18. The IGF-I values obtained by the ELISA were highly correlated (Pearson $r = 0.97$) with values obtained by radioimmunoassay after acid chromatography. All assays were carried out in a blinded manner, and quality control samples were included within assay runs. Average intra-assay coefficients of variation for IGF-I, IGF-II, and IGFBP-3 were 4.9%, 3.0%, and 9.0%, respectively.

19. To evaluate the effect of our blood collection methods on IGF-I levels, we compared IGF-I and IGFBP-3 levels in blood samples that were processed and serum frozen immediately after venipuncture (the usual collection and processing methods) to samples that were stored as heparinized whole blood for 24 and 36 hours before processing (mimicking our collection conditions). The mean IGF-I and IGFBP-3 values were almost identical and the interclass correlations between results of the two collection methods were 0.98 for IGF-I and 0.96 for IGFBP-3, indicating that our collection methods did not adversely affect sample integrity.

20. To examine how well a single measurement of IGF-I represents levels over time, we collected two blood samples each from 16 people, 8 weeks apart (time 1 and time 2). The correlation between blood levels taken at time 1 and time 2 was 0.65.

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The Role of Visual Experience in the Development of Columns in Cat Visual Cortex

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The role of experience in the development of the cerebral cortex has long been controversial. Patterned visual experience in the cat begins when the eyes open about a week after birth. Cortical maps for orientation and ocular dominance in the primary visual cortex of cats were found to be present by 2 weeks. Early pattern vision appeared unimportant because these cortical maps developed identically until nearly 3 weeks of age, whether or not the eyes were open. The naïve maps were powerfully dominated by the contralateral eye, and experience was needed for responses to the other eye to become strong, a process unlikely to be strictly Hebbian. With continued visual deprivation, responses to both eyes deteriorated, with a time course parallel to the well-known critical period of cortical plasticity. The basic structure of cortical maps is therefore innate, but experience is essential for specific features of these maps, as well as for maintaining the responsiveness and selectivity of cortical neurons.

Many investigations of the role of experience in the development of the brain's representation of the external world have focused on the visual cortex, where there is abundant evidence that neural activity plays a role in organizing the connections among neurons during normal development, and where rapid plasticity can easily be induced during a critical period in early life (1). In the adult visual cortex, single cells are selective for the orientation of the edges of objects in the world, and they commonly respond to stimulation of one eye better than to stimulation of the other (2). These cells are organized into columns aligned perpendicular to the surface of the cortex, and the columns are arranged into maps. Within a column, cells have similar optimal stimuli, and the preferred stimulus for neighboring columns typically changes gradually and progressively across the cortex.

We studied the development of maps for orientation and ocular dominance (eye preference) using imaging of intrinsic optical signals (3) and single-unit microelectrode recordings in the primary visual cortex of normal and binocularly deprived (BD) cats. BD cats were deprived of patterned visual experience by bilateral lid suture from before the time of eye opening. In both normal and BD cats, maps of stimulus orientation (Fig. 1) formed before the end of the second postnatal week (4). Nine microelectrode penetrations in four cats at postnatal day 15 (P15) or younger confirmed that single neurons were selective for the orientation depicted in the optical maps, even at these early ages. Independent stimulation of the left and right eyes produced similar maps of preferred orientation—for example, the similar sequences of colors from head to tail along the white arrows drawn at the same position on the two eyes' orientation maps in a normal cat at P14 (Fig. 1A) and in a BD cat at P19

(Fig. 1B). Thus, consistent with conclusions drawn from earlier studies (5), the visual cortex contains a single orientation map common to the two eyes even when pattern vision is prevented during development.

A measure of the similarity between the orientation maps resulting from stimulation of the two eyes (6) is plotted as a function of age for both normal and BD cats in Fig. 1C. During the first 3 weeks of life, findings in normal and BD cats are indistinguishable from one another, and the two eyes' maps become increasingly similar (similarity greater than randomized in both normals and BDs, $P < 0.001$). At about P21, visual experience begins to make a difference (similarity greater in normals than in BDs, $P < 0.0001$). At this time, maps through the two eyes in normal cats become and remain nearly identical to one another, whereas in the BD cats, maps begin to deteriorate and the two eyes' maps become less similar (Fig. 1D).

A striking feature of the responses in all the young cats, which persists in the BD cats, is that responses to stimulation of the eye contralateral to the hemisphere under study are markedly stronger and more selective than are responses to stimulation of the ipsilateral eye. This difference between the two eyes is apparent in Fig. 1A and Fig. 2A shows it is also true for the other hemisphere of the same cat. The ipsilateral eye responses increase and become nearly identical to those through the other eye in normal cats at about 3 weeks of age (Fig. 2B), and they continue to become stronger thereafter (Fig. 2C). The asymmetry between responses to the two eyes is also present in young BD cats (Fig. 2D), and its persistence is apparent in the BD cases shown in Figs. 1B and 2E.

In normal cats, the bias toward the contralateral eye measured from the optical maps (7) was greatest during the first 3 weeks (Fig. 3D) and became much smaller thereafter. In the BD cats, however, the bias persisted. Microelectrode recordings con-

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firmed the strong bias toward the contralateral eye in both normal and BD young cats [Fig. 3E, consistent with previous results (8–10)]. The more balanced nature of the adult cortical response is well established in the literature (2).

The role of visual experience is demonstrated further by changes in orientation selectivity during development. In both normal and BD cats, selectivity increased during the first 3 weeks of life (Fig. 3A). For this measure also, patterned visual experience begins to make a difference during the fourth week of life, when the selectivity in BD cats begins to deteriorate. By the seventh week of life, responses in BD cats were weak and poorly selective (Figs. 2F and 3A). These findings show that pattern vision is required for the maintenance of orientation selectivity rather than for the initial development of the orientation map (11).

The measurement of selectivity also clearly revealed the differences between responses to stimulation of the two eyes. In young normal cats, maps through the ipsilateral eye were much less selective until P21 ($P < 0.0001$), when they caught up to and remained as selective as those through the other eye (Fig. 3B, $P > 0.1$). BD cats were similar to normals until P21 ($P > 0.3$), but maps through the ipsilateral eye never improved to equal those through the other eye (Fig. 3C, $P < 0.01$), and both deteriorated between the fourth and seventh weeks.

The period of life during which the maps deteriorate in the absence of visual experience corresponds closely to the well-known "critical period" of susceptibility to the effects of unilateral eye closure (12, 13). During this critical period, even monocular deprivations of brief duration can cause an acute loss of responses to and input from the deprived eye (12–15). This coincidence provides evidence that the critical period is a time during which visual experience helps to organize the cortex in normal development.

Anatomical inputs representing the two eyes are thought initially to make intermingled functional connections in layer IV before the beginning of the third week of life (10). Segregation of the inputs into ocular dominance columns is first reported to be evident anatomically at P22 (10), and blocking retinal activity beginning before P16 prevented this segregation (16). It was therefore surprising that the optical maps obtained at even 2 weeks of age showed a pattern of alternating eye preference reminiscent of ocular dominance columns (Fig. 4) (17), despite the clear overall dominance of contralateral responses. Microelectrode penetrations targeted into areas seen in the optical maps to be most strongly biased

toward one eye or the other confirmed that these patterns accurately portrayed the organization of cortical responses. In all penetrations targeted into areas most strongly dominated by the contralateral eye, ipsilateral eye responses were absent or weak. In contrast, all penetrations targeted into areas of strongest optical response to the ipsilateral eye revealed neurons dominated by or responding well to the ipsilateral eye. The summary of targeted penetrations in four cats at P12, P14, P14, and P15 shows that responses to the two eyes in the cortex have the periodic pattern evident in the optical map (Fig. 4D), a full week before anatomical studies have indicated the existence of a segregation of inputs from the two eyes.

The picture that emerges from our experiments is of a young cortex in which contralateral eye input is everywhere, perhaps as a result of the earlier development of the contralateral visual pathways (18), but ipsilateral eye input is, at least functionally, patchy. Although we know that the arbors of the individual geniculate cells that convey visual information to the cortex are widespread and generally sparse up to P22 (19), newer anatomical techniques may reveal that inputs from many geniculate cells serving the ipsilateral eye are clustered together as early as P14. Alternatively, an organization of physiological responses among the cortical cells might account for the pattern of alternating eye preference without such
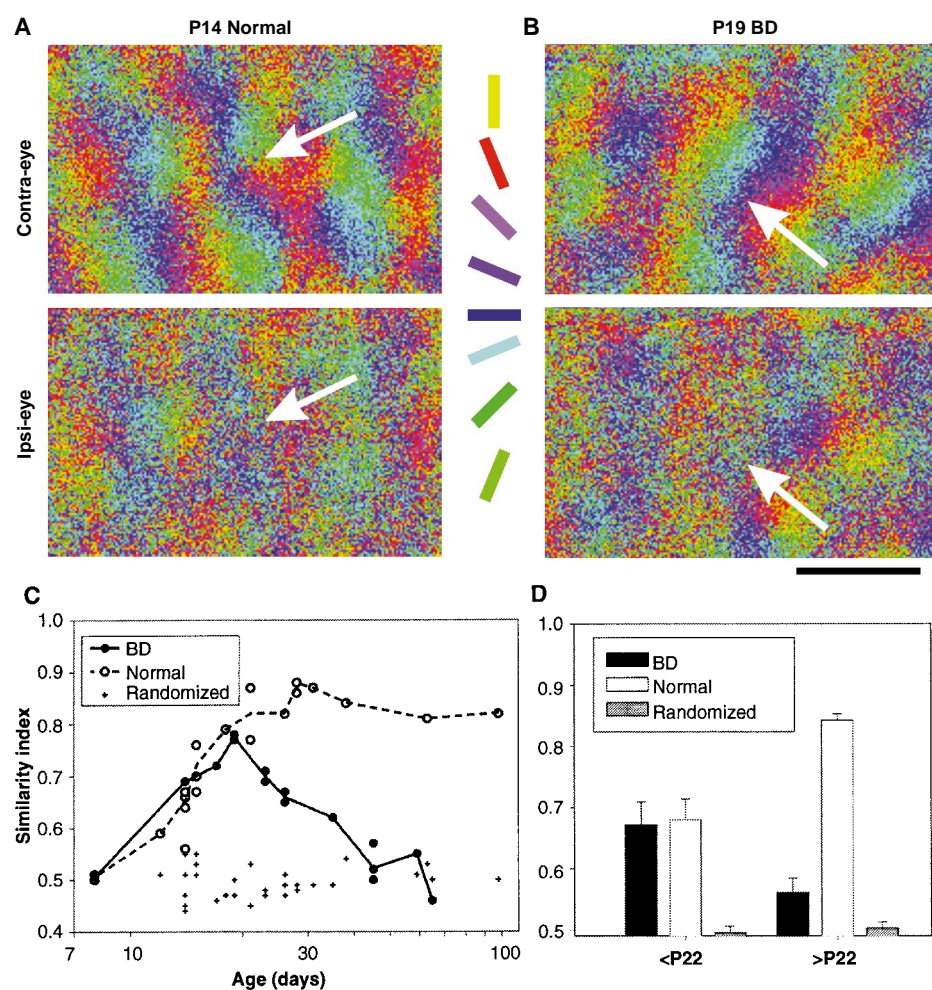

Fig. 1. Maps of stimulus orientation match between the two eyes even when cats are reared without patterned visual experience. (**A**) Orientation maps, obtained separately by stimulation of the contralateral or ipsilateral eye, from a young (P14) cat with normal visual experience. Color of each pixel indicates stimulus orientation producing the greatest optical response; colored bars show scale. (**B**) Orientation maps from the two eyes of a young (P19) BD cat. White arrows in (A) and (B) are drawn at the same location in both eyes' maps. Scale bar (black), 1 mm. (**C**) The similarity between orientation maps from the two eyes, as measured by an index (23) for which two unrelated maps would have a similarity of 0.5 and identical maps would have a similarity of 1.0. The abscissa is shown on a logarithmic scale. Significance is indicated by comparison with randomized data, computed from the real data by rotating the orientation map from one eye by 180°. (**D**) Summary of the similarity indices from all normal cats (20 hemispheres from 15 cats) and BD cats (15 hemispheres from 10 cats), grouped by age and rearing condition, compared with the randomized data.

early clustering of thalamocortical inputs. Results from two other experiments support the conclusion that upper layer cortical or-ganization may precede and guide rather than follow thalamocortical organization. Monocular deprivation for 1 to 2 days at the peak of the critical period causes a marked reorganization of cortical responses to the two eyes, which foreshadows the major ana-
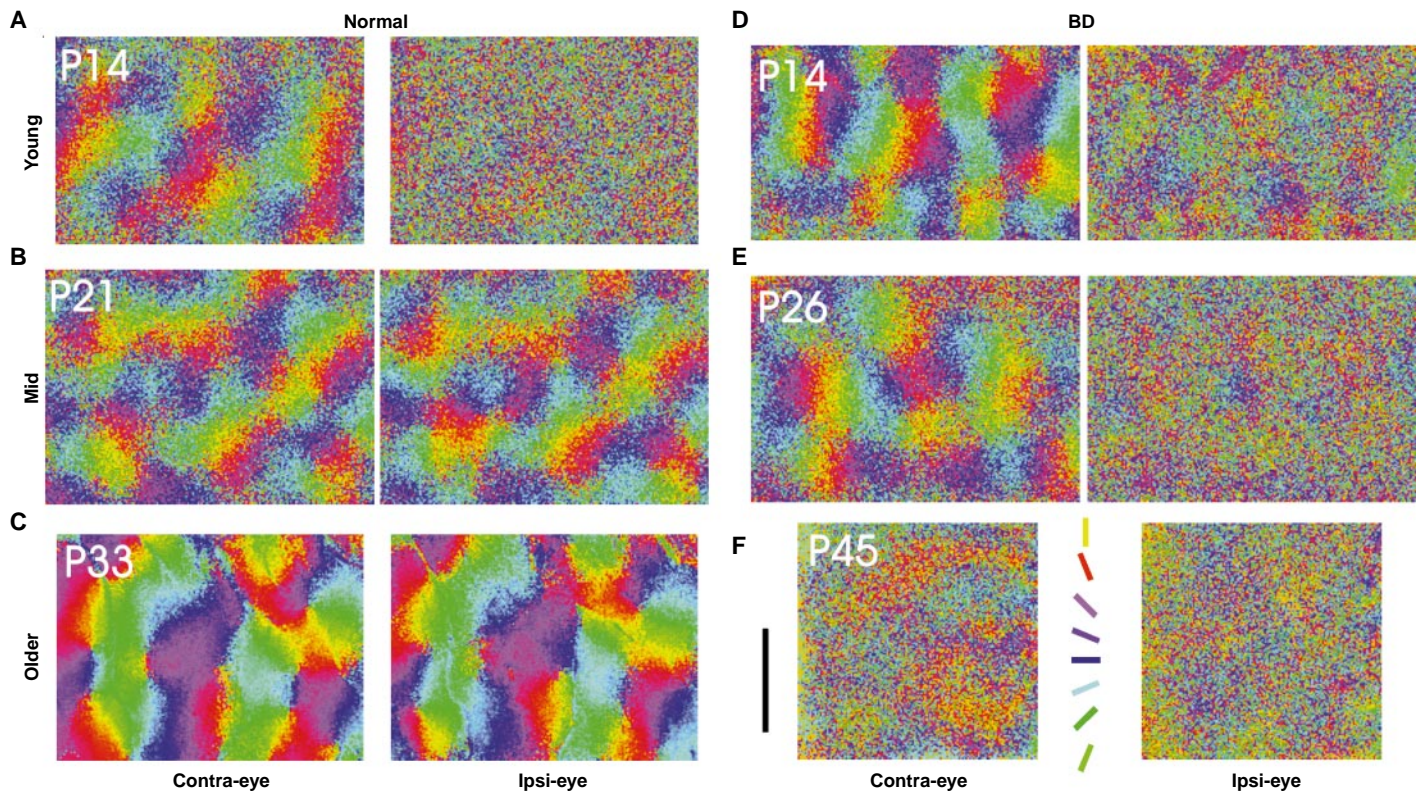

Fig. 2. Development of orientation maps in normal and BD cats. (**A**) Maps shown are from the opposite hemisphere of the cat depicted in Fig. 1A. (**B**) Maps from a slightly older normal cat are similar in the two eyes, and they become more selective (**C**) by the end of the first postnatal month. (**D**) Orientation maps in a young BD cat. (**E**) Unlike normals, the initial contralat-eral bias persists in BD cats, until in old BD cats (**F**), the orientation map deteriorates significantly and is difficult to detect. Nonetheless, ocular dom-inance ratio maps from such older BD cats show a strong pattern (*26*), indicating that responses are present but are not orientation selective. Scale bar (black), 1 mm.
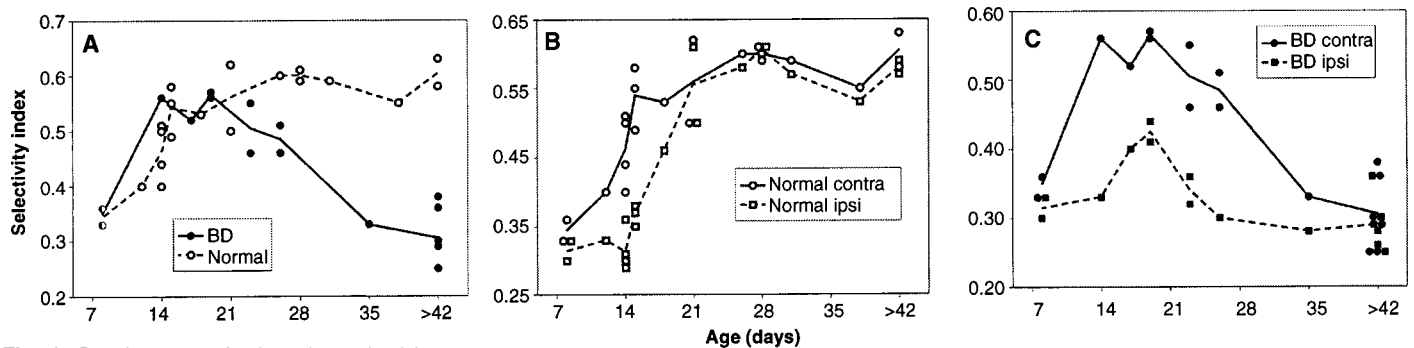
Fig. 3. Development of orientation selectivity and elimination of contralateral bias. (**A**) Comparison of orientation selectivity as a function of age for the contralateral eye of cats reared under normal and BD conditions. Selectivity is mea-sured using an index of orientation tuning (*17*). (**B** and **C**) Development of orientation selectivity for contralateral and ipsilateral eyes of normal (B) and BD (C) cats. (**D**) Eye dominance index (*7*) in normal and BD cats at young and older ages. The difference between normal and BD cats is significant at the older ages ($P < 0.0001$). (**E**) Single-unit responses in young normal and BD cats also show a strong contralateral bias. Re-sponses from five cats in the present study and three from an earlier study (*16*) are summarized.
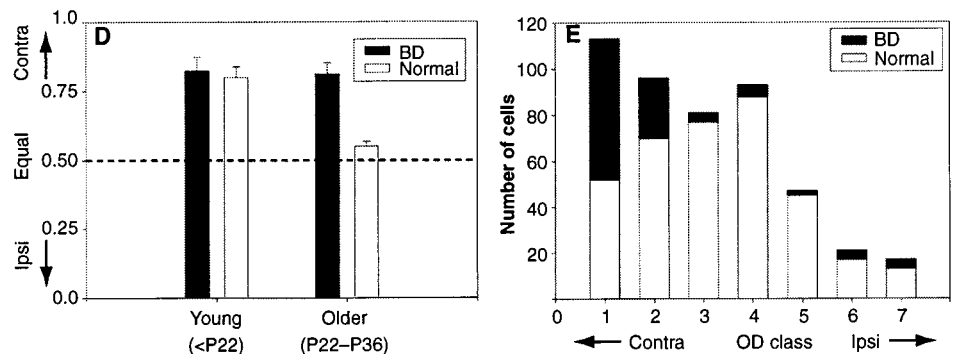
tomical reorganization of thalamocortical inputs that soon follows (*14*, *20*). The patchy corticocortical connections that connect columns of similar orientation preference in adult cats form in the ferret nearly a week before orientation selectivity, and orientation columns are evident (*21–23*).

Models of the development of orientation and ocular dominance columns have conventionally assumed that the two eyes' inputs to the cortex start out in a diffuse pattern but are of more or less equal strength (*24*). Our findings have two implications for such models. First, segregation of ocular dominance columns takes place from an initial condition of overall contralateral eye dominance rather than equality. It is difficult to understand how a purely Hebbian mechanism can account for both the segregation and the equalization of the two eyes' inputs. Second, the early contralateral dominance provides a natural explanation

for the presence of a single set of orientation columns that comes to be shared by the two eyes, because the template for the orientation columns has been established before responses to the ipsilateral eye become strong. In essence, the ipsilateral eye may just go along for the ride, perhaps sustained by waves of spontaneous activity (*25*), until the time at which patterned visual experience is necessary for continued normal development of the orientation maps. Interestingly, the ocular dominance pattern persists despite the devastating effects of continued binocular deprivation (*16*, *26*). These findings will be challenging to incorporate into a unified formal model rich enough to exhibit the segregation, equalization, and matching of the two eyes' orientation maps.

Our findings widen the distinction between the roles of neural activity and sensory experience. Neural activity in the visual sys-

tem has recently been shown to be necessary for a number of processes that take place in utero or before eye opening (*21*, *22*, *27*), when visual experience is not possible; now we see that patterned visual experience may play no role for a time in development even when it is present. These observations support the hypothesis that similar processes may underlie the development of the cortex in animals born at such different stages of maturity of the visual system as ferret, cat, monkey, and human.

Our findings, while consistent with many earlier observations (*5*, *8*, *15*, *28*), provide an overall view of the interaction between innate and experiential factors in the development of the cortex. Pattern vision appears to play no role in the establishment of the positions of the orientation and ocular dominance columns in the visual cortex. Orientation columns emerge, independent of patterned visual experience, during the second postnatal week, and patches of ipsilateral eye responses appear by early in the third week. Experience is then essential for the next stage, in which responses to the two eyes become strong, more selective, and nearly equal by the beginning of the fourth week. With continued binocular deprivation, ipsilateral eye responses never become strong or very selective. Experience remains necessary for the maintenance of responsiveness and selectivity, which in its absence degrade over the course of the well-known critical period. Other cortical areas may have similar sequences of experience-independent and experience-dependent development (*29*).
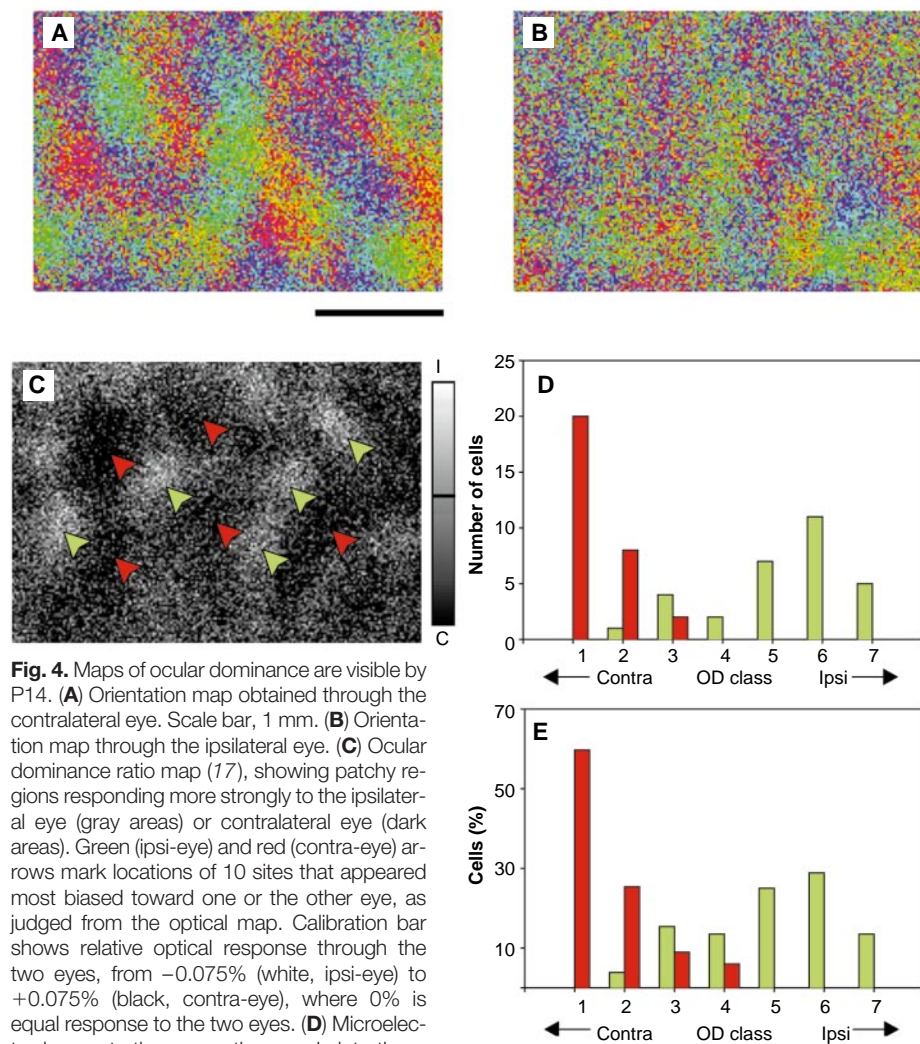
Fig. 4. Maps of ocular dominance are visible by P14. (**A**) Orientation map obtained through the contralateral eye. Scale bar, 1 mm. (**B**) Orientation map through the ipsilateral eye. (**C**) Ocular dominance ratio map (*17*), showing patchy regions responding more strongly to the ipsilateral eye (gray areas) or contralateral eye (dark areas). Green (ipsi-eye) and red (contra-eye) arrows mark locations of 10 sites that appeared most biased toward one or the other eye, as judged from the optical map. Calibration bar shows relative optical response through the two eyes, from −0.075% (white, ipsi-eye) to +0.075% (black, contra-eye), where 0% is equal response to the two eyes. (**D**) Microelectrode penetrations were then made into these sites without knowledge of the eye preference in the optical maps. Histograms show ocular dominance of single units (six cells per penetration) assessed on the conventional 1-to-7 scale (*2*), grouped by eye preference from the ocular dominance map [(colors as in (C)]. (**E**) Summary of similar targeted penetrations made in four cats, age P15 and younger ($n = 52$ cells for ipsi-targeted, $n = 67$ for contra-targeted).

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7. An index of eye dominance ranging from 0 (exclusively ipsilateral) to 1 (exclusively contralateral) was computed from each pixel of the unfiltered, blank screen–normalized images by comparing the response strengths to stimulation of the two eyes at the orientation producing the greatest response. This index is

derived from the procedure used to determine ocular dominance in single-unit recordings, where the responses to optimal stimuli in the two eyes are compared. It is different from the conventional ocular dominance ratio maps (17), which compare the average rather than the optimal responses; such maps can show an ocular dominance pattern even when orientation maps are poor (Fig. 2F) or when one eye is dominant [see Fig. 4C or figure 1 in (14)].

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Inhibitory Cerebello-Olivary Projections and Blocking Effect in Classical Conditioning

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The behavioral phenomenon of blocking indicates that the informational relationship between the conditioned stimulus and the unconditioned stimulus is essential in classical conditioning. The eyeblink conditioning paradigm is used to describe a neural mechanism that mediates blocking. Disrupting inhibition of the inferior olive, a structure that conveys unconditioned stimulus information (airpuff) to the cerebellum prevented blocking in rabbits. Recordings of cerebellar neuronal activity show that the inferior olive input to the cerebellum becomes suppressed as learning occurs. These results suggest that the inferior olive becomes functionally inhibited by the cerebellum during conditioning, and that this negative feedback process might be the neural mechanism mediating blocking.

Current thinking about associative learning has been profoundly influenced by the phenomenon of blocking initially reported by L. J. Kamin in 1968 (1). In a typical blocking experiment, one conditioned stimulus (CS) ("A") is first extensively paired with an unconditioned stimulus (US) (A−US). Then a second CS ("B") under-

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goes a compound conditioning with A and the same US (AB−US). Later, when B is tested, virtually no (or very little) conditioning has occurred to B. However, if A was previously not (or weakly) conditioned with the US, then B (as well as A) accrues substantial associative strength during the compound conditioning phase. Thus, conditioning to B during the compound conditioning is inversely proportional to the magnitude of previous conditioning to A. The blocking effect suggests that if a US is already fully predicted by one stimulus, and if the addition of a new stimulus provides no new information about the US, then the

US will not activate or support the learning process responsible for establishing a new CS-US association (2).

Although blocking has been examined extensively at the behavioral level (3) and several neural models (4) have been proposed, one or more underlying neural mechanisms have yet to be identified. Because the essential neural circuitry involved in classical conditioning of eyeblink or nictitating membrane response in the rabbit has been well characterized (5, 6), this paradigm is ideal for examining the blocking phenomenon at the neuronal level (Fig. 1). Typically, eyeblink conditioning occurs when a CS (for example, tones or lights) is paired with a US (for example, airpuffs), which elicits an unconditioned response (UR; a reflexive eyelid closure). Through CS-US association formation, the animal learns to exhibit a conditioned response (CR) to the CS that mimics the UR, precedes the US in onset time, and peaks at about the time of US onset.

Several lines of evidence suggest that the inferior olive provides the "reinforcing" US input to the cerebellum, which supports eyeblink conditioning (7–11). For example, eyeblink conditioning will develop to a tone CS when using inferior olive stimulation as the US (instead of a peripheral US) (8), and lesioning the inferior olive in previously trained animals results in extinction (9, 10) or abolition (11) of CRs with continued CS-US presentations. An interesting property of neurons in the inferior olive is that they show evoked neural activity to the airpuff US (12) and the periorbital stimulation US (13) during the initial stage of CS-US training (before the animal exhibits any CRs), but not when the animals perform CRs during CS-US trials.

Employing a single-unit recording technique, we examined the complex spike responses of cerebellar Purkinje cells (which receive climbing fiber inputs from the inferior olive) (14) over the course of the behavioral training (15, 16). Recordings were centered over Larsell's HVI, because many Purkinje neurons in this region respond to CS and US presentations (5) and because HVI is importantly involved in eyeblink conditioning (6). Of the 54 cells recorded in rabbits, 31 were recorded in lobule HVI, 12 in anterior lobe (HV), 6 in HVIIA (crus I), and 5 in paramedian lobule (Fig. 2). Sixteen of these cells (10 in HVI, 5 in anterior lobe, 1 in HVIIA) exhibited specific evoked complex spike activity in response to US onset during US-only trials. Of these, 5 were recorded early in training (before animals performed any CRs) and also responded to the US with complex spikes on paired trials, whereas 11 were recorded in trained (CR-performing) animals and did not respond to