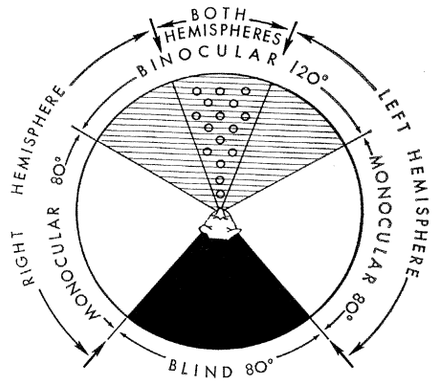

Fig. 2. The visual field of the cat and its relationships with the eyes and the cerebral hemispheres. The figure is partially imitated from Duke-Elder (*12*). The visual callosal mechanisms described in Fig. 1 allow both hemispheres to receive projections from an area of the visual field extending 20° on either side of the vertical meridian. The extension of the bihemispheric region of the visual field is inferred from the data of Berlucchi *et al.* (*2*) showing that some visual receptive fields of callosal units are as large as 20°.

finding, incidental to this study, of some visual cortex neurons totally unexcited by visual stimuli.

In accord with the data of Choudhury *et al.* (*3*) the nine units which were binocularly driven were found in a cortical strip that, following the cortical maps of Otsuka and Hassler (*10*), was at the boundary between areas 17 and 18. With each binocularly driven unit, the visual receptive field mapped upon stimulation of the contralateral, "callosal" eye was remarkably similar to that mapped upon stimulation of the ipsilateral, "geniculocortical," eye. Both receptive fields lay at the same longitude on the nasal side of the vertical meridian, which actually marked the temporal border of each field; as a result, the binocular receptive field of each one of these units covered an area across the vertical meridian, whose surface equaled the sum of the surfaces of the two monocular receptive fields. These units had receptive fields within or in the close neighborhood of the areas centralis up to approximately 10° along the vertical meridian. The size, orientation, and organization of the "callosal" receptive fields were also very similar to those of the corresponding "geniculocortical" fields. Monocular field sizes of these units varied from approximately 1° by 2° to 9° by 9°. All the binocularly driven cells showed field and response characteristics of the "complex" type (see *5*), displaying, however, some abnormalities, as mentioned above. Responses to stimulation presented in the "callosal" field were in general less brisk and more fatiguable than responses to equal "geniculocortical" stimuli. Simultaneous presentation of appropriate stimuli in both receptive areas of a binocular unit resulted in a response more intense than that obtained by stimulation of either area alone.

Although nine units are too small a sample to allow exclusion of other possible callosal-geniculocortical interactions, the results definitely show that some visual cortex neurons are linked with the eyes not only through the specific geniculocortical pathway, but also through an indirect callosal pathway.

Further, there is an orderly arrangement between specific and commissural inputs to the same visual cortex neuron, so that the receptive fields resulting from them, besides sharing the same organizational properties, add in visual space and give origin to a functionally homogeneous receptive area crossing the vertical meridian.

Figure 1 shows the experimental situation in the present study. It demonstrates how a visual cortical cell could be activated from both eyes, through a direct geniculocortical pathway and an indirect geniculocortico-callosal route, respectively.

Figure 2 presents a schematization of the relationships between visual space, eyes, and brain, taking into account the callosal mechanisms presented here.

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7. It has been suggested (*11*) that in the cat, callosal fibers reaching the visual cortex originate not only from contralateral visual cortical areas, but also from contralateral lateral geniculate bodies. The existence of such crossed geniculocortical pathways has not been confirmed in a recent anatomical investigation [M. E. Wilson and B. G. Cragg, *J. Anat.* **101**, 677 (1967)]. The callosal visual interactions between the hemispheres revealed by recent electrophysiological work (*2*, *3*) appear to be mediated by a corticocortical pathway only.
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16 November 1967

Tetraploid DNA Content of Purkinje Neurons of Human Cerebellar Cortex

Abstract. *Microspectrophotometric analysis of single cells in Feulgen preparations revealed tetraploid amounts of DNA in Purkinje neurons of human cerebellar cortex.*

A brief preliminary report of the finding of tetraploid quantities of DNA in Purkinje neurons of normal human cerebellum has been published (*1*). This study was based on microspectrophotometric measurements of Feulgen preparations by the conventional plug technique. (I use "tetraploid" only with reference to DNA content, since the true status of the chromosomes is unknown.) I now report later measurements on the same cell type with a Deeley-type Barr and Stroud integrating microdensitometer (*2*). This instrument incorporates a scanning device that minimizes distributional error; extinction is summated as the scanning progresses, so that a direct measurement of total absorption is provided in arbitrary units for a single whole nucleus.

Material consisted of tissue blocks from cerebellar hemispheres of five brains obtained at necropsy from cadavers demonstrated to be free of evidence of neurologic disease; it was fixed in a 1 : 3 mixture of glacial acetic acid and 95-percent ethanol. After 4 to 6 hours of fixation, tissues were dehydrated and embedded in paraffin. For measurement of DNA content in Purkinje cells, sections were cut at 18 μ in order to ensure whole nuclei in sufficient numbers. A 10-μ section was prepared for measurement of gran-

ule cells of cerebellar cortex. A section of kidney, fixed and processed identically and cut at 10-μ thickness, was mounted as a control tissue on each slide adjacent to the section of cerebellum.

The Feulgen reaction was carried out with a 12-minute exposure to 1*N* HCl at 60°C (*3*). Hydrolysis was omitted from one section in each set, which served as a Feulgen control section. Absorption was measured with the integrating microdensitometer at a wavelength of 550 mμ; determinations were obtained from 30 Purkinje neurons per specimen. Cells were chosen at random, except that only uncut nuclei, free of overlapping cells, were analyzed. Each determination was the average of three readings. For every specimen, control measurements were obtained from ten oligodendrocytes of cerebellar white matter in the same 18-μ section as the Purkinje cells; further control values were determined in ten granule cells of cerebellar cortex (in 10-μ sections) and in 15 to 20 renal tubular epithelial cells in the section of kidney mounted with the cerebellum. Moreover, random determinations were carried out on Feulgen preparations of two specimens of human liver (20 cells each), processed in a manner like that described, in order to establish the tetraploid DNA level in a tissue other than brain.

The data appear in Fig. 1. Control measurements of oligodendrocytes from all five specimens are combined in Fig. 1A; those from renal tubular epithelium, in Fig. 1B; the values in both types of cells fall into a single, diploid class. Hepatic cells with lowest DNA content (combined data, Fig. 1C) are also diploid; a second group of hepatic cells is tetraploid (Fig. 1C), having approximately twice the diploid amount of DNA.

Results with granule and Purkinje neurons of cerebellum appear separately for each specimen (Fig. 1, D–H). In every instance the granule and Purkinje cells constitute two separate and distinct DNA classes: Mean values for granule cells are at the diploid level; mean DNA for Purkinje neurons is approximately twice the diploid level and similar to tetraploid liver values, indicating a tetraploid content of DNA.

As a further check on the data, a hydrolysis series was performed on one specimen of cerebellum. The results (Table 1) reveal unimodal distribution; they are comparable to hydrolysis curves obtained from other human cells by use of a similar temperature and concentration of HCl (*4*).

Three aspects of the finding of tetraploid DNA levels in Purkinje neurons are especially noteworthy:

1) In contrast to polyploid cells in other organs such as liver parenchyma, these data suggest that, at least in the areas of cerebellar hemisphere examined, tetraploidy is universal among Purkinje cells, since only a single DNA class was demonstrated.

2) Nuclei of Purkinje neurons are strikingly pale structures when stained with the usual nuclear dyes, unlike cells with higher ploidy in other tissues. In general, polyploid nuclei are larger than nuclei of diploid cells in the same tissue, and their depth of

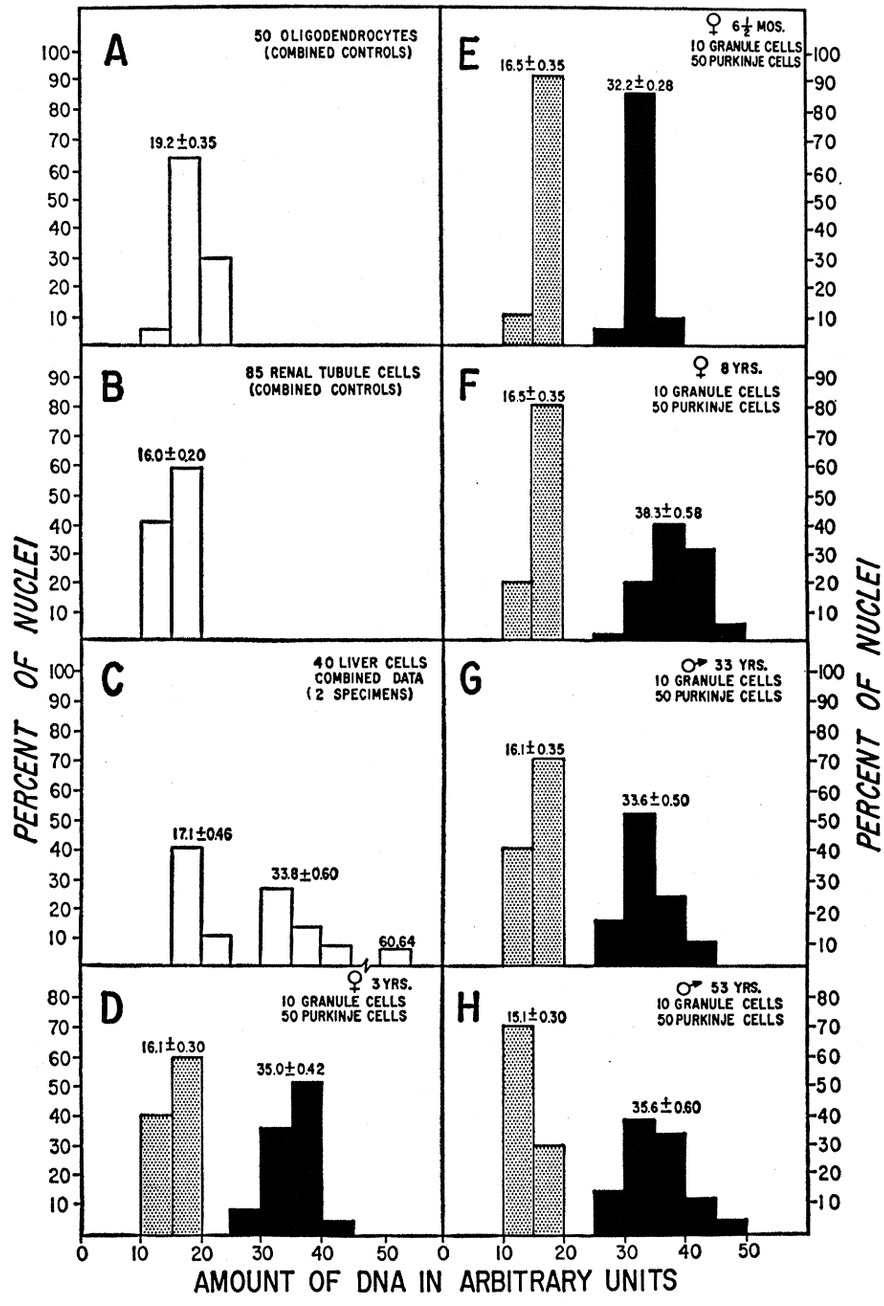

Fig. 1. Content of DNA (arbitrary units) in various cell types of human cerebellum, kidney, and liver. (D–H) Dotted columns represent cerebellar granule cells; solid columns, Purkinje cells. Numbers above columns are means and standard errors, except for the last column to the right of C, in which the numbers are true values obtained from two octaploid nuclei.

Table 1. Effect of variation of time of hydrolysis in the Feulgen reaction on DNA content of Purkinje neurons.

Time (min)	Mean DNA (5 cells)	S.E., ±
10	24.9	0.53
12	28.8	1.17
15	27.4	0.53
18	24.9	.85
21	20.2	1.08
25	16.8	0.67
30	10.6	.86

staining is equivalent to or greater than that of diploid forms. The pallor of Purkinje cell nuclei is readily explained by their enormous volume, however, which is some fivefold to tenfold greater than the volume of most diploid nuclei, and four to five times that of tetraploid cells of liver.

3) Ages of patients from whom specimens were obtained ranged from 6½ months to 53 years. In the Purkinje neuron, tetraploidy is thus present in infancy and appears to be stable thereafter; in contrast, polyploidy in the cells of human liver develops after infancy and is an age-related phenomenon (*5*).

The significance of tetraploidy is not known. The Purkinje cell is a large neuron with an elaborate and extensive dendritic tree; it provides a complex, integrative action as the sole efferent neuron of the cerebellar cortex. In keeping with the concept that DNA is the basic determinant of cellular synthetic processes and ultimately of cellular function, the doubled content of genetic material in the mature, differentiated Purkinje cell may be expected to achieve expression in the metabolic machinery of this neuron.

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Visual Pigment Fluorescence

Abstract. *The fluorescence of cattle rod outer segments (dried) and of rhodopsin in solution lies in the range of 575 to 600 millimicrons with a quantum efficiency of 0.005 if excitation is in the visible band near 500 millimicrons. The emission is abolished by bleaching at −196°C but can be reversibly regenerated by irradiation with light of longer wavelength (600 millimicrons). This behavior reflects the known interconversion of rhodopsin to prelumirhodopsin at this temperature.*

In the initial photoprocess relating to vision, the primary function of the exciting photon is to convert the chromophore related to 11-*cis*-retinal to the all-*trans* isomer (*1*). Such a structural change appears to induce a protein rearrangement which in some unknown way could lead to membrane depolarization and complete the transduction process. A number of mechanisms to account for this transfer of information have been proposed (*2*). However, experimental evidence in favor of one mechanism over others has not been presented.

If we examine the initial photoprocess in more detail, we find that there must be some dissipative process to allow the excited state to return to the ground state (*3*). However, it is not at all clear by which mechanism this occurs. Possible mechanisms are: a reemission process (such as fluorescence or phosphorescence), radiationless transfer, exciton formation, or simply dissipation as heat. The problem is compounded in that the dissipative process itself may be the triggering mechanism, and the bleaching phenomena may be only a process controlling sensitivity (*4*).

To investigate this point more closely, we began a study of the emission characteristics of intact rod outer segments and of rhodopsin extracts obtained in 2-percent digitonin. Previous investigations of visual pigment fluorescence have dealt with the emission characteristics of the products of bleaching rather than rhodopsin. The first fluorescent species described (*5*) is a pigment (probably pigment 465) formed after the decay of metarhodopsin (probably meta II). There are apparently no reports of a fluorescence from any earlier species, including rhodopsin. If a rhodopsin fluorescence does exist, according to Hagins, it might be in the near infrared and would thus be difficult to detect. On the other hand, our experiments indicate that a rhodopsin fluorescence does exist and occurs closer in wavelength (575 mμ) to its absorption maximum (500 mμ) than was expected.

Rod outer segments were obtained from frozen retinas of cattle (*6*). The segments were isolated by a sucrose flotation method similar to that described by Wald and Brown (*7*). Digitonin solutions (2 percent) were prepared from these rods and filtered through calcium phosphate gel (*8*). As an index of purity, the ratio of the absorbancies at 400 and 500 mμ was found to fall in the range of 0.24 to 0.30, an indication of fairly pure preparations.

Fluorescence measurements were obtained with a modified version of the Aminco-Bowman spectrophotofluorometer with the R136 photomultiplier detector. When a high-intensity monochromator (*6*) and xenon light source were substituted for the Aminco source, they gave doubly monochromatized excitation light. A marked reduction in scattering resulted with this modification, and a further reduction was obtained with the insertion of variable band-pass interference filters of the appropriate wavelength cutoffs. Wavelength standardization was obtained by a series of compounds of known fluorescence activity. Intensity corrections applied to correct the spectra were obtained with the YSI bolometer. We then estimate that, for these rather broad emission spectra, the wavelength maxima are probably not accurate to more than ± 10 mμ.

The samples were placed in 3-mm cylindrical quartz tubes maintained in position in a quartz Dewar flask. To avoid large self-absorption distortions in the emission spectra, we kept the concentrations of the rhodopsin and digitonin samples quite low (in the 10^{-5} to $10^{-6}M$ range). For the low-temperature measurements on rhodopsin, the solvent used was a mixture of glycerol and water (50 : 50). All manipulations of light-sensitive materials were carried out with only deep-red illumination. The observed emis-