TABLE 1 Mean allele frequencies for Mdh^{100} and Hk^{100} in five honey bee populations

Population	$f(Mdh^{100})$	N	$f(Hk^{100})$	N
European subspecies	0.18 (a)	20	1.00 (d)	27
New World Europeans	0.23 (a)	84	0.95 (d)	40
Neotropical Africans				
Mexican	0.70 (b)	56	0.63 (e)	56
non-Mexican	0.92 (c)	21	0.45 (f)	191
African *A. m. scutellata*	0.99 (c)	25	0.29 (f)	15

Mean allele frequencies (f) for Mdh^{100} and Hk^{100} in five honey bee populations, weighted by number of hives sampled (N). Frequencies of Mdh^{100} and Hk^{100} are significantly independent among populations (Mdh: G, the likelihood ratio chi-square, =137.21; Hk: $G=146.6$; 4 d.f., 1 $P<0.001$); frequencies with the same letter (a–f) are not significantly different ($P>0.05$, simultaneous procedure for test of independence[34]). Frequencies presented include original and previously published data. New data: Mdh in European *A. m. mellifera* and *A. m. carnica* (collected 1987 by O.R.T. and D.R.S.); Mdh and Hk in European populations from Costa Rica (1986), in neotropical African populations from Mexico (1988) and in South African *A. m. scutellata* (1986). Previously published data: Mdh in *A. m. ligustica*[35], New World Europeans from the United States[36] and Brazil[30], neotropical Africans from Brazil[17], and *A. m. scutellata*[17]; Hk in European *A. m. ligustica* and *A. m. carnica*[32] and New World Europeans from the United States and Mexico[32]; Mdh and Hk in New World Europeans from the United States[31] and neotropical African populations from Costa Rica[31]. Samples from Africanized apiaries were avoided where possible. There is no significant difference in allele frequencies between European subspecies and New World Europeans, nor between long-established (non-Mexican) neotropical African populations and *A. m. scutellata*. There are significant differences between Mexican and long-established (non-Mexican) populations of neotropical African bees. Although there may be gene flow from the European to the African population (through drones) when the African population initially invades new territory, allele frequencies in long-established neotropical African populations are not different from those of South African *A. m. scutellata*.

continent. In addition, allele frequencies of biparentally inherited allozymes (malate dehydrogenase (Mdh), and hexokinase (Hk) distinguish African *A. m. scutellata* from European subspecies[18,30–32] and do not differ significantly between South African *A. m. scutellata* and long-established neotropical African populations (Table 1). Thus, an essentially African population is expanding in neotropical habitats through migration and colonization of new territory by African females. The paucity of European mtDNA in the feral neotropical African populations may be due to selection against some trait(s) of European or European/African hybrids in tropical habitats, to competitive interactions between European and African bees or to other factors.

The invasion of the New World by African bees provides an unusually clear example of asymmetrical gene flow between two conspecific populations. There is no evidence that the feral neotropical African population has become extensively 'Europeanized'. Future research into the management of neotropical African honey bees should emphasize the factors that limit gene flow from European to African populations and the potential competitive interactions between neotropical African bees and the large North American feral European population. □

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Associative long-term depression in the hippocampus induced by hebbian covariance

Patric K. Stanton* & Terrence J. Sejnowski*

Department of Biophysics, Johns Hopkins University, Baltimore, Maryland 21218, USA

A BRIEF, high-frequency activation of excitatory synapses in the hippocampus produces a long-lasting increase in synaptic strengths called long-term potentiation (LTP)[1]. A test input, which by itself does not have a long-lasting effect on synaptic strengths, can be potentiated through association when it is activated at the same time as a separate conditioning input[2–4]. Neural network modelling studies have also predicted that synaptic strengths should be weakened when test and conditioning inputs are anti-correlated[5–8]. Evidence for such heterosynaptic depression in the hippocampus has been found for inputs that are inactive[2,9] or weakly active[3] during the stimulation of a conditioning input, but this depression does not depend on any pattern of test input activity and does not seem to last as long as LTP. We report here an associative long-term depression (LTD) in field CA1 that is produced when a low-frequency test input is negatively correlated in time with a high-frequency conditioning input. LTD of synaptic strength is also produced by activating presynaptic terminals while a postsynaptic neuron is hyperpolarized. This confirms theoretical predictions[8] that the mechanism for associative LTD is homosynaptic and follows a hebbian covariance rule[7].

We searched for conditions under which the stimulation of a hippocampal pathway, rather than its inactivity, could produce, depending on the pattern of stimulation, either long-term

* Present addresses: Departments of Neuroscience and Neurology, Albert Einstein College of Medicine, 1410 Pelham Parkway South, Bronx, New York 10461, USA (P.K.S.); Computational Neurobiology Laboratory, The Salk Institute, 10010 N. Torrey Pines Road, La Jolla, California 92037, and Department of Biology, University of California at San Diego, La Jolla, California 92093, USA (T.J.S.).

depression or potentiation of synaptic strengths. The conditioning stimulus (Fig. 1), based on the finding that bursts of stimuli at 5 Hz are optimal in eliciting LTP[10], was applied to Schaffer collateral/commissural fibres; the test stimulus was applied to a subicular input on the opposite side of the recording site. Each shock of the test input was either superimposed on the middle of each burst of the conditioning input (in phase) or occurred symmetrically between the bursts (out of phase).

Extracellular, evoked-field potentials were recorded from the apical dendritic and somatic layers of CA1 pyramidal cells. The low-frequency train of test stimuli was first applied alone and did not itself induce long-lasting changes. The conditioning site was then stimulated alone, which elicited homosynaptic LTP of the conditioning pathway but did not significantly alter amplitude of responses to the test input. When test and conditioning inputs were activated in phase, there was an associative potentiation of the test input synapses (Fig. 2a). Both the population excitatory postsynaptic potential (e.p.s.p.) and population action potential were significantly enhanced.

By contrast, when test and conditioning inputs were applied out of phase, we observed an associative long-term depression

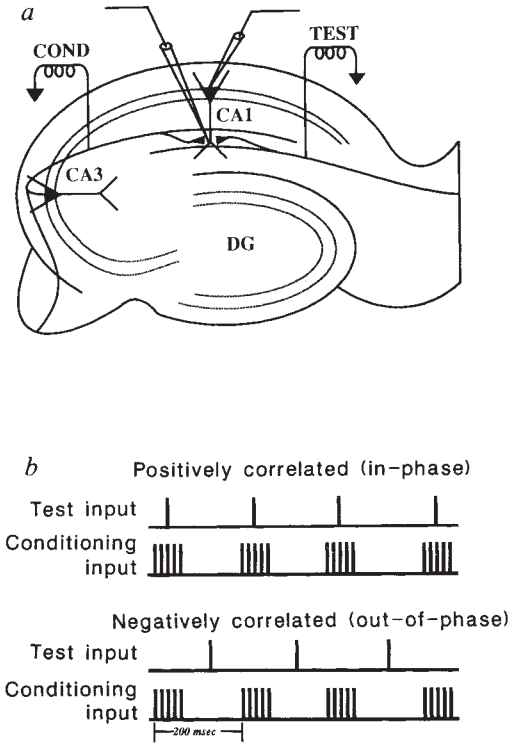

FIG. 1 Hippocampal slice preparation and stimulus paradigms. *a*, Schematic diagram of the hippocampal slice *in vitro* showing recording sites in the CA1 pyramidal cell somatic and dendritic layers, and stimulus sites activating Schaffer and commissural collaterals (COND) and subicular afferents (TEST). *b*, Schematic diagram of stimulus paradigms used. Conditioning input stimuli, four trains of 100-Hz bursts. Each burst had 5 stimuli and the interburst interval was 200 msec. Each train lasted 2 seconds and had a total of 50 stimuli. Test input stimuli, four trains of shocks at 5-Hz frequency, each train lasting for 2 seconds. When these inputs were in-phase, the test single shocks were superimposed on the middle of each burst of the conditioning input, as shown. When the test input was out of phase, the single shocks were placed symmetrically between the bursts.

METHODS. Hippocampal slices (400 μm thick) were prepared by standard methods[14] and incubated in an interface slice chamber at 34–35 °C. Extracellular recording electrodes were filled with 2 M NaCl and had 1–5 MΩ resistance. Intracellular micropipettes were filled with 2 M potassium acetate and had 70–120 MΩ resistance. Tips of bipolar glass-insulated, platinum wire, stimulating electrodes were 50-μm in diameter; all electrodes were prepared by standard methods[14].

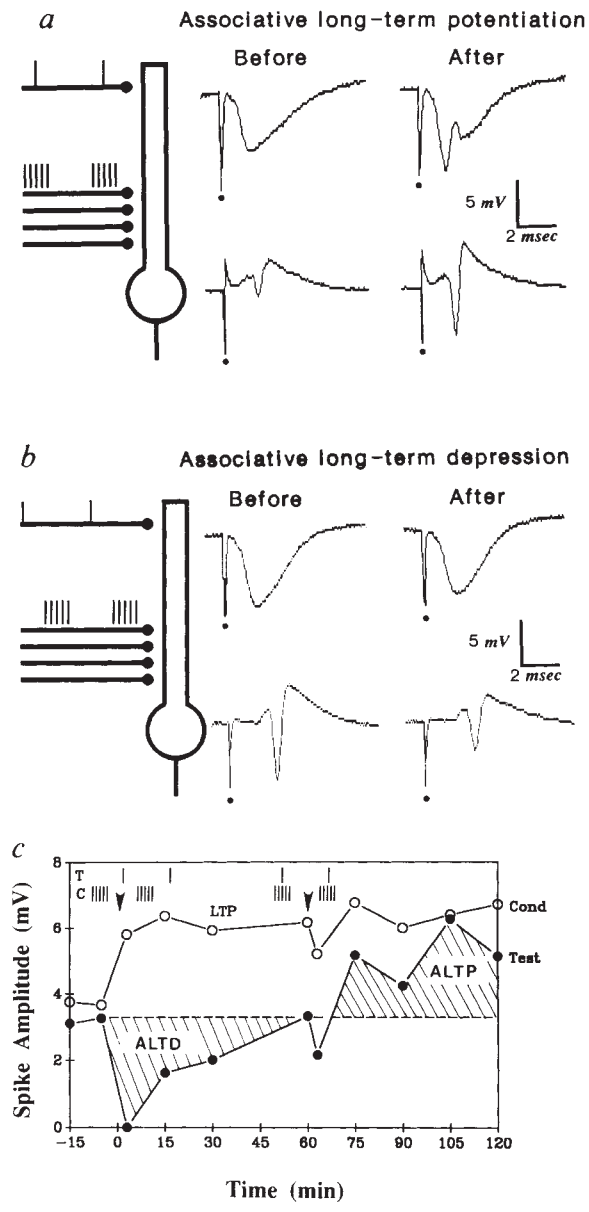

FIG. 2 Illustration of associative long-term potentiation (LTP) and associative long-term depression (LTD) using extracellular recordings. *a*, Associative LTP of evoked e.p.s.ps and response of the population action potential in the test input. Test reponses are shown before and 30 min after application of test stimuli in phase with the coactive conditioning input. *b*, Associative LTD of evoked e.p.s.ps and reponses of population spikes in the test input. Test responses are shown before and 30 min after application of test stimuli out of phase with the coactive conditioning input. *c*, Time course of the changes in population spike amplitudes for a typical experiment. Inset (top) shows the stimulus patterns for the test (T) and conditioning (C) inputs, and arrows show the time of stimulation. Single responses from the conditioning input (open circles), show that the high-frequency bursts (5 pulses of frequency 100 Hz at 200-msec intervals; see Fig. 1) elicited synapse-specific LTP, independent of other input activity. Single responses from the test input (filled circles) show that stimulation of the test pathway, out of phase with the conditioning one, produced associative LTD (ALTD) of this input. In phase stimulation of the same pathway elicited associative LTP (ALTP). In 20 slices the average change in the maximum initial slope of the population e.p.s.p. was +49.8 ± 7.8%, and the average increase of the population action potential amplitude in 14 slices was +65.4 ± 16.0%. (Results given as mean percent change ± s.e.m.) The average reduction of the population spike during associative LTD in 10 slices was −46.5 ± 11.4%, with a smaller decrease in the average maximum initial slope of the population e.p.s.p. of −13.8 ± 3.5% in 13 slices. The duration of associative LTD was at least 30 min and up to 3 h following stimulation. The amplitude and duration of associative LTD or LTP could be increased by stimulating input pathways with more trains of shocks.

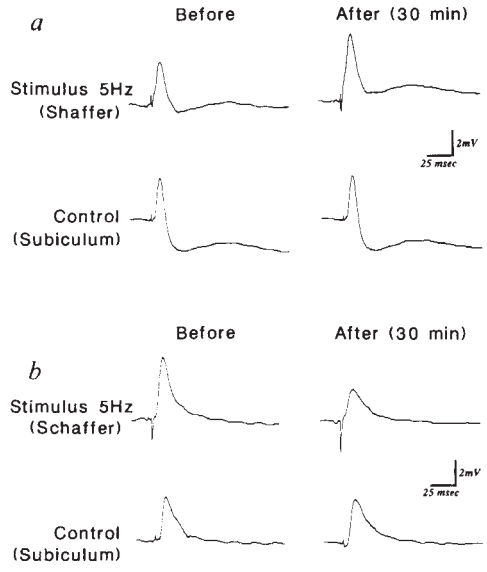

FIG. 3 Pairing of postsynaptic hyperpolarization with stimulation of synapses on CA1 hippocampal pyramidal neurones produces LTD specific to the activated pathway, while pairing of postsynaptic depolarization with synaptic stimulation produces synapse-specific LTP. *a*, Intracellular evoked e.p.s.ps are shown at stimulated (Stimulus 5 Hz, Schaffer) and unstimulated (Control, Subiculum) pathway synapses before and 30 min after pairing depolarizing current injection with 5-Hz synaptic stimulation (the constant +2.0 nA current produced a 20-mV depolarization of the soma without synaptic stimulation). The stimulated pathway exhibited associative LTP of the e.p.s.p., whereas the control, unstimulated input showed no change in synaptic strength. (RMP = −65 mV; R_N = 35 MΩ). *b*, Intracellular e.p.s.ps evoked at stimulated and control pathway synapses before and 30 min after pairing a 20-mV hyperpolarization at the cell soma with 5-Hz synaptic stimulation (the constant −1.0 nA current produced a 20-mV hyperpolarization of the soma in the absence of synaptic stimulation). The input (Stimulus 5 Hz), activated during the hyperpolarization, showed associative LTD of synaptic evoked e.p.s.ps, while synaptic strength of the silent input (control) was unaltered (RMP = −62 mV; R_N = 38 MΩ). The cell fired action potentials during the depolarizing current injection, but not during injection of the hyperpolarizing current. In five cells, a depolarizing current injection paired with the test input led to an average increase of +110 ± 31.3%, while a control input inactive during the stimulation only changed by +15.5 ± 5.4%. In five different cells, prolonged hyperpolarizing current injection paired with the same test stimuli led to an average decrease of −57.6 ± 6.8% in the stimulated pathway, but a change of only −13.4 ± 8.4% in the unstimulated pathway. The resting membrane potential did not change following stimulation. In a previous study, hyperpolarizing current applied during high-frequency synaptic stimulation blocked LTP, but LTD of the synaptic input was not reported[16]. The input stimulus, however, was typically 30 Hz or higher compared to the 5 Hz used in our experiment, so that the dendritic membrance potential during synaptic stimulation was probably significantly more positive at the 30-Hz rate.

of the test input synapses (Fig. 2*b*). There was a marked reduction in the population spike with smaller decreases in the population e.p.s.p. Note that the stimulus patterns applied to each input were identical in these two experiments, and only the relative phase of the test and conditioning stimuli was altered. With these stimulus patterns, synaptic strength could be repeatedly enhanced and depressed in a single slice (Fig. 2*c*). The covariance model[7] predicts that stimuli with no covariance should produce no change in synaptic strengths. A test input containing an equal number of in phase and out of phase stimuli mixed together produced no net change in synaptic strength in five slices. Thus, the associative LTP and LTD mechanisms seem to be balanced in both strength and duration.

The simultaneous depolarization of the postsynaptic membrane and activation of glutamate receptors of the N-methyl-D-aspartate (NMDA) subtype leads to LTP induction[11-13]. Associative LTP is thought to depend on the spread of current from conditioning synapses to test synapses in the dendritic tree[2-4]. Consistent with this hypothesis, we found that the NMDA receptor antagonist 2-amino-5-phosphonovaleric acid (AP5, 10 μM) blocked the induction of associative LTP by the in phase stimuli in CA1 pyramidal neurones (the average change in population spike amplitude, only −7.3 ± 10. 4% in five slices). By contrast, the application of AP5 to the bathing solution at the same concentration did not affect associative LTD (average change in population spike amplitude, −42.9 ± 5.7% in five slices). Thus, the induction of associative LTD does not require the activation of the NMDA receptor.

In a second series of experiments, intracellular recordings from CA1 pyramidal neurones were made using standard techniques[14]. An in-phase stimulus produced an increase in peak e.p.s.p. amplitude (49.2 ± 7.1% in six cells) and a lowered action potential threshold in the test pathway, as reported previously[4]. Conversely, an out-of-phase stimulus elicited a long-lasting reduction of the e.p.s.p. (−29.4 ± 5.5% in six cells) and reduced ability of the test input to elicit firing of action potentials. As in the extracellular experiments, the test input alone produced no long-lasting alterations in intracellular e.p.s.ps or firing properties, whereas the conditioning input alone yielded specific increases of the e.p.s.p. of the strong pathway without altering e.p.s.ps elicited by test input stimulation.

A test stimulus that is out of phase with a conditioning stimulus, arrives when the postsynaptic neurone is hyperpolarized as a consequence of inhibitory postsynaptic potentials and after hyperpolarization from mechanisms intrinsic to pyramidal neurones. This suggests that postsynaptic hyperpolarization coupled with presynaptic activation may trigger LTD. To test this hypothesis, we injected current through intracellular microelectrodes to hyperpolarize or depolarize a CA1 pyramidal neuron while stimulating a synaptic input. Pairing the injection of depolarizing current with the test input led to LTP of those synapses (Fig. 3*a*), whereas a control input inactive during the stimulation did not change, in agreement with previous experiments in which a higher stimulus frequency was used[15-17]. Conversely, prolonged, hyperpolarizing current injection, paired with the same test stimuli, led to induction of LTD in the stimulated pathway (Fig. 3*b*), but not in the unstimulated pathway. The application of either depolarizing current, hyperpolarizing current, or the test 5 Hz synaptic stimulation alone did not induce long-term alterations in synaptic strengths. Thus, hyperpolarization and simultaneous presynaptic activity is sufficient for the induction of LTD in CA1 pyramidal neurones. Further experiments are underway to determine whether this mechanism can fully account for associative LTD observed with out of phase inputs.

These experiments identify a novel form of hebbian synaptic plasticity in the hippocampus and confirm predictions made from a covariance model of information storage in neural networks[7,8]. Unlike previous reports of synaptic depression in the hippocampus[2,3,9], the plasticity is associative, long-lasting, and is produced when presynaptic activity occurs while the postsynaptic membrane is hyperpolarized. The chronic hyperpolarization of neurones in visual cortex leads to a long-term depression of the active, but not inactive, inputs from the lateral geniculate nucleus[18]. Thus, the hebbian covariance mechanisms present in the hippocampus may also be found elsewhere in the central nervous system[19,20]. □

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Local positional cues in the neuroepithelium guide retinal axons in embryonic *Xenopus* brain

William A. Harris

Department of Biology, B-022, and Center for Molecular Genetics, University of California at San Diego, La Jolla, California 92093, USA

GROWING retinal axons home to their distant target, the tectum, even when they are displaced from their normal pathway[1]. This argues for long-range guidance mechanisms in the embryonic brain. Growth cones may orientate to diffusible attractants released from the target, as proposed in other systems[2,3], or they may use a stable distribution of positional information in the neuroepithelium[1]. To distinguish between these possibilities, small pieces of the presumptive optic tract, through which retinal axons will normally grow, were rotated by ~90° either clockwise or counterclockwise. When the retinal axons later encountered the rotated neuroepithelium, they also turned clockwise or counterclockwise, in correspondence with the direction of rotation. This demonstrates that long-range navigation of retinal axons in the vertebrate brain is based partly on stable, local positional factors, rather than on remote diffusible factors.

The general protocol for these experiments is outlined in Fig. 1. After exposing the embryonic brain by removing the epidermis and the optic vesicle on the right side, a piece of neuroepithelium, about 100 μm in diameter, containing ~200 cells of the right presumptive optic tract from a stage 26–28 *Xenopus* embryo[4] (before axons leave the eye[5]) was excised, labelled with Hoechst dye (Hoechst no. 33258, Sigma), and reimplanted either in the normal orientation (controls), or in rotated orientations (experimentals). The right optic vesicle and epidermis were re-attached and the animals were allowed to recover until they reached stage 37/38–41 (when axons first reach the target[5]), at which time they were fixed, and the retinal fibres originating from the left eye were labelled with DiI (1, 1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; Molecular Probes), a fluorescent lipophilic dye. DiI has recently been shown to be an excellent marker for labelling axonal projections in the post-mortem brain[6]. To test for the stability of graft orientation, a small crystal of DiI was used to mark a corner of the transplant in a series of experiments (Fig. 2*a*). It was found that 37 of 44 grafts clearly maintained their grafted orientations (Fig. 2*b*). Of the remaining seven, the orientations of four were ambiguous, and the other three seemed to have healed into the host in an unintended orientation. Whether the transplant healed incorrectly, or whether the crystal of DiI was dislocated in these cases is not certain. What is clear, however, is that in most cases (≥84%) the graft seemed to maintain its operated orientation. For the experimental series, DiO (3,3′-dioctadecyloxacarbocyanine perchlorate; Molecular Probes) or carbon particles were used as the orientation markers, in order not to interfere with the fluorescence of the DiI-labelled fibres. DiO crystals and carbon particles, even though they were reliable during the initial transplantations, were not useful as long-term orientation markers. The carbon particles become indistinguishable in the background of developing pigment cells; the DiO crystals were usually extruded from the neuroepithelium, as DiO apparently does not stick to the tissue as well as DiI. Whole-mounted embryonic brains with early optic tract rotations revealed normal surface contours. Rarely (<10% of cases), the rotations produced minor morphological aberrations in the developing brain, probably as a result of poor wound healing; these were discarded from the analysis.

Axons growing through control transplants (that is, removed, stained and reimplanted in their normal orientation) usually grew towards the tectum along trajectories that seemed similar to the trajectories taken by axons in unoperated animals (compare Figs 2*c* and *d* and Fig. 3*a*), although no quantitative comparison between these two conditions was made. Axons growing through rotated transplants (Figs 2*e*, *f* and 3*c*, *d*, *e*), however, veered clockwise or counterclockwise on entering the rotated tissue. Quantitative analysis of the results (Fig. 4) showed mostly non-deflected trajectories in operated control animals, whereas experimental groups showed trajectories that deviated in the predicted directions. Often the pathway deflections were

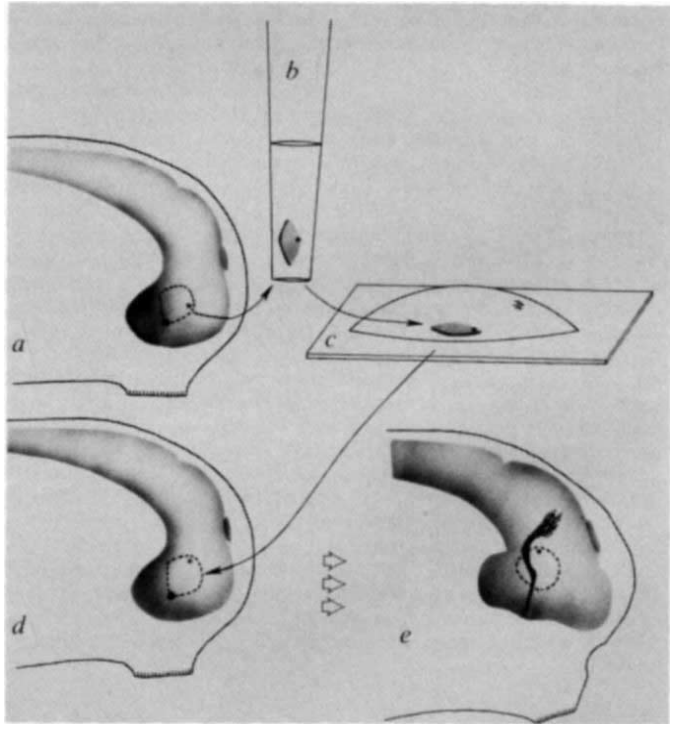

FIG. 1 Procedure of rotation experiment. *a*, Right side of stage-26 embryonic brain (shaded object) at time of surgery. Dashed lines indicate region of graft to be removed. Star indicates orientation marker; *b*, Spemann pipette transferring tissue to labelling solution; *c*, graft incubating in drop of Hoechst dye; *d*, graft in new counterclockwise orientation; *e*, optic fibre trajectory through graft.

METHODS. To mark the orientation of the graft, a small crystal of DiO, DiI or a carbon particle was embedded into the anterior corner of the diamond. Staining was in 200 μM Hoechst in Steinberg's solution[17] for 10 min. The tissue was washed twice and repositioned in the brain either in its normal position (controls) or rotated approximately 90° clockwise or counterclockwise (experimentals). After a survival period of up to two days, the embryos were fixed in 4% paraformaldehyde and the optic fibres growing out of the left eye were labelled by placing crystals of DiI on the fixed retinal surface[6]. All samples were photographed as single and double exposures to show the relationship of the Hoechst (graft) and DiI (fibres) fluorescence, and then traced from projected slides to reconstruct line drawings for further analysis.