TABLE 1 Mean allele frequencies for Mdh<sup>100</sup> and Hk<sup>100</sup> in five honey bee populations

<table>
<thead>
<tr>
<th>Population</th>
<th>f (Mdh&lt;sup&gt;100&lt;/sup&gt;)</th>
<th>N</th>
<th>f (Hk&lt;sup&gt;100&lt;/sup&gt;)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>European subspecies</td>
<td>0.18 (a)</td>
<td>20</td>
<td>1.00 (d)</td>
<td>27</td>
</tr>
<tr>
<td>New World Europeans</td>
<td>0.23 (a)</td>
<td>84</td>
<td>0.95 (d)</td>
<td>40</td>
</tr>
<tr>
<td>Neotropical Africans</td>
<td>0.70 (b)</td>
<td>56</td>
<td>0.63 (e)</td>
<td>56</td>
</tr>
<tr>
<td>Mexican</td>
<td>0.92 (c)</td>
<td>21</td>
<td>0.45 (f)</td>
<td>191</td>
</tr>
<tr>
<td>African a. m. scutellata</td>
<td>0.99 (c)</td>
<td>25</td>
<td>0.29 (f)</td>
<td>15</td>
</tr>
</tbody>
</table>

Mean allele frequencies (f) for Mdh<sup>100</sup> and Hk<sup>100</sup> in five honey bee populations, weighted by number of hives sampled (N). Frequencies of Mdh<sup>100</sup> and Hk<sup>100</sup> 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). 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<sup>16</sup>, New World Europeans from the United States<sup>17</sup> and Brazil<sup>18</sup>, neotropical Africans from Brazil<sup>19</sup> and A. m. scutellata<sup>20</sup>; Hk in European A. m. ligustica and A. m. carnica<sup>21</sup> and New World Europeans from the United States<sup>22</sup> and Mexico<sup>23</sup>; Mdh and Hk in New World Europeans from the United States<sup>24</sup> and neotropical African populations from Costa Rica<sup>25</sup>. 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<sup>16, 17, 20</sup> 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.


ACKNOWLEDGMENTS: Samples of Africanized bees were collected off (Mexican bees). Further samples were provided by the U.S.D.A. Honey Bee Breeding, Genetics and Physiology Laboratory, Baton Rouge, Louisiana (Venezuelan bees); and R. Morse, D. De Jong, C. Henderson and the Department of Genetics, University of Sao Paulo, Ribeira Preto, Brazil (Brazilian bees). Samples of A. m. scutellata were provided by R. Cweve, R. Hagen and L. Watsler gave criticism of the manuscript, and R. Hagen provided statistical advice. This research was supported by the NSF (O.R.S. and W.M.B.) and by the NIH (W.M.B.).

ASSOCIATIVE LONG-TERM DEPRESSION IN THE HIPPOCAMPUS INDUCED BY HEBBIAN COVARANCE

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A BRIEF, high-frequency activation of excitatory synapses in the hippocampus produces a long-lasting increase in synaptic strengths called long-term potentiation (LTP)<sup>1</sup>. 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<sup>2</sup>. Neural network modelling studies have also predicted that synaptic strengths should be weakened when test and conditioning inputs are anti-correlated<sup>3</sup>. Evidence for such heterosynaptic depression in the hippocampus has been found for inputs that are inactive<sup>4</sup> or weakly active<sup>5</sup> 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<sup>6</sup> that for associative LTD is homosynaptic and follows a hebbian covariance rule<sup>7</sup>.

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...
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\textsuperscript{10}, was applied to Schaffer collateral/commissural fibres; the test stimulus was applied to a subiculum 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.

![Diagram](image-url)

**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 stimulus, 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 stimulus, four trains of shocks at 5-Hz frequency, each train lasting for 2 seconds. When these input 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\textsuperscript{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\textsuperscript{14}.

![Diagram](image-url)

**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 responses are shown before and 30 min after application of test stimuli in phase with the active conditioning input.

**b** Associative LTD of evoked e.p.s.ps and responses 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 active conditioning input.

Time course of the changes in population spike amplitudes for a typical experiment. Insert (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.
AssOCIATIVE LTP is thought to depend on the spread of current from conditioning synapses to test synapses in the dendritic tree. 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. 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. 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.p.s 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.p.s 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 LTD of those synapses (Fig. 3a), whereas a control input inactive during the stimulation did not change, in agreement with previous experiments in which a higher stimulus frequency was used. Conversely, prolonged, hyperpolarizing current injection, paired with the same test stimulus, led to induction of LTD in the stimulated pathway (Fig. 3b), 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. Unlike previous reports of synaptic depression in the hippocampus, the plasticity is associative, long-lasting, and is produced with 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. Thus, the hebbian covariance mechanisms present in the hippocampus may also be found elsewhere in the central nervous system.
Local positional cues in the neuropath epithelium guide retinal axons in embryonic Xenopus brain

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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 neuropath epithelium\(^4\). To distinguish between these possibilities, small pieces of the presumptive optic tract, through which retinal axons will normally grow, were rotated by \(-90^\circ\) either clockwise or counterclockwise. When the retinal axons later encountered the rotated neuropath epithelium, 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 neuropath epithelium, about 100 \(\mu\)m in diameter, containing \(\sim 200\) 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 (experiments). 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\(^2\)), at which time they were fixed, and the retinal fibres originating from the left eye were labelled with Dil (1,1-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate; Molecular Probes), a fluorescent lipophilic dye. Dil 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 Dil was used to mark a corner of the transplant in a series of experiments (Fig. 2a). It was found that 37 of 44 grafts clearly maintained their grafted orientations (Fig. 2b). Of the remaining seven, the orientations of four of them were ambiguous, and the other three seemed to have healed into the host in an unintended orientation. Whether the transplant healed in an incorrect form, or whether the crystal of Dil 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'-dioctadecylxocarbocyanine perchlorate; Molecular Probes) or carbon particles were used as the orientation markers, in order not to interfere with the fluorescence of the Dil-labelled fibres. Dio crystals and carbon particles, even though they were reliable during the initial transplantations, were not useful as long-term orientation markers. These carbon particles became indistinguishable in the background of developing pigment cells; the Dio crystals were usually extruded from the neuropath epithelium, as Dio apparently does not stick to the tissue as well as Dil. 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 2c and d and Fig. 3a), although no quantitative comparison between these two conditions was made. Axons growing through rotated transplants (Figs 2e, f and 3c, 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

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**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, Dil or a carbon particle was embedded into the anterior corner of the diamond. Staining was in 200 \(\mu\)M Hoechst in Steinberg's solution\(^7\) for 10 min. The tissue was washed twice and repositioned in the brain either in its normal position (controls) or rotated approximately \(90^\circ\) clockwise or counterclockwise (experiments). 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 Dil on the fixed retinal surface\(^8\). All specimens were photographed as single and double exposures to show the relationship of the Hoechst (graft) and Dil (fibres) fluorescence, and then traced from projected slides to reconstruct line drawings for further analyses.