to recall of field A at +5.5 hours. The regions are shown on the normalized MRI of a typical participant (*18*). (**A**) Transverse view of an area in the left posterior parietal cortex (peak location at –30, –78, 54; BA 7; Z = +4.95; late A versus recall A) and the peak location's changes in rCBF with respect to the null field (mean 95% CI). Shown also are the changes in rCBF from the group of participants that learned a novel field B at +5.5 hours. (**B**) Transverse view of an area of activation in the left dorsal premotor cortex (–50, –16, 60; BA 6; Z = +3.74; late A versus recall A). (**C**) Sagittal view of an area of activation in the right anterior cerebellar cortex (18, –60, –20; Z = +4.93; early A versus recall A). The increased activations in these regions were specific to recall of the recently acquired IM of field A.

of visual and somatic information (*38*). Indeed, parietal lobe lesions produce apraxia, an impairment of skilled movements in the absence of elementary sensory or motor deficits. Motor memory deficits in apraxic patients suggest a loss of a component of the IM (*39*).

The results presented here suggest that the representation of a motor skill is reorganized in the brain shortly after an IM has been acquired. Although this reorganization does not affect task performance, it may contribute to increased stability of the representation of the motor skill.

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14. Right-handed, healthy male participants (*n* = 16, age = 23.6 ± 3.2 years) were recruited and gave written consent. Participants lay in a supine position in a dimly lit sound-attenuated room, on the gurney of a GE 4096+ PET tomograph. A catheter was placed in the left cubital vein for injection of the radioisotope. Each man gripped the handle of a robot manipulandum with his right hand. The robot is a portable, light-weight, very low friction planar mechanism equipped with two pairs of optical position and velocity encoders, a force transducer at the handle, and brushless dc motors that deliver torques independently to each joint (*8*). Participants viewed a monitor that displayed a cursor corresponding to the handle's position. The task was to take the handle to a series of targets. Participants were instructed that they should reach for the displayed target and that their movement time should be within 500 ± 50 ms (targets were at 10 cm). A target randomly appeared in one of eight directions (Fig. 1B). The target turned blue if a participant reached it too late, red if he reached it too soon, and "exploded" if the reach was in time. One second after a target was reached, the next target appeared. The experiment was approved by the Johns Hopkins University Joint Committee on Clinical Investigation.
15. A scan produced 15 brain image slices at a resolution of 6.1 mm in each direction. Emission scans were attenuation-corrected with a transmission scan before each session. Twenty seconds before each scan, 62 mCi $H_2^{15}O$ was administered [M. E. Raichle *et al.*, *J. Nucl. Med.* **24**, 790, (1983)]. Accumulated radioactivity in the 90 s after initiation of the scan was used as an index of rCBF. Scans were acquired at 10-min intervals. The motor task was initiated 1 min before administration of the bolus and continued until completion of the scan. Participants practiced in the field for 5 min between scans 6 and 7 and rested between all other scans. Arterial blood was not sampled. Blood flow data reported here are changes in units of flow relative to the mean of the flow acquired for the whole brain.
16. Field A refers to the force field $f = V_A \dot{x}$, shown in Fig. 1D, where *f* is a force vector acting on the handle of the robot, $\dot{x}$ is the handle's velocity, and V_A = {{0,13}, {–13,0}} N · s/m. Field B was defined as $f = V_B \dot{x}$ where $V_B = -V_A$.
17. Other measures of motor output, including total force, mechanical work, and integrated electromyogram, are correlated with movement length during learning of a force field.
18. Images were realigned and normalized with SPM96 software [K. Friston *et al.*, *Hum. Brain Mapp.* **2**, 189 (1995)]. The scans from each participant were realigned with the first image used as a reference. A T1 weighted magnetic resonance image (MRI) was coregistered to the mean PET image for each participant and then stereotactically transformed to a standard MRI template in the Talairach and Tournoux space. The resulting transformation matrix was applied to the PET images. The normalized PET images were smoothed with an isotropic Gaussian filter (full width at half maximum of 12 mm). The normalized MRI scans were combined to generate a population-specific anatomic atlas. Based on a distance measure [R. P. Woods, S. R. Cherry, J. C. Mazziotta,

J. Comput. Assist. Tomogr. **115**, 565 (1992)] between the individual MRIs and the atlas, the median brain among the population of participants was labeled as typical. Smoothed, normalized PET data were analyzed with the use of SPM software with a multisubject block design, two replications per condition, and an analysis of covariance global normalization. Participant and global brain activity were two covariates of no interest, and the conditions of the task were the covariates of interest. The search volume was from $z = -30$ mm to $z = 60$ mm and did not allow a complete view of the cerebellum. In the parametric test, the contrasts represented the average movement length per condition. In the subtraction tests, the contrasts were −1 and 1 (or 1 and −1) for the conditions of interest. We considered as significant regions where voxel-level Z values exceeded 4.4 so that the corrected $P < 0.05$. We also considered as significant regions in the sensorimotor system that we had selected a priori, where voxel-level Z values exceeded 3.09. These regions were the primary sensorimotor, the premotor, and the supplementary motor areas, the striatum, and the cerebellum.

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20. Principal component analysis [K. J. Friston, C. D. Frith, R. S. J. Frackowiak, *Proc. R. Soc. London Ser. B* **254**, 47 (1993)] of the data in session 1 showed that changes in rCBF in regions of the left BA 18, thalamus, and right BA 46 were highly correlated. The images from each participant were projected through the eigenimage that represented this network. The result was a score that was minimum in the random field condition and maximum during early learning. This suggests that the network was least active when the task was most novel (participants experienced a field for the first time in the random condition) yet unlearnable, and was most active only when the task was being rapidly learned. Therefore, it is likely that the increase in BA 46 (Fig. 2B) in early learning versus the random condition is learning-related and not due to confounding factors such as the novelty or attention requirements of the task.
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Drosophila Mitotic Domain Boundaries as Cell Fate Boundaries

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Fate determination in *Drosophila* embryos is evidenced by the appearance of mitotic domains. To identify fate or fates of cells, individual cells in mitotic domains 2, 8, and 15 were marked and monitored through development. Comparison of the different fates indicated that domain boundaries are cell fate boundaries. Cells were marked by expression of GAL4-dependent transgenes after photoactivation of a caged GAL4VP16 analog that had its DNA binding activity inhibited with a photolabile blocking reagent. Caged GAL4VP16 was also used to induce gene expression in *Xenopus* embryos. Thus, photoactivated gene expression is a versatile tool for spatiotemporal control of gene expression.

To control the temporal and spatial expression of selected genes at the single-cell level for the purpose of fate mapping and genetic manipulation, we devised a method for "caging" the DNA binding activity of GAL4VP16, a potent transcriptional activator. Caging is a form of photo-reversible chemical modification that has been used in the light-mediated activation of molecules such as adenosine 5′-triphosphate, Ca^{2+}-chelators, and actin (*1*). Caged GAL4VP16 was produced by modifying lysine residues of purified GAL4VP16 (*2*) with the amine-reactive compound 6-nitroveratrylchloroformate (NVOC-Cl) (*3*). GAL4VP16 DNA binding activity was abolished after a 30-min incubation with 2 mM NVOC-Cl under mildly basic conditions (Fig. 1A). More than 50% of the initial binding activity was recovered by irradiating the caged GAL4VP16 with a low-intensity, long-wavelength (365 nm) ultraviolet (UV) lamp.

Caging of GAL4VP16 with 0.5 mM

NVOC-Cl, which modified about 8 of the 14 GAL4VP16 lysines (*4*), completely inhibited in vivo transcriptional activation in *Drosophila* embryos (*5*). This level of caging did not affect GAL4VP16 DNA binding activity in vitro (Fig. 1A). It is not known why the lower level of caging inhibited in vivo activity (*6*). Inhibition of the transcriptional activity of caged GAL4VP16 could be reversed in vivo with 365-nm light from a 100-W mercury lamp shone through a microscope objective via the epi-fluorescence light path of a standard inverted microscope. Experiments with *Drosophila* embryos required 3 to 4 s of irradiation (*7*) for maximal photoactivation.

We determined the efficiency of GAL4VP16-mediated photoactivated gene expression by quantitating the fluorescence of coinjected RGPEG (*8*), a fluorogenic β-galactosidase (β-Gal) substrate, in embryos that contained a GAL4-dependent *lacZ* construct ($UAS_G lacZ$) (Fig. 1B). GAL4VP16 was usually injected at a concentration of 0.2 mg/ml or less (*9*). Concentrations of unmodified or caged GAL4VP16 greater than 0.4 mg/ml caused developmental defects. This may have resulted from squelching, where general transcription factors bound to the acidic domain of unbound GAL4VP16 (*10*). Injection of RGPEG alone or with caged GAL4VP16, but not followed by irradiation,

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