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Source: *Science*, New Series, Vol. 203, No. 4387 (Mar. 30, 1979), pp. 1353-1355
Published by: American Association for the Advancement of Science
Stable URL: https://www.jstor.org/stable/1747326
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be high because of complications caused by ACh rebinding. The experiments without neostigmine give values between −60 and −70 mV.

6. It seems possible that two ACh molecules bind to the receptor. In this case, Eq. 2 will be altered to $[A]^2/[A']^2 - 1 = K_I[I]$, and Eq. 3 will be unaltered. The value of $K_I$ obtained from Eq. 2 will be larger and this will give a different estimate for the surface potentials. Nevertheless, all the experiments in Table 1 still yield negative surface potentials if the revised Eq. 2 is correct.
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$$\sigma^2 = \frac{\epsilon kT}{2\pi}[i^+][\exp(Q\psi kT) + \exp(-Q\psi kT) - 2] + [i^{2+}][2\exp(Q\psi kT) + \exp(-2Q\psi kT) - 3]$$

where $\sigma$ is the membrane surface charge density (charges per square centimeter), $\psi$ is the surface potential (millivolts), $[i^+]$ and $[i^{2+}]$ are the concentrations of univalent and divalent ions (moles per cubic centimeter), $k$ is the Boltzmann constant, $T$ is temperature in degrees Kelvin, $Q$ is the charge on the electron, and $\epsilon$ is the permittivity of water.


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11. We thank D. Attwell, D. H. Jenkinson, and G. R. Strichartz for helpful discussions. Supported by grants HL-20558 and NINCDS-10320 from PHS and a grant from the Muscular Dystrophy Association. I.C. is a recipient of a research career development award from the National Heart, Lung, and Blood Institute.

18 July 1978; revised 11 October 1978


## Lateral Geniculate Nucleus in Dark-Reared Cats: Loss of Y Cells Without Changes in Cell Size


*Abstract.* *In cats reared in the dark from birth until 4 months of age, the dorsal lateral geniculate nucleus contained few normal Y cells in either the binocular or monocular segments. Although most of the neurons appeared to be normal X cells unaffected by light deprivation, many cells with abnormal receptive field and response characteristics were encountered. These effects were permanent, since 1 to 2 years of normal visual experience following initial light deprivation did not lead to any functional recovery. The sizes of cell bodies in cats reared in the dark were similar to those of normal animals, an indication that changes in geniculate cell physiology need not be related to changes in cell size.*


The cat's retino-geniculo-cortical pathways have been subdivided on the basis of electrophysiological criteria into at least two subsystems. These subsystems, called the X and Y pathways, are composed of retinal ganglion X and Y cells that project to geniculate X and Y cells (*1–3*). These neurons in turn project to the visual cortex. Compared with X cells, Y cells generally (i) have axons that conduct more rapidly, (ii) respond to more rapidly moving visual stimuli, (iii) are more phasic in their response to standing contrast, (iv) have larger receptive fields, and (v) display less linear response summation to visual stimuli.

During postnatal development, Y cells in the dorsal lateral geniculate nucleus are more susceptible to visual deprivation than are geniculate X cells. For instance, if the lids of one or both eyes of a kitten are sutured shut just after birth and maintained in this manner for several months, there is a significant reduction in the percentage of geniculate Y cells encountered with microelectrodes in the laminae receiving input from the sutured eye or eyes (*4–6*). In contrast, the development of Y cells in the retina appears to be unaffected by lid suture (*7*). Although eyelid suture essentially eliminates patterned visual input, the amount of light striking the retina is reduced during development by only 1 to 2 log units (*8*). To determine the effects of total light deprivation on the development of geniculate X and Y cells, we raised four cats from birth to 16 to 18 weeks in complete darkness. No light source of any sort including infrared was introduced into the rearing area during this period.

At the end of the dark-rearing period, single geniculate neurons were recorded extracellularly in two of the kittens (DR cats), and the two remaining animals were given 1 to 2 years of normal visual experience prior to recording (DR-LR cats). We used tungsten microelectrodes to isolate and study 156 single units in laminae A and A1 of the four dark-reared cats. Electrodes were placed near the rostro-caudal middle of the nucleus, and thus all receptive fields were within a few degrees of the horizontal zero parallel. Details of recording and criteria used for classifying cells as X or Y have been reported (*1, 2, 4, 7*).

All cells encountered in the binocular segment of laminae A and A1 of normal adult cats can be classified as X or Y cells, and they are sampled with roughly the same frequency (Fig. 1A) (*9*). By contrast, in the A laminae of DR and DR-LR cats, X cells comprised the vast majority of the total (Fig. 1A). Only a few of the neurons could be classified as normal Y cells, and about a fifth of the cells had abnormal response properties and could not be classified as normal X or Y cells (*10, 11*). No difference was found between animals dark-reared with or without additional normal visual experience. Furthermore, no significant interanimal variability was observed among the four animals of our experiment, since the range in percentage of Y cells was 0 to 7 percent.

The ratio of X and Y cells encountered in the A laminae depends on the eccentricity from the vertical meridian of the receptive fields sampled, which in turn relates to the medio-lateral location of the electrode within the lateral geniculate nucleus (Fig. 1B) (*4*). The effects of monocular and binocular eyelid suture differ not only in the ratio of X and Y cells encountered, but also in these ratios as a function of eccentricity (*4*). Cats with monocular lid suture have few Y cells throughout the deprived, binocular segment of the nucleus, yet have a normal ratio of Y cells in the deprived, monocular segment. Such evidence suggests that the Y cell loss observed in cats with monocular lid suture results from an imbalance in visual input between the two eyes (binocular competition) and not from deprivation per se (*4, 12*). In comparison with normal animals, cats with binocular lid suture suffer a reduction of Y cells throughout the nucleus, including the monocular segment. A similar but more pronounced effect is seen in DR and DR-LR animals (Fig. 1B). In these animals, we found a reduced percentage of Y cells throughout the nucleus. Moreover, we also found that the percentage of Y cells in cats reared in the dark was reduced in comparison with cats with binocular lid suture ($P < .01$, $\chi^2$ test). In fact, the percentage of Y cells encountered in the binocular segment of animals reared in the dark is essentially as low as that reported for the binocular segment of monocularly deprived cats. However, the monocular segment of cats with monocular lid suture differs from that of cats reared in the dark (Fig. 1B).

In addition to the electrophysiological experiments, we also measured the cross-sectional areas of 100 cells in each DR and DR-LR animal. These samples were taken from a zone in the binocular segment of lamina A near the rostro-caudal middle of the nucleus (typically from sections just rostral or caudal to those containing electrode tracks), and only cells with nucleoli in the plane of the section were measured. Medio-laterally, the sample zone was in that area of lamina A which represents the



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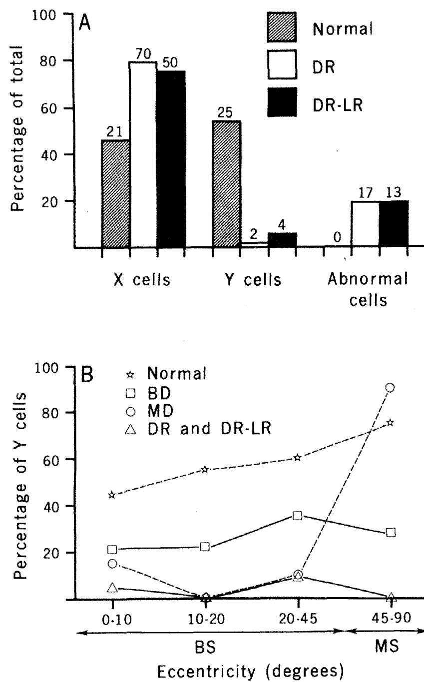


Fig. 1. Cell types in the dorsal lateral geniculate nucleus. (A) Percentages of X cells, Y cells, and abnormal cells (*11*) in normal cats (*9*), two dark-reared cats (*DR*), and two dark-reared cats that received subsequent normal visual experience (*DR-LR*). The number of cells in each group is shown above each bar. Note both the decreased percentage of Y cells and the number of abnormal cells in animals reared in the dark compared with normal cats. (B) Comparison of percentage of Y cells in normal cats, cats in which the eyelids were binocularly sutured (*BD*) (*4*), the deprived laminae of cats with monocularly sutured lids (*MD*) (*4*), and the four cats reared in the dark for this experiment. The data are broken down into groups on the basis of the eccentricity in the visual field of the receptive fields sampled. Abbreviations: *BS*, binocular segment (the central portion of visual field, which can be viewed by both eyes); *MS*, monocular segment (the peripheral portion of visual field, which can be viewed only by the ipsilateral eye). The number of units sampled and mapped at each eccentricity group for the dark-reared cats are 82 (0° to 10°), 25 (10° to 20°), 25 (20° to 45°), and 14 (45° to 90°). Ten additional units could not be mapped because responses to visual stimuli were poor or absent (*11*).

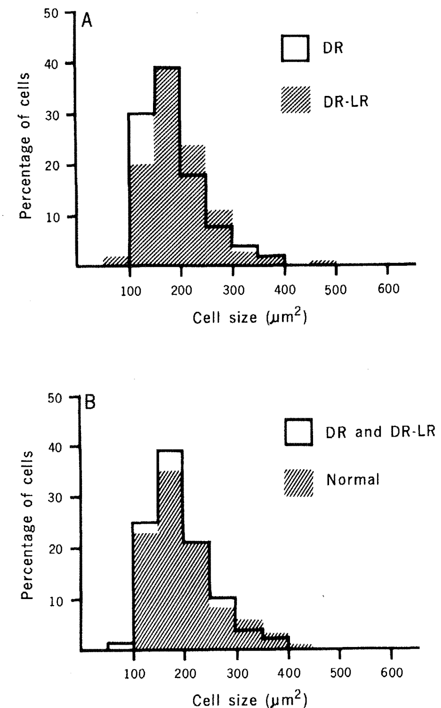


Fig. 2. Cross-sectional area of cells from the dorsal lateral geniculate nucleus of dark-reared and normal cats; 100 cells for each animal were measured. (A) Comparison of cell sizes from two cats reared in the dark (*DR*) and two also reared in the dark but that received subsequent normal visual experience (*DR-LR*). No statistical differences were found (Mann-Whitney U test, $P > .1$). (B) Comparison of cell sizes of the four cats from (A) and four normal cats. No statistical differences were found (Mann-Whitney U test, $P > .1$). Both histograms represent pooled data for all the animals in each condition, and no detectable interanimal variability occurred in these measures.

peripheral 5° to 10° of visual field (*13*). For comparison we also made equivalent measurements in four normal adult cats (*14, 15*). The DR and DR-LR cats did not differ in their cell body sizes (Fig. 2A). Furthermore, when these data are combined and compared with the measurements from normal adult cats, again no difference is observed (Fig. 2B). A similar result has been reported by Kalil (*15*), who presented evidence that the growth of geniculate cells in cats reared in the dark is slower than normal, but that cell growth continues until the neurons achieve and maintain normal size at 16 weeks of age.

The mean cell size in deprived geniculate laminae of cats with monocular lid suture is smaller than normal (*16*). It has thus been necessary to acknowledge that the reduction in the percentage of recorded Y cells may be due to a change in electrode sampling characteristics as a consequence of the change in cell sizes. Since the geniculate cell size distributions in the dark-reared cats appear to be normal, however, it seems unlikely that the observed loss of geniculate Y cells in these animals can be explained by changes in electrode sampling characteristics brought about by abnormal cell sizes. Although this possibility cannot be completely excluded, at least five alternative explanations can account for the apparent loss of Y cells. (These explanations are not mutually exclusive.) (i) Some of the cells destined to become Y cells might fail during rearing in the dark to develop properly and thus become abnormal. This hypothesis is supported by the observation of a trend in cats reared in the dark toward a greater percentage of abnormal geniculate cells with increasing receptive field eccentricity ($P < .05$, $\chi^2$ test based on the ratio of abnormal cells in each of the eccentricity groups illustrated in Fig. 1B); more geniculate Y cells are normally found with increasing receptive field eccentricity (Fig. 1B). However, even if all of the abnormal cells seen in the cats reared in the dark are considered to be Y cells, the percentage of geniculate X cells remains too high for this possibility to account for all the missing Y cells (Fig. 1A). (ii) Although there is no evidence to suggest missing neurons, and cell densities in our histological material seems normal, some of the presumptive Y cells may be physically missing from the nucleus. (iii) Some of the presumptive Y cells may have developed as X cells (*17*). (iv) Some of the Y cells may be present but unresponsive. (v) Finally, although suturing the lid permits qualitatively normal development of retinal ganglion cells (*7*), the more total deprivation imposed by rearing in the dark may not. Rearing in the dark may selectively retard development of Y cells in the retina; since retinal input largely determines geniculate cell physiology, the result would be fewer geniculate Y cells.

Our results support the conclusion from studies of cats with sutured lids that geniculate Y cells are markedly affected during postnatal development by visual deprivation (*4*–*6*). The more severe effects of rearing in the dark compared with binocular lid suture suggest that even diffuse illumination of the retina can play an important role in the development of some geniculate Y cells. Furthermore, extensive normal experience following 4 months of dark-rearing does not alter in any obvious way the established reduction in normal geniculate Y cells. Therefore, in terms of development of the lateral geniculate nucleus, dark-rearing during the first 16 to 18 postnatal weeks appears to produce permanent functional alterations not matched anatomically by any apparent changes in cell size. Such a dissociation between physiological and morphological effects suggests that the loss of Y cells in visually deprived animals is a true functional loss and not merely the result of an electrode sampling bias created by changes in cell sizes.

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9. These data were obtained from normally reared cats in our laboratory according to the identical techniques used for the dark-reared cats (*4*).
10. The cells classified as normal in the cats reared in the dark, in addition to having qualitatively normal receptive field properties, also responded to shock of the optic chiasm within an expected latency range. From our sample of normal neurons, the mean ($\bar{X}$) latency ± standard deviation (S.D.) for Y cells was 1.3 ± 0.2 msec, and for X cells was 2.0 ± 0.2 msec. In the cats reared in the dark, the latency for Y cells was 1.3 ± 0.2 msec, for X cells it was 2.1 ± 0.3 msec, and for the 21 abnormal cells that responded to optic chiasm shock, the latencies included the entire range seen normally (range, 1.0 to 2.5 msec; $\bar{X}$ ± S.D., 1.8 ± 0.4 msec).
11. The 30 abnormal cells encountered in the four cats reared in the dark can be further subdivided into the following categories. (i) Eight cells appeared to be Y cells on the basis both of latency to electrical stimulation to the optic chiasm and of responses to moving stimuli, but they had abnormal spatial summation properties. That is, they displayed a clear "null position" (zero response) to a stimulus that bisected the receptive field along a line separating zones flashing on and off 180° out of phase with one another (*2*). This can be taken as evidence for linear spatial summation. Such cells, which otherwise had properties of Y cells, were never seen in normal cats. (ii) The opposite was seen in four neurons that appeared to be normal X cells except that no null position could be found. Such cells are occasionally seen in normal cats, however, and could be the result of artifacts such as eye movements (*2*). To consider these as normal X cells would in no way affect our conclusions; instead, the inability to record Y cells would seem more dramatic. (iii) Four cells with Y-like receptive fields had abnormally long latencies to optic chiasm shock (> 1.8 msec), and such cells were never seen in normal cats. (iv) Eight cells responded poorly and six cells did not respond to visual stimuli. Such abnormal cells as described here were also seen in other studies of deprived Y cells [T. T. Norton, V. A. Casagrande, S. M. Sherman, *Science* **197**, 784 (1977); (*6*)].
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18. Supported by NIH grant EY 01565 and NIH research career development award EY 00020 (S.M.S.), NIH grant EY 01331 (R.K.), and NIH postdoctoral fellowship EY 05077 (K.E.K.).

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31 July 1978; revised 6 October 1978


## Magnetite in Freshwater Magnetotactic Bacteria


Abstract. *A previously undescribed magnetotactic spirillum isolated from a freshwater swamp was mass cultured in the magnetic as well as the nonmagnetic state in chemically defined culture media. Results of Mössbauer spectroscopic analysis applied to whole cells identifies magnetite as a constituent of these magnetic bacteria.*


Iron-containing bacteria from diverse aquatic environments which orient and swim in a preferred direction in weak (0.1 gauss) magnetic fields (magnetotaxis) have been described (*1*). Cellular iron is localized in crystals (100 by 150 nm) within these bacteria. Kalmijn and Blakemore (*2*) demonstrated geomagnetic orientation by similar bacteria in saltmarsh sediments. These workers subsequently obtained evidence through cell remagnetization studies that the bacteria exhibited properties of single domain ferromagnets (*3*). Thus, the directed swimming response of magnetic bacteria to geomagnetism is a direct one, clearly different from electromagnetic induction exhibited by elasmobranch fishes (*4*).

Definitive studies of the chemical nature of iron in magnetic bacteria have not been possible because the organisms have not been available in pure culture. Recently, Blakemore (*5*) isolated a freshwater magnetotactic bacterium. In this report, we describe the results of Mössbauer spectroscopic analyses of magnetic and nonmagnetic whole cells of this isolate cultured in chemically defined media.

The organism was an unclassified magnetotactic spirillum (Fig. 1) designated strain MS-1. It was isolated from sediments of Cedar Swamp, Woods Hole, Massachusetts, and appears to be a new bacterial species by criteria separate from its magnetic properties. Characterization, taxonomy, and details of culturing this organism have been studied (*6*). Cells of the organism were cultured under microaerobic conditions (the $O_2$ atmosphere over the cultures was initially 6 to 7 $\mu M$) in a liquid medium containing filtered bog water with succinic acid and sodium nitrate as the principal sources of carbon and nitrogen, respectively. Subsequently, a chemically defined medium lacking bog water was employed. Iron was supplied in this latter culture medium to a final total iron concentration of 1.6 mg/liter, as ferric sulfate and ferric quinate. Results of atomic absorption spectrophotometric analyses indicated that magnetic cells contained 1.5 percent of their dry weight as iron. The cells contained an average of 22 intracellular crystals, each approximately 50 nm on a side (Fig. 1).

After prolonged culture of strain MS-1 in a medium with less iron, cells grew nonmagnetically. They did not align with stationary external magnetic fields or rotate in response to reversal of the ambient field. From such a culture, a homogeneously nonmagnetic population of cells was obtained by standard microbiological cloning procedures. Nonmagnetic cells lacked intracellular crystals present in magnetic cells and contained less than one-tenth the amount of iron of magnetic cells. In other respects, the two types were similar. Nonmagnetic cells were maintained in a chemically defined medium identical to that used for magnetic cells, except that ferric quinate was deleted. The total iron content of this medium was 3.6 $\mu M$.

Cells were mass cultured at 30°C in glass carboys having a 10-liter capacity. They were harvested by continuous flow centrifugation (15,000 rev/min) at 10°C. Cell yields were (wet weight) 0.2 to 0.5 g per liter. Harvested cells were washed three times in distilled water and lyophilized. They were not exposed to magnetic fields stronger than those normally associated with general laboratory conditions (such as a-c motors, pumps, and electrical lines) during growth, harvest, or preparation for analyses.

Mössbauer spectra at room temperature were obtained with 350-mg samples of freeze-dried cells grown under various conditions. Cells analyzed included (i) magnetic cells grown in medium containing bog water, (ii) magnetic cells grown in chemically defined medium containing 29 $\mu M$ iron, and (iii) nonmagnetic cells grown in chemically defined medium containing 3.6 $\mu M$ iron.

No discernible $\gamma$-ray absorption greater than 0.2 percent was observed in nonmagnetic cells (Fig. 2a). The Mössbauer spectrum of magnetic cells grown in medium containing bog water (data not shown) was identical to that of magnetic cells cultured in chemically defined medium (Fig. 2b). The spectrum of Fig. 2b can be characterized as being due primarily to iron in magnetite (*7*). There are, however, two significant differences between the spectrum in Fig. 2b and the spectrum of stoichiometric magnetite (Fig. 2c). These are (i) an extra absorption area close to $v = 0$ (Fig. 2b), which



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