together, our results suggest that IFN-α might represent a potential target for therapeutic intervention in SLE.

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15. After informed consent (IRB no. 0199017), blood was obtained from pediatric patients who satisfied diagnostic criteria of the American College of Rheumatology (ACR) for SLE (median age = 15; range: 7 to 18) and controls (children visiting the clinic for reasons other than autoimmunity or infectious disease, median age = 12; range: <1 to 17), and from age-matched patients with dermatomyositis or juvenile arthritis. Subsets of DCs and monocytes were determined using DC kit and/or CD14 monoclonal antibody (mAb) (Becton Dickinson). Disease activity was assessed by the SLEDAI determined the day of blood draw. SLE patients were evaluated in three groups on the basis of the steroid regimen: (i) either newly diagnosed or not on steroid regimen [neither intravenous (i.v.) pulse nor oral (per os p.o.)]; (ii) not on steroid pulse (but on p.o. dose); or (iii) the blood sample was taken a minimum of 2 weeks after the last steroid pulse (>2 weeks post), and the median time post-pulse was 4 weeks (range 2 to 52 weeks). The remaining treatment included Cytoxan and/or Plaquenil. Patients with JDM were on a steroid regimen (both pulse and p.o. doses), and the blood was evaluated at a median of 6 weeks post pulse (range 2 to 52 weeks). The remaining treatments included methotrexate and/or Plaquenil.
16. $CD14^+$ monocytes were purified from blood by depletion using monoclonal antibodies and Dynabeads and were cultured in six-well plates (1×10^6/well) for 3 days with GM-CSF (100 ng/ml) + IL-4 (20 ng/ml) or lupus serum (25%) or autologous serum (25%). On day 3, cells were stained for flow cytometry. For proliferation assays, DCs were cultured at graded doses with 1×10^5 total $CD4^+$ or naïve $CD4^+CD45RA^+$ allogeneic T cells for 5 days. Cells were pulsed with 0.5 mCi [^{3}H]thymidine per well (NEN). The antigen capture activity was determined by incubating the human lymphocyte antigen HLA-DR–labeled DCs with COLO829 melanoma cell line killed by γ-irradiation (150 Gy) labeled with DNA dye 7-AAD (SIGMA) for 1 hour at 37°C (or on ice as a control). The capture was analyzed by flow cytometry. To assay autologous T cell proliferation, DCs were loaded with killed cells for 4 hours and cultured at graded doses with 1×10^5 $CD4^+$ T cells for 5 days.
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23. Purified monocytes were cultured with autologous serum, without or with 100 UI/ml IFN-α, without or with neutralizing antibody against IFN-α or control antibody. After 3 days, cells were harvested and tested for their ability to stimulate MLR. Serum samples were assayed for IFN-α using an ELISA from BioSource (Camarillo, CA). For blocking experiments, serum samples were incubated for 30 min with the neutralizing antibody (BioSource) before cell contact.
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Choice Behavior of *Drosophila* Facing Contradictory Visual Cues

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We studied the underlying neural mechanism of a simple choice behavior between competing alternatives in *Drosophila*. In a flight simulator, individual flies were conditioned to choose one of two flight paths in response to color and shape cues; after the training, they were tested with contradictory cues. Wild-type flies made a discrete choice that switched from one alternative to the other as the relative salience of color and shape cues gradually changed, but this ability was greatly diminished in mutant (*mbm[1]*) flies with miniature mushroom bodies or with hydroxyurea ablation of mushroom bodies. Thus, *Drosophila* genetics may be useful for elucidating the neural basis of choice behavior.

Most animals can make a rapid and rational choice among alternative behaviors by assessing the advantages and disadvantages on the basis of previous experience, but the underlying neural mechanism is largely unknown (*1*). Studies of simple organisms have made important contributions to our understanding of the cellular and molecular basis of learning and memory, as well as other cognitive functions (*2–5*). Here, we used the visual learning paradigm developed by Wolf and Heisenberg (*6*) to examine choice behavior in *Drosophila*. Individual flies were presented with two conflicting cues, which they had been previously conditioned to follow, and their choice behavior was examined.

An individual fly in a flight simulator was trained to associate a particular visual pattern with a punishment (heat). In a typical protocol, the fly was first examined during a test period (three 2-min blocks) for its directional preference for various patterns in the flight arena. This was followed by two training sessions (two 2-min blocks each, spaced by one 2-min test block) during which the heat was switched on whenever a particular pattern entered the frontal 90° sector of the fly's visual field. The posttraining test sessions consisted of four 2-min blocks without heat application. In experiments in which wild-type Berlin (WTB) flies (*5*) were trained to follow a color cue (*7*), the flies showed no color preference between a green T and a blue T on a dark background during the pretraining test (preference index $PI_{1-3} \sim 0$) (*6*) (Fig. 1A). After the training to associate the heat punishment with the blue T, they exhibited persistent preference for the green T (positive PI_{9-12}). The flight path angular histograms in Fig. 1B (average of 26 flies) depict, at 0.5-min intervals, the relative amount of time spent by the flies in different directions between –90° and +90° relative to the location of the green T. However, when the same experiment was carried out with colored Ts on a white background, the flies failed to learn a preferred color T (Fig. 1C). White background illumination may result in a loss of color sensitivity of fly photoreceptors (*8*); thus, a dark background was used in all experiments. In the absence of color cues, the WTB flies could also learn to choose correctly between a white upright T and an inverted T (Fig. 1D). Thus, WTB flies can use either color or shape cue alone in visual learning.

The *Drosophila* eye is differentially sensitive to green and blue light (*9*). We made

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use of this fact to investigate whether the fly indeed uses color rather than light intensity in its visual learning of colored patterns. The WTB flies were trained to follow a high-intensity blue T (Bh) instead of a low-intensity green T (Gl). After the training, the flies were tested for their preference between a high-intensity green T (Gh) and a low-intensity blue T (Bl). The light intensity was set quantitatively equal between blue and green lights at high and low intensities, respectively, and the intensity of Gh was set above that of Bl, and that of Bh above Gl, at all wavelengths (Fig. 1E). Posttraining tests showed that the flies chose to follow the color cue in favor of the low-intensity blue T. Similar preference for color rather than intensity cue was found when the flies were trained to prefer Gh over Bl or to prefer Gl over Bh (Fig. 1E). Thus, the flies were indeed using color vision in visual learning in the present experimental protocol.

Can flies use color and shape cues simultaneously in visual learning? If so, can memory be selectively retrieved independently by a single cue, and is there an overshadowing effect of one cue over the other during training? To address these questions, we trained flies to choose between a green upright T and a blue inverted T, with color intensity (CI) set at a maximal value of 1.0 (*7*). After training with the double cues, the flies were tested with a single cue (Fig. 2A). For memory retrieval with the color cue, flies were tested for preference between a green upright T and a blue upright T. For retrieval with the shape cue, flies were tested for preference between a white upright T and a white inverted T. Significant retrieval of memory was found in both cases (Fig. 2, B and C), with PI values close to those observed after single-cue training (Fig. 1, A and D). Furthermore, the shape cue was retrieved equally well over the entire range of CI from 1.0 to 0 (Fig. 2D), suggesting the absence of any overshadowing of the shape cue by the color cue for both WTB and *mbm*^{1} mutant flies (described below). In addition, over a large range of CI values (1.0 to 0.4), the color cue could also be retrieved (Fig. 2E), which suggests that there was no overshadowing of the color cue by the shape cue (*10*). Thus, flies can simultaneously use two types of cues for visual learning, and each cue can be used for memory retrieval independently.

The use of multiple cues in visual learning may result in a dilemma for the fly when it encounters conflicting information. We presented a "color/shape" dilemma to the fly after the training by reversing the matching rules for the color and shape of T patterns. The fly was first trained to follow the green upright T and avoid the blue inverted T. During the posttraining test, the green upright T was changed to a green inverted T, whereas the blue inverted T became a blue upright T. The fly may choose the green inverted T on the basis of color, or may choose the blue upright T on

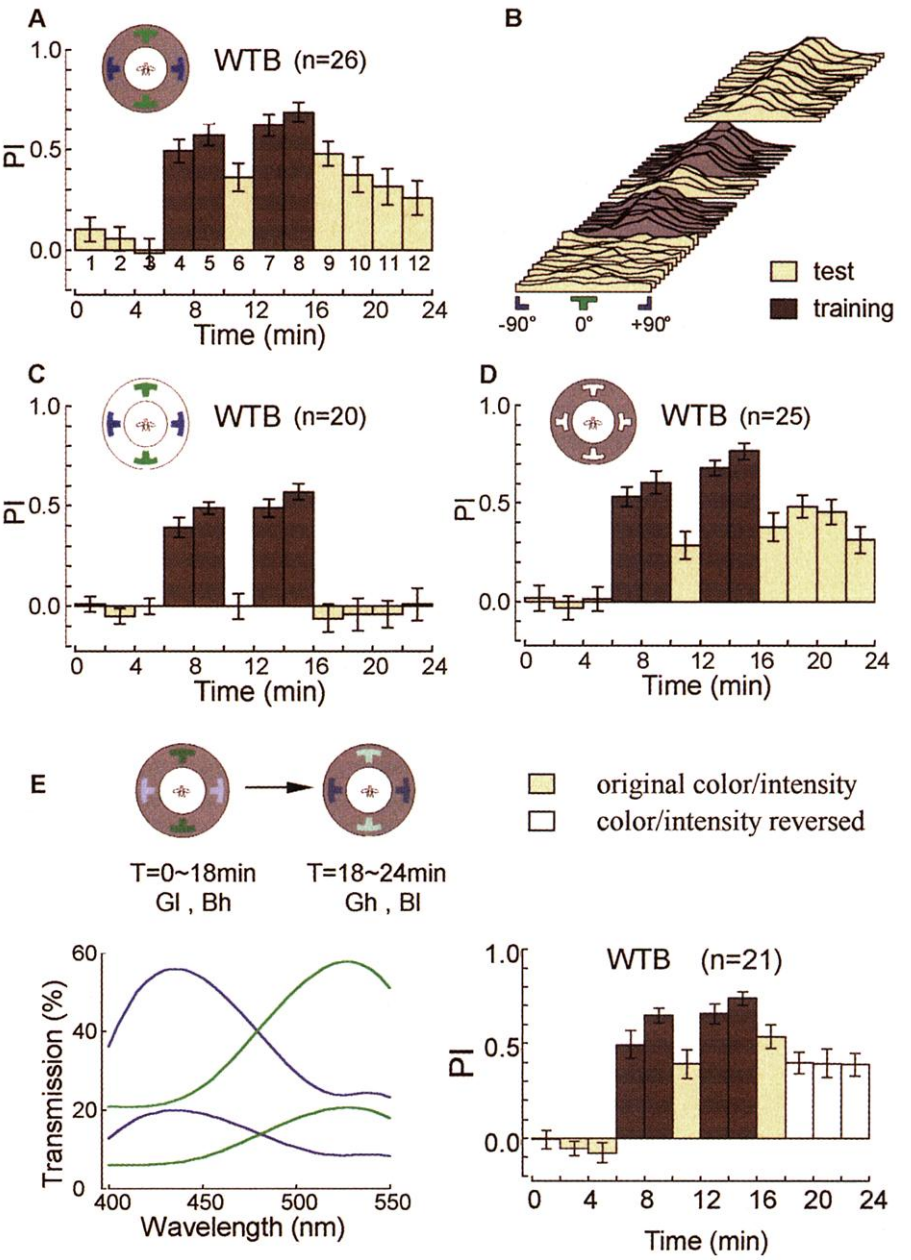

Fig. 1. Visual learning of *Drosophila* using single visual cues. (**A**) WTB flies were trained to prefer a green upright T over a blue upright T in the flight simulator. Histograms of preference index (PI) showed mean positive values during training (PI_{4-5} and PI_{7-8}, SEM, n = 26 flies) and after training (PI_6 and PI_{9-12}). Similar results were found when the flies were trained to prefer a blue T over a green T. (**B**) Flight path angular histograms of the relative time spent by flies in different flight directions between –90° and +90° relative to the location of the green upright T, estimated over a 0.5-min interval for each histogram and averaged for 26 flies [same set as in (A)]. (**C**) Failure of visual learning for a colored cue on a white background (n = 20). PI showed high positive value during training (PI_{4-5} and PI_{7-8}), but memory retention near zero after training (PI_6 and PI_{9-12}). (**D**) Visual learning with the shape cue. The flies were trained to prefer a white upright T over a white inverted T on a dark background. Histograms of PI showed positive values during and after training (n = 25). (**E**) The flies use the color rather than the intensity of the colored light for visual learning. The flies were trained to prefer color cues of high or low intensity; after training, they were tested with color cues with intensity values reversed. Left panel: Spectra of blue and green light and the relative values used for the high- and low-level intensities. Right panel: Histograms summarizing the data for all experiments using color/intensity reversal during the posttraining test. The positive values of PI_{10-12} (n = 21) indicate flight behavior in favor of the color cue.

the basis of shape. In making a choice, the fly must evaluate the relative weight of the color and shape cues. We found that the outcome of the fly's choice depended critically on the color intensity of the patterns during the test. When color intensity was high (CI = 1.0), the WTB flies made their choice according to the color cue, whereas at a lower color intensity (CI = 0.7) the WTB flies chose to follow the shape cue, with a preference for the blue upright T. At CI = 0.8, however, the flies showed equal preference for color and shape cues, which suggests that these cues exerted equal weight in choice behavior at this particular CI.

To further examine the choice behavior, we trained WTB flies to follow a green upright T and to avoid a blue inverted T at CI = 0.8, thus allowing learning of color and shape cues with "equal" weight. The flies were then tested at different levels of color intensity (CI = 1.00 to 0.00) with color and shape cues reversed. Figure 3A depicts the mean PI_{10-12} as a function of CI, obtained from a total of 165 WTB flies. When the intensity of the color cue was strong during the posttraining test, the flies made their choice in favor of the color. However, they chose to follow the shape

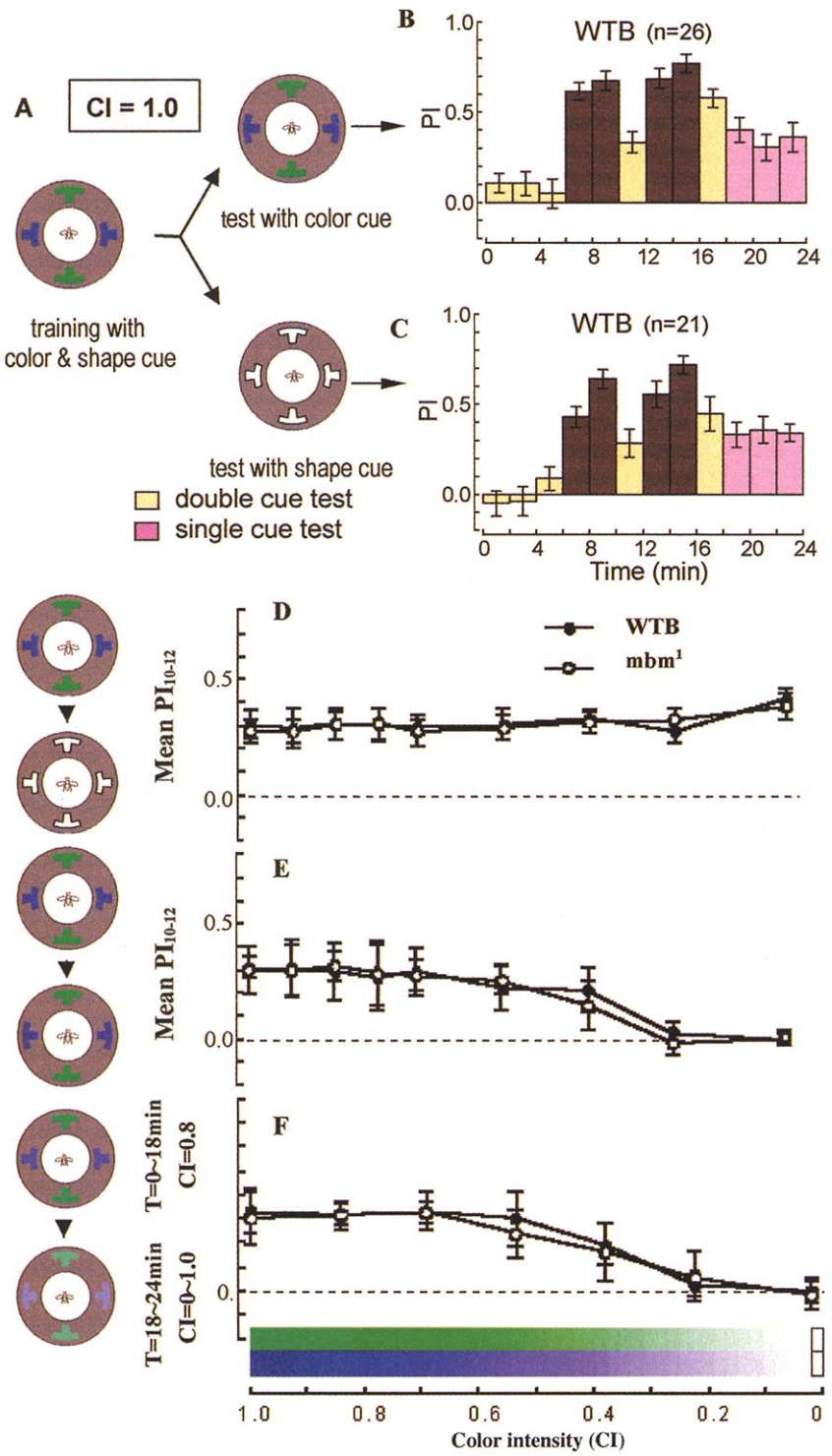

Fig. 2. Visual learning using double cues and the test of the overshadowing effect. (**A**) WTB flies were trained with the color and shape cues at a high color intensity (CI = 1.00) and were tested with a single cue during the posttraining session at the same CI. (**B**) After training to prefer a green upright T over a blue inverted T, flies were tested for their behavior based only on the color cue (to choose between a green upright T and a blue upright T). The PI_{10-12} values for WTB flies were positive (n = 26). (**C**) After training to prefer a green upright T over a blue inverted T, flies were tested for their behavior based only on the shape cue (to choose between a white upright T and a white inverted T). The PI_{10-12} values for WTB flies were positive (n = 21). (**D**) The experiment described in (C) was repeated for WTB (n = 131) and *mbm*^{1} (n = 93) flies over the entire range of CI values (1.0 to 0). The number of flies for each CI value tested ranged from 10 to 25. No difference was found between WTB and *mbm*^{1} flies (P = 0.66, Student's paired t test). (**E**) The experiment described in (B) was repeated for WTB (n = 96) and *mbm*^{1} (n = 80) flies over the range of CI from 1.0 to 0. No difference was found between WTB and *mbm*^{1} flies (P = 0.89, Student's paired t test). (**F**) Control experiments that examined the color sensitivity and the role of light intensity in the choice behavior of WTB and *mbm*^{1} flies. The flies were trained with both color and shape cues at CI = 0.80 (as in Fig. 3A) and were presented with only the color cue (upright green T versus upright blue T) at CI = 1.0 to 0 during the posttraining test (T = 18 to 24 min). The dependence of PI_{10-12} on CI was identical for WTB (n = 54) and *mbm*^{1} (n = 55) flies (n = 8 to 10 for each CI value).

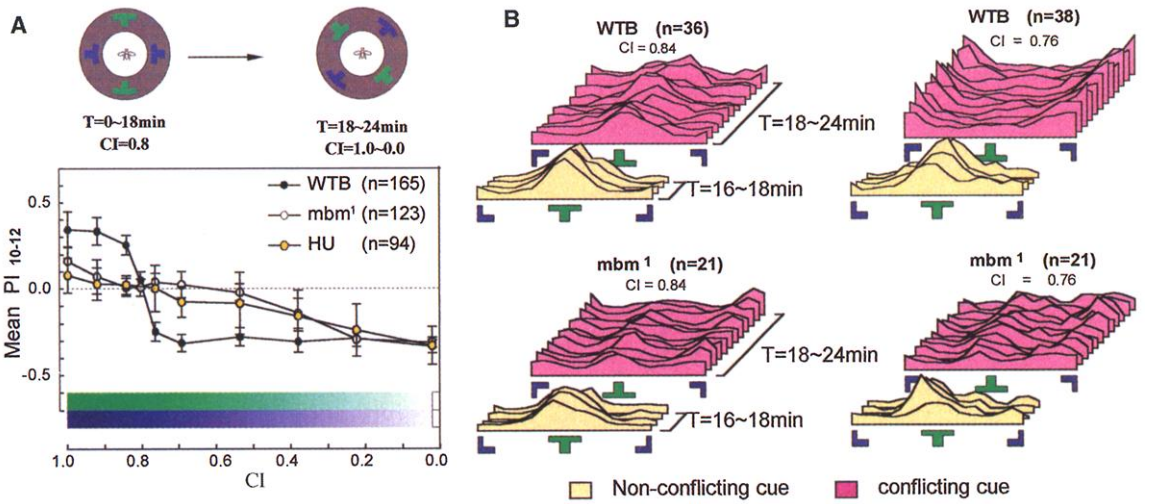

Fig. 3. Choice behaviors of WTB, *mbm¹*, and HU-treated flies facing the "color/shape dilemma." (**A**) The flies were trained at CI = 0.8 to prefer a green upright T over a blue inverted T, but were presented with conflicting cues (green inverted T and blue upright T) during the posttraining session over the entire range of CI values (1.00 to 0.00). Data points represent mean PI_{10-12} values (SEM; n = 10 to 38 for each point). (**B**) Angular histograms during posttraining sessions for WTB and *mbm¹* flies. The flies were presented with original nonconflicting cues during the first posttraining test session (T = 16 to 18 min), followed by conflicting cues in subsequent test sessions (T = 18 to 24 min). Data belong to the same set as shown in (A).

cue when the color intensity dropped below CI = 0.80. These WTB flies exhibited a sharp and complete transition in flight behavior within a narrow range of CI values from 0.84 to 0.76. Beyond the transition zone, the mean PI_{10-12} exhibited a relatively constant positive or negative value, suggesting stable behavior in accordance with color or shape cue, respectively. The existence of a discrete transition point in the flight behavior suggests that the fly can make firm and stable choices on the basis of small differences in the relative salience of competitive cues.

To gain insight into the underlying neural mechanism of choice behavior in *Drosophila*, we have examined the role of mushroom bodies (MBs), structures proposed to endow the insect with a degree of "free will" or "intelligent control" over instinctive actions (*11*). MBs are involved in multimodal sensory processing (*12*) and context generalization (*5*), and so may be involved in making choices. We found that *mbm¹* mutant flies, which have miniature MBs, exhibited a choice behavior distinctly different from that of WTB flies (Fig. 3A). There was no sharp transition in flight behavior of *mbm¹* flies as CI was gradually reduced (Fig. 3A), indicating indecisive choice-making over a wide range of color intensity (CI = 0.92 to 0.52) when conflicting cues were presented. Similar results were observed for flies in which the MB neuroblasts were ablated by applying the cytostatic drug hydroxyurea (HU) during the early first larval instar (*13*) (Fig. 3A). The absence of MBs in HU-treated flies [P-GAL4 line OK107 crossed to UAS-GFP (*14*)] was confirmed by fluorescence microscopy. The flight path angular histograms of *mbm¹* flies during the posttraining test (T = 18 to 24 min) with conflicting cues showed equally strong preference for the color and shape cues (Fig. 3B), which suggests that the flies failed to make a firm choice between two alternatives. A posttraining test (T = 16 to 18 min) with the original nonconflicting cues showed that *mbm¹* flies exhibited normal learning and memory (Fig. 3B).

The difference in choice behavior was not due to any difference in color sensitivity between WTB and *mbm¹* flies. When the flies were trained with double cues but tested after training with only the color cue, the resulting PI_{10-12} showed a gradual reduction for tests with CI = 1.0 to 0, with no difference between WTB and *mbm¹* flies (Fig. 2F). Furthermore, over the critical range of CI = 1.0 to 0.6, both WTB and *mbm¹* flies showed normal flight behavior based on the color cue, indicating that the difference in color sensitivity cannot account for the difference in choice behavior.

In summary, we have shown that (i) flies can make discrete and firm choices among behavioral alternatives on the basis of small differences in the saliency of visual cues; (ii) the behavioral choice is not based on overshadowing or selective visual attention, but is due to a process that can assess the relative saliency of conflicting visual cues; and (iii) MBs are the likely site for such choice behavior. Hence, our results support the notion that the core function of MBs in insects is to mediate intelligent behavior (*11*).

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6. The flight simulator and the operant conditioning procedure have been described (*15–17*). A single fly was fixed to a torque meter to measure its yaw torque, placed in the center of a vertical rotating arena (which was illuminated from behind), and allowed to control the angular velocity of the arena with its own yaw torque by a negative feedback mechanism. This arrangement allows the tethered fly to stabilize and choose its flight orientation with respect to the arena by adjusting its yaw torque. The angular position (i.e., the fly's flight orientation with respect to the visual landmarks) of the arena was detected and stored continuously in a computer for later evaluation of the flight behavior. The arena was divided into four quadrants with the visual patterns at their respective centers. For conditioning, a beam of infrared light, controlled by a computer-driven electric shutter, was directed at the fly as an instantaneous source of heat whenever the fly was heading into a quadrant with a particular pattern for association, and was intercepted when the fly oriented toward a different pattern not for association. During the tests before and after the training, the heat was switched off. Measurements of flight behavior were normally made during three sessions: The pretraining session comprised three consecutive 2-min test blocks during which the fly flew in a closed loop without heat application. The standard training session consisted of two consecutive 4-min training blocks spaced by one 2-min test block. The posttraining test session consisted of four consecutive 2-min test blocks during which heat was switched off and the trained fly was tested with either original cues or altered cues, or both. Flight behavior was evaluated by the preference index, defined as $PI = (t_1 - t_2)/(t_1 + t_2)$, with t_1 and t_2 indicating the time the fly spent fixating to the quadrant without heat and with heat application, respectively.
7. The color T-patterns were printed on inkjet transparency films with a color printer (Epson PHOTO710). The transmission of color films was calibrated quantitatively with a spectrophotometer (Cary 5E). For convenience, we have defined a color intensity (CI) scale from 1 to 0, which corresponds linearly to color brightness (CB) from 0.5 to 1.
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Rapid Increase in Clusters of Presynaptic Proteins at Onset of Long-Lasting Potentiation

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A change in the efficiency of synaptic communication between neurons is thought to underlie learning. Consistent with recent studies of such changes, we find that long-lasting potentiation of synaptic transmission between cultured hippocampal neurons is accompanied by an increase in the number of clusters of postsynaptic glutamate receptors containing the subunit GluR1. In addition, potentiation is accompanied by a rapid and long-lasting increase in the number of clusters of the presynaptic protein synaptophysin and the number of sites at which synaptophysin and GluR1 are colocalized. These results suggest that potentiation involves rapid coordinate changes in the distribution of proteins in the presynaptic neuron as well as the postsynaptic neuron.

Although there is consensus that the induction of plasticity in the CA1 region of the hippocampus usually involves Ca^{2+} influx through postsynaptic *N*-methyl-D-aspartate (NMDA) receptor channels, there is less agreement about the expression of the plasticity (*1–3*). Recent studies have provided strong support for postsynaptic mechanisms (*4*), including changes in the surface membrane expression of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) glutamate receptors in synaptic clusters or "puncta" that label for GluR1 (*4–7*). However, there is also evidence that long-lasting potentiation may involve presynaptic mechanisms as well (*1–3*). To investigate such presynaptic changes, we examined immunoreactivity for GluR1 and a presynaptic vesicle-associated protein, synaptophysin, in cultured hippocampal neurons.

Hippocampal neurons in culture can undergo activity-dependent long-lasting synaptic plasticity with many of the key features of long-term potentiation (LTP) or long-term depression (LTD) in the CA1 region of hippocampus in slices or in vivo (*5, 8–11*). Application of glutamate to the cultures can also produce long-lasting potentiation or depression (*6, 12*). Brief (~1 min) application of glutamate in 0 Mg^{2+} saline (to allow opening of NMDA receptor channels) produced a rapid and long-lasting increase in the amplitude of excitatory postsynaptic currents (EPSCs) at synapses between pairs of hippocampal neurons in dissociated cell culture (*13*) (Fig. 1A). Glutamate also produced a rapid and long-lasting increase in the frequency of spontaneous miniature EPSCs (mEPSCs) (Fig. 1B) with no change in their amplitude (average 5 to 30 min after glutamate, 102 ± 6% before glutamate; average before glutamate, 8.8 ± 0.3 pA). Furthermore, the glutamate-induced increase in mEPSC frequency was blocked when the bath solution contained the NMDA receptor antagonist D-aminophosphovalerate (D-APV), consistent with the idea that glutamate acts by stimulating postsynaptic NMDA receptors.

Brief application of glutamate also produced an increase in the number of GluR1-immunoreactive (IR) puncta in cultures fixed 5 min after the glutamate application, compared to control cultures from the same batch that were fixed 5 min after saline application (average from all such experiments, 166 ± 18% of control, n = 26 and 25 dishes, t(49) = 3.45, $P < 0.01$) [*S1* (*14*)] (Fig. 2, A and B). Although the size and fluorescence intensity of the GluR1 puncta varied considerably, there was no difference in these parameters between control and glutamate-treated cultures (intensity, 96 ± 2% of control; size, 101 ± 2%, control average = 4.2 ± 0.2 μm^2), suggesting that the increase in number was not due to an overall shift in size or intensity that made more puncta detectable.

We double-labeled the cultures with an antibody against synaptophysin, a vesicle-associated protein used as a presynaptic marker. In control cultures, some but not all (32.7 ± 2.2%) of the synaptophysin puncta colocalized with GluR1 puncta (appearing as yellow or, more often, as adjacent red and green puncta) and therefore might participate in functional glutamatergic synapses. The remaining synaptophysin-IR puncta may form synapses with other types of receptors or may be nonsynaptic. As for GluR1-puncta, brief exposure to glutamate produced increases in the number of synaptophysin-IR puncta [204 ± 17%, t(49) = 5.93, $P < 0.001$] and sites where synaptophysin and GluR1 were colocalized [282 ± 46%, t(49) = 3.81, $P < 0.001$]. Moreover, for both GluR1-IR and synaptophysin-IR puncta, glutamate produced a greater increase in the number of puncta that were colocalized than puncta that were not [GluR1, 275 ± 44% versus 143 ± 15%, t(25) = 3.99, $P < 0.001$; synaptophysin, 288 ± 46% versus 174 ± 11%, t(25) = 2.84, $P < 0.01$] (*S2*). Like GluR1-IR, there was no change in the intensity (97 ± 2% of control) or size (104 ± 3%, control average = 4.5 ± 0.2 μm^2) of the synaptophysin-IR puncta.

The glutamate-induced increases in the number of synaptophysin-IR puncta, GluR1-IR puncta, and sites where they were colocalized were all blocked by the NMDA antagonists D-APV (Fig. 2, A and B) or MK801 [100 μM; synaptophysin, 159 ± 13% versus 99 ± 7%, n = 5 and 6, t(9) = 4.43, $P < 0.01$; GluR1, 126 ± 34% versus 89 ± 10%; colocalized, 163 ± 24% versus 91 ± 16%, t(9) = 2.60, $P < 0.05$]. These results suggest that, like the potentiation of mEPSC frequency, the increase in synaptophysin-IR puncta may depend on activation of postsynaptic NMDA receptors. Furthermore, like the potentiation, the increase in the number of synaptophysin-IR puncta was maintained at nearly the same level 30 min after the glutamate application [192 ± 23% of control, n = 6 and 5, t(9) = 2.98, $P < 0.05$], consistent with a role in maintenance of the potentiation (*S3*).

To verify that these changes were not particular to the pre- and postsynaptic markers examined, we also demonstrated that brief application of glutamate produced increases in the number of puncta that labeled for the presynaptic proteins synapsin I (Fig. 2, C and D) and

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