E. I. Stiefel, W. E. Newton, *J. Am. Chem. Soc.* **100**, 2748 (1978). The cobalt compound was used as a model for Se-Fe EXAFS. Since the phase and amplitude parameters for Co-Se do not differ significantly from Se-Fe parameters, cobalt model compounds of similar oxidation state can be used in place of iron model compounds for the analysis (*11*). No well-characterized Fe-Se model compounds were available for this study.

9. Briefly, the important points of an EXAFS analysis are as follows. For selenium, the x-ray absorption coefficient increases sharply at 12,655 eV. This is caused by the absorption of x-ray energy and the transition of a core electron from the selenium 1*s* orbital to higher energy valence and continuum states (K-edge). Fine structure above the K-edge results from the interference between outgoing photoelectron waves (from electrons ejected to continuum states) and backscattered waves from neighboring atoms. When EXAFS data are converted to momentum space **k**, the fine structure is directly related to the sum of sinusoidal oscillations for each shell of neighboring atoms. The frequency of each oscillation is $[2kR_{\mathrm{Se-X}} + \phi(\mathbf{k})_{\mathrm{Se-X}}]$, where $R_{\mathrm{Se-X}}$ is the bond length between selenium and neighboring atom X and $\phi(\mathbf{k})_{\mathrm{Se-X}}$ is the phase function specific to selenium and atom X (since the frequency is directly related to the bond length, the EXAFS contribution from an iron atom at twice the distance of the first shell of oxygen atoms will have a frequency twice that of the EXAFS contribution of the first shell). After the $\phi(\mathbf{k})$ values have been determined from "model" compounds of known structure, the bond lengths and coordination number for a given shell can be determined by a least-squares fit of a theoretically calculated curve to the experimental EXAFS data. The measure of the goodness of fit is given by the minimization function value F, defined as $F = \{\Sigma[k^3(\chi_{\mathrm{obs}} - \chi_{\mathrm{calc}})^2]/n\}^{1/2}$, where χ_{obs} and χ_{calc} are the observed and calculated EXAFS, respectively, and n is the number of data points.
10. G. Sposito, *ACS Symp. Ser.* **323**, 217 (1986).
11. B. K. Teo and P. A. Lee, *J. Am. Chem. Soc.* **101**, 2815 (1979).

12. We gratefully acknowledge the support of the Environmental Protection Agency (EPA R-809561) and the National Science Foundation (CHE 8512129 and EAR 8513488). The Stanford Synchrotron Radiation Laboratory is supported by the U.S. Department of Energy and the Biotechnology Resource Program of the National Institutes of Health.

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New Routes to Early Memories

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Stimulation of one side of the olfactory system during training with odor-milk pairings in neonatal rats results in their ability to recall an odor memory by using the trained but not the untrained side of the brain. In 12-day-old rats, olfactory learning can be recalled by stimulation of either the trained or untrained side. The development of bilateral recall reflects the maturation of olfactory commissural pathways that provide access to the olfactory memory stored on the contralateral side. Furthermore, the commissural pathways need not be present at the time of memory formation but can establish new and specific access to already existing olfactory memories.

NORMALLY, THE RIGHT AND LEFT sides of the brain are in communication through a series of commissures, fiber systems that cross the midline to form reciprocal connections between corresponding bilateral structures. Sectioning the major commissure, the corpus callosum, causes animals to behave in many situations as if they have two separate brains (*1*). With hemispheres disconnected, for example, each half of the brain can exhibit relatively independent perception, learning, and memory when the inflow of sensory information is restricted to one side. We have recently shown that newborn rats behave as a "natural split-brain preparation" in an olfactory learning protocol (*2*). Specifically, in rats younger than 12 days of age, the memory for a learned odor is confined to one side of the brain if a single naris is stimulated during pairings of an odor and milk reward. Such pups show increased preference for the odor in a choice task when tested with the same naris that was open during training; they show no such preference when tested with the other naris open. In contrast, when pups 12 days of age or older are trained unilaterally, they show learned preference with either naris open. This bilateral response develops simultaneously with the maturation of specific components of the anterior commissure (*3*), the olfactory system's crossed projection pathway. Indeed, the anterior commissure pathways are crucial for bilateral olfactory learning in older pups because cutting them before training restores unilateral conditioning (*2, 4*). However, even in older pups showing bilateral preference, the olfactory memories are still stored unilaterally, as demonstrated by a unilateral preference when the commissure is sectioned after training.

Although these findings indicate that commissural fibers can provide access to unilateral olfactory memories and confirm that the functional maturation of these pathways occurs between 6 and 12 days after birth, they raise an additional question. Do the newly formed crossed connections gain access to already formed memories, previously inaccessible to one side of the brain, or does bilateral access require existence of connections at the time of learning? To study this question we examined the recall of unilateral learning in 12-day-old rat pups that had been trained at 6 days of age. Unilateral training thus occurred in the absence of commissural projections to target layers in the contralateral anterior olfactory nucleus and piriform cortex (*3*). Pups were then tested at 12 days, when projections providing bilateral access had developed. To ensure that any bilateral access observed was the result of new access to memories (and not, for example, a duplication of learning on the untrained side), we sectioned the anterior commissure in some pups to confirm unilateral storage of the olfactory preference memories.

Forty-eight 6-day-old pups (*5*) received ten training trials consisting of a 20-second exposure to cedar odor, provided to one naris, starting 15 seconds before and continuing during the brief 5-second infusion of milk (*6, 7*). Cedar-milk pairings occurred every minute for 10 minutes. Milk rewards were delivered through small cannulas installed in pups' mouths (*7, 8*). We confined odor to one naris by inserting a soft rubber plug into the opposite nostril (*9*). After the conditioning, the nose plug was switched to the other naris for half of the pups and was sham-switched (removed and reinserted into the same naris) for the other half. Half an hour after pups had received the training treatment, they were tested for their response to cedar odor in a two-choice preference test (*10*) in which they were allowed to spend time over cedar-scented or unscented shavings. The number of seconds spent over

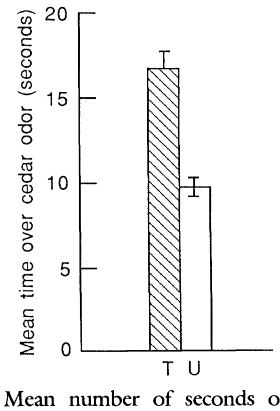

Fig. 1. Mean number of seconds over cedar during each preference test (maximal score, 30 seconds) as a function of naris open during the preference test administered at 6 days of age. T, trained naris; U, untrained naris. Error bars represent SEM. This finding of unilateral olfactory learning at 6 days of age replicates the results of our previous studies (*2*).

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*On 24 October 1987, Dr. Kucharski died in an automobile accident.

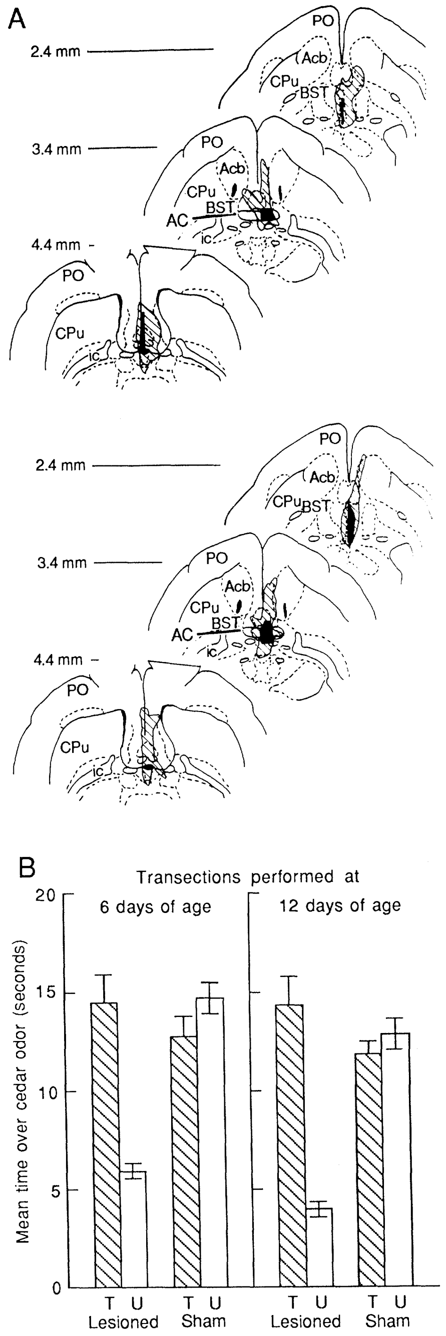

Fig. 2. Bilateral access to early, unilaterally stored memories for sham-lesioned pups but not for pups that received transection of the anterior commissure. (**A**) Reconstruction of lesions (and associated necrotic tissue) from subjects that received transections of the anterior commissure either after training at 6 days of age (top) or prior to testing at 12 days of age (bottom). The lesions are reconstructed on diagrams derived from Paxinos and Watson [plates 56, 58, and 60 (*11*)]. The striped regions correspond to lesions sustained by one or more animals, and the black regions correspond to lesions sustained by all animals. In all cases, the anterior commissure was transected. In addition, some animals sustained damage as far rostral as the nucleus accumbens and as far caudal as the third ventricle. The side of lesion was counterbalanced (right or left) in this study; however, for schematic purposes, the lesions are represented on the right side. Abbreviations: AC, anterior commissure; Acb, accumbens nucleus; BST, bed nucleus of stria terminalis; ic, internal capsule; CPu, caudate putamen; PO, primary olfactory cortex. (**B**) Mean number of seconds over cedar during each preference test for lesioned and sham control pups as a function of experimental group (conditioned or naïve controls) and naris open during the preference tests.

cedar was recorded during each test session. After this first preference test, nose plugs were switched and, 0.5 hour later, pups were given a second test to assess their preference with the other naris open. This testing sequence was repeated when these same pups were 12 days of age, again in a counterbalanced order. Half of the pups from each of the six litters received sham surgery, while the other half received transections of the anterior commissure (*11*). Surgery was performed after training at 6 days of age or prior to testing at 12 days of age.

Pups showed unilateral preference during the initial test of learning at 6 days of age (Fig. 1), spending 81% more time over cedar when tested with the trained naris open than when tested with the untrained naris open [$F(1,5) = 33.23$, $P < 0.01$; littermates' scores were averaged so that number of litters represents the unit of analysis]. In contrast, when the preference tests were repeated at 12 days of age, the pups that received sham surgery showed bilateral learned preference. They spent an equivalent amount of time over cedar when tested with the trained (mean number of seconds over cedar during each test ± SEM, 13.2 ± 2.0) or untrained naris open (14.7 ± 1.5) and showed an increased preference for cedar compared to the control animals (*12*). Thus, 12-day-old pups could access the previously unavailable memory when tested with the untrained naris.

On the other hand, animals that received transections of the anterior commissure (*11*) (Fig. 2A) either just after training at 6 days [$F(1,5) = 19.36$, $P < 0.01$] or just prior to testing at 12 days [$F(1,5) = 32.49$, $P < 0.01$] showed a greater preference for cedar when tested with the trained naris open (14.8 ± 1.1) than when tested with the untrained naris open (5.1 ± 0.7) (Fig. 2B). This finding confirmed that memories were still stored unilaterally on the trained side (*13*) and further indicated that the unilateral representation of the memory trace does not change during development. If the memory trace had become bilaterally represented, then these transections would have been ineffective in restoring unilateral conditioning. These data thus confirm the role of the anterior commissure in providing access to the unilaterally represented early memories and suggest that it is the formation of new connections by this pathway that creates new access routes to the earlier stored olfactory memories.

A capacity to access contralateral olfactory memories develops postnatally. The timing of this change is telling. After the last stage of anterior commissure development, when fibers have extended into the anterior olfactory nucleus and piriform cortex, rats become capable of bilateral retrieval of the learned information. If fibers are prevented from communicating with these areas, animals cannot retrieve the information. Other crossed projections carried in the anterior commissure (for example, fibers from anterior olfactory nucleus to the contralateral olfactory bulb) are not likely to be involved in the developmental change in memory access because they appear to mature before 6 days of age (*3*). Thus, the anterior olfactory nucleus and piriform cortex are implicated as structures that, once innervated, provide critical sites for, or pathways to, olfactory preference memories. Further, these pathways can provide access to preexisting memories even though they were not functional at the time of memory formation and are unlikely to have ever experienced the stimulus odor after becoming functional.

The finding that unilaterally represented memories remain unilateral even after bilateral retrieval processes have developed is consistent with experiments (*2*) that indicated that memories for conditioned olfactory preferences are stored unilaterally (when olfactory input is restricted to one naris during conditioning), even in older pups with mature commissural systems, and are accessed bilaterally via the anterior commissure. There have been other reports of unilaterally, rather than bilaterally, stored memories that are accessed via commissural systems (*14*). Indeed, it has been suggested that one function of commissural systems is to restrict memories to a single side of the brain (*15*). By this account, the commissural system provides bilateral access to memories but prevents bilateral representation of memories and so increases the mnemonic storage capacity of the brain. Our results demonstrate the natural development of access to a unilaterally stored memory, provide a demonstration of new and specific access to earlier learning, and point to neural regions involved in one type of olfactory memory.

REFERENCES AND NOTES

1. R. E. Myers, *Brain* **79**, 358 (1956); R. W. Sperry, *Science* **133**, 1749 (1961).
2. D. Kucharski and W. G. Hall, *Behav. Neurosci.*, in press; D. Kucharski, I. B. Johanson, W. G. Hall, *Behav. Neural Biol.* **46**, 472 (1986).
3. J. E. Schwob and J. L. Price, *J. Comp. Neurol.* **223**, 177 (1984).

4. See H. Teitelbaum [*J. Comp. Physiol. Psychol.* 76, 51 (1971)] for experiments with adult rats testing the location of olfactory memories.
5. The subjects were six litters of 6-day-old offspring of Charles River CD strain rats mated in our laboratory ($n = 6$). Pregnant females were housed in individual cages (45 by 24 by 20 cm) containing wood-chip bedding. Water and Purina Lab Chow pellets were continuously available. Pups were removed from their mothers 24 hours before training and testing. During the deprivation period, they were housed individually in a warm (32.5° ± 0.5°C), moist (70 to 90% relative humidity) incubator.
6. Just before training, we emptied the pups' bladders by gently stroking the anogenital region with a soft, sable brush. Then pups were placed in a clear, plastic container (11 by 6 by 11 cm) in a warm test incubator (32° to 34°C). The incubator was constructed from a 57-liter glass aquarium covered with a roof of hinged Plexiglas panels (*7*). To deliver the cedar odor stimulus to a pup, we pumped a light stream of air through plastic tubing into a sealed 25-ml flask containing 1 ml of cedar wood oil. A piece of plastic tubing exiting from the flask extended into the pup's test container. Activating the pump immediately introduced an air stream bearing cedar odor into the pup's environment. A blower in the test container rapidly removed scented air.
7. W. G. Hall, *Science* 205, 206 (1979).
8. The oral cannulas were installed at least 30 minutes prior to training (*7*). This required less than 10 seconds, and pups appeared to habituate rapidly to the presence of the cannula.
9. The plug was made from a 0.6- to 0.7-cm piece of Silastic tubing (Dow Corning; 0.067-cm outside diameter for 6-day-olds; 0.094-cm outside diameter for 12-day-olds) with a thread knotted and inserted into the lumen to block air flow and as an aid in removal. The plug was installed after coating the tip with petroleum jelly. Installation could be accomplished in a few seconds. Pups appeared to adapt to the presence of the plug quickly and paid it little attention. The naris that was occluded during training was counterbalanced between litters. The nose plug was installed at least 30 minutes prior to training or testing.
10. For testing, pups were placed in a container that had fresh bedding on one side and fresh bedding scented with cedar odor on the other side (approximately 0.5 ml of cedar wood oil was added to 85 g of bedding). Each preference test consisted of five 30-second trials. For each trial, the pup was placed lengthwise in the middle of a start zone (direction of placement was alternated from trial to trial), and the cumulative amount of time over cedar was recorded. The pup was scored as being over cedar if its snout was out of the start zone and in the cedar shavings area. The subjects were completely counterbalanced in each litter for order of testing (trained or untrained naris open during first test) and direction of first placement in the testing apparatus. No more than one pup from a litter was assigned to a counterbalanced condition; however, as there were no differences for order of testing (that is, animals spent an equivalent amount of time over cedar when tested with the trained naris open regardless of whether the test occurred before or after the test with the untrained naris open; animals spent an equivalent amount of time over cedar when tested with the untrained naris open regardless of whether the test occurred before or after the test with the trained naris open), the littermates' scores were averaged for these conditions to create a single litter mean for analysis. Thus, we treated litters as the experimental unit throughout these experiments.
11. To transect the anterior commissure (either 1 hour after conditioning at 6 days or 4 hours before retention testing at 12 days) we anesthetized the pups with methoxyflurane (Metofane). The skull was exposed and a burr hole made just lateral to the midsagittal suture and just posterior to bregma. The transection was made with a 30-gauge hypodermic needle (0.9 mm long), rounded at the tip and inserted through the hole perpendicular to the animal's skull. The needle was swung in the sagittal plane forward through 30° of arc. The needle was withdrawn, and the wound was closed and dressed. Side of needle insertion (just right or just left of the suture) was counterbalanced between litters. After the test, animals were killed, and the brains were removed and cut horizontally in 20-μm sections. Damage to the anterior commissure was investigated in Nissl stained sections. Area of damage was traced onto atlas figures [G. Paxinos and C. Watson, *The Rat Brain in Stereotaxic Coordinates* (Academic Press, New York, 1982)]. For the sham-operated pups, the needle was inserted only into the cortex. Littermates' scores for a particular treatment group were averaged so that the number of litters remained the unit of analysis.
12. Control values were obtained from previous studies. These studies replicate the general finding that 12-day-old pups exhibit conditioning when tested with the trained or untrained naris open. Conditioned pups spent substantially more time over cedar than naïve controls, and the values obtained for conditioned pups were nearly identical to those reported in our present experiment. The preference test scores obtained in the present experiment for animals tested with the trained or untrained naris open are shown in Fig. 2B with the data further partitioned according to the time when separate groups received sham surgery for comparison with pups that received transections of the anterior commissure.
13. In our control studies we have trained rats at 6 days of age and tested them at 12 days of age for unilateral conditioning, but without any previous testing at 6 days of age. These animals responded bilaterally when tested at 12 days of age, precluding the argument that the testing session administered at 6 days itself contributed to the "transfer" effect when pups were retested for 6-day retention. Previous investigations have also included various other conditioning controls (including backward-conditioning groups), and no differences have been found between any of these groups with this conditioning protocol, although differences were detected between each of the control conditions and the experimental condition. Finally, conditioning has also been found to be specific to the odor that was paired with milk reward.
14. M. S. Gazzaniga, *Exp. Neurol.* 8, 14 (1963); S. Watanabe, *Behav. Brain Res.* 17, 163 (1985).
15. N. Negrao and R. W. Doty, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 28, 647 (1969); R. W. Doty and N. Negrao, in *Handbook of Sensory Physiology: Central Processing of Visual Information*, R. Young, Ed. (Springer Verlag, Berlin, 1973), vol. 7, part 3B, pp. 543–582.

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A Novel Thyroid Hormone Receptor Encoded by a cDNA Clone from a Human Testis Library

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The c-*erb*A gene belongs to a multigene family that encodes transcriptional regulatory proteins including the v-*erb*A oncogene product, steroid hormone receptors, and the vitamin D_3 receptor. A v-*erb*A DNA probe encoding the DNA-binding region of the v-*erb*A protein was used to screen a human complementary DNA testis library. One of the clones isolated, *erb*A-T-1, was found to encode a 490–amino acid protein (*erb*A-T). The *erb*A-T polypeptide shows high homology with the proteins encoded by both the chicken c-*erb*A and the human c-*erb*A-β genes but is most closely related to the chicken gene. The chicken c-*erb*A and the human c-*erb*A-β genes encode high-affinity receptors for thyroid hormone, and here it is shown that the *erb*A-T protein binds specifically to 3,5,3′-triiodo-L-thyronine with a dissociation constant of $3.8 \pm 0.2 \times 10^{-10}M$. These data imply that more than one thyroid hormone receptor exists in humans and that these receptors might have different tissue- and gene-activating specificities.

THE *erb*A GENES ENCODE A CYSteine-rich domain that shows high homology with the putative DNA-binding domain of steroid hormone receptors (*1–8*). The amino acid sequence of this domain is almost fully conserved for the glucocorticoid receptors of the mouse, rat, and human (*9, 10*) and for the estrogen receptors of the chicken and the human (*11*). The chicken (*7*) and human *erb*A (*8*) proteins, which are both thyroid hormone receptors (*7, 8*), share only 90% homology in this region and may therefore correspond to different genes encoding proteins with similar but not identical functions. To isolate further members of the *erb*A gene family, we used a 600-bp Ava I–Pst I DNA fragment from v-*erb*A (*12*) encoding the cysteine-rich domain to screen human complementary DNA (cDNA) libraries. Two cDNA clones, which hybridized strongly with the ^{32}P-labeled v-*erb*A probe, were isolated from a testis library (*13*). Both clones also hybridized with a probe from the 3′ end of v-*erb*A. We subcloned and sequenced two Eco RI inserts of the larger of these clones (*erb*A-T-1) (*14*). The 2112-bp-long sequence of *erb*A-T-1 contains a long open reading frame of 490 amino acids with an initiator methionine codon at nucleotide 334 and a terminator codon at nucleotide 1804 (*15*).

The predicted polypeptide encoded in the

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