the YD triggered an equatorward reorganization of zonal circulation over North America (*16*). Other zonal belts might be displaced southward, including the Intertropical Convergence causing increased zonal circulation in the Southern Hemisphere. Such enhancement in the Southern Hemisphere, however, is inconsistent with the absence of a YD dust spike in Antarctic ice records (*17*), suggesting that the event, if present, was not marked by enhanced windiness.

Another possible cause of the YD, reduced production of North Atlantic deep water, has the most pronounced climatic response in the Southern Hemisphere, because transfer of heat to mixed layers in the Southern Ocean is reduced (*18*), and sea-ice should grow. The 3.3°C mean annual cooling in the Southern Hemisphere predicted by one simulation of this process (*18*) is incompatible with our record.

Processes that yield either a largely North Atlantic signal (for example, iceberg or freshwater caps over the North Atlantic without changes in deepwater formation) or global processes that yield small thermal changes (a reduction of the greenhouse gas CO_2 by 50 ppm would cause a 1°C decline) are consistent with the absence of a marked thermal signal from NZ.

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Callosal Window Between Prefrontal Cortices: Cognitive Interaction to Retrieve Long-Term Memory

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A perceptual image can be recalled from memory without sensory stimulation. However, the neural origin of memory retrieval remains unsettled. To examine whether memory retrieval can be regulated by top-down processes originating from the prefrontal cortex, a visual associative memory task was introduced into the partial split-brain paradigm in monkeys. Long-term memory acquired through stimulus-stimulus association did not transfer via the anterior corpus callosum, a key part interconnecting prefrontal cortices. Nonetheless, when a visual cue was presented to one hemisphere, the anterior callosum could instruct the other hemisphere to retrieve the correct stimulus specified by the cue. Thus, although visual long-term memory is stored in the temporal cortex, memory retrieval is under the executive control of the prefrontal cortex.

The primate inferior temporal cortex, located at the final processing stage of visual object perception (*1*), plays an important role in recall as well as storage of visual memory; inferotemporal neurons can be dynamically activated by retrieval of visual long-term memory in monkeys (*2*), and electric stimulation of this region results in imagery recall in humans (*3*). The neural network that enables such imagery recall in cognition has not been established. A likely component is the prefrontal cortex, which has been implicated in executive processes such as planning, working memory, and memory retrieval (*4, 5*). A conventional approach by means of lesion to the prefrontal cortex often produces devastating cognitive impairments (*4*). On the other hand, the capacity for interhemispheric transfer through the anterior corpus callosum (CC), the callosal window between prefrontal cortices (*6, 7*), would positively highlight executive processes undertaken by the prefrontal cortex. So far, there has been little evidence for what transfers via the anterior CC (*8, 9*), whereas it has been established that posterior callosal fibers between sensory cortical areas (*7*) provide channels for communication in each modality (*6, 8, 10, 11*). In a clinical report of an epileptic patient who had undergone selective posterior callosotomy (*12*), although sensory stimuli lateralized to the nondominant right hemisphere could not be transferred for naming, semantic features of these stimuli somehow could be described by the expressive language system of the left hemisphere. This observation leads to a hypothesis that top-down processes originating from the prefrontal cortex can regulate retrieval of long-term memory from the modality-specific posterior association cortex, even in the absence of direct sensory input. To test this hypothesis, we examined in partial split-brain monkeys whether the prefrontal cortex can instruct, through the anterior CC, the contralateral hemisphere to retrieve long-term memory when sensory interaction between posterior cortical areas is prevented (Fig. 1A).

Monkeys underwent two-stage scheduled commissurotomy (Fig. 1B) (*13*). In the first operation, we transected occipito-temporal

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visual commissural fibers (*7*)—the splenium (SP) of the CC and the anterior commissure (AC). At this posterior-split stage, we could evaluate communication between prefrontal cortices via the anterior CC. After behavioral testing, the second operation was performed to sever the anterior CC for full-split control experiments (see below). The extent of callosal lesions for individual animals, based on magnetic resonance imaging (MRI) and histological data (*14, 15*), is summarized (Fig. 2A). MRI after the first operation showed that, as intended, SP and AC were split and the anterior CC was left intact in all the posterior-split monkeys (Fig. 2B). Histological data obtained after the second operation ensured that the anterior CC as well as SP and AC were divided at the full-split stage (Fig. 2C). In sections stained for myelin, commissural lesions were evident and the residual transected fibers looked atrophic and demyelinated (Fig. 2D). Slight unintended lesions were found in the right anterior cingulate gyrus and area preoptica medialis. The fornix was bilaterally intact. Interhemispheric cortico-cortical connections were further analyzed with retrograde fluorescent tracers (*15*). After diamidino yellow (DY) was injected in anteroventral portions of unilateral inferotemporal cortex, no labeled neurons were detected in the contralateral homotopic areas in the posterior-split preparation (Fig. 2E), in marked contrast to the unoperated control (Fig. 2F). However, injections of fast blue (FB) in the lateral prefrontal areas at the posterior-split stage produced extensive contralateral labeling in the supragranular layers (Fig. 2G), which was absent after the full-split surgery (Fig. 2H). These results confirmed that commissural fibers between prefrontal cortices were connected and alive in posterior-split monkeys, whereas those between visual cortical areas were selectively disrupted.

Two behavioral experiments were carried out. In the first experiment, we found that visual stimulus-stimulus association learning (*16*) did not transfer in posterior-split monkeys. In this task, the monkeys were required to memorize associations between arbitrarily assigned cue and choice pictures. On each trial, after a sequential presentation of cue

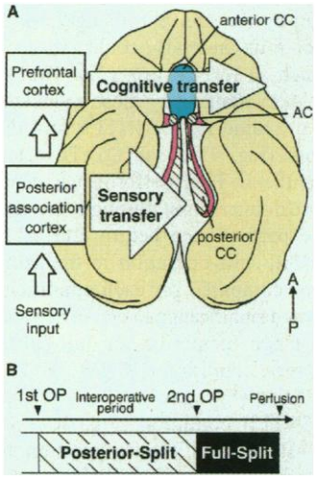

Fig. 1. (**A**) Ventral view of a monkey brain illustrating the experimental design. The posterior CC and AC (both colored red), which interconnect occipito-temporal visual areas, were selectively transected (hatched), while the anterior CC (blue) between prefrontal cortices was left intact. This preparation allowed dissociation of mnemonic processes undertaken by prefrontal and posterior association cortices. A, anterior; P, posterior. (**B**) Schedule of two-stage commissurotomy in operated animals drawn to time scale. At the first operation, AC and the posterior CC were transected. At the second operation, the remaining anterior CC was transected. Monkeys were behaviorally tested in two stages: posterior-split stage [hatched, also shown in (A)] and full-split stage (filled).

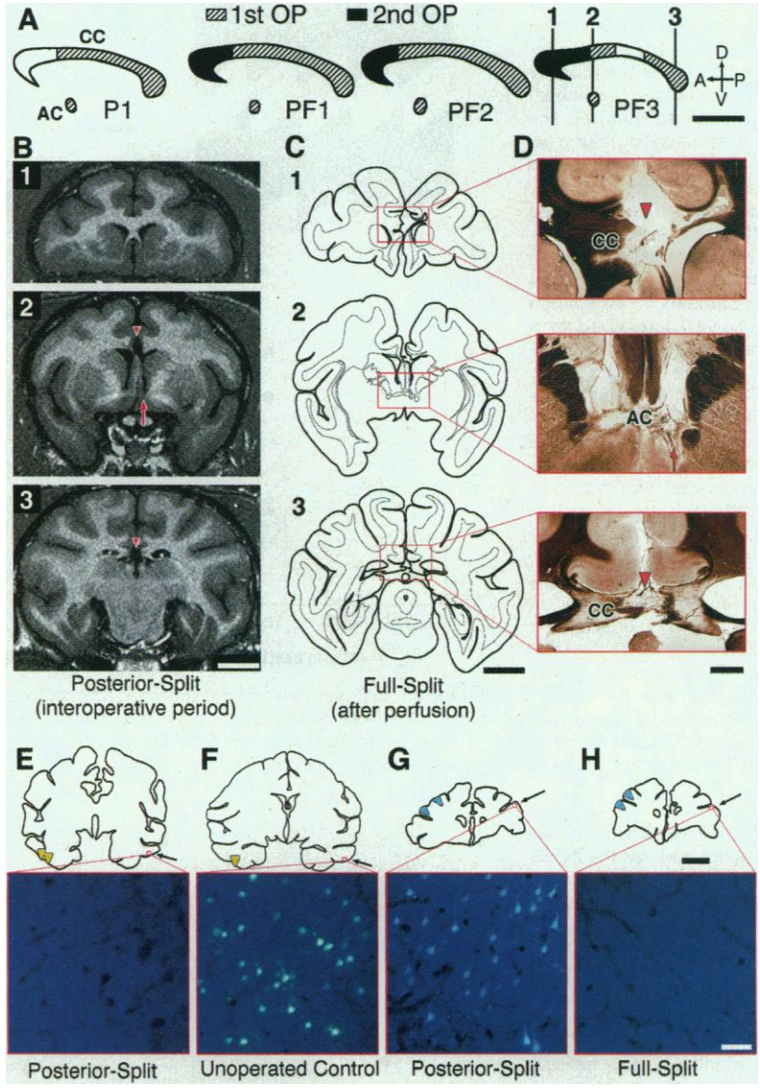

Fig. 2. (**A**) Extent of the first (hatched) and second (filled) commissurotomy in each operated animal. One posterior-split monkey (P1) did not undergo the second surgery and was used for tracer experiments (E and G). Three monkeys (PF1 to PF3) underwent two-stage commissurotomy (*13*). Numbers indicate coronal slice levels in (B) and (C). D, dorsal; V, ventral; A, anterior; P, posterior. Scale bar = 10 mm. (**B**) Representative MRIs from monkey PF3 during the interoperative period. Lesions of CC (arrowheads) and AC (arrow) are marked. Scale bar = 10 mm. (**C**) Line drawings of coronal sections from PF3 after perfusion (*15*). Scale bar = 10 mm. (**D**) Fiber-stained coronal sections, showing enlarged areas enclosed by red rectangles in (C). Scale bar = 2 mm. (**E** to **H**) Yellow (DY) and blue (FB) arrowheads in upper diagrams show injection sites of tracers in the left hemisphere (*15*). Small red squares (arrows) indicate the loci of dark-field fluorescent photomicrographs enlarged below. In the posterior-split monkey (P1), retrogradely labeled cells were not observed in the right inferotemporal areas (E) but were abundant in the right prefrontal areas (G). In the full-split monkey (PF1), prefrontal labeling was absent (H). Upper diagram, scale bars = 10 mm; lower photomicrograph, scale bar = 50 μm.

and choice stimuli during fixation, the subject must select one of the choices specified by the cue with saccade (Fig. 3, A and B). In the intrahemispheric (INTRA) condition, the information necessary to recall the visual stimulus-stimulus association was lateralized to a single cerebral hemisphere (*17*). The monkeys were trained to reach criterion in one hemisphere and then tested in the opposite hemisphere until the criterion was reachieved (*18*). In the unoperated group, the experience of original learning in the first hemisphere facilitated relearning in the second hemisphere (Fig. 3C, left). However, there was no apparent learning improvement in the posterior-split group (Fig. 3C, right). Analysis of variance (ANOVA) revealed a significant interaction between monkey group and learning effect ($F_{(1, 5)} = 19.71$; $P < 0.007$). In the unoperated controls, significantly fewer trials were needed in the second than in the first hemisphere [$t = 4.62$; degrees of freedom (df) = 2; $P < 0.05$, two-tailed t test], whereas in the posterior-split animals there was no significant difference ($t = 0.67$; df = 3; $P > 0.5$). As an index to estimate the effect of learning transfer, a percentage saving score was calculated for each stimulus set (*19*). The average saving scores for the unoperated controls were significantly above zero ($t = 12.99$; df = 2; $P < 0.006$), showing nearly perfect transfer (Fig. 3D). On the other hand, those for the posterior-split animals were not significantly different from zero ($t = 0.27$; df = 3; $P > 0.8$). Thus, long-term memory of visual stimulus-stimulus association learned in one hemisphere did not transfer to the other hemisphere via the anterior CC.

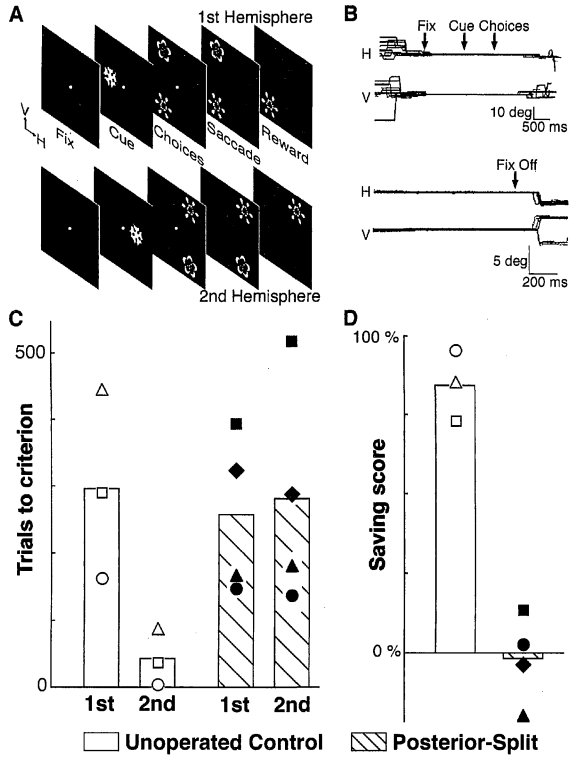

Fig. 3. (**A**) Visual stimulus-stimulus association task for the INTRA conditions. One cue and subsequently two choice stimuli were presented to the same visual hemifield while the monkey maintained fixation. The animal must saccade to one of the choices instructed by the cue. Because the animal continued to fixate during the stimulus presentation and then saccaded straightforward to one of the targets (B), all the information necessary to recall the visual stimulus-stimulus association was exclusively lateralized to a single hemisphere in this condition (*17*). The monkey was trained to reach criterion in one hemisphere. When the performance reached criterion in the first hemisphere, then the identical stimulus set was tested in the second hemisphere for transfer of learning (*18*). (**B**) Horizontal (H) and vertical (V) eye positions of a posterior-split monkey aligned at the onset ("Fix" in upper traces) and offset ("Fix Off" in lower traces) of the fixation spot. Data sampling rate is 250 Hz. (**C**) Number of trials to criterion for the first and second hemisphere in normal (open bars, open symbols) and posterior-split (hatched bars, filled symbols) monkeys. Each symbol represents data from an individual animal, the average score for four stimulus sets. (**D**) Average saving scores for normal and posterior-split monkeys. Symbols are as in (C).

In the second experiment, an interhemispheric (INTER) version of a visual stimulus-stimulus association task (Fig. 4A) was introduced. In the INTER condition, choice stimuli were presented to the opposite side of the cue (*16*). Because the monkeys' fixation and saccade were just as accurate as in the INTRA condition (Fig. 4B), the cue and choice stimuli were received by separate cerebral hemispheres. The two hemispheres must then communicate with each other, moment to moment (*20*), to select the correct choice specified by the cue. Surprisingly, all of the posterior-split animals could successfully solve such an INTER task (*21*). The performance level attained by these monkeys was almost the same for the INTER and INTRA conditions (Fig. 4, C and D). To determine whether the INTER performance depended on cortical interaction through the anterior CC, the performance before and after the second full-split operation in the same animal was compared for each condition (*21*). There was a significant interaction between operative stage (posterior- or full-split) and hemispheric condition (INTER, left INTRA, right INTRA) ($F_{(2, 10)} = 52.79$; $P < 0.0001$). In the INTER condition (Fig. 4C), performance after the full-split operation fell at chance and was significantly lower than at the posterior-split stage ($t = 18.78$; df = 2; $P < 0.003$). This ruled out the possibility that peripheral cuing strategy or subcortical commissural interaction (*22*) might account for the INTER performance. The drop in the INTER performance could not be attributable to surgical damage affecting general mnemonic ability, because the INTRA performance was not significantly altered after the second operation (left: $t = 0.54$; df = 2; $P > 0.6$; right: $t = 0.72$; df = 2; $P > 0.5$) (Fig. 4D). We conclude that the anterior CC is able to support cognitive interaction necessary to recall visual stimulus-stimulus association.

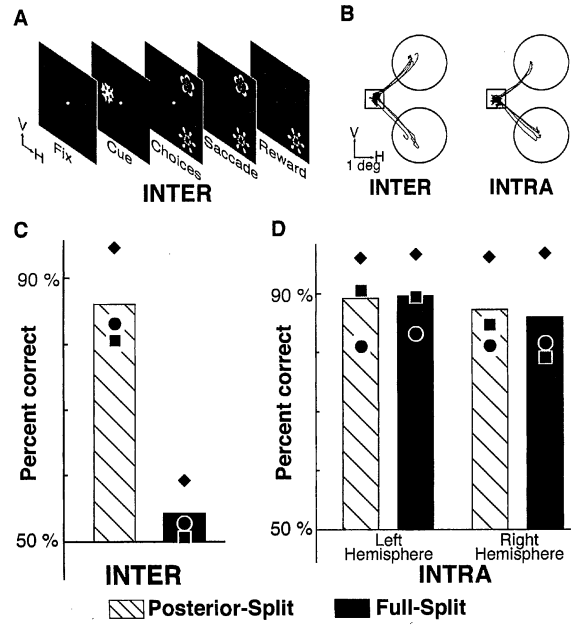

Fig. 4. (**A**) Visual stimulus-stimulus association task for the INTER condition. The cue and choice stimuli were sequentially presented to separate hemispheres. (**B**) Eye trajectories during fixation and saccade for the INTER (left) and INTRA (right) conditions in a posterior-split monkey. The central square represents the 1° × 1° fixation window. Circles indicate locations where the choice stimuli were presented. H, horizontal; V, vertical. (**C** and **D**) Performance for the INTER (C) and INTRA (D) conditions at the posterior-split (hatched bars) and full-split (filled bars) stages. In posterior-split monkeys, the INTER performance was almost the same for the INTRA performance. After the full-split operation, the INTER performance fell at chance and the INTRA performance was not altered. Symbols represent data from individual animals.

The finding that visual long-term memory acquired through stimulus-stimulus association learning does not transfer between prefrontal cortices (Fig. 3, C and D) has two implications. First, this suggests that visual associative long-term memories are primarily stored in the inferotemporal cortex (*2, 3, 23, 24*) and that the prefrontal cortex cannot make up for this function. Therefore, deficits in visual associative learning observed after periarcuate lesions (*25*) would be ascribed not to loss of long-term memory but rather to dysfunction of executive processes. Second, consistent with previous behavioral, anatomical, and neurophysiological data (*26*), this result indicates that the visual image of cue or choice per se did not transfer in posterior-split monkeys. After transection of SP and AC, the monkeys were visually split. Nevertheless, cognitive unity could be maintained through the callosal window between prefrontal cortices. The nature of the signal carried by this callosal bundle should be characterized by further studies with the current paradigm.

The uncinate fascicle, bidirectional temporo-frontal cortical pathway is necessary for visual associative learning in monkeys (*27*). A distributed cortical network along this pathway, which also might be involved in object working memory (*4, 5*), would regulate retrieval of visual long-term memory from the inferotemporal cortex (*1–3, 23, 24*) even in the absence of direct visual input. Consistent with this view, human functional neuroimaging studies have revealed that prefrontal areas are activated in various memory retrieval tasks (*28*). Thus, in primates, prefrontal and posterior association cortices should play essentially different roles in retrieval and storage of long-term memory.

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13. Seven monkeys (*Macaca fuscata*) were used. Before the start of the behavioral training, four animals underwent section of the splenium of the CC and the AC and served at first as the posterior-split group. After the behavioral experiments, one monkey was immediately injected with tracers and dedicated to histological analyses (*15*). Three of the posterior-split monkeys underwent further section of the remaining anterior part of the CC and then served as the full-split group. Three animals served as unoperated controls. Surgery was carried out with sodium pentobarbital anesthesia (25 mg per kilogram of body weight per hour, intravenously) under sterile conditions. In the posterior-split operation, at least one-third of CC from the posterior end of the splenium was aspirated. The lateral ventricle was entered at the level of the interventricular foramen, and AC was exposed and cauterized (*24*). In the full-split operation, the callosal lesion was extended anteriorly from the level of the AC to the rostrum of the CC.
14. We used a 1.5 T inversion recovery sequence (slice thickness = 2 mm, in-plane resolution = 0.4 × 0.4 mm^2, repetition time = 2 s, echo time = 29 ms, inversion time = 0.5 s, field of view = 100 × 100 mm^2) [K. Sakai *et al., Magn. Reson. Med.* **33**, 736 (1995)] to obtain coronal and sagittal MRIs in all the posterior-split monkeys.
15. At the end of the experiment, the animals were perfused with 4% paraformaldehyde in phosphate buffer (pH 7.4). Adjacent series of sections (50 μm) were stained with cresyl violet or stained for myelin with the modified Gallyas silver technique. Two full-split, one posterior-split, and one unoperated control monkey were injected with the retrograde fluorescent tracers FB and DY 14 to 16 days before perfusion. With a 1-μl Hamilton syringe, nine sites surrounding the anterior middle temporal sulcus in the inferotemporal cortex were injected with DY (2%, 0.25 to 0.5 μl), and 12 to 14 sites in the ventrolateral, dorsolateral, and lateral orbital prefrontal areas were injected with FB (3%, 0.25 to 0.5 μl). For these animals, a series of sections were examined by computerized microscopy (Zeiss, KS-400) for the presence of fluorescently labeled cells.
16. Figures 3A and 4A illustrate the behavioral tasks. Procedures for the visual stimulus-stimulus association task were as described in detail in (*2, 23, 24*), except that in this study the animals must maintain fixation during parafoveal presentation of the visual stimuli and respond with saccade. Visual stimuli were monochrome Fourier descriptors extending approximately 2° × 2°. While the monkey fixated within 0.4° to 0.6° of a spot on a computer monitor, one cue (for 0.7s) and two choice stimuli (0.5 to 1.0 s) were sequentially presented 2.5° lateral to the fixation spot. In the INTRA condition (Fig. 3A), all the stimuli were presented to the identical visual hemifield. In the INTER condition (Fig. 4A), choices were presented to the opposite side of the cue. When the fixation spot was turned off, the animal saccaded to one of the choices. The animal was rewarded when he correctly selected the choice stimulus instructed by the cue. Incorrect trials were followed with additional correction trials, which were not included in the data analysis. If the monkey failed to maintain fixation, the trial was aborted. Eye position was monitored with the scleral search coil method [S. J. Judge, B. J. Richmond, F. C. Chu, *Vision Res.* **20**, 535 (1980)]. For one control animal, a measurement system with a charge-coupled device camera was also used [K. Matsuda, *Neurosci. Res.* **20**, S270 (1996)].
17. The visual stimulus presented to one visual hemifield (*16*) must be lateralized to the contralateral hemisphere in the current paradigm, because each visual hemifield is both anatomically and functionally represented in the contralateral hemisphere except for a strip of 1° or narrower, if any, at the vertical meridian [J. Stone, J. Leicester, S. M. Sherman, *J. Comp. Neurol.* **150**, 333 (1973); M. Sugishita, C. R. Hamilton, I. Sakuma, I. Hemmi, *Neuropsychologia* **32**, 399 (1994); R. Fendrich and M. S. Gazzaniga, *ibid.* **27**, 273 (1989)]. Restriction of visual input to one hemisphere is further confirmed by our own findings that learning of visual long-term memory was not transferred in posterior-split animals and that full-split animals could not perform the INTER task above chance (see text).
18. Before measurement of learning transfer, acquisition of at least three stimulus sets was required for each left and right INTRA condition. A transfer test was then carried out with four new stimulus sets in a daily 200-trial session for 1 to 3 days until a criterion level was reached. The criterion was defined as 80% or more correct responses for two consecutive 20-trial blocks within a session. The averaged number of trials for individual animals to reach the criterion was statistically analyzed by a repeated-measures ANOVA and post hoc *t* tests.
19. The saving score for each stimulus set was calculated as follows: saving score = (TC1 − TC2)/(TC1 + TC2) × 100; TC1 and TC2, trials to criterion for the first and second hemisphere, respectively. The range of the score is from −100% to +100%, where 100% indicates perfect transfer and 0% means no transfer.
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21. After completion of the first experiment, the posterior-split animals were shaped into the INTER condition and trained with the same four stimulus sets that were used for the learning transfer test (*18*). It took 285 ± 105 trials (mean ± SE; $N = 3$) to reach the criterion in the INTER condition. In performance test, the averaged score over two consecutive 100-trial sessions was recorded for each of the four stimulus sets per animal on three conditions: left INTRA, right INTRA, and INTER. After the full-split surgery, performance for these same stimulus sets was again tested for every condition. For the INTER condition, however, blocks of 10 INTER trials and of 5 INTRA trials were alternated to maintain motivation. The averaged score for individual animals in each condition was statistically analyzed with repeated-measures ANOVA and post hoc *t* tests.
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26. Previous behavioral studies (*6, 8, 10*) indicated that visual discrimination learning did not transfer after transection of SP and AC, which is consistent with the topography of the forebrain commissural fibers (*7*). Neurophysiological evidence (*11*) also confirmed that SP and AC exclusively support transfer of visual signals in anesthetized monkeys.
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29. Experiments were conducted in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* and with the regulations of the University of Tokyo School of Medicine. This work was supported by grants from Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists to I.H. and T.I. and by a grant-in-aid for Specially Promoted Research from the Ministry for Education, Science, and Culture of Japan (07102006) to Y.M. We thank S. Konishi and M. Kameyama for collaboration; H. Niki for helpful discussion; C. Hamada for statistical assistance; T. Kitamura, J. Yamada, M. Yukie, and M. Yoshida for histological advice; and T. Kirino for encouragement.

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Coupled Gating Between Individual Skeletal Muscle Ca^{2+} Release Channels (Ryanodine Receptors)

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Excitation-contraction coupling in skeletal muscle requires the release of intracellular calcium ions (Ca^{2+}) through ryanodine receptor (RyR1) channels in the sarcoplasmic reticulum. Half of the RyR1 channels are activated by voltage-dependent Ca^{2+} channels in the plasma membrane. In planar lipid bilayers, RyR1 channels exhibited simultaneous openings and closings, termed "coupled gating." Addition of the channel accessory protein FKBP12 induced coupled gating, and removal of FKBP12 uncoupled channels. Coupled gating provides a mechanism by which RyR1 channels that are not associated with voltage-dependent Ca^{2+} channels can be regulated.

Intracellular Ca^{2+} release channels, present in the endoplasmic (or sarcoplasmic) reticulum of virtually all cells, are integral to diverse signaling pathways that require translation of electrical or biochemical extracellular signals into intracellular activation of Ca^{2+}-dependent molecules. The Ca^{2+} release channels in skeletal muscle comprise four 565-kD type 1 RyR subunits and four molecules of the 12-kD protein FKBP12 (*1*). FKBP12, which stabilizes the RyR1 complex and enables the four subunits to open and close coordinately (*2*), is a member of the immunophilin family of cis-trans peptidyl-prolyl isomerases that serve as cytosolic receptors for immunosuppressant drugs including rapamycin and FK506 (*3*).

Recombinant RyR1 expressed in insect (Sf9) cells in the absence of FKBP12 forms channels with multiple subconductance states, consistent with a defect in coordination of the activity of the four channel subunits (*2*). Addition of FKBP12 to recombinant RyR1 stabilizes the channel complex, resulting in the formation of channels with full conductance (*2*). This stabilizing effect is reversed by treating the channels with rapamycin or FK506 to remove FKBP12 from RyR1 (*2*).

The cytosolic domain of RyR1 projects into the space that separates the transverse tubule (T-tubule) and the sarcoplasmic reticulum (SR). A cytosolic domain of the α1 subunit of voltage-dependent Ca^{2+} channels (VDCCs) in the T-tubule is required for activation of RyR1 during excitation-contraction (E-C) coupling (*4*). Fragments of this domain can activate or inactivate RyR1 (*5–7*), indicating that E-C coupling may involve a protein-protein interaction between the two types of Ca^{2+} channels. Clusters of four VDCCs in the T-tubule overlie only every other RyR1 channel (*8*). Thus, a cytosolic loop from a VDCC is directly apposed to each subunit of only half of the RyR1 channels.

Recombinant RyR1 coexpressed with FKBP12 in Sf9 cells formed Ca^{2+}-activated Ca^{2+} channels that exhibited stable openings to 4 pA in planar lipid bilayers (Fig. 1A) (*2*). A current amplitude histogram (Fig. 1D) revealed two discrete peaks corresponding to closed channels (0 pA) and openings to the full amplitude of a single channel (4 pA). In some experiments (9 of 44), two channels opening and closing (gating) independently in the same bilayer were observed (Fig. 1B). In these experiments, one channel opened to the 4-pA level and a second channel was clearly apparent, opening independently of the first. A current amplitude histogram (Fig. 1E) revealed three discrete peaks corresponding to closed channels (0 pA) and openings to the full amplitude for one channel (4 pA) or for two channels (8 pA).

The single-channel properties of recombinant RyR1 coexpressed with FKBP12 were identical to those of native RyR1 from SR vesicles (*2*). The native RyR1 exhibited the typical current amplitude of 4 pA (Fig. 2, A and B). In some experiments (12 of 56), two channels were observed in the bilayer (Fig. 2B); channel openings to the 4-pA level and a second channel opening to 8 pA were apparent. A current amplitude histogram (Fig. 2E) revealed three discrete peaks corresponding to closed channels (0 pA) and openings to 4 and 8 pA.

In ~10% of experiments with either recombinant (4 of 44) (Fig. 1C) or native (5 of 56) (Fig. 2C) RyR1, channels were observed that opened to 8 pA, twice the normal current amplitude. Current amplitude histograms (Figs. 1F and 2F) revealed two discrete peaks corresponding to closed channels (0 pA) and openings to 8 pA. RyR1 channels exhibit a conductance of ~100 pS when Ca^{2+} (50 mM) is the current carrier at 0 mV (*9*). The conductances were 93 ± 18 pS for the single-amplitude openings and 180 ± 20 pS for the double-amplitude openings (Fig. 1G).

If both the 4-pA and the 8-pA openings represented activity of two independent RyR1 channels in the bilayer, then the binomial distribution of open probabilities would provide a calculated open probability ($P1_{calc}$) for the 4-pA current equal to the experimental value *P1*. The probability of the 4-pA openings in Fig. 1B predicted by a binomial distribution ($P1_{calc}$) equalled the experimentally observed value *P1* ($P > 0.05$, Student's *t* test). The same analysis applied to the open probabilities of currents in Fig. 1C showed that $P1_{calc}$ did not match the experimentally observed value for *P1* ($P < 0.001$, Student's *t* test). The failure of a binomial distribution based on the open probability of the 8-pA currents (*P2*) in Fig. 1C to predict the open probability of the 4-pA currents (*P1*) indicates that the 8-pA currents did not result from openings of two independent channels (*10*). Thus, the gating of two channels in Fig. 1C was likely coupled. Application of the binomial distribution is limited by the fact that it cannot distinguish between the presence of two interdependent cooperative channels each exhibiting a 4-pA current only, and that of a single channel with current amplitudes of 4 and 8 pA. However, if the actual current amplitude for RyR1 is 8 pA, the conductance of the channel would be exactly twice that measured for RyR1 in previous studies (*2, 11, 12*). Moreover, the 8-pA cur-

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