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Ganglioside Structures and Distribution: Are They Localized at the Nerve Ending?

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Gangliosides generally provide a small portion of the complex carbohydrate content of cell surfaces. An exception is the central nervous system where they comprise up to 5–10% of the total lipid of some membranes. This tissue is unique in that the quantity of lipid-bound sialic acid exceeds that of the protein-bound fraction. Over 30 different molecular species have been characterized to date. These range in complexity from sialosylgalactosyl ceramide with 2 sugars to the pentasialoganglioside of fish brain with 9 carbohydrate units. Virtually all cellular and subcellular fractions of brain that have been carefully examined contain gangliosides to one degree or another, but the majority of brain ganglioside is located in the neurons. Their mode of distribution within the neuron has not been entirely clarified by subcellular studies. Calculations based on reported values for axon terminal density and synaptosomal ganglioside concentration in the rat reveal that nerve endings contribute less than 12% of total cerebral cortical ganglioside. It is concluded that the plasma membranes of neuronal processes contain most of the neuronal ganglioside. These and other considerations suggest the possibility that gangliosides may be distributed over the entire neuronal surface.

Key words: **gangliosides; glycosphingolipids; oligosaccharide structures; nervous system; neurons; subcellular distribution**

Gangliosides and other glycolipids usually comprise a small proportion of the total glycoconjugates that exist on the surface of cells. Major exceptions to this general rule are found in the central nervous system of mammals where the quantity of lipid-bound sialic acid exceeds that of protein-bound sialic acid, and where the myelin membrane contains substantial glycolipid but only a minor amount of glycoprotein. Similarities in the oligosaccharide structures of gangliosides and sialoglycoproteins have led to speculation that

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both groups may subserve similar membrane functions. In containing a single oligosaccharide chain per molecule and a ceramide unit in place of a peptide, glycosphingolipids such as the gangliosides comprise simpler and hence more easily characterized structures than the glycoproteins.

The discovery that gangliosides are receptors for a variety of bacterial toxins and possibly some viruses (reviewed in Refs. 1 and 2) has renewed interest in their possible role as receptors for natural agonists. Demonstration of regions of peptide homology between the B chain of cholera toxin and the β subunits of such glycoprotein hormones as thyrotropin, luteinizing hormone, human chorionic gonadotrophin, and follicle-stimulating hormone has led to the proposal that these proteins might utilize a common mechanism based on ganglioside receptors for transporting subunits across and possibly within the plane of the membrane (2–4). More generally, gangliosides might have as one of their functions the transfer of information from the exterior to the interior of the cell.

If this is indeed a correct view of ganglioside function the large variety of oligosaccharide structures present in both neural and extraneural tissues could provide the specificity required for recognition and binding of an equally large variety of biologically active peptides. At the same time it may be pointed out that several other functions have been proposed, particularly in the central nervous system (CNS) where the high concentration of gangliosides in neuronal elements has led to much speculation regarding a possible role in nerve conduction and/or synaptic transmission. Aside from intriguing hints that gangliosides help to retain the excitability of isolated cerebral tissues (5), very little evidence has come forth to support this or any other hypothesis concerning their role in the CNS. It seems likely that a variety of functions could be involved, consistent with the widespread distribution of these substances throughout the myriad components of the nervous tissue and the impressive diversity of structure that is now apparent. Following a review of ganglioside structures some aspects of their CNS distribution will be critically examined, particularly in regard to the question of localization within the neuron.

STRUCTURES

The distinguishing feature of gangliosides, as opposed to the large group of neutral glycosphingolipids, is the presence of one or more sialic acid units in the oligosaccharide chain. These generally occupy a terminal position, being linked to either galactose or another sialic acid. Some 17 or 18 different sialic acids have been found in nature (6) but only a few of these have been detected thus far in gangliosides. The most commonly occurring forms are N-acetylneuraminic acid (NAN) and N-glycolylneuraminic acid (NGN). The former is virtually the only type found in brain gangliosides of most mammals, although in some cases (e.g., bovine) a small percentage of NGN has been detected (7, 8). Both NAN and NGN, and often acylated forms of each, are found in extraneural tissues, the types and amounts showing considerable species specificity. NAN is the only sialic acid that has been reliably identified in man.

Glycosphingolipids may be divided into 2 general categories based on the carbohydrate immediately linked to ceramide. The first category is derived from galactosylceramide (Fig. 1). It is a relatively small family with a single ganglioside ($G_7 = G_{M4}$). The large majority of glycosphingolipids, including virtually all gangliosides except G_7, are derived from the second family originating with glucosylceramide (Fig. 2). This family diverges into 4 major branches from lactosyl ceramide, based on the nature of the third

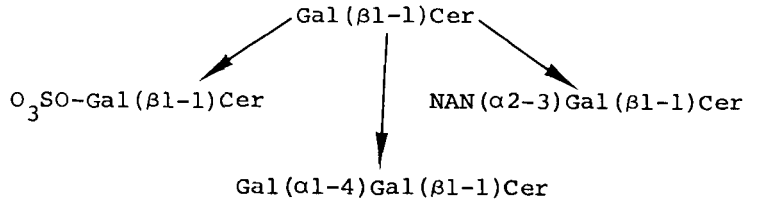

Fig. 1. Family of glycosphingolipids derived from galactosylceramide.

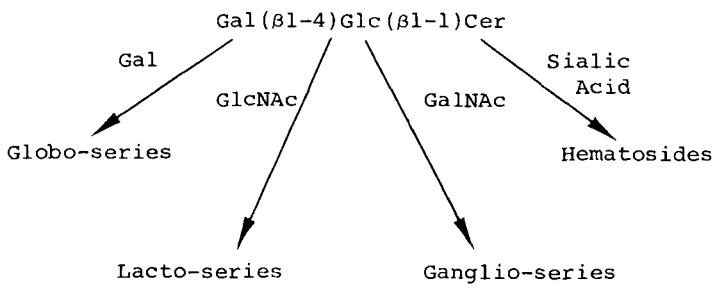

Fig. 2. Family of glycosphingolipids derived from glucosylceramide.

attached sugar. The globo series (following the currently adopted nomenclature, see Ref. 9) has galactose as the third sugar and includes such substances as globoside I (10, 11) and Forssman glycolipid (12):

GalNAc (β1-3) Gal (α1-4) Gal (β1-4) Glc (β1-1) Cer
Globoside I

GalNAc (α1-3) GalNAc (β1-3) Gal (α1-4) Gal (β1-4) Glc (β1-1) Cer
Forssman glycolipid

The added galactose in most cases has an α-glycosidic linkage of the type shown, but it has also been found to have a different linkage (e.g., β1-4) in certain of the blood group A-active fucolipids of hog gastric mucosa (13–15). The globo series is the only one of the 4 groups depicted in Fig. 2 which has not yet been found to include gangliosides.

The lacto, ganglio, and hematoside series (Fig. 2) contain most of the 30-odd gangliosides characterized to date. Their structures are summarized in Tables I–III along with some of the sources from which they were obtained. The tissues listed are representative rather than comprehensive. Animal species are indicated in some instances where this appears to be a factor affecting structural specificity.

Hematosides (Table III) are defined in the broad sense as those gangliosides which lack hexosamine. This widely used term arose from the fact that they were originally isolated from erythrocytes (16), but they have since been detected in virtually every

TABLE I. Structures of Vertebrate Gangliosides. Ganglio-N-Glycose Series

Structure	Symbol[a]	Source	Reference
Monosialo			
1. GalNAc (β1-4) Gal (β1-4) Glc (β1-1) Cer 3 ↑ α 2 NAN	G_{M2}	normal brain Tay-Sachs brain	83 84
2. GalNAc (β1-4) Gal (β1-4) Glc (β1-1) Cer 3 ↑ α 2 NGN	G_{M2}(NGN)	spleen, kidney, (bovine)	17
3. Gal (β1-3) GalNAc (β1-4) Gal (β1-4) Glc (β1-1) Cer 3 ↑ α 2 NAN	G_{M1}	normal brain G_{M1} gangliosidosis spleen, kidney adrenal medulla	85 86 17 87
4. Gal (β1-3) GalNAc (β1-4) Gal (β1-4) Glc (β1-1) Cer 3 ↑ α 2 NGN	G_{M1}(NGN)	spleen, kidney (bovine)	17
5. Gal (β1-3) GalNAc (β1-4) Gal (β1-4) Glc (β1-1) Cer 2 3 ↑ α ↑ α 1 2 Fuc NAN		brain (bovine) testis (boar)	88 89
6. Gal (β1-3) GalNAc (β1-4) Gal (β1-4) Glc (β1-1) Cer 2 3 ↑ α ↑ α 1 2 Fuc NGN		liver (bovine)	17
Disialo			
7. GalNAc (β1-4) Gal (β1-4) Glc (β1-1) Cer 3 ↑ α 2 NAN (8←2α) NAN	G_{D2}	brain	90,91
8. Gal (β1-3) GalNAc (β1-4) Gal (β1-4) Glc (β1-1) Cer 3 3 ↑ α ↑ α 2 2 NAN NAN	G_{D1a}	brain adrenal medulla muscle retina	92–95 87 112 119
9. Gal (β1-3) GalNAc (β1-4) Gal (β1-4) Glc (β1-1) Cer 3 3 ↑ α ↑ α 2 2 NAN NGN	G_{D1a} (NAN/NGN)	brain (bovine) adrenal medulla (bovine)	88 87
10. Gal (β1-3) GalNAc (β1-4) Gal (β1-4) Glc (β1-1) Cer 3 3 ↑ α ↑ α 2 2 NGN NAN	G_{D1a} (NGN/NAN)	brain (bovine)	88

TABLE I. Structures of Vertebrate Gangliosides. Ganglio-N-Glycose Series (continued)

Structure	Symbol[a]	Source	Reference
Disialo (continued)			
11. Gal (β1-3) GalNAc (β1-4) Gal (β1-4) Glc (β1-1) Cer 3 3 ↑ α ↑ α 2 2 NGN NGN	$G_{Dla}(NGN)_2$	liver, spleen, kidney (bovine)	17
12. Gal (β1-3) GalNAc (β1-4) Gal (β1-4) Glc (β1-1) Cer 3 ↑ α 2 NAN (8←2α) NAN	G_{D1b}	brain retina	92, 96–98 119
13. GalNAc (β1-4) Gal (β1-3) GalNAc (β1-4) Gal (β1-4) Glc (β1-1) Cer 3 3 ↑ α ↑ α 2 2 NAN NAN		brain	99
Trisialo			
14. Gal (β1-3) GalNAc (β1-4) Gal (β1-4) Glc (β1-1) Cer 3 3 ↑ α ↑ α 2 2 NAN (8←2α)NAN NAN	G_{T1a}	brain (human)	100
15. Gal (β1-3) GalNAc (β1-4) Gal (β1-4) Glc (β1-1) Cer 3 3 ↑ α ↑ α 2 2 NAN NAN (8←2α)NAN	G_{T1b}	brain	92, 96
16. Gal (β1-3) GalNAc (β1-4) Gal (β1-4) Glc (β1-1) Cer 3 ↑ α 2 NAN(8←2α)NAN(8←2α)NAN	G_{T1c}	brain (fish)	29
Tetrasialo			
17. Gal (β1-3) GalNAc (β1-4) Gal (β1-4) Glc (β1-1)Cer 3 3 ↑ α ↑ α 2 2 NAN (8←2α) NAN NAN (8←2α) NAN	G_{Q1b}	brain (human)	31, 101
18. Gal (β1-3) GalNAc (β1-4) Gal (β1-4) Glc (β1-1) Cer 3 3 ↑ α ↑ α 2 2 NAN NAN (8←2α) NAN (8←2α) NAN	G_{Q1c}	brain (fish)	29
Pentasialo			
19. Gal (β1-3) GalNAc (β1-4) Gal (β1-4) Glc (β1-1) Cer 3 3 ↑ α ↑ α 2 2 NAN(8←2α) NAN NAN (8←2α) NAN (8←2α) NAN	G_{P1}	brain (fish)	29

[a]The symbols are those of the Svennerholm system (Ref. 102). Additional symbols beyond those originally proposed have been added in a manner thought to be consistent with the system as a whole. Where more than one type of sialic acid is present in the same molecule the first designated within ( ) is that most distal from ceramide.

TABLE II. Structures of Vertebrate Gangliosides. Lacto-N-Glycose Series

Structure	Source	Reference
1. Gal (β1-4) GlcNAc (β1-3) Gal (β1-4) Glc (β1-1) Cer 3 ↑ α 2 NAN	erythrocytes (human) peripheral nerve brain spleen, kidney, liver muscle plasma	79, 20 19 19 17 19 19
2. Gal (β1-4) GlcNAc (β1-3) Gal (β1-4) Glc (β1-1) Cer 6 ↑ α 2 NAN	spleen, kidney, liver	17
3. Gal (β1-4) GlcNAc (β1-3) Gal (β1-4) Glc (β1-1) Cer 3 ↑ α 2 NGN	spleen, kidney (bovine)	17, 103, 104
4. Gal (β1-4) GlcNAc (β1-3) Gal (β1-4) Glc (β1-1) Cer 3 3 ↑ α ↑ α 2 1 NAN Fuc	kidney (human)	105
5. Gal (β1-4) GlcNAc (β1-3) Gal (β1-4) Glc (β1-1) Cer 3 ↑ β 1 GlcNAc (4←1β) Gal (3←2α) NAN	spleen (human)	106

TABLE III. Structures of Vertebrate Gangliosides. Hematoside Series

Structure	Symbol[a]	Source	Reference
1. NAN (α2-3) Gal (β1-1) Cer	G_{M4}	human brain	34, 83, 107, 108
		chimpanzee brain	109
		avian brain	110
2. NAN (α2-3) Gal (β1-4) Glc (β1-1) Cer	G_{M3}	brain	90, 108
		erythrocytes	111
		spleen, kidney, liver	17,121
		adrenal medulla	87
		muscle	112
		plasma	113,120
		platelets	118
3. NGN (α2-3) Gal (β1-4) Glc (β1-1) Cer	G_{M3}(NGN)	erythrocytes (equine)	16, 114, 115
		spleen (bovine)	17
		adrenal medulla (bovine)	87
4. OAc-NGN (α2-3) Gal (β1-4) Glc (β1-1) Cer		erythrocytes (equine)	116
5. NAN (α2-8) NAN (α2-3) Gal (β1-4) Glc (β1-1) Cer	G_{D3}	brain, normal	83
		brain, SSPE	117
		spleen, kidney, liver	17, 121
		platelets	118
		retina	119,123
		placenta	122
6. NGN (α2-8) NAN (α2-3) Gal (β1-4) Glc (β1-1) Cer	G_{D3} (NGN/NAN)	spleen, kidney, liver (bovine)	17
7. NAN (α2-8) NGN (α2-3) Gal (β1-4) Glc (β1-1) Cer	G_{D3} (NAN/NGN)	spleen, kidney, liver (bovine)	17
8. NGN (α2-8) NGN (α2-3) Gal (β1-4) Glc (β1-1) Cer	G_{D3} $(NGN)_2$	erythrocytes (cat)	124, 125
		spleen, kidney, liver (bovine)	17

[a]See footnote to Table I.

vertebrate tissue and often occur as the major ganglioside(s). Brain contains only minor amounts of the mono- (G_{M3}) and disialosyl (G_{D3}) types and variable amounts of sialosylgalactosyl ceramide ($G_7 = G_{M4}$) depending on species (see below).

Several gangliosides have been detected in the lacto series (Table II), most of these occurring in visceral tissues such as liver, kidney, and spleen (17). Two basic tetraglycosyl structures occur in this series:

Gal (β1-3) GlcNAc (β1-3) Gal (β1-4) Glc (β1-1) Cer
lacto-N-tetraosylceramide
Type I

Gal (β1-4) GlcNAc (β1-3) Gal (β1-4) Glc (β1-1) Cer
lacto-N-neotetraosylceramide
Type II

Virtually all the well-characterized gangliosides of this kind are based on lacto-N-neotetraosylceramide (Type II). A ganglioside containing the Type I structure was initially described in bovine spleen and kidney by Wiegandt (18), but his later report on these substances (17) failed to include this particular structure. Wiegandt also pointed out in his second study (17) that one monosialosyl derivative of lacto-N-neotetraosylceramide ($G_{Lntet}1b$ by his nomenclature) had been incorrectly identified as a disialosyl derivative in the earlier study (18). The structure with NAN linked (α2-3) to terminal galactose of lacto-N-neotetraosylceramide was shown to be the major ganglioside in human peripheral nerve (19) and erythrocytes (20) but only a minor type in human brain (19). To date it is the only glucosamine-containing ganglioside detected in brain.

Most of the known gangliosides, including the large majority in brain, belong to the ganglio series (Table I) with N-acetylgalactosamine as the third sugar. Since sialic acid is also substituted on the same galactose, these all contain branched structures. Biosynthetically, sialic acid is attached first to lactosylceramide followed by N-acetylgalactosamine. These and subsequent sugars were shown to be added sequentially by a series of glycosyltransferases, believed to function as a coordinated complex (21).

Mammals generally contain 4 major brain gangliosides, of which G_{M1} forms the basic structural unit (Fig. 3). The 3-dimensional projection shown is based on manipulation of molecular models, which indicated reduced steric crowding when the sialic acid group is perpendicular to the linear carbohydrate chain. When attached by the α-ketosidic linkage demonstrated for gangliosides (22), the sialic acid of G_{M1} would thus tend to be shielded to some extent by the adjacent sugars, while the additional sialic acids of G_{D1a}, G_{D1b}, and G_{T1b} would experience less shielding. A small difference in the acidity of these groups has been correlated with differences in their chromatographic behavior on ion-exchange resins (23).

Treatment of the di- and trisialogangliosides of Fig. 3 with neuraminidase converts these to G_{M1}, the sialic acid of which is resistant to this enzyme except in the presence of detergent (24). Despite such resistance the sialic acid of G_{M1} is linked through an α-ketosidic bond of the same configuration as the reactive sialic acids, and its resistance has been ascribed to steric hindrance by the adjacent hexosamine (25, 26). When such interference is absent, as in G_{M3}, sialic acid becomes quite reactive to the enzyme. It may be noted that the disialosyl grouping, NAN(2-8)NAN, such as is found in G_{D1b} and G_{T1b}, reacts very sluggishly with neuraminidase in comparison to the NAN(2-3)Gal unit present in G_{D1a}, G_{M3}, etc. This disialosyl unit, known for a long time to be present in gangliosides, was only recently detected in glycoproteins (27).

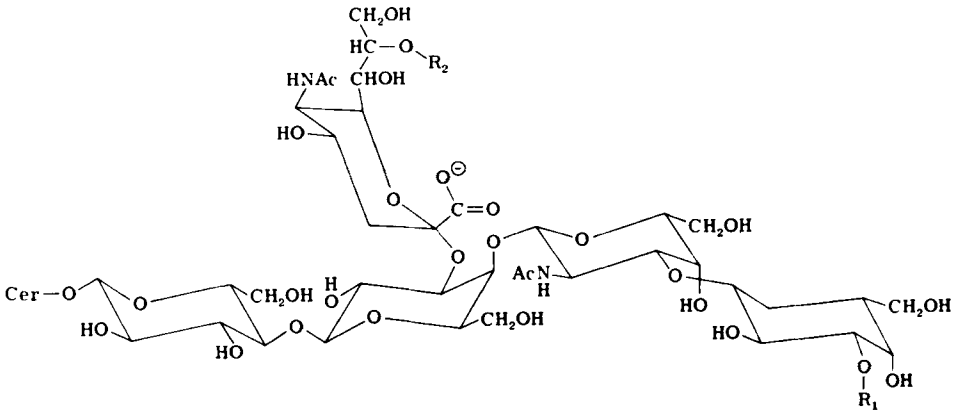

Fig. 3. Structures of the 4 major gangliosides of mammalian brain. G_{M1}: $R_1 = R_2 = H$; G_{D1a}: R_1 = NAN, $R_2 = H$; G_{D1b}: $R_1 = H$, R_2 = NAN; G_{T1b}: $R_1 = R_2$ = NAN.

While the 4 gangliosides depicted in Fig. 3 comprise the major brain types of most mammals, lower vertebrates have a different pattern in which more complex polysialogangliosides predominate and monosialo species such as G_{M1} are barely detectable. Fish brain was thus found to have a tetra- and a pentasialoganglioside as the 2 major forms (28, 29) while a similar pattern was suggested for carp, perch, and frog (30). The structure proposed (29) for the tetrasialoganglioside of fish brain (entry 18, Table I) differs from that of the tetrasialoganglioside of human brain (entry 17, Table I). The latter was recently shown (31) to have a disialosyl NAN(2-3)NAN unit attached to each galactose, corresponding to a structure proposed earlier by Klenk (32) on the basis of incomplete evidence. Attachment of the disialosyl unit to terminal galactose was also detected in a newly characterized trisialoganglioside G_{T1a} of human brain (33).

It should be pointed out that certain other vertebrates depart from the typical mammalian brain pattern in having considerable amounts of sialosylgalactosyl ceramide ($G_7 = G_{M4}$). In human white matter this was shown to be the third most abundant ganglioside on a molar basis (34), while recent work has revealed an unusually high concentration of this ganglioside in chicken and pigeon brains (35). In all cases it appears to be localized primarily in myelin.

Space does not permit detailed discussion of the lipophilic constituents of these glycosphingolipids. Some general trends may be noted, however, a principal one being the presence of substantial amounts of C_{20} long-chain bases in CNS gangliosides and little if any in extraneural species. Fatty acid differences have also been reported. Gangliosides from certain peripheral nerve sources provided an interesting hybrid of CNS and extraneural properties (36). Ganglioside G_7 from human myelin had unique lipophilic components that closely resembled those of myelin cerebrosides (34). Lipophilic composition has been considered in recent reviews of ganglioside biochemistry (37–40).

Although gangliosides are generally considered to be characteristic lipids of the vertebrates, a few types have been isolated from various species of Echinodermata. They were first detected in the gametes of the sea urchin Pseudocentrotus depressus (41), and most subsequent studies have employed either gametes or gonads of various echinoderms. This was the only one of many marine invertebrate phyla examined which was found to contain gangliosides (41, 42). The simplest of these had the structure NAN (2-6) Glc (1-1)

Cer (43). Virtually all the analyzed fractions contained glucose as the only carbohydrate besides sialic acid. One such substance from the gonads of Echinocardium cordatum contained an unusual sialic acid with a sulfate on the 8-hydroxyl of NAN (44). Disialogangliosides containing one (43) and 2 (45) glucose units have also been detected. The lipophilic constituents were shown to include phytosphingosine and α-hydroxy fatty acids, thus differing from most vertebrate gangliosides.

DISTRIBUTION

Early distribution studies (46, 47) recognized the substantially higher concentration of gangliosides in gray matter compared to white, and this finding has since been confirmed many times. The inference from this that gangliosides are uniquely neuronal constituents has been refuted by more recent studies in several laboratories which have revealed their presence in virtually every cell type and most subcellular fractions of the CNS. Like glycosphingolipids in general, they appear to be ubiquitous in vertebrate tissues and a few were detected in certain invertebrates as well (see above). However, the fact that the concentration in brain so greatly exceeds that of most other organs – approximately 15-fold when comparing gray matter to liver, for example – has served to focus special interest on their distribution and behavior in this organ.

While it now appears highly probable that the majority of brain ganglioside is localized in the neurons, their precise distribution within these complex cells is far from clear. When gangliosides were first quantified in isolated neuronal perikarya their concentration turned out to be surprisingly low: 1.24 μg NAN/mg protein for the rat in the study of Norton and Poduslo (48), and 2.88 μg NAN/mg protein for the rabbit in the study of Hamberger and Svennerholm (49). Both groups reported the astrocyte level to be higher than that of neurons from the same source (3.45 and 5.38 μg NAN/mg protein from rat and rabbit, respectively). It was suggested (48) that since astrocytes have a higher ratio of surface area to volume than neuronal perikarya their higher ganglioside content could reflect the greater quantity of plasma membrane. An alternative explanation proposed that these astrocyte values are artifically high due to contamination by nerve ending membranes (49, 50). In any case it is apparent that the cell bodies of these 2 cell types account for only a small part of total gray matter ganglioside.

This conclusion is supported by quantitative estimations utilizing the above concentrations and reported values for neuronal and glial numbers. The rat somatosensory cortex has been estimated (51, 52) to contain 1.32×10^8 neurons/g[1], and with a protein weight of 246 pg/neuronal cell body (135) the perikaryal protein amounts to 32.5 mg/g cortex. Using a ganglioside concentration of 0.8 μg NAN/mg protein obtained for rat neurons (136) the total contribution from this source is calculated as 26 μg NAN/g cortex. Similar calculations for glia, based on a density of 53×10^6 cells/g cortex (51, 52), a protein content of 307 pg/cell (48), and a ganglioside concentration of 2.1 μg NAN/mg protein (136), yield values of 16.3 mg glial protein/g rat cortex and 34.2 μg NAN/g rat cortex. These values pertain to glial cell bodies and the processes associated during their isolation. The content of the processes shorn off during isolation cannot be estimated at present. The result here based on the ganglioside value for astroglia should be considered an upper limit estimate since the cell count included oligodendroglia and microglia as well. Oligodendroglia, which are less numerous than astrocytes in the cortex, contain less ganglioside (53) and hence would tend to lower the above estimate. If the claim of synaptic membrane contamination is true this would further contribute to an overestimation of the glial contribution.

[1] All references to a gram of cortex denote fresh weight values.

Since ganglioside content of rat cerebral cortex is approximately 880 μg NAN/g (54) – slightly higher than the value (849 μg NAN/g) for whole rat brain (8) – it is evident that the contribution from neuronal plus glial cell bodies is probably less than 10% of the total. By inference, therefore, a large portion of cortical ganglioside resides in the neuronal (and to a lesser extent glial) processes. This was suggested by an earlier study (55) in which wet microdissection was employed to demonstrate that neuropil trimmed from an area adjacent to neurons (and therefore rich in neuronal processes) contained the highest concentration while untrimmed neuropil containing mainly glial cells had the lowest. Cleaned neurons were intermediate. More recently, microchemical analysis of sectioned layers of human and rat cortex demonstrated maximal ganglioside in regions of high concentrations of dendritic and axonal plexuses and their synaptic articulations (52).

An important question yet to be addressed, however, is the mode of ganglioside distribution within the processes. The idea has gained currency in recent years that the axon terminals are the major loci of ganglioside concentration. Support for this idea came from studies reporting appreciable ganglioside levels in isolated synaptosomes and, most particularly, synaptic plasma membranes. Critical examination of the data, however, reveals that these structures do not account for a major portion of neuronal ganglioside.

Published values for ganglioside content of synaptosomes and synaptic plasma membranes are summarized in Tables IV and V, respectively. The wide variations, particularly in membrane values, probably reflect to some extent differences in purity of the various preparations stemming from different isolation techniques. In a critical review Morgan (56) has estimated that synaptosomes are generally isolated in 40–50% purity while synaptic plasma membranes prepared by his method (57) are estimated as approximately 50–80% pure. Population selection is another possible source of variation since some laboratories (58, 59) have reported higher ganglioside content in "cholinergic" as opposed to "noncholinergic" synaptic membranes. Species differences might be an additional factor. Reports dealing with ganglioside levels in several components of the nervous system have been summarized in a recent review (60).

The approximate contribution of nerve endings to total cortical ganglioside may be calculated as follows. The ganglioside concentration in whole synaptosomes is taken as 9.0 μg NAN/mg protein, the average of the 5 values reported for rat (Table IV). The range (7.0–10.3) for this species was not excessive. The reliability of this value depends of course on the nature of the contaminants and their ganglioside content. Microsomes are usually considered a major source of contamination (56, 61) and since this heterogeneous fraction has a high ganglioside content (62–65) their presence would tend to elevate synaptosomal values. Mitochondria or mitochondrial membranes would have the opposite effect because of their low ganglioside content. Glial contamination is another possible factor whose magnitude has not been established.

In regard to nerve-ending density, values of 12.6×10^{11} (66) and 14×10^{11} (67, 68) were reported as the number of axon terminals per cubic centimeter of mature rat cortex. Clementi et al. (69), using a polystyrene bead tagging procedure, reported a value of 4×10^{11} for the number of synaptosomes produced per gram of guinea pig cortex on homogenizing under defined conditions. Since a certain percentage is destroyed by homogenization, the true number of axon terminals should be greater. Cragg's more recent figure of 21×10^{11} will be used here; this value, an average for rat visual and frontal cortex, was obtained by electron microscopy (70). Cragg also obtained a value of 0.452 μm for the average diameter of axon terminals in the same areas. The calculated volume (0.048 μm^3) in conjunction with a density (from gradient centrifugation) of 1.15, yields a weight of 0.055 pg/nerve ending. The 21×10^{11} axon terminals in a gram of rat cortex would thus

TABLE IV. Ganglioside Content of Synaptosomes

Species	μg NAN per mg protein	Reference
ox	16.3	126
rabbit	13.8	49
rabbit	11.9	127
rat	10.3	128
rat	9.7	129
rat	9.2	59
rat	9.2	64
rat	7.0	130
guinea pig	8–9	65
human (infant)	7.0	131
human (adult)	7–10	131

Several of the above values have been recalculated from the original data.

TABLE V. Ganglioside Content of Synaptic Plasma Membranes

Species	μg NAN per mg protein	Reference
guinea pig	16–18	132
rat ("cholinergic")	16.7	58
rat ("noncholinergic")	7.3	58
rat ("cholinergic")	45.2	59
rat ("noncholinergic")	18.5	59
rat	44.6	133
rat	19.3	134

weigh 116 mg and of this amount it may be assumed that approximately 10% is protein. Utilizing the above ganglioside concentration one obtains a calculated value of 104 μg NAN as the contribution of all nerve endings in 1 g of rat cortex.[2] This is roughly 12% of the total (880 μg NAN/g cortex). Since the calculation was based on whole synaptosomes it does not depend on the concentration in synaptic plasma membranes, about which there is still considerable uncertainty (Table V).

These results are summarized in Table VI. Such elements as epithelial cells, ependymal cells, myelin, and capillaries, which would probably contribute little, and a portion of astroglial processes are not included. The calculations indicate that neuronal and glial cell bodies plus nerve endings account for only about 50% of the protein and 20% of the

[2]This result is considered an upper limit estimate because of the relatively high values employed for ganglioside concentration and axon terminal density. A recent study in our laboratory (unpublished) indicated the ganglioside concentration of carefully washed rat brain synaptosomes to be significantly below the value of 9.0 μg NAN per mg protein, taken as an average from Table IV. Several of the synaptosome preparations represented in Table IV were not washed in a manner required to free the particles of microsomal contamination (57, 61), and this may have resulted in artificially high ganglioside concentrations.

TABLE VI. Contributions of Subfractions to Total Ganglioside of Rat Cerebral Cortex

	Protein mg/g cortex	Ganglioside concentration μg NAN/mg protein	Ganglioside quantity μg NAN/g cortex
Neuronal cell bodies	32.5	0.8	26.0
Glial cell bodies	16.3	2.1	34.2
Nerve endings	11.6	9.0	104.4
	60.1		164.6
Total/g cortex	123		880
Remainder	63		715

ganglioside in a gram of rat cerebral cortex. The large majority of cortical ganglioside would therefore reside in neuronal processes exclusive of axon terminals. The calculations are, of course, dependent on the reliability of the reported measurements of cell number, nerve-ending density, ganglioside concentrations, etc., but it may be noted that considerable variation in 1 or more of these parameters would be possible without altering the basic conclusion.

It is not possible at this stage to estimate the relative ganglioside content of dendrites and axons, the major components of the neuropil. Assuming that these processes contain in equivalent proportions the 715 μg ganglioside NAN and 63 mg protein unaccounted for ("remainder") in Table VI, the average concentration would be 715/63 = 11.3 μg NAN/mg protein, a value higher than that of whole synaptosomes. From these considerations one might speculate that the ganglioside content in plasma membranes of processes may be comparable to or greater than that of synaptosomal plasma membranes. Extending this concept to include the cell body (see below) the possibility exists that gangliosides may be distributed over a substantial part of the neuronal surface. This plasma membrane pool would likely comprise half or more of total neuronal ganglioside, additional though smaller pools being present in endoplasmic reticulum, cytoplasm, mitochondria, and possibly other intracellular compartments. As noted above, the microsomal fraction which is thought to originate from a diversity of plasma and reticular membranes has a rather high ganglioside content (62–65), approaching in some studies the value for synaptic plasma membrane. Our own studies (137) have verified this similarity between microsomes and synaptic plasma membranes, although the ganglioside concentrations in these preparations were well below the maximum value appearing in Table V. Owing to the uncertainties cited above as well as other potential methodological pitfalls, the precise concentrations of gangliosides in such membranes remain in some doubt.

To calculate the approximate ganglioside content of the perikaryal plasma membrane, one can utilize data which correlates surface area with protein content. This was found in the case of erythrocytes (71, 72) to be 0.37 μg protein per square centimeter (10^8 μm^2) of surface area, and it is assumed here that the neuronal membrane has roughly the same percentage of protein and hence the same protein to area ratio. A spherical neuron with a diameter of 18 μm has a surface area of 1,017 μm^2, while 1.32×10^8 neurons (the amount in a gram of rat cortex, Ref. 52) would have a total area of 1.34×10^{11} μm^2 corresponding to 495 μg protein. If it is assumed that 50–80% of cell body ganglioside (26 μg NAN/g rat cortex, Table VI) is localized in the plasma membrane, the ganglioside concentration of

the latter is calculated to be 26–42 μg NAN/mg protein. These calculated values, while admittedly dependent on a number of assumptions, do appear to fall within the range of values reported for synaptic plasma membranes (Table V) and suggest the possibility that ganglioside concentration may approach uniformity over certain portions of the neuronal membrane. It is conceivable, in light of considerations discussed previously, that dendritic membrane gangliosides could also fall in the same concentration range. Axonal membranes, owing to the presence of myelin and altered surface characteristics, may or may not prove unique in this regard.

The hypothesis that gangliosides are distributed over a large part of the neuronal surface is consistent with current concepts of a fluid mosaic model of membranes (73, 74). If the neuronal plasma membrane can be viewed as a continuum, individual lipids inserted into the perikaryal membrane, for example, would be expected to diffuse into the adjoining plasma membranes of the processes, assuming the absence of diffusional barriers. The rate of lateral diffusion can be quite rapid, e.g., the diffusion constant for phospholipid molecules in sarcoplasmic reticulum has been estimated at 6×10^{-8} cm^2/sec (75). If the constant for neuronal membranes is comparable a molecule would diffuse 1 mm in about a day, a rate considerably more rapid than the turnover time of brain gangliosides (76, 77). Such a mechanism would therefore allow equilibration of surface molecules between the neuron and a number of its processes, while the more remote axonal and synaptic regions would be expected to receive ganglioside through the demonstrated mechanism of rapid axonal transport (78, 79). Lateral diffusion might then function to equalize ganglioside concentrations in these distal membranes as well. The shorter axons and associated terminals could receive and equilibrate their membrane gangliosides by a combination of lateral diffusion and axonal flow.

If such equilibration does in fact occur over large portions of the neuronal surface this should be reflected in similarity of ganglioside composition as well as concentration. It may therefore be significant that the ganglioside patterns of neurons and synaptosomes were similar when analyzed in the same laboratory (49), although somewhat different patterns were found for the synaptosomes themselves in other laboratories (59, 133). Comparison of microsomes, synaptosomes, and synaptic plasma membranes revealed similar ganglioside compositions, again when the analyses were carried out in the same laboratory (59). Whether the synaptic junction itself would participate in such equilibration, or present local barriers to diffusion, is open to question. It was previously shown (80) that the extrajunctional (axon terminal) synaptic membrane conforms to the fluid mosiac model in that at least some of its membrane-bound components exhibit lateral mobility, but certain other synaptic components were later found (81) to have greatly restricted mobility. Inhomogeneity of ganglioside content was suggested by the results of one study (79) showing the synaptic junctional complex to have significantly less of this lipid than the adjoining membrane, although the finding of no difference in another study (82) leaves the question open. In any case, the area encompassed by synaptic thickenings, where restricted mobility would be most likely to occur, comprises a minority of the total neuronal membrane. The hypothesis of ganglioside distribution over much if not all the neuronal surface will hopefully be amenable to testing with the aid of improved methods for isolating membranes of the perikaryon and processes.

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