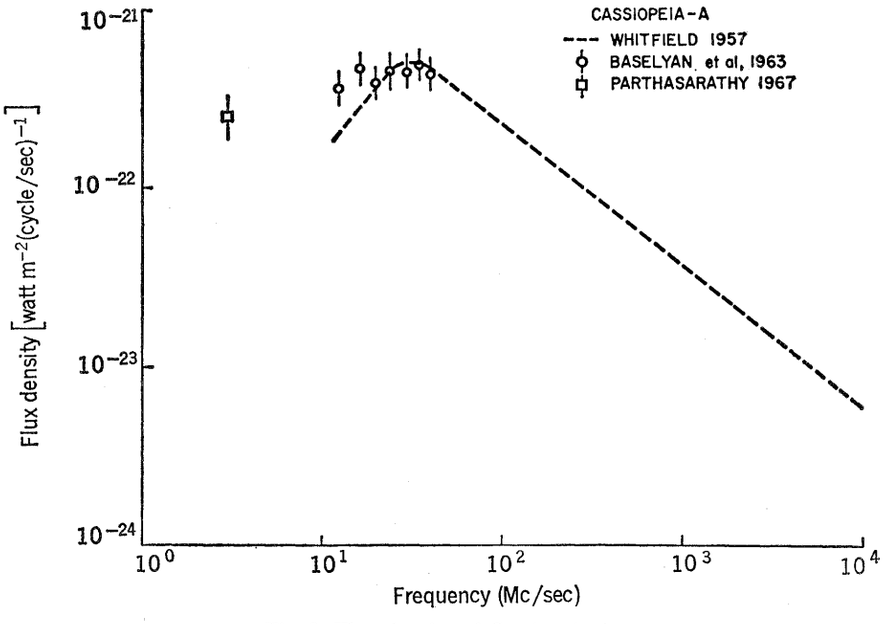

Fig. 2. Flux density of Cassiopeia-A.

density from a source of about 4×10^{-22} watt m^{-2} $(cy/sec)^{-1}$. The Cassiopeia-A can be readily recognized at 2215 to 2315 hours on 8 November, in both modes, at 1945 to 2015 hours on 9 November, at least in the O-mode, and at 2245 to 2330 hours on 16 November, in the O-mode. The pattern is absent at other times either because of high critical frequencies (for example, on 8 November) or because excessive scintillations (for example, on 21 November) nearly destroyed the sine-wave-like trend. From the patterns where the O- and E-mode levels were nearly equal (for example, at about 2300 hours, 8 November, about 2000 hours, 9 November), the flux density of Cassiopeia-A could be derived as $(2.5 \pm 1.0) \times 10^{-22}$ watt m^{-2} $(cy/sec)^{-1}$.

Figure 2 shows our 3-Mc/sec data along with those at higher frequencies. The dashed curve (*2*), a mean of several observers, clearly underestimates the low-frequency flux, since the 16.5-Mc/sec flux included a datum (*6*) which is low by an order of magnitude. Data of Baselyan *et al.* (*3*) from the Soviet arctic are compatible with our 3-Mc/sec data. Bridle (*7*) has obtained a somewhat lower flux, 2.8×10^{-22} at 10 Mc/sec, than Baselyan *et al.*

Numerical estimates reveal that the source is unlikely to be intrinsically a poor emitter below about 30 Mc/sec; the decline in the spectrum is more plausibly due to attenuation along the path. The 3-Mc/sec flux is about 12 decibels weaker than the value that may be obtained by extrapolating the trend at the frequencies $\gtrsim$ 50 Mc/sec. To convert this attenuation into the "emission measure," $\int N_e^2 ds$ (where N_e = electron density per cubic centimeter in the ionized hydrogen clouds, and ds is measured in parsecs),

$$A \text{ (db)} = 1.3 \times 10^{-3} T_e^{-3/2} \lambda^{2.1} \int N_e^2 \, ds$$

where λ is in centimeters and T_e is electron temperature. With $T_e \approx 10^4$, we obtain the "emission measure" to be $\approx$ 100. Since Cassiopeia-A is located at about 3×10^3 parsecs, this could mean either that absorption occurred uniformly along the path, with $N_e \approx$ 0.18, or that a single, typical, H-II cloud of $N_e \approx 10$ and diameter 1 parsec intervened in the path. As pointed out by several workers [see, for example, Bridle (*7*)], one can readily derive an integrated emission measure along the 3 kiloparsecs from about 50 to 200 by assuming plausible values of N_e and T_e.

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Gangliosides in Isolated Neurons and Glial Cells

Abstract. *Gangliosides occur in much greater amounts in clean isolated neurons and in the neuropil teased from immediately around the neuron cell body and dendrites than in isolated clumps of glial cells. Since the zone of neuropil adjacent to neurons is richest in terminal axons and synaptic endings, these findings indicate a specific concentration of these sialoglycolipids in synaptic membranes.*

Within the central nervous system there is considerable indirect evidence of a specific neuronal localization of gangliosides, a group of complex acidic membrane sialoglycosphingolipids (*1*). Brain regions rich in neurons have a high ganglioside content. The distribution of these lipids in subcellular fractions of adult and developing rat brain shows the highest concentration in nerve-ending particles and certain synaptic or dendritic membrane fractions (*2–4*). Fractions of synaptosome ghost-membrane recently isolated (*4*) contain the highest relative specific concentrations not only of gangliosides but also of acetylcholinesterase, adenylcyclase, and a particulate phosphodiesterase. Although gangliosides are present in white matter and occur in small concentrations in purified myelin fractions, it appears they are not constituents of myelin (*5*) but rather of the axon, probably the axolemma (*6*). Deposition of gangliosides during development occurs predominantly before myelination, corresponding to the period of active neuronal increase and expansion of dendritic surface area (*2*). There is no relationship between ganglioside content in various brain regions and the quantitative distribution of neurohormones such as acetylcholine, catecholamines, or 5-hydroxytryptamine. The distribution of these lipids in brain parallels more closely that of γ-amino-

butyric acid (*1*). Gangliosides are not constituents of synaptic vesicles (*2*, *4*). To obtain more direct evidence on ganglioside localization in brain, we performed analyses for these lipids in samples of neurons, neuropil immediately adjacent to the neuron cell bodies and dendrites, and glial cell clumps isolated from Deiter's vestibular nucleus of the brain stem of the ox.

The techniques used for the isolation of single neurons and glial cell clumps were similar to those of Hydén (*7*). With a Stadie blade, thin slices of tissue were cut through the lateral vestibular nuclei of fresh ox brain stems and the slices were moistened with Krebs-Ringer solution without glucose. In the ox, the lateral vestibular nuclei lie in the medulla on the floor of the fourth ventricle and extend dorsally to the inner border of the inferior cerebellar peduncles. Under a dissecting microscope (× 100), neurons and surrounding glial cells were picked out of the nucleus with a Nikrothal-L stainless steel wire (15 μ in diameter) made into a spatula. Methylene blue could not be used to assist in neuron identification since it interfered in the fluorometric reaction for ganglioside determination. Each isolated neuron together with a mass of surrounding neuropil and glial cells attached to it was placed in a small drop of Krebs-Ringer solution on a glass slide. The neuropil and glial cell mass were then carefully teased away from the neuron. Four types of samples were collected for

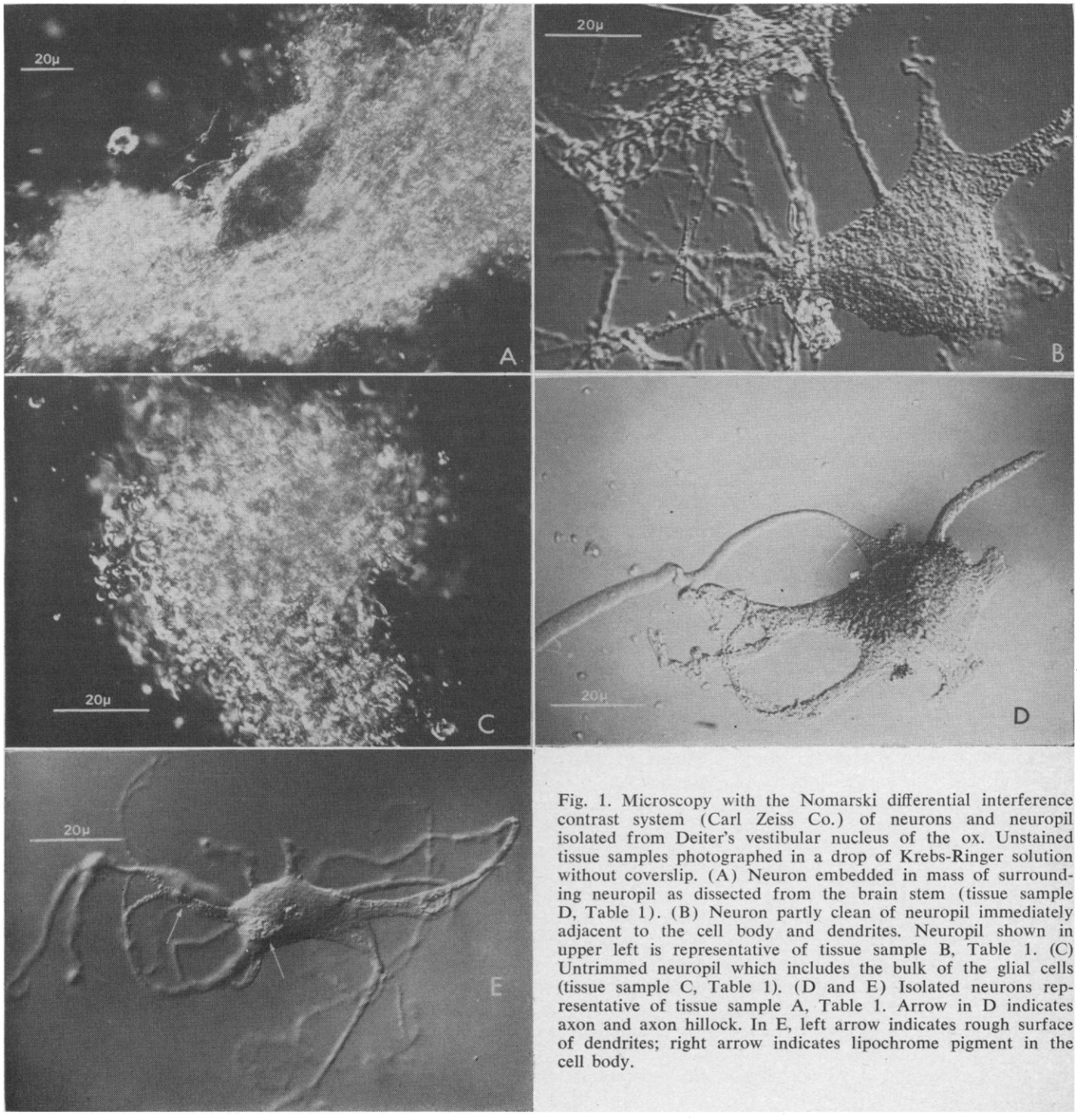

Fig. 1. Microscopy with the Nomarski differential interference contrast system (Carl Zeiss Co.) of neurons and neuropil isolated from Deiter's vestibular nucleus of the ox. Unstained tissue samples photographed in a drop of Krebs-Ringer solution without coverslip. (A) Neuron embedded in mass of surrounding neuropil as dissected from the brain stem (tissue sample D, Table 1). (B) Neuron partly clean of neuropil immediately adjacent to the cell body and dendrites. Neuropil shown in upper left is representative of tissue sample B, Table 1. (C) Untrimmed neuropil which includes the bulk of the glial cells (tissue sample C, Table 1). (D and E) Isolated neurons representative of tissue sample A, Table 1. Arrow in D indicates axon and axon hillock. In E, left arrow indicates rough surface of dendrites; right arrow indicates lipochrome pigment in the cell body.

subsequent ganglioside analyses: (i) cleaned neurons with part of their dendrites still intact, (ii) neuropil samples trimmed from immediately around the neuronal perikarya and dendrites, (iii) untrimmed neuropil which included the bulk of glial cells, and (iv) the complete tissue sample as it was removed from the vestibular nucleus; that is, a single neuron with a mass of neuropil and glial cells (Fig. 1, A–E). On a glass slide separate piles of each of these samples, representing 50 to 70 isolations, were collected together in distilled water; the water was removed from around them with a fine glass capillary, and the samples were lyophilized. The dried tissue samples were weighed on a quartz-fiber balance constructed to weigh between 1 and 10 μg to the nearest 0.1 μg (*8*). The weight of an individual neuron was calculated by dividing the total dry weight by the number of neurons dissected. For 305 isolated neurons the average weight of each was 36 ng. These results are in good agreement with those of other workers (*7, 8*). Gangliosides in the dried tissue samples were extracted with a mixture of chloroform, methanol, and water (16 : 18 : 1, by volume) at 60°C for 1 hour in sealed microtubes. The gangliosides were then partitioned three times into an aqueous upper phase (*9*). Additions and removals were performed with a 100 μl Hamilton syringe. The combined upper phases in a second series of microtubes were dried in a vacuum, and the concentration of *N*-acetylneuraminic acid was determined fluorimetrically (*10*) by reaction with 3,5-diaminobenzoic acid to form the highly fluorescent quinaldines. A preparation of *N*-acetylneuraminic acid recrystallized five times was used as standard (*2*). Each fluorescent measurement was made in 200 μl with a microcell attachment to the Turner fluorometer. Accurate determinations could be made between 10 and 100 ng of *N*-acetylneuraminic acid.

The morphological appearance of the isolated neurons and glial cell masses (Fig. 1, A–E) was clearly demonstrated with the Zeiss differential interference contrast system of Nomarski (*11*). Glial cells were not always clearly distinguishable within the neuropil removed from around the neurons, and this sample clearly contained besides glial cells many strands which probably represented pieces of myelinated and unmyelinated axons and also small pieces of dendrites. It was not possible to remove all contaminating dendritic branches and terminal axons without totally disintegrating the glial cell clumps. The neuropil immediately adjacent to the neurons, but not including the greater mass of glial cells, appeared as a meshwork of fine terminal axons and unidentifiable strands, some of which are almost certainly processes from glial cells (Fig. 1B). A wide variation in the numbers of the main dendritic branches were seen in the isolated neurons (Fig. 1, D–E). The dendrites were covered by small knobs or excrescences which gave the surface a rough appearance (Fig. 1E). These probably represent terminal boutons or spines. The surface of the axon is smooth.

Table 1. Concentrations of gangliosides in isolated neurons, neuropil, and glial clumps from Deiter's vestibular nucleus of the ox brain stem. (A) Cleaned neurons, (B) neuropil trimmed from area adjacent to neurons, (C) untrimmed neuropil containing the bulk of glial cells, and (D) single neuron with neuropil and glial cells attached. Glycolipid *N*-acetylneuraminic acid (NANA) values were converted to gangliosides based on a NANA content of 28.5 percent for purified unfractionated ox brain gangliosides.

Tissue samples	Experiments (No.)	Samples per experiment (Av. No.)	Mean contents, gangliosides ± S.D. (mg/g dry wt.)
A	5	61	10.7 ± 4.2
B	4	61	13.0 ± 3.9
C	2	50	1.5, 2.9
D	4	50	3.1 ± 2.0

The concentration of gangliosides was as high, if not higher, in the neuropil taken from just around the neuron cell body and dendrites as it was in the clean neurons (Table 1). However, samples which contained a single neuron within the bulk of surrounding glial cells had a much lower ganglioside content, almost as low as isolated clumps of glial cells. Thus increased numbers of glial cells in the samples decreased the ganglioside content on a dry-weight basis. It is likely that gangliosides present in the isolated glial samples are due to contamination by pieces of dendrites and axon terminals. Since even the adjacent neuropil is contaminated to some extent with ganglioside-free glial elements (*12*), the nonglial elements in this tissue sample (Table 1B), which are predominantly nerve terminals and torn-off endings, would have an even higher ganglioside content if all of the glial tissue could be removed. A grossly dissected block of tissue from the medulla oblongata contains 2 to 3 mg of gangliosides per gram of tissue (dry weight). Thus the ganglioside content of clean isolated neurons and the adjacent neuropil is five to six times greater than that for the medulla. The conclusion from this study, that the highest concentration of gangliosides is within the membranes of axon terminals and endings, gives further support to the evidence obtained in other studies that gangliosides are important specific membrane lipids of synaptic complexes.

Our experience has been that it is technically almost impossible to separate pure glial cells from the neuropil. Since the neuropil adjacent to neurons is extremely rich in terminal axons and their synaptic connections, it seems worthwhile to consider the possibility that the reciprocal or different metabolic and chemical characteristics of isolated neurons and glial cells (*13*) might in part be due to differences in the chemical and metabolic characteristics of neuronal perikarya and terminal axons and boutons.

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