Human brain gangliosides in development, aging and disease

IVICA KRAČUN[1]*, HARALD ROSNER[2], VALERIJA DRNOVŠEK[1], MARIJA HEFFER-LAUC[1], ČEDOMIR ČOSOVIĆ[1] and GORDAN LAUC[1]

[1]*Department of Chemistry and Biochemistry, School of Medicine, University of Zagreb, Republic of Croatia, Yugoslavia and* [2]*Institute of Zoology, University of Hohenheim, Stuttgart, Germany*

ABSTRACT In this study, brain gangliosides in prenatal and postnatal human life and Alzheimer's disease were analyzed. Immunohistochemically, the presence of the «c»-series of gangliosides (GQ1c) was only registered in the embryonic brain at 5 weeks of gestation. Biochemical results indicated a two-fold increase in ganglioside concentration in the human cortex between 16 and 22 weeks of gestation. The increasing ganglioside concentration was based on an increasing GD1a ganglioside fraction in all regions analyzed except in the cerebellar cortex, which was characterized by increasing GT1b. During prenatal human development, regional differences in ganglioside composition could only be detected between the cerebrum («a»-pathway) and the cerebellum («b»-pathway). Between birth and 20-30 years of age, a cerebral neocortical difference of ganglioside composition occurred, characterized by the lowest GD1a in visual cortex. Analyzing the composition of gangliosides in cortical regions during aging, they were observed to follow region-specific alterations. In the frontal cortex, there was a greater decrease in GD1a and GM1 than in GT1b and GD1b, but in the occipital (visual) cortex there was no change in individual gangliosides. In hippocampus, GD1a moderately decreased, whereas other fractions were stable. In the cerebellar cortex, GD1b and GT1b fractions decreased with aging. In Alzheimer's disease, we found all ganglio-series gangliosides (GM1, GD1a, GD1b, GT1b) to be decreased in regions (temporal and frontal cortex and nucleus basalis of Meynert) involved in pathogenesis of disease. In addition, in Alzheimer's disease we found simple gangliosides (GN2, GM3) to be elevated in the frontal and parietal cortex, which might correlate accelerated lysosomal degradation of gangliosides and/or astrogliosis occurring during neuronal death.

KEY WORDS: *gangliosides, human brain, development, aging, Alzheimer's disease*

Introduction

Gangliosides of the so-called ganglio-series (GM1, GD1a, GT1a, GD1b, GT1b, GQ1b; Designation according to IUPAC-IUB Recommendations, (1977) *Eur. J. Biochem. 79:* 11-21) are typical brain glycosphingolipids of all vertebrates situated on the outer membrane leaflet of the neurons (Ledeen, 1985).

By increasing the phylogenetic scale, the pattern of brain gangliosides changes: (1) by accretion of less sialylated gangliosides, (2) by switching of «c» (GT3, GT1c, GQ1c, GP1c) via «b» (GQ1b, GT1b, GD1b) to the «a-series» (GD1a, GM1) of gangliosides, and (3) by the appearance of pronounced regional differences in the ganglioside composition in man (Suzuki, 1965; Kracun *et al.*, 1984; Hilbig and Rahmann, 1987; Rosner and Rahmann, 1987). Gangliosides are involved in neuronal differentiation and synaptogenesis (Dreyfus *et al.*, 1980; Rosner, 1982), known also from genetic errors of ganglioside degradation (gangliosideosis) characterized by the appearance of aberrant neuronal plasticity (meganeurites) (Purpura and Suzuki, 1976). It is still not clear how the gangliosides are involved in formation and maintenance of neuronal plasticity. However, it seems that gangliosides realize their functional role on neuronal membranes via connections with the protein-kinase system and phosphorylation of membrane proteins (Chan, 1989).

Gangliosides of the developing human brain

By biochemical and immunohistochemical methods we analyzed distinct cellular layers of different cortical regions of the developing human brain (subplate layer (SL) and cortical plate (CP) from 5

Abbreviations used in this paper: Ganglioside abbreviations follow the nomenclature system of L. Svennerholm (1963): (GM3) II^3, NeuAc-Lac-Cer; (GM1) II^3, NeuAc-$GgOse_4$-Cer; (GD1a) IV^3, NeuAc-, II NeuAc-$GgOse_4$-Cer; (GD1b) II $NeuAc_2$-$GgOse_4$-Cer; (GT1b) IV^3 NeuAc-, II^3 $NeuAc_2$-$GgOse_4$-Cer; (GQ1b) IV^3 NeuAc2-, II3 $NeuAc_2$-$GgOse_4$-Cer.

***Address for reprints**: Department of Chemistry and Biochemistry, School of Medicine, University of Zagreb, 41000 Zagreb, Republic of Croatia, Yugoslavia. FAX: 41-424.001

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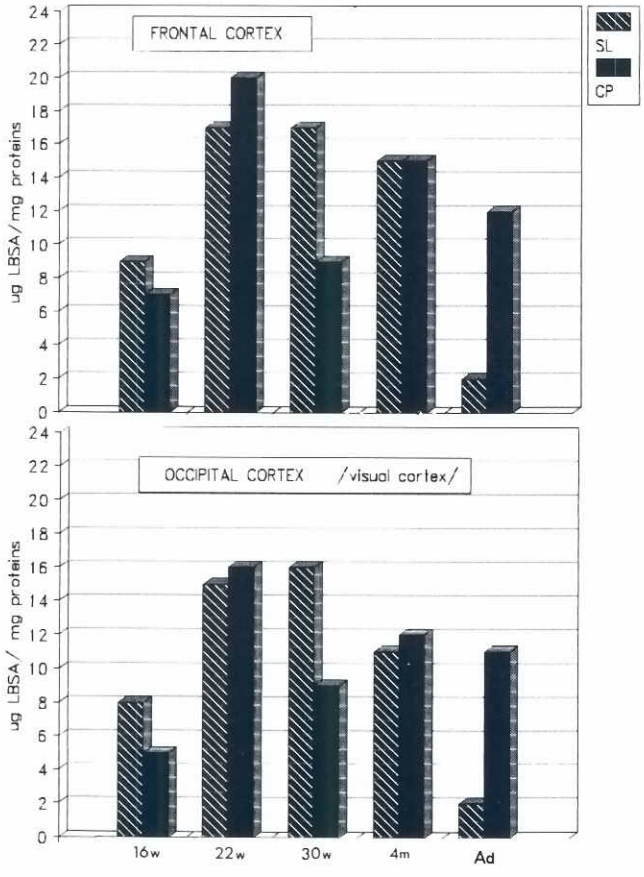

Fig. 1. Ganglioside concentrations (μg lipid-bound sialic acid, LBSA/mg proteins) in human cortical layers (CP and SL) of the developing, postnatal and adult human brain. *± Standard error (2-8 determinations). SL, subplate layer; CP, cortical plate; Ad, adult (40 years of age); m, months; w, weeks.*

weeks of gestation to the adult and senescent stage (90 years of age).

Immunohistochemically, we identified gangliosides in different cortical regions of the fetal, postnatal and adult brains by using monoclonal antibodies against GM3, GD3, GD2, GD1b, and GQ1c (Drnovsek and Kracun, 1991, in preparation).

In the 5-week-old embryonic human brain, the presence of the «c-series» of gangliosides (mainly GQ1c) recognized by monoclonal antibodies Q211 (Henke-Fahle, 1983) was found. Later, after 16 weeks of gestation, no «c-series» of gangliosides were detected. This correlates well with the hypothesis that the «c-series» of gangliosides are also expressed in the early stage of the developing human brain confirming the biogenetic rule (Avrova, 1971) on short repetition of phylogeny in ontogeny since lower vertebrates are enriched in the «c-series» of gangliosides.

Further, GD3 gangliosides in the ventricular layer of the developing fetal pallium at 17 weeks of gestation was detected, correlating well with the evidence that proliferating cells in the embryonic nervous tissue express GD3 gangliosides (Rosner *et al.*, 1985, 1988).

Rapid increase of GD1a ganglioside in SL and CP during rapid cortical synaptogenesis occurring in the human brain between 16 and 30 weeks of gestation (Sidman and Rakic, 1973; Kostovic and Krmpotic-Nemanic, 1976) was found (Figs. 1 and 2). We observed that fetal cortical layers representing the main site of cortical synaptogenesis, the so-called «subplate layer» (SL) (Kostovic and Molliver, 1974), was characterized by a higher concentration of GD1a than cortical plate of the frontal and occipital cortex, respectively. This information on higher GD1a in SL than in CP of human fetal neopallium at 30 weeks of gestation correlated well with neuroanatomical evidence on the SL cells to be the most differentiated neurons of the cerebral cortex in this developmental interval (McConnell *et al.*, 1989).

Our observation on rapid accumulation of ganglioside GD1a during the human brain «growth spurt» is in accordance with previous results (Vanier *et al.*, 1971; Yusuf *et al.*, 1977; Martínez

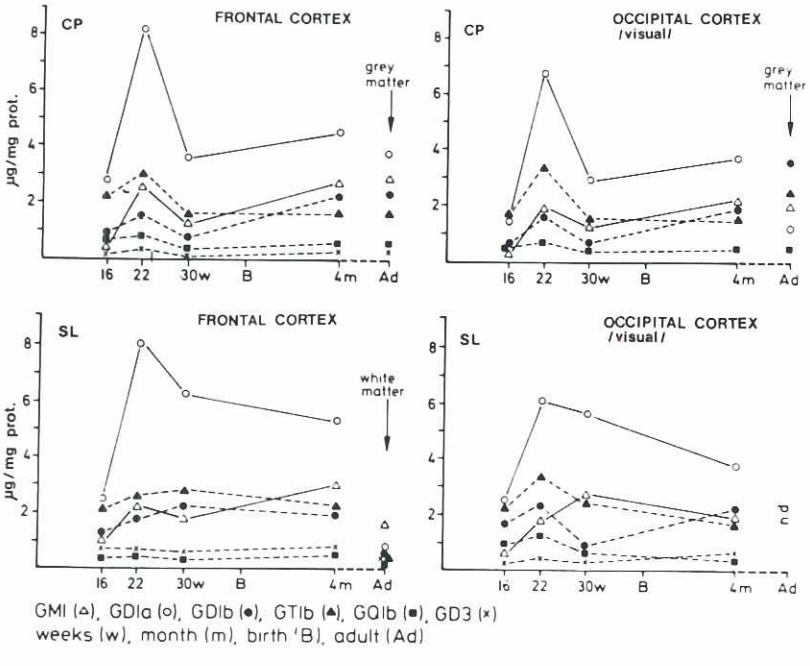

Fig. 2. Ganglioside composition (μg LBSA/mg proteins) of the cortical layers (CP,SL) of the frontal and occipital cortices. *Individual gangliosides are marked according to the legend below the figure. B, birth; n.d., not determined. For abbreviations also see Fig. 1.*

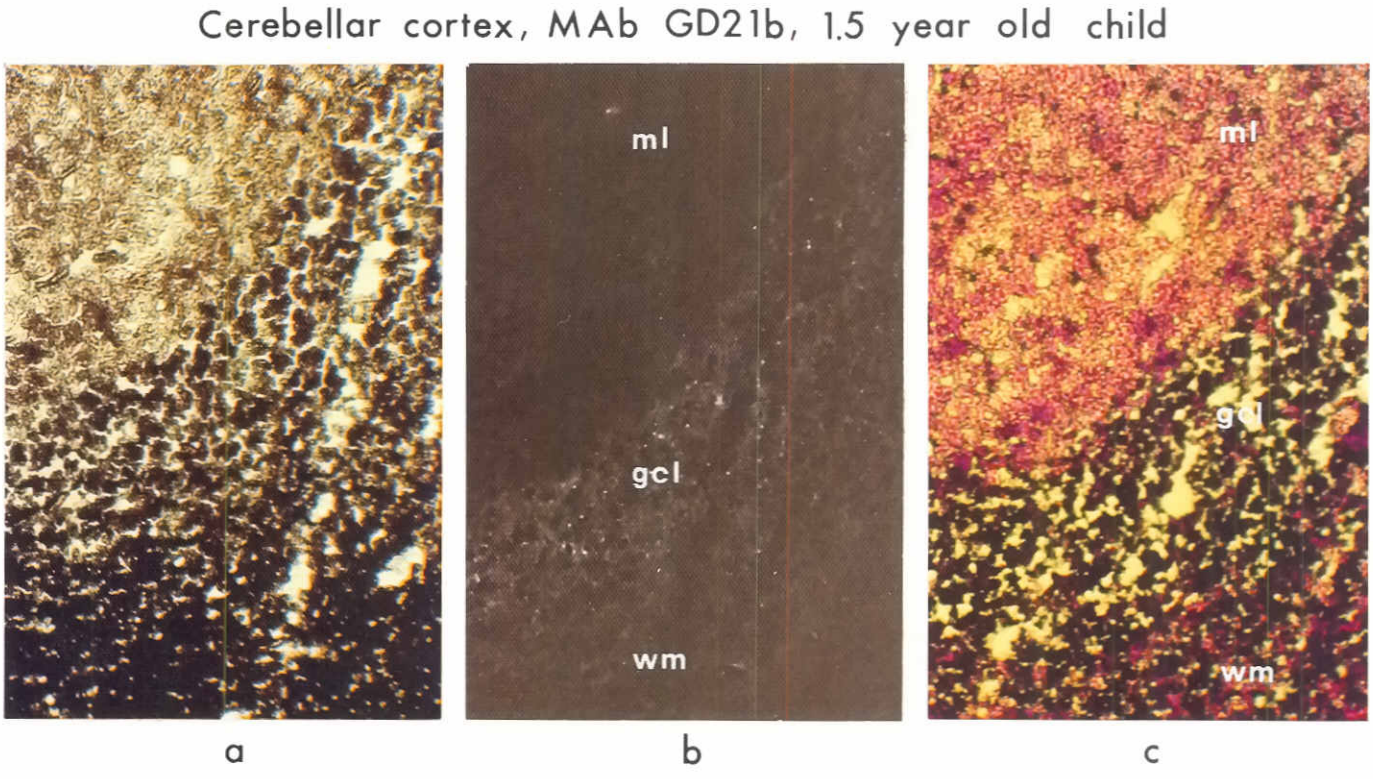

Fig. 3. Immunohistochemical distribution of the GD1b ganglioside in the cerebellar cortex of a 1.5 year old child (b). *Adjacent sections were silver impregnated by the method of Liesegang le Vay (Cruz* et al., *1984)* **(a)** *and Nissl-stained* **(c).** *ml, molecular layer; gcl, granular cell layer; wm, white matter*

and Ballabriga, 1978; Kracun *et al.*, 1986; Svennerholm *et al.*, 1989). Prenatal neocortical differences in ganglioside patterns were not observed (Figs. 1 and 2), however, the archicortex (hippocampus) by a high proportion of GD1a and the cerebellar cortex by a high proportion of «b-series» gangliosides (GQ1b, GT1b and GD1b) were recognized (Fig. 6).

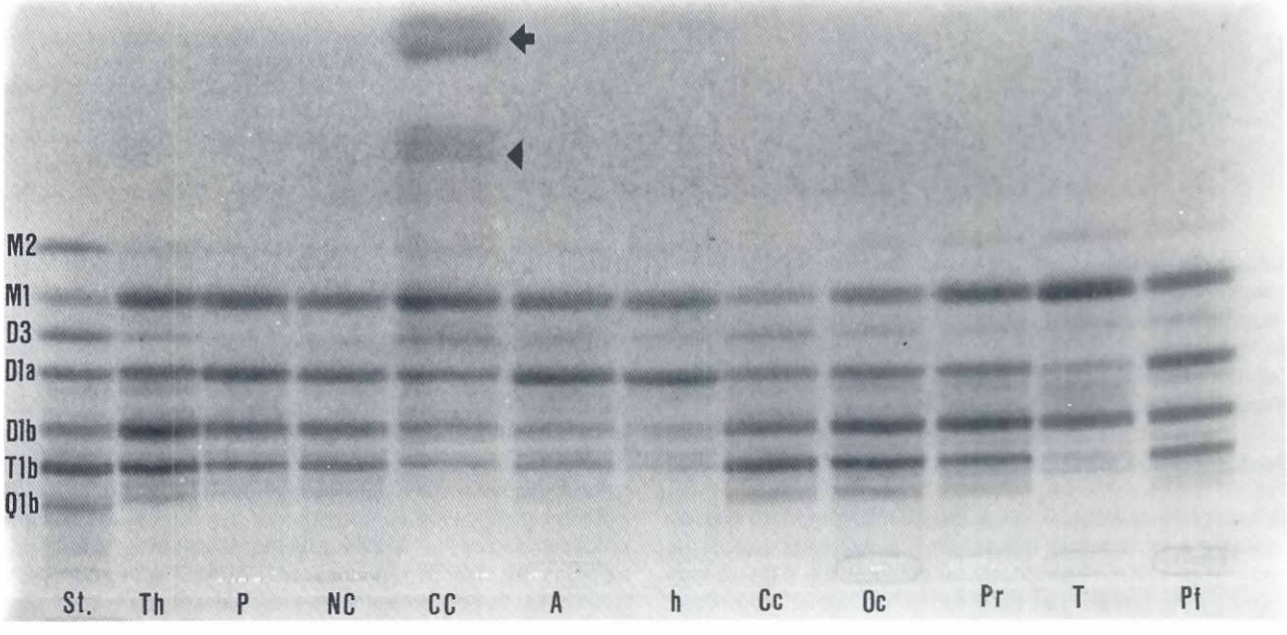

Fig. 4. Thin layer chromatographic separation of gangliosides extracted from different brain regions of the adult human brain (40 years of age). *St, standard ganglioside mixture; arrow, asialolipids; arrowhead, GM4. For abbreviations see Fig. 5.*

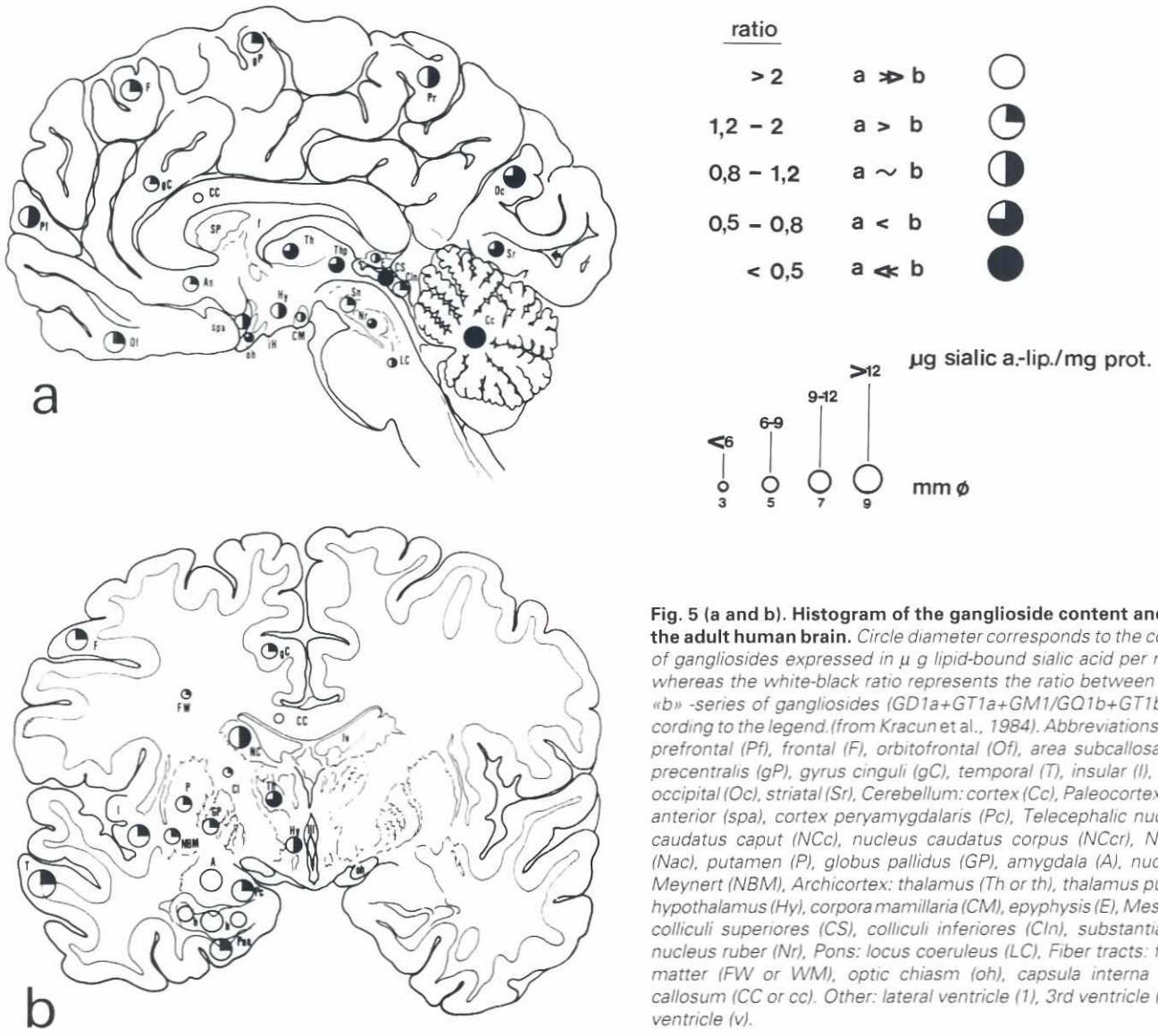

Fig. 5 (a and b). Histogram of the ganglioside content and pattern in the adult human brain. *Circle diameter corresponds to the concentration of gangliosides expressed in µ g lipid-bound sialic acid per mg proteins, whereas the white-black ratio represents the ratio between the «a» and «b» -series of gangliosides (GD1a+GT1a+GM1/GQ1b+GT1b+GD1b) according to the legend.(from Kracun* et al., *1984). Abbreviations: Neocortex: prefrontal (Pf), frontal (F), orbitofrontal (Of), area subcallosa (As), gyrus precentralis (gP), gyrus cinguli (gC), temporal (T), insular (I), parietal (Pr), occipital (Oc), striatal (Sr), Cerebellum: cortex (Cc), Paleocortex: s.perforata anterior (spa), cortex peryamygdalaris (Pc), Telecephalic nuclei: nucleus caudatus caput (NCc), nucleus caudatus corpus (NCcr), N.accumbens (Nac), putamen (P), globus pallidus (GP), amygdala (A), nucleus basalis Meynert (NBM), Archicortex: thalamus (Th or th), thalamus pulvinar (Thp), hypothalamus (Hy), corpora mamillaria (CM), epyphysis (E), Mesencephalon: colliculi superiores (CS), colliculi inferiores (CIn), substantia nigra (Sn), nucleus ruber (Nr), Pons: locus coeruleus (LC), Fiber tracts: frontal white matter (FW or WM), optic chiasm (oh), capsula interna (CI), corpus callosum (CC or cc). Other: lateral ventricle (1), 3rd ventricle (III), fornix (f) ventricle (v).*

In comparison to other brain regions of the fetal, postnatal and adult human brain, GD1b was found to be highly expressed in the cerebellum within the distinct cytoarchitectonic compartment (Fig. 3). This immunoreactivity most probably corresponds to mossy fibers of the granular cell layer (Figs. 3 a-c), correlating well experimental data in mutant mice (Seyfried *et al.*, 1984).

Gangliosides of the adult human brain (40-50 years of age)

In the adult human brain we analyzed 40 different brain regions and registered a tremendous difference in ganglioside concentrations and composition, respectively. In general, the highest concentration of gangliosides was detected in the human neocortex, showing a shift from «a» (GD1a, GM1) to «b» (GQ1b, GT1b, GD1b) pathway gangliosides in an anterio-posterior direction. In this way, the frontal cortex showed a prevalence of GD1a and GM1 gangliosides, whereas the visual cortex revealed a prevalence of GD1b and GT1b gangliosides (Figs. 4 and 5). The neocortical difference of the ganglioside composition between the frontal and visual cortices developed after birth (until 20-30 years of age)(Fig. 6). This difference could have developed during functional cortical postnatal maturation, corresponding to the cytoarchitectonic difference between these cortical areas since the visual cortex is a typical heterotypic granular cortex, whereas the frontal cortex is a typical heterotypic agranular or homotypic cortex (Mountcastle, 1979). The different ganglioside composition of the frontal and visual cortex characterized by low GD1a within the visual cortex is not surprising because the chemical «polarity» between these two cortices is very well known. For instance, the monkey's visual cortex contains a high binding activity to muscarinic and benzodiazepine receptors, but low opiate receptors, whereas the frontal cortex is quite the opposite (Divac *et al.*, 1981). Therefore, it would not be surprising

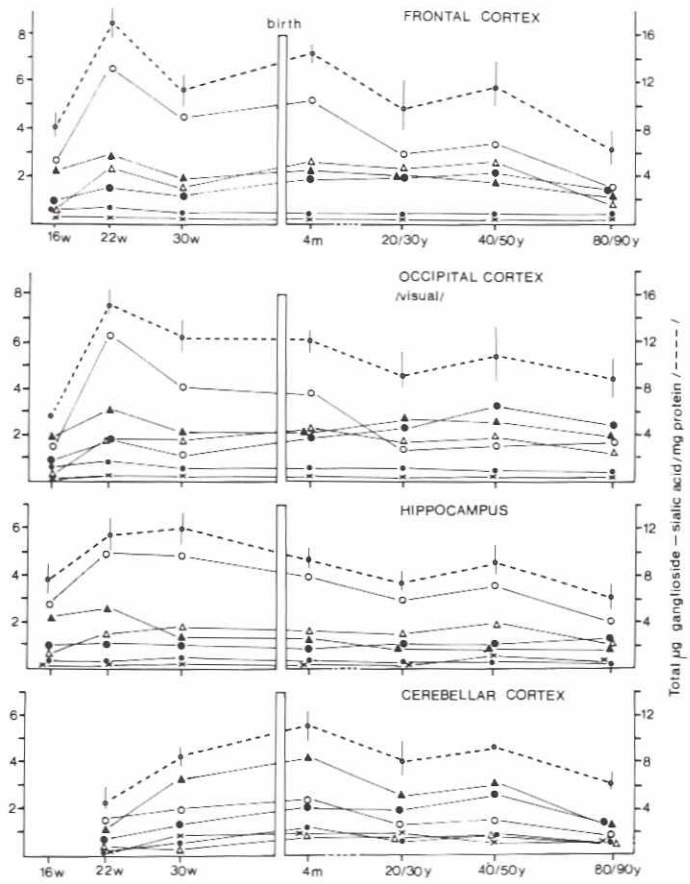

Fig. 6. Variation of total and individual gangliosides (µg lipid bound sialic acid/mg proteins) in human cortex prenatal development, postnatal maturation and aging. *(—), total gangliosides. (——), individual gangliosides. For abbreviations of gangliosides see Fig. 2. Note: in brains of 16, 22 and 30 weeks of gestation and 4th postnatal month, values are expressed as standard error of mean, whereas in brains 20/30 (n=5), 40/50 (n=5) and 80/90 (n=5) years of age values are expressed as mean standard deviation.*

if ganglioside metabolism were also different in the frontal and visual types of neurons.

The basis of regional differences of the ganglioside pattern in the adult human brain was the different «a»:»b» ratio of gangliosides (GD1a+GT1a+GM1:GQ1b+GT1b+GD1b) ranging from 2.0 in the hippocampus and 0.3 in the cerebellar cortex. We hypothesized that this heterogeneity is based mainly upon a different cytoarchitectonic pattern (neuron : glia ratio; number of (non)myelinated fibers, density of synapses, types of neurons, etc.).

However, it is not infrequent for phylogenetically old regions to favor one-pathway gangliosides, *i.e.* archicortex (hippocampus) and amygdala «a» pathway (GD1a, GM1) and cerebellar cortex «b» pathway gangliosides (GQ1b, GT1b, GD1b and GD3). In addition, findings in GM2 gangliosidosis have contributed to the hypothesis on a differential ganglioside metabolism in the human brain since both the accumulation of ganglioside GM2 and residual ß-hexosaminidase activity (Bolhuis *et al.*, 1987) as well as neuronal degeneration (Escola, 1961) markedly vary between different brain areas.

Human brain gangliosides during aging

By analyzing four cortical regions (frontal, visual, hippocampus, cerebellar cortex) of 15 human brains ranging between 20-90 years of age, different behavior of gangliosides in different cortical regions was found (Fig. 6). In the frontal cortex, a decrease in ganglioside concentrations, more GD1a and GM1 than GD1b and GT1b contributing to a decrease in «a»:»b» ratio, were registered. In the occipital cortex (visual area), both ganglioside content and ganglioside composition were stable with a permanent prevalence of «b-series» gangliosides. In the archicortex (hippocampus), the prevalence of «a-series» (GD1a and GM1) was found to be a specific feature of immature, mature and senescent human hippocampus. Nevertheless, comparing brains at 20 and 30 years of age, a moderate decrease in GD1a and GM1 in hippocampus was detected. On the other hand, the cerebellar cortex showed a decrease in «b-series» gangliosides (GT1b and GD1b) during human aging. Therefore, it seems that aging changes in ganglioside composition in different human and brain regions are regionally specific. Segler-Stahl *et al.* (1983) and Svennerholm *et al.* (1989), analyzing the whole human brain and frontal pole, respectively, did not recognize these differences and suggested more decreased GD1a and GM1 as a metabolic phenomenon of gangliosides in the senescent human brain based upon a new model of ganglioside synthesis (Pohlenz *et al.*, 1988). We believe that changes in cortical gangliosides during human aging follow region-specific cytoarchitectonic changes (McNeill, 1983) rather than a shift in biosynthesis of gangliosides from an «a» to «b» pathway. Namely, during human brain aging a significant increase in the glial population with a decrease in the neuron to glia ratio (Uemura *et al.*, 1978) and increase in the number of oligodendrocytes (Hansan and Glees, 1973) was observed. Further, in the precentral frontal gyrus of the human newborn brain, dominant cells in external and internal granular layers are granular cells, whereas in the senescent brain small pyramidal neurons prevail (Brody, 1955). Brody's study demonstrated that the fewest changes in the number of neurons occurred in sensory areas, correlating well with our observations of ganglioside composition in the visual cortex. We would like to emphasize the different sensitivities of different neuronal populations which might have a different ganglioside metabolism. Therefore, ganglioside changes during human aging were expected to be regionally characteristic.

Human brain gangliosides in Alzheimer's disease (AD)

By analyzing regional distribution of gangliosides in Alzheimer's disease (five human brains suffering from dementia more than 6 years, age 55-65 years), we detected a significant decrease in ganglioside concentrations ($P<0.05$, Mann-Whitney's test) in frontal and temporal cortices and basal telencephalon (when gangliosides are expressed as nmol LBSA/mg DNA) and in the frontal cortex and frontal white matter when expressed in nmol LBSA/g fresh weight (Fig. 7a and b). These differences in ganglioside expression were most probably influenced by different neuronal degeneration to glial proliferation occurring in AD.

In ganglioside composition, complex ganglio-series gangliosides (GM1, GD1a, GD1b, GT1b) were decreased, but there was no significant change in the «a»/»b» ratio of gangliosides, except for the

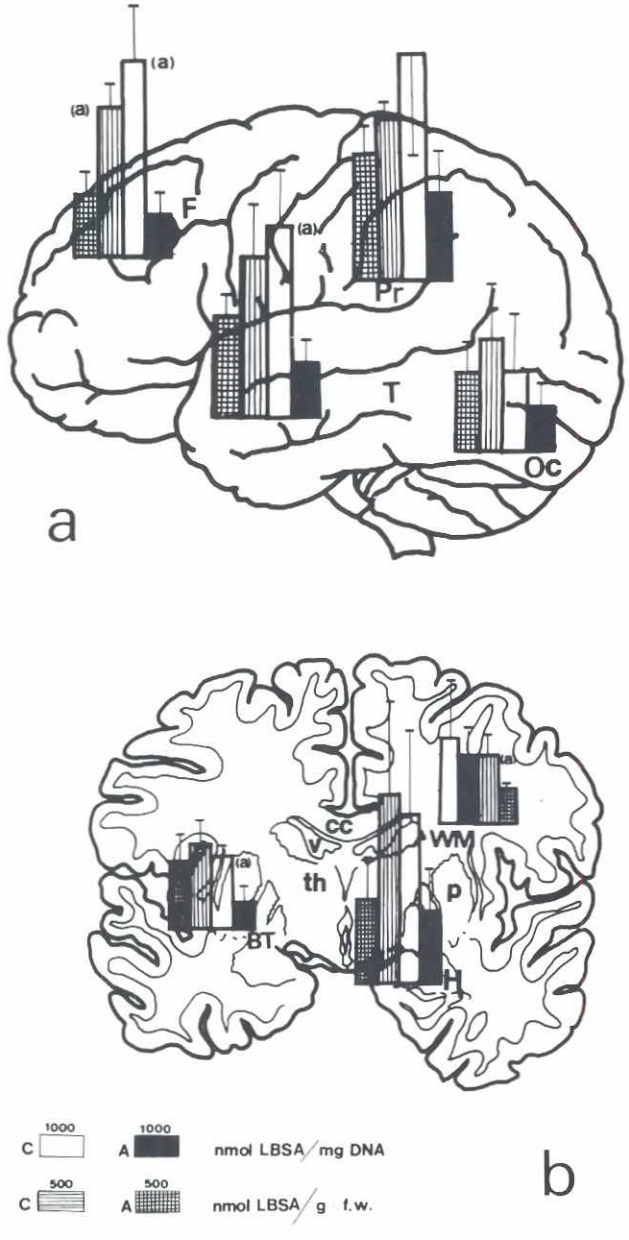

Fig. 7. (a and b) *Histogram of ganglioside concentrations in different brain regions of Alzheimer's disease (n=5) in comparison to control brains. Gangliosides are expressed in nmol lipid-bound sialic acid per mg DNA and per g fresh weight). (a) Significant difference P<0.05 (Mann-Whitney test). For abbreviations see Fig. 5 (BT corresponds to NBM).*

TABLE 1

REGIONAL DISTRIBUTION OF GANGLIOSIDE COMPOSITION (NMOL LIPID-BOUND SIALIC ACID, LBSA, PER mg DNA) IN ALZHEIMER'S DISEASE (AD) AND AGE-MATCHED CONTROL BRAINS (n=3).

Brain region	nmol LBSA/mg DNA								a/b
	GT1b	GD1b	GD1a	GD3	GM1	GM2	GM3	GM4	
Frontal cortex (Brodmann's area 10.11)									
C	902	1054	1008	-	745	9	10	-	0.89
	(331)	(370)	(439)		(257)	(5)			(0.08)
AD	126*	183*	156*	61	175*	34*	16	15	1.07
	(72)	(120)	(129)		(9)	(5)		(0.34)	
Parietal cortex (Brodmann's area 40)									
C	1020	1094	1159	-	1102	-	-	-	1.14
	(529)	(826)	(551)		(539)				(0.33)
AD	298	313	320	77	335	46	50	34	1.23
	(251)	(229)	(232)	(63)	(216)	(29)	(24)	(25)	(0.45)
Temporal cortex (Brodmann's area 21)									
C	608	628	1469	47	760	-	-	-	1.80
	(38)	(351)	(1011)	(26)	(321)				(0.61)
AD	224*	268*	300*	21	174*	2	-	-	0.98*
	(130)	(181)	(139)	(11)	(76)				(0.23)
Occipital (visual) cortex (Brodmann's area 17)									
C	288	420	224	85	316	27	28	15	0.69
	(158)	(243)	(128)	(53)	(226)	(20)	(12)		(0.19)
AD	209	272	135	36	151	15	4	9	0.64
	(115)	(162)	(44)	(9)	(79)	(0.6)		(0.15)	
Basal telencephalon (Meynert's nucleus)									
C	340	306	327	91	390	54	81	27	1.10
	(22)	(34)	(46)	(21)	(52)	(21)	(24)	(0.09)	
AD	134*	122*	151*	34*	152*	26*	32*	15	1.12
	(98)	(76)	(104)	(27)	(111)	(26)	(22)		(0.15)
Hippocampus									
C	540	614	1261	258	1191	93	26	15	2.16
	(323)	(440)	(767)	(152)	(764)	(66)	(11)	(10)	(0.32)
AD	370	361	981	66	692	75	28	18	2.24
	(195)	(113)	(545)	(57)	(400)	(42)	(12)	(12)	(0.73)
Frontal white matter									
C	204	124	357	131	582	15	82	91	2.30
	(161)	(101)	(320)	(112)	(504)	(18)	(21)	(57)	(1.10)
AD	255	119	301	188	368	34	51	104	1.78
	(115)	(65)	(113)	(50)	(187)	(14)	(50)	(70)	(0.30)

a/b - ratio of GD1a+GM1/GT1b+GD1b.
Neocortical samples were isolated according to Brodmann's subdivision (1909).
* P<0.05 Mann-Whitney test () SD
a/b - ratio of GM1+GD1a / GT1b+GD1b
(*) Monoclonal antibodies against GD3, GD2, GD1a were from Biotechnik (Dr. B. Pallmann, Munich), whereas monoclonal antibody against GM3 was the generous gift of Dr. J. Johnson (Munich). Monoclonal antibody Q211 against the «c»-series of gangliosides was obtained in Prof. H. Rosner's Lab.

temporal cortex where the «a»/»b» ratio was decreased in AD brains in comparison to controls (Table 1). In addition, the frontal and parietal cortex exhibited an elevated concentration of 'simple' gangliosides (GM3, GM2). The observations of decreased concentrations of the ganglio-series gangliosides in frontal and temporal cortices and basal telencephalon (Crino *et al.*, 1989; Kracun *et al.*, 1990) are similar to the neuroanatomical evidence concerning more pronounced neuronal degeneration in these brain regions in AD (Terry *et al.*, 1981).

In conclusion, by analyzing different human brain regions (cortical areas and subcortical nuclei) in development, adult stage, senescence and Alzheimer's disease we found the ganglioside composition to be compartmentalized in favor of the «a»- or «b»-pathway gangliosides and selectively vulnerable to aging processes and disease. We suppose that different cellular metabolisms and subcellular distribution of gangliosides in different types of neurons (efferent, afferent, interneurons) as well as different neuronal sensitivity in the human brain might explain our findings. However, this hypothesis has to be proved.

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