labeled cells were also seen closely attached to the exposed surfaces of the cerebellums of the hosts. In the interior of the cerebellar cortex, however, labeled cells were not seen at this time, which indicated that free [^{3}H]thymidine was not available to the host tissue. In the rats that survived for 2 to 4 days after surgery the transplanted tissue was still identifiable. The following observations were made in this tissue. Extensive regions in the transplants were full of degenerated nerve cells and were characterized by intense phagocytic activity. In these regions very few intensely labeled cells were seen. Smaller isolated regions that appeared normal were seen and, in these, tagged cells with intense to moderate accumulation of label were present. Furthermore, in some regions of the transplants large numbers of streaming, spindle-shaped migratory cells were seen (Fig. 2). In these streams of migrating cells a few were intensely labeled, some lightly labeled, and many were unlabeled. Judging from the varying patterns of labeling over these cells and their intimate relationship with the surviving external granular layer of the transplants, we assumed that they represented an extension of the external granular layer of the transplants and that many of these had multiplied a number of times. Such streams of migrating cells, which followed tortuous courses, appeared to penetrate the host tissues either through the medullary layer or through the external granular layer of the cerebellum of the hosts.

In the brains of the animals that survived for 10 to 16 days after surgery, there remained no trace of the transplanted tissue. However, close to the site of transplantation in many regions of the host cerebellum a varied number of well-labeled cells was seen in the internal granular layer and in the molecular layer (Figs. 3 and 4). In addition to these intensely and moderately labeled cells, a large number of lightly labeled cells were found in these regions. These observations suggested that some intensely labeled cells had proliferated after having migrated into the host tissues. Although the identity of the donor cells could not be determined when they were intensely labeled, examination of the lightly labeled cells suggested that the donor cells or their progeny became differentiated in the cerebellar cortex of the hosts into granule and basket cells.

These results indicated that transplantation of the precursors of neurons is possible in the maturing nervous system of postnatal mammals. At least two factors facilitated this process. First, the precursors of nerve cells of the donor tissue, because of their active migratory behavior, escaped from the local degenerative processes as they migrated into the host tissue. This observation suggested that the transplantation of harvested undifferentiated cells of the external granular layer devoid of other cerebellar tissue should further facilitate transplantation. Second, the ongoing neurogenetic processes in the host cerebellum, whereby the primitive cells that migrate in large numbers become incorporated into the evolving architecture of the cerebellar cortex, facilitates the acceptance of exogenous cells of similar properties. This consideration suggested that depletion of the local supply of competing migratory cells by x-irradiation (*6*) may further facilitate acceptance of the donor cells.

The uses of this technique in studying some of the principles of neurogenesis, particularly in mammals, must be obvious. For instance, it has been debated for some time whether the undifferentiated cells of the external granular layer are pluripotent and can differentiate into neurons as well as glia, and whether some inherent properties of the multiplying cells or the time of their origin and the milieu in which they differentiate determine whether they will become basket, stellate, or granule cells. Indeed, can a cell destined to become a granule cell in the cerebellar cortex become differentiated into a granule cell in the hippocampus with totally different chemical and morphological properties?

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Shift in Binocular Disparity Causes Compensatory Change in the Cortical Structure of Kittens

Abstract. *Kittens were raised with prisms in front of their eyes which introduced a vertical disparity. At 4 months of age the disparity necessary to maximally stimulate a sample of binocular cortical cells was determined. The distribution of optimal disparities was abnormal, and shifted in a direction which would tend to compensate for the prism-induced disparity.*

Ample evidence has been amassed that the environment exerts a degree of control over the development of the nervous system (*1–5*), making it necessary to suppose that activity in a nerve fiber can effect the functional connections made by that fiber. It has been proposed that an ingrowing axon tends to make contact with a cell when the activities in the axon and the target cell are correlated (*6*). Synapses already present are strengthened by the existence of such a correlation, while synapses that exhibit weak or no correlation between pre- and postsynaptic activity either decay or are displaced by axon terminals with higher correlations. Axon terminals which either alone or in concert with others exert a large degree of control over a postsynaptic neuron will tend to increase in their control to an even greater extent. As a result, axons with highly correlated activities among each other (such as those receiving inputs from nearly the same part of the visual field) will tend to

form and/or maintain contacts with common postsynaptic cells even in the absence of direct communications among the ingrowing axons, because the correlation between the activity of the postsynaptic neuron and each presynaptic terminal will be higher than if the activities of the presynaptic axons were uncorrelated. Below I report some results from an experiment designed to test the notion that correlation of activity among presynaptic endings determines permanent contacts on common postsynaptic cells in the cortex of the developing cat.

In reference to a normal cat's visual system one can speak of corresponding retinal points, regions in the two eyes which regard the same portion of the visual field, are stimulated with similar light intensities over time, and consequently have highly correlated activities in their neural projections. A careful determination of the binocular receptive fields of a sample of cortical cells in a cat indicates that the projection from corresponding points in the visual field is not exact: different cells are optimally stimulated by patterns having slightly different disparities between the two eyes (*7–11*). If vertical disparity is defined relative to a horizontal line joining the projections of a retinal landmark, such as the tops of the optic disks, the vertical disparity necessary to maximally stimulate a sample of cortical cells having receptive fields in the region of the visual axis has a mean of zero and a range of about $\pm 1.2°$. If a shift is imposed in retinal correspondence in kittens by placing deviation prisms before their eyes, a compensatory change in cortical structure might occur such that the distribution of disparities in a sample of binocularly driven cells would be abnormal. Such a change could only be ascribed to a change in the correlation of activity among axons.

Therefore, kittens were raised in such a manner that practically their entire visual experience consisted of viewing the environment through a pair of prisms which introduced either 2 or 4 prism diopters of vertical disparity between the two eyes (1 prism diopter $= 0.01$ radian $= 0.573°$). The vertical direction was chosen in preference to the horizontal for three reasons: the range of optimal disparities for cortical stimulation is smaller, the possibility of compensation by changes in relative eye position is smaller, and it is relatively easy to establish absolute vertical disparities in relation to retinal landmarks, while it is extremely difficult to do the same in the horizontal direction (*7, 8, 11*).

At 8 days of age the animals were placed in the dark except for a short exposure each day (approximately 2 minutes during cage cleaning). At 14 days the animals were anesthetized with sodium pentobarbital (30 mg/kg) supplemented with Xylocaine (Astra) infiltrated locally, and an implant of soft plastic, molded to conform to the shape of the skull, was sutured under the scalp. Two nylon screws, fixed in the implant, projected through the skin at the midline to serve as attachment points and alignment guides for the prisms. The prism "eyeglasses" were cut down from lightweight acrylic plastic ophthalmic prisms. They were drilled, tapped, and attached with screws to a thin sheet of phosphor bronze which had been bent and cut to conform approximately to the shape of the kitten's head and to position the prisms in front of the eyes. Two holes were drilled in the plate to fit onto the screws protruding through the scalp. The metal sheet holding the prisms was covered with a soft dental molding compound (Coe-Soft, Coe Laboratories, Chicago), fitted onto the head, allowed to set, removed, and trimmed. Thus each kitten had a custom-molded "cap" on which the prisms were mounted. The threads on the nylon screws in the scalp served largely to hold the cap in place, but this was supplemented by placing a coating of library paste on the underside of the cap and pressing it firmly onto the head. When necessary, a little water injected under the cap with a syringe served to loosen it adequately for removal. The result was an appliance which was well tolerated and which could, in general, remain on an active kitten for an hour or so but which, when stressed, would detach itself rather than tear the implant from the scalp.

The kittens were maintained in a light-tight, ventilated cage which was in turn placed in a darkroom. Each day the kittens were removed one at a time from the cage and the prisms were mounted before their eyes. Initially this operation was attempted under infrared light with an image-converter viewing system, but the method was abandoned when it became evident that accidental loss of the glasses during activity would account for a much greater time of exposure to light without the prisms than would the few seconds necessary to mount and demount the prisms in the first place. The animals were only allowed in the light when under the direct supervision of the experimenter, and prism glasses that were dropped were immediately replaced. The animals were allowed to play together with as little interference as possible while in the light, and the environment was "enriched" with various objects to play with or climb on. An average of 1 hour per day was spent in the light, and it is estimated that each animal spent at least 99.5 percent of that time viewing through the prisms.

Up until the kittens were about 8 weeks old their behavior did not alter when the prisms were accidentally dislodged. After this period, the exact duration of which varied from animal to animal, loss of the prism glasses caused an immediate cessation of activity, cowering, and freezing. This is interpreted as indicating that some sort of adaptation to the prisms had taken place, and had the additional virtue of making capture of the kitten and replacement of the prisms relatively easy. At 4 months of age each animal was tested for amblyopia by covering one eye at a time and observing its behavior during play, as well as testing visual placing reactions. Under these conditions the only deficit observed was a loss of accuracy in judging distances while jumping, which can be attributed to the use of monocular vision.

A chronic procedure was used to record data in order to maximize the ultimate cell sample size through repeated testing. The animals were given large doses (at least 5 mg/kg) of trifluomeprazine tranquilizer until they did not resist restraint or respond to pinching of an ear, maximally forceful separation of the toes, or other painful stimuli. Visual and locomotor activity were still in evidence, however. They were then anesthetized with fluothane, and the scalp, ear canals, infraorbital region, upper jaw, and the skin of the posterior portion of the hind legs were liberally infiltrated with a long-lasting local anesthetic (Zyljectin, Abbott). They were then placed in a stereotaxic instrument designed for research on the visual system. The scalp was incised, and a small craniotomy was performed over the projection of the visual axis to the area 17-18 border of the left hemisphere. The dura was reflected and the exposed cortex was covered with an agar-saline mixture. The lids were re-

tracted, pupils dilated, and corneas anesthetized by instilling 10 percent phenylephrine HCl, atropine sulfate, and Tetracaine (Tilden Yates) into the eyes. The corneas were covered with zero-power contact lenses and the eyes were retinoscopically refracted to the screen distance (73 cm) with supplementary lenses.

The animals were then given an initial dose of paralytic agent consisting of gallamine triethiodide (15 mg/kg) and *d*-tubocurarine (3 mg/kg) intraperitoneally. Upon cessation of breathing the anesthetic was discontinued, the trachea was intubated, and artificial respiration was begun. Heart rate was monitored continually by an auditory signal, and the electrocorticogram was occasionally checked by looking at the signal from the microelectrode without the spike filter in place. The heart rate was completely regular and the electrocorticogram showed occasional sleep patterns, indicating that even though

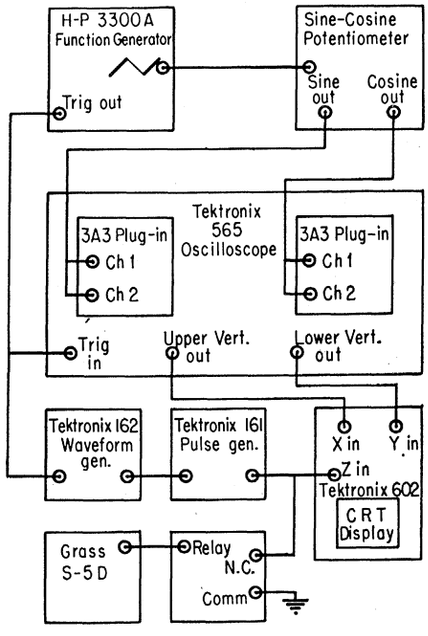

Fig. 1. Block diagram of apparatus which generates two independently adjustable lines for the determination of binocular receptive field positions. Sine-cosine potentiometer adjusts the angle of orientation of the lines by varying the relative amplitude of the triangle waves being fed to the *x* and *y* inputs. Electronic switches in the 3A3 plug-ins (set in the alternating trace mode) provide two traces, adjustable horizontally and vertically with the position controls on the preamps. The duration of the unblanking pulse from the 161 pulse generator determines the length of the line, while by varying the delay of the unblanking pulse the line segment can be shifted parallel to its axis. Grounding and momentarily ungrounding the *z*-axis input with a relay flashes the lines when desired.

not anesthetized in the conventional sense, the animals were experiencing no stress. Paralysis was maintained by hourly injections of gallamine (8 mg/kg) and curare (1 mg/kg) intramuscularly in the region of the hind leg previously infiltrated with local anesthetic. Each experiment was limited to 8 hours, after which the animals were anesthetized with sodium pentobarbital (20 mg/kg) and the wound was closed. Recovery was generally slow because of the potentiation of the barbiturate by the tranquilizer.

Single cell recordings were made with tungsten microelectrodes lowered into the cortex through the agar cover. Visual stimuli for determining the positions of cortical receptive fields were generated on a cathode ray tube (CRT) with a P-7 (blue) phosphor by the apparatus shown in Fig. 1. This device produced two lines of variable length but constant luminance (1.0 log footlambert against a background of −1.0 log footlambert) (1 footlambert = 1.076 mlam) which could be rotated to any angle of orientation. The positions of the two lines were independently adjustable, and the lines could be either presented continuously or flashed. The divergence of the visual axes which occurs under paralysis (*7*, *8*, *12*) allowed the two eyes to be stimulated separately by a single display. Occasionally a small-value deviation prism was placed before one eye to facilitate positioning of receptive fields within the area of the CRT face.

After the isolation of a cell and the confirmation of its singleness by observation on an oscilloscope, its activity was monitored by ear and the following procedure was carried out. First, the orientation preference of the cell was determined by moving a long line across the screen at various orientations. The line was then shortened, the length of the receptive field determined, and the length of the stimulus adjusted to fill the receptive field. Finally the line was located so as to maximally stimulate the cell through one eye. Next the second line (of the same length and orientation) was adjusted to maximally stimulate the cell through the other eye. The two lines were then flashed simultaneously and small adjustments were made in line position in an attempt to maximize the summated excitation of the cortical cell, which is a very sensitive indicator of the position of optimal disparity (*7*, *9*).

Cells for which binocular facilitation could not be clearly induced were accepted only if well-defined receptive fields could be found in each eye, in which case the stimulus lines were placed in the centers of the two response areas. These criteria for cell mapping were used to reduce the possibility of experimenter bias influencing the results.

As soon as the determination was completed, the projections of the tops of the optic disks were observed with an ophthalmoscope and marked on a Lucite screen which surrounded the CRT display and was inscribed with a 1-cm grid. The mean change in relative vertical position of the optic disks between each determination was 0.34°. In the event of a large change in relative disk position from the previous determination, the cell response was redetermined. The position of the lines on the CRT display was recorded photographically, and this information, together with the difference in elevation of the optic disks, allowed calculation of the vertical disparity of the cell (*7*, *8*). If the center of the receptive field of the right eye was above that of the left when corrected for eye position, the disparity was assigned a positive value.

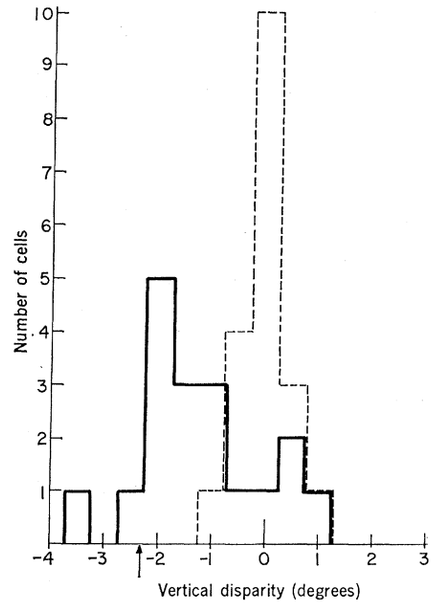

Fig. 2. The dashed line indicates the distribution of disparity sensitivities of 19 cells from the cortex of three normal cats. The solid line is the distribution of disparity sensitivities of 18 cells from the cortex of two cats raised with 4 prism diopters of vertical disparity between the two eyes. Arrow indicates the expected modal value of the test distribution (solid line) if complete compensation were to take place.

The results of some of these determinations are shown in Fig. 2. The dashed line represents 19 cells from three normal cats. The mean and range agree well with the more extensive determinations available in the literature (*7*, *8*, *11*). The solid line represents 18 cells from two cats that were raised with two 2-diopter prisms in front of their eyes (4 diopters total disparity), base up on the right eye and base down on the left. (The results from the cats that were raised with only 2 diopters of disparity between their eyes suggest a similar shift, but insufficient data have been collected to warrant inclusion at present.) A *t*-test for the difference between the means of the two distributions was found to be significant ($P < .01$). The distribution of the disparities for the test cats is skewed, and the deviation of the modal value is less than 2.3°, the value which would be expected if complete compensation had taken place. There are three possible explanations for the less than complete compensation: the short period of time spent in the light without the prisms may exert a large influence; there may have been a partial compensation by the animals through changes in relative eye position while wearing the prisms; or a disparity of 4 prism diopters may exceed the limit set by the preexisting cortical structure (*13*). If the mechanism of cortical plasticity during maturation acts only by selecting synapses for preservation from a set of synapses present at birth without establishing new contacts, then such a limit must exist. Nonetheless, the results obtained to date favor the hypothesis that correlated activity in presynaptic endings can determine the formation or maintenance of permanent cortical connections, or both. Three of the test animals remain alive and are available for further study.

These results are consistent with the claim that the specification of the ipsilateral retinal projection to the tectum of *Xenopus* is dependent upon visual input (*2*). Cells receiving stimulation from the same portion of the visual field, but through different eyes, apparently grow onto common tectal cells despite manipulation of the eyes before metamorphosis (as by rotation of one eye through 180° about the visual axis). The work on *Xenopus* could not be confirmed in the frog (*14*), where the formation of the ipsilateral retinotectal projection is independent of visual input. However, correlated activities among fibers from corresponding points on the two retinas seem to be necessary for the maintenance of the ipsilateral connections once they are formed.

The principle of controlling functional contacts by the correlations in activity among fibers can equally well account for many of the other demonstrations of the effect of the visual environment on the developing brain. It has recently been shown that the distribution of orientation selectivities of cortical neurons in cats depends upon the orientation of the spatial structure viewed during the time they were kittens (*3*). If an eye views only horizontal lines, cells in the lateral geniculate nucleus (LGN) receiving inputs along horizontal lines in the visual field will be stimulated in a highly correlated way each time the eye moves, while cells receiving inputs along lines of other orientations will correlate less in activity among each other as the deviation from horizontal increases. Similar considerations can account for the failure of an eye deprived of patterned visual input to drive any of the cortical cells which have discrete receptive fields (*4*), whereas in a normal cat most such cells can be driven from either eye (*12*). All of the LGN axons receiving projections from the deprived eye are apparently displaced from sites on cortical cells by axons receiving projections from the exposed eye, where patterned input can produce high correlations of activity among nearby cells.

If the eyes are alternately occluded during kittenhood, few binocularly driven cells are observed (*5*) because LGN neurons receiving input from one eye have normal levels of interaxonal correlation, but the correlation in activity between cells from the two eyes is zero. Therefore a cortical cell tends ultimately to be controlled entirely by input from one eye or the other, but rarely receives effective inputs from both. In the case of artificial squint produced by sectioning the medial rectus muscle (*5*), which has the same effect on cortical physiology as the alternate occlusion of the eyes, two additional factors must be taken into account. First, the cutting of the eye muscle produces an ocular imbalance (paralytic strabismus) such that the angle between the visual axes of the two eyes varies with the angle of regard, so that no pair of points on the two retinas receives correlated inputs for an extended period of time. Second, unlike *Xenopus*, the kitten possesses an organized, binocular cortical system at birth (*13*) before any possible specification or respecification by visual experience can take place. Such a preexisting structure can be expected to impose a limit on the flexibility of the cortex in accommodating to shifts in retinal correspondence, and the large deviations introduced by extraocular muscle section may have exceeded this limit.

A final point of interest is that the production of shifts in cortical correspondences by optical means may be a useful experimental model of anomalous retinal correspondence. Patients with this defect do not fixate an object with both foveas, yet have stereopsis and single vision. Surgical correction of the fixation abnormality in maturity usually results in diplopia. It is speculated that anomalous correspondence develops from a concomitant strabismus of small magnitude during maturation.

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