used in this study. Those for the assessment of anomalies ranged from .73 to .96.

12. Use of the mechanical activity recorder is described by R. Q. Bell [*J. Exp. Child Psychol.* **6**, 302 (1968)].
13. The nursery school sample was composed of children both with and without anomaly scores computed shortly after birth. When the nursery school sample with newborn anomaly scores was compared with the nursery school sample without such scores, there were no significant differences in the distributions of scores for the variables used in this study or in the magnitude of the correlations with the anomaly score.
14. A manual giving detailed definitions of all the variables is available upon request.

4 May 1977; revised 5 December 1977

Interaction of Critical Periods in the Visual Cortex of Kittens

Abstract. *The critical period for modifying the preferred direction in cat cortical units occurs earlier than that for monocular deprivation. The independence of the effects of these two types of deprivation from each other was tested by rearing six kittens with both reverse suture and reversed directional deprivation. The kittens were placed in a drum rotating in one direction with one eye open at ages 2½ to 5 weeks; the drum rotation was reversed and the other eye opened when they were 5 to 12 weeks old. Recordings were then made in the visual cortex. The results were the sum of the effects of reverse suture and reversal of directional deprivation: most cells were driven by the eye that was open second, and most unidirectional cells preferred the direction to which the animals were exposed first. Consequently, many unidirectional cells preferred the first direction but were driven by the eye open second—a combination that the animal never saw during rearing. There was also an effect of ocular deprivation on directional properties and vice versa: reverse suture reduced the overall percentage of unidirectional cells, just as directional deprivation has been shown to affect the ocular dominance histogram. This result suggests that the same cells may be affected by both forms of deprivation.*

The critical periods for different kinds of visual deprivation are not the same (*1*). In animals reared with only the left eye open until 8 to 10 weeks and only the right eye open after that, nearly all the cells in the visual cortex will be driven by the left eye, whereas reverse suture at an earlier age leads to a substantial number of cells driven by the right eye. Thus, the critical period for monocular deprivation in the kitten is largely over by 8 to 10 weeks (*2*). Similar experiments with directional deprivation (animals reared in a drum with vertical stripes continuously rotating around them to the left until a certain age, and subsequently to the right) show that the critical period for this kind of deprivation is largely over by 5 to 6 weeks. In fact, the critical periods are sufficiently different that in a monocularly deprived animal with reverse suture at 5 weeks, more than three-quarters of its cells will be dominated by the eye that was open second, whereas in a directionally deprived animal with reversal of direction at 5 weeks, twice as many cells will prefer movement in the direction seen first, as will prefer movement in the direction seen second (*1*).

We have now studied the interaction between two kinds of visual deprivation. We reared a group of kittens that were both monocularly and directionally deprived, with reversals at 5 weeks. The results were compared with those from (i) normal kittens, (ii) one previous group of animals that had experienced only reverse suture, and (iii) another group that was only directionally deprived. Two questions arise. (i) Are the results of reverse suture plus directional deprivation with reversal of direction simply the sum of the results of these two deprivations taken separately? (ii) Does reverse suture affect the properties of directionally sensitive cells, and does directional deprivation affect the ocular dominance histogram?

In order to discuss the possible outcomes of the experiment, let us consider a kitten that, until the age of 5 weeks, has only its left eye open and is exposed to a leftward-moving set of stripes. At 5 weeks, the right eye is opened and the left eye sutured shut; from then until 12 weeks, the kitten sees only a pattern of stripes moving to the right. When the kitten is not being exposed to the moving stripes it is in the dark. Our previous experiments on monocular deprivation with reverse suture suggest that the majority of the cells should be dominated by the right eye (which was open second), and the experiments on directional deprivation with reversal at 5 weeks suggest that the majority of cells with directional sensitivity would prefer movement to the left (the direction that the kitten was exposed to first) (*1*). This is the result one would expect if the effects of the two kinds of deprivation simply sum. In a kitten so treated, the majority of cells would be specific for leftward movement seen through the right eye—a combination that the kitten was never exposed to simultaneously during its rearing. On the other hand, if there is some interaction between the two types of deprivation, one would expect a different result.

We reared six kittens from three litters with such a double deprivation. In general, cells in the left cortex tend to be dominated by the right eye and prefer rightward movement (*1*, *3*, *4*); consequently, we reared kittens with all four possible combinations of deprivation (left eye, left direction first; left eye, right direction first; and so forth). The kittens were kept in the dark until they were 2½ weeks old, then one eye was sutured shut and they were exposed to moving stripes for 1 hour per day until they were 5 weeks old. After reverse suture at 5 weeks, they were exposed to drum movement in the opposite direction until 12 weeks. They were then kept in the dark until recordings were made. The total amount of visual experience was approximately 50 hours, which should be adequate to develop a normal cortex in the absence of any other kind of deprivation (*5*).

Since various combinations of left and right were used in the rearing, it was not necessary to record from both sides of the cortex, and all recordings were made in the left visual cortex. We did not know the circumstances of rearing until all the kittens in a litter had been studied, although we could usually guess which eye had been opened second. Precautions taken to avoid statistical artifacts from the columnar organization of the cortex were (i) long penetrations down the medial bank of the lateral gyrus and (ii) movement of the electrode for at least 150 μm after a cell was characterized before looking for another cell (*1*, *6*). Other recording procedures have been described (*1*).

Cells were assigned to one of seven ocular dominance categories (*3*). On the basis of their responses to moving stimuli, they were also characterized as unidirectional, bidirectional, omnidirectional, or visually unresponsive (*1*); unidirectionality was defined by preference for a particular direction of movement, with little or no response for movement at 180° to this. We also noted the rate of spontaneous activity, preferred speed of movement, position of responses to leading and trailing edges of a bar, the extent of the receptive field plotted with stationary flashed spots, and the existence or

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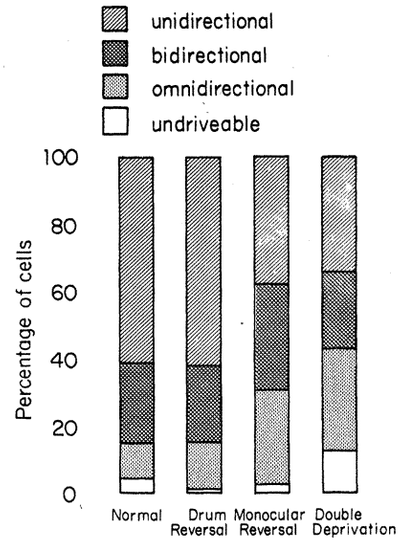

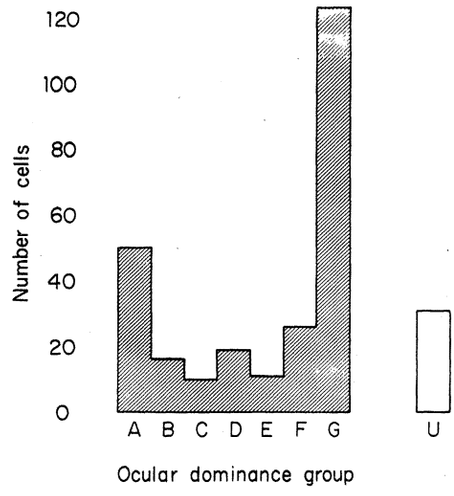

Fig. 1 (left). Percentages of unidirectional, bidirectional, omnidirectional, and unresponsive cells in normal cats and cats reared under various kinds of deprivation. Drum reversal: animals reared with stripes moving around them in one direction from 2½ to 5 weeks and in the reverse direction from 5 to 12 weeks. Monocular reversal: animals reared with an eye sutured shut from 2½ to 5 weeks, the other eye sutured shut from 5 to 12 weeks. Double deprivation: one eye sutured plus continuously moving stripes, with reversal at 5 weeks. Sample sizes: double deprivation, 300 cells; monocular reversal, 125 cells: drum reversal, 233 cells; normal animals, 129 cells. Fig. 2 (right). Ocular dominance histogram for cells in the visual cortex of doubly deprived kittens. Histograms were drawn separately for each kitten and then transposed according to whether the left or the right eye was open first. Group A cells driven exclusively by the first eye, group C cells driven exclusively by the second eye, group B cells dominated very strongly by the first eye, and so forth. Group U cells were visually unresponsive.

absence of a preferred orientation for a stationary flashed bar.

A total of 300 cells were examined in the six kittens. The most striking effect was a large increase in the percentage of omnidirectional and visually unresponsive cells and a corresponding decrease in the percentage of unidirectional cells. We found only 100 of the 300 cells (33 percent) that were unidirectional, compared to 78 of 129 (60 percent) in normal animals. The value in normal animals is similar to that found by other investigators (*7*). In contrast, the percentage of omnidirectional cells was increased from 9 percent (12 of 129) in normal animals to 29 percent (88 of 300) in doubly deprived ones, and the percentage of unresponsive cells was increased from 4 percent (6 of 129) to 12 percent (37 of 300). The percentage of bidirectional cells changed little—23 percent (68 of 300) in doubly deprived animals, compared with 24 percent (31 of 129) in normal ones.

The reduction in the percentage of unidirectional cells appears to be a result of the reverse suture (Fig. 1). We analyzed our previous results again (*1*) and found that kittens reared with both eyes open in a rotating drum, with reversal of direction at 5 weeks, showed a normal percentage of unidirectional cells (141 of 233 or 60 percent), whereas kittens that were monocularly deprived, with reverse suture at 5 weeks, and free to look around the room showed a reduced percentage of unidirectional cells (46 of 125 or 37 percent).

In this respect, reverse suture at 5 weeks has an effect somewhat like binocular deprivation, which several investigators have reported increases the percentage of nonspecific and unresponsive cells and reduces the percentage of specific ones (*8*). In particular, binocular deprivation dramatically reduces the percentage of direction selective cells (*9*). Blakemore and van Sluyters (*2*) and Movshon (*10*), in their work on the effects of reverse suture at various ages, noted an increase in the percentage of unresponsive and nonspecific cells, but they did not comment on the decrease in unidirectional cells that we report here. They did emphasize a decrease in the percentage of orientation-sensitive cells and either a divergence between the preferred orientations in the two eyes or the lack of a preferred orientation in one.

Of those cells that were unidirectional in our doubly deprived kittens, most preferred movement in the direction that they were exposed to first. Excluding those cells with preferred directions within 30° of the vertical, there were 73 unidirectional cells: 48 of these preferred movement in the first direction, 25 in the second. This ratio, approximately 2 : 1, is the same as that found in kittens which were directionally deprived with reversal at 5 weeks but which had both eyes open. Thirty-three cells preferred movement in the first direction and were dominated by the eye open second, compared with only nine preferring movement in the second direction and dominated by the eye open first.

The ocular dominance histograms in these kittens showed that 160 cells were dominated by the eye open second compared with 76 dominated by the eye open first (Fig. 2). This ratio is not far from that (89 : 30) which we found in kittens reared with monocular reversals at 5 weeks but which were free to look around the room (*1*).

We conclude that the effects of reverse suture and directional deprivation sum with each other. Monocular deprivation with reversal at 5 weeks leads to a visual cortex in which the majority of cells (68 to 77 percent) are dominated by the eye open second: this is also true of the double deprivation. Directional deprivation with reversal at 5 weeks results in a visual cortex in which the majority (66 to 69 percent) of the unidirectional cells prefer movement in the direction seen first; this is also true of the double deprivation. Double deprivation thus leads to a large number of cells that prefer the first direction but are driven by the second eye. However, reverse suture by itself does affect directional sensitivity—it leads to a substantial reduction in the percentage of cells which are unidirectional—just as directional deprivation by itself affects eye dominance—it leads to a reduced percentage of binocular cells (*1*). We suggest that this occurs because some of the same cells in the cortex are involved in both binocularity and directional sensitivity.

N. W. Daw
N. E. J. Berman*
M. Ariel

Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, Missouri 63110

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* Present address: Department of Physiology and Biochemistry, Medical College of Pennsylvania, Philadelphia 19129.

31 May 1977; revised 7 September 1977

HeLa Marker Chromosomes, Chang Liver Cells, and Liver-Specific Functions

Nelson-Rees and Flandermeyer have (*1*) indicted the Chang liver cell (*2*) as a HeLa cell contaminant. They have concluded that, regardless of designation, the Chang cell should be considered a de facto strain of HeLa. These authors based their conclusion on the following: (i) the electromobility of glucose-6-phosphate dehydrogenase (G6PD) and phosphoglucomutase of the Chang cell were similar to those of the HeLa cell; (ii) the Chang cell contained a complex of rearranged chromosomes or markers described for HeLa cell cultures; and (iii) there was no Y chromosome in the Chang cell. It is unfortunate that these authors failed to mention that Kaighn and Prince (*3*) had found the Chang but not the HeLa cell capable of producing serum albumin and fibrinogen, and that Bausher and Schaeffer (*4*) had demonstrated tyrosine aminotransferase activity in the Chang cell.

In 1953 to 1954 I made many attempts to cultivate cells from a variety of human tissues on the simplistic assumption that a specific differentiated cell might support in vitro the growth of a specific virus (for example, the human hepatocyte might support the growth of the human hepatitis virus). Since the goal was merely to obtain a sufficient number of cells that would support the growth of certain viruses under study, no effort was made to record the sex, race, age, and medical diagnosis of the tissue donor. The liver specimen, from which the Chang cell was derived (*2*), was obtained during biopsy from a patient undergoing exploratory laparotomy.

Since there is no record of the sex and race of this tissue donor, the absence of a Y chromosome and the presence of G6PD and phosphoglucomutase with specific electromigration patterns (similar to those found predominantly among the black race) cannot be used as evidence for indicting the Chang liver cell, because the tissue donor could be a black woman. Therefore, the indictment by Nelson-Rees and Flandermeyer is based solely on the morphologic appearance of chromosomes.

Ludueña *et al.* (*5*) have presented evidence that another protein characteristic of, but perhaps not unique to, differentiated liver cells (liver alkaline phosphatase) is synthesized by the Chang but not by the HeLa cell. There are now on record three groups of investigators who have found proteins characteristic of differentiated human liver cells in or secreted by the Chang liver cell. Other reported differences between these two cell lines include susceptibility to aflatoxin B_1 (*6*) and the total and epinephrine-sensitive adenyl cyclase activities (*7*).

In view of these reports, I ask the following questions: If the Chang cell is derived from a culture of HeLa cell and not from a human liver biopsy as reported (*2*), what is the probability that the Chang but not the HeLa cell contains more than one liver-specific protein? Is chromosomal morphology sufficiently dependable to be used as the sole criterion in tracing the origin of an established line of human cells? We are all aware of the seriousness of cross-culture cell contamination in research involving cell cultures. But, to indict a cell line as a HeLa cell contaminant on insufficient evidence may be counterproductive.

R. SHIHMAN CHANG
Department of Medical Microbiology, University of California, Davis 95616

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9 August 1977

"Chang liver cells" were first suspected of being HeLa cells in 1966 on enzymatic grounds (*1*). In 1974, Chang brought to my laboratory a culture of the "liver cells" for karyologic analysis. Our results were discussed with Chang and with Sussman, a co-author of the paper by Ludueña cited by Chang in his comment, and were summarized by us (*2*). Our results were confirmed by Lavappa *et al.* (*3*) on the "Chang liver cells" at the American Type Culture Collection (ATCC) (*3*). We indicted the cells as being HeLa cell contaminants because they possess a group of chromosomes originally described by Miller *et al.* (*4*) for HeLa cells. These "markers" consist of chromosomes whose banding patterns coincide with those of portions of specific human chromosomes, however rearranged (translocations, misdivision, nondisjunction). Besides these "Miller markers," many HeLa strains share other identical markers which serve to characterize closely related strains of HeLa or the culprits in HeLa contamination of other cultures. In fact, we communicated to Chang that some markers that we observed in the "liver cells" were identical to some observed in the HeLa-contaminated cultures from Russian laboratories (*5*). In every culture analyzed to date, the cells that exhibit "Miller markers" and others also lack a Y chromosome and produce type A (fast moving) G6PD.

A sample of "Chang liver cells" supplied by the ATCC was studied recently by O'Brien (*6*) for additional enzyme polymorphism. The cells exhibited characteristics identical to HeLa and to three other now well-known HeLa strains—H.Ep-2, KB, and J111—in the electrophoretic resolution of seven relatively polymorphic, human gene-enzyme systems previously studied by him [see (*7*)]. According to O'Brien the genotype frequency of HeLa, based on allelic frequencies of the seven tested enzyme loci in natural populations, is 0.013; or, as concerns all cells studied by him, the probability that another cell line would express the same genotype is .05.

Thus, while there is no record of sex, age, race, and medical diagnosis of the tissue donor for the original liver culture, the results of up-to-date karyology and enzymology speak more convincingly for its being now a strain of HeLa cells through the common occurrence of cross-cell-contamination than that of a liver derivative of which no other human line exists in spite of many initiation attempts. As to the liver functions detected in this strain of HeLa, we have re-

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