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Neuropathology provides clues to the pathophysiology of Gaucher disease

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Abstract

To better understand the pathogenesis of brain dysfunction in Gaucher disease (GD), we studied brain pathology in seven subjects with type 1 GD (four also exhibited parkinsonism and dementia), three with type 2 GD and four with type 3 GD. Unique pathologic patterns of disease involving the hippocampal CA2-4 regions and layer 4b of the calcarine cortex were identified. While these findings were common to all three GD phenotypes, the extent of the changes varied depending on the severity of disease. Cerebral cortical layers 3 and 5, hippocampal CA2-4, and layer 4b were involved in all GD patients. Neuronal loss predominated in both type 2 and type 3 patients with progressive myoclonic encephalopathy, whereas patients classified as type 1 GD had only astrogliosis. Adjacent regions and lamina, including hippocampal CA1 and calcarine lamina 4a and 4c were spared of pathology, highlighting the specificity of the vulnerability of selective neurons. Elevated glucocerebrosidase expression by immunohistochemistry was found in CA2-4. Hippocampal $^{45}Ca^{2+}$ uptake autoradiography in rat brain was performed demonstrating that hippocampal CA2-4 neurons, rather than CA1 neurons, were calcium-induced calcium release sensitive (CICR-sensitive). These findings match recent biochemical studies linking elevated glucosylceramide levels to sensitization of CA2-4 RyaR receptors and 300% potentiation of neuronal CICR sensitivity. In two patients with type 1 GD and parkinsonism, numerous synuclein positive inclusions, similar to brainstem-type Lewy bodies found in Parkinson disease, were also found hippocampal CA2-4 neurons. These findings argue for a common cytotoxic mechanism linking aberrant glucocerebrosidase activity, neuronal cytotoxicity, and cytotoxic Lewy body formation in GD.
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Keywords: Gaucher disease; Hippocampus; Glucocerebrosidase; Calcarine cortex; Astrogliosis; Neuronal loss; Synuclein; Lewy body; Parkinsonism

Introduction

Gaucher disease (GD) is a clinically and pathologically heterogeneous disorder caused by a deficiency of the lysosomal enzyme glucocerebrosidase [1]. As a result, there is accumulation of glycolipid substrates, primarily glucocerebroside and glucosylsphingosine [2]. Patients with GD can be categorized into three general, clinical [2,3], and biochemical phenotypes [4,5]. Type 1 GD is the most common form and classically the CNS is not affected [6,7]. The onset of signs in type 2 GD (also called acute neuronopathic GD) is in infancy while type 3 GD (chronic neuronopathic GD) may commence at any age [8].

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The neuronopathic form [9] of Gaucher disease (NPGD) was described 32 years after Philippe Gaucher first described the disease in his doctorate thesis [10]. The most consistent neuropathologic finding reported in NPGD was the periadventitial accumulation of lipid-laden macrophages (Gaucher cells) [2,11–14]. Significant neuronal loss with crumpled, shrunken-atrophic neurons [11,13] has been described in the basal ganglia, nuclei of the midbrain, pons and medulla, cerebellum, dentate nucleus and hypothalamus [13,15,16]. Other reports identified cerebral cortical laminar necrosis of the third and fifth cortical layers [11,12,16], neuronal loss with astrogliosis in layer IV, Gaucher cells in the visual cortex and occipital–temporal lobes [17,18], as well as parenchymal Gaucher cells lying free within cerebral cortex, especially in the occipital lobes [12,14,15]. Non-specific gray and white matter gliosis [12,13,18] and microglial proliferation have been noted [13,15]. Neuronal storage of glucosylceramide (GlcCer) [12,13] has also been described but the identification of GlcCer tubular structures in neurons is rare [19].

Type 1 GD by definition spares the CNS. However, there are reports of clinical and pathological CNS findings in patients who would be considered type 1 GD [7,20,21]. In addition, Parkinson disease or parkinsonian symptoms in conjunction with type 1 GD have been reported as an incidental association [14,22,23], but are the subject of more focused investigations recently [24–28].

In this study, we sought to identify specific patterns of neuronal injury that might provide clues to underlying pathogenic mechanisms in GD and to find possible mechanisms common to the pathogenesis of both Parkinson disease and GD. Intrigued by the descriptions of regional laminar neuronal susceptibility and astrogliosis, we set out to determine whether specific patterns of selective neuronal vulnerability underlie the different GD phenotypes. We hypothesized that a detailed neuropathologic investigation would elucidate clues to the pathogenesis of NPGD.

Methods

Neuropathological material

We performed neuropathologic evaluations of 14 subjects with Gaucher disease (Table 1), including 10 males and four females ranging in age from 3 years to 86 years. Diagnosis of Gaucher disease was made by noting glucocerebrosidase deficiency (data not shown) and mutations of the glucocerebrosidase gene (Table 1). Three patients had type 2 GD, including two with "classic" rapidly progressive disease (Patients 8 and 9) and one with a slower disease progression (Patient 10).

Four patients had type 3 GD. One patient died at a very early stage of his disease while undergoing a bone marrow transplant for GD (Patient 11), and two patients had progressive myoclonic encephalopathy (PME) (Patients 12 and 14).

Seven patients were classified as having type 1 GD. Three lacked neurologic symptoms (Patients 1–3), and four had a form of parkinsonism and dementia (Patients 4–7). The encephalopathy of Patient 5 included horizontal supranuclear gaze palsy, a feature of NPGD.

Complete brain samples including cortical, subcortical white matter, cingulate gyrus, temporal, parietal, calcarine cortex, striatum, thalamus, hippocampal, cerebellar, midbrain and brainstem sections or hemibrains were obtained in 12 of the 14 patients (Patients 1–11, 14). Cerebral cortical sections were evaluated in all 14 patients; some sections were not available from Patients 6, 12, and 13 as noted in Table 1.

Special staining and immunohistochemistry

Standard hematoxylin and eosin, periodic acid–Schiff (PAS), alcian blue, and Bielschowsky stains were performed using 9 μm sections (Luna Reference Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd edition, edited by Lee G. Luna, HT (ASCP), McGraw-Hill, New York, New York, 1968). Modified alcian blue 8GX/PAS was performed as previously described [29]. Standard ABC immunohistochemical methods were performed by automated Dako Autostainer, Universal Staining System. Antibody titrations included glial fibrillary acidic protein (GFAP) (Dako, Carpinteria, CA, USA) at 1:4000 dilution, synaptophysin (Dako) at 1:50 dilution, synuclein (Novacastra, Newcastle, UK) at 1:320 dilution, and ubiquitin at (Dako) 1:300 dilution. Anti-glucocerebrosidase monoclonal antibody 8E4 was provided by Dr. J.M. Aerts [30]. 8E4 immunohistochemistry was performed manually by Envision HRP (Dako). Modifications included primary and secondary antibody incubations under a warm water steam bath for 30 min/incubation. 8E4 titers were performed in the range of 1:50–1000, with the best 8E4 staining results at 1:200 dilution.

Autoradiography

Histochemical analysis of $^{45}Ca^{2+}$ uptake was performed as described previously [31]. Briefly, 20 mm frozen rat brain sections were allowed to thaw before being pre-incubated in permeabilization buffer containing 100 mM KCl, 10 mM HEPES, pH 7.3, 3% polyethylene glycol (PEG), 1 mM DTT, and 10 mM digitonin for 10 min at 25 °C. Sections were then transferred into uptake buffer containing 20 mM HEPES (pH 7.3, KOH), 75 mM K_2oxalate, 3% PEG, 10 mM phosphocreatine, 10 U/ml creatine phosphokinase, 5 mM NaN_3, 0.2 mM total Ca^{2+}, 0.1 mCi/ml $^{45}Ca^{2+}$, 1 mM $MgCl_2$, 2 mM ATP, and 10 mM DTT at 37 °C. The free Ca^{2+} concentration

Table 1
Clinical, genetic, and pathologic findings in patients with Gaucher disease

Patient	Gaucher disease	Sex/age of death	Clinical phenotype	Genotype	Brain sections	Hippocampus CA2-4	Calcarine cortex, Layer 4b	Cerebral cortex, Layer 5, focally layer 3	Other significant neuropathology
1	Type 1	M/69 Y	Non-neurologic	N370S/Q350X	All	Astrogliosis	Astrogliosis	Astrogliosis	Sensorimotor cortex layer 5 astrogliosis
2	Type 1	M/58 Y	Non-neurologic	N370S/c.208delC	All	Astrogliosis	Astrogliosis	Astrogliosis	
3	Type 1	M/86 Y	Non-neurologic	N370S/N370S	All	Astrogliosis	Astrogliosis	Astrogliosis	Ballooning of CA2 pyramidal cell neurons
4	Type 1	F/62 Y	Parkinsonism, dementia	N370S/?	All	Astrogliosis	Astrogliosis	Astrogliosis	DLBD with cortical LB, PD
5	Type 1	F/53 Y	Parkinsonism, dementia, myoclonus, horizontal supranuclear gaze palsy	D409H/ L444P + duplication	All	Astrogliosis	Astrogliosis	Astrogliosis	Brainstem-type Lewy bodies in hippocampal CA2-4 pyramidal cell neurons
6	Type 1	M/75 Y	Parkinsonism, dementia	N370S/N370S	All except brainstem, midbrain was present	Astrogliosis	Astrogliosis	Astrogliosis	Brainstem-type Lewy bodies in substantia nigra
7	Type 1	M/54 Y	Parkinsonism, dementia	N370S/N370S	All	Astrogliosis	Astrogliosis	Astrogliosis	Brainstem-type LB in hippocampal CA2-4 pyramidal cell neurons and cortical LB
8	Type 2	F/15 Mo	Typical progression	Rec*Nci*I/L444P	All	Neuronal loss	Neuronal loss	Neuronal loss	
9	Type 2	F/3 Y	Typical progression	R257Q/L444P	All	Neuronal loss	Neuronal loss	Neuronal loss	
10	Type 2	M/3.5 Y	Milder type 2	F213I/S107L	All	Neuronal loss	Neuronal loss	Neuronal loss	
11	Type 3	M/4 Y	Saccadic paresis, developmental delay, no myoclonus	L444P/ G202R + duplication involving the pseudogene	All	Astrogliosis of CA4-3	No pathology	Focal mild astrogliosis of layers 3 and 5	Died 10 days post-BMT at an early phase of Type 3 GD, large somatosensory amplitude without overt myoclonus [35]
12	Type 3	M/7.5	PME	Rec*Nci*I/V394L	Cerebral motor cortex only	Not evaluated	Not evaluated	No astrogliosis or neuronal loss in the motor cortex	No astrogliosis in sensorimotor cortex
13	Type 3	M/18 Mo	Died of aspiration	Y304C/L444P	Cerebral and calcarine cortex only	Not evaluated	Neuronal loss	Neuronal loss	
14	Type 3	M/14	Severe systemic disease and PME	G377S/g.5245delT	All	Neuronal loss	Neuronal loss	Neuronal loss	

M, male; F, female; Y, years; Mo, months; Brain sections: All, standard brain sections to include hippocampus, Calcarine, cerebral cortex and white matter, subcortical regions, midbrain, and brainstem. DLBD, diffuse Lewy body dementia; LB, Lewy body; PD, Parkinson disease; BMT, bone marrow transplant; PME, progressive myoclonic encephalopathy. Parkinsonism indicates the presence of rigidity, bradykinesia, and occasional tremor or postural instability.

was adjusted to 0.3 mM with a Ca^{2+}-selective electrode. Incubations were performed in plastic slide mailing vessels (Evergreen, Los Angeles, CA) and buffer volumes were adjusted to give ~100 mg/ml protein in the assay. After incubation, slides were removed from uptake buffers and transferred into wash buffers containing 100 mM KCl, 10 mM K_2oxalate, 3% PEG, 5 mM $MgCl_2$, 10 mM HEPES–KOH (pH 7.3), and 2 mM EGTA at 4 °C. After washing in this buffer for 10 min, sections were dried under a cool air stream blown from one edge of the slide with careful, gentle vacuum aspiration of excess fluid from the other side and then were apposed to β-particle sensitive film (BetaMax; Amersham, Arlington Heights, IL) and developed in Kodak D-19 developer after 12–24 h exposure. Inositol triphosphate (IP_3) or caffeine was included in the uptake assay as indicated to identify calcium pools released via the IP_3 receptor and ryanodine receptor, respectively.

Results

Neuropathologic findings of selected brain regions from the 14 patients together with patient genotypes are summarized in Table 1. A range of glucocerebrosidase gene mutations was identified. It is noteworthy that most with type 1 GD had at least one allele with N370S, that was hitherto considered a "neuroprotective" allele. The most characteristic consistent and specific neuropathologic findings were the selective vulnerability of hippocampal CA2-4 pyramidal neurons, as well as neurons of the calcarine cortex layer 4b and cerebral cortex layer 3 and 5 (Table 1). Pathologic changes in the three brain regions were present in every Gaucher brain we examined. However, there were qualitative and quantitative differences between the GD types. Patients with GD types 2 and 3 had prominent neuronal loss and astrogliosis, whereas those with type 1 GD had astrogliosis without significant neuronal loss.

Hippocampal pathology in Gaucher disease; H&E and GFAP studies

The hippocampus had neuropathological findings in the CA2, CA3, and CA4 regions with relative sparing of the CA1 region (Figs. 1A and B) in all patients with GD. In patients with type 2 GD, the CA2 region had severe neuronal loss and dense fibrillar and gemistocytic astrogliosis (Fig. 1D, compare with 2D). CA3 and CA4 had moderate neuronal loss and astrogliosis (Fig. 1A). There was no detectable neuronal loss or increased astrogliosis in CA1 (above background gliosis).

Among subjects with type 1 GD, the CA2 region had marked astrogliosis with moderate astrogliosis of CA3-4. There was no increase in astrogliosis of CA1 (Fig. 1B) detectable above the low background level.

In all patients with GD, excepting Patient 11, there was a low level of background gliosis that was most prominent in the brainstem and striatum, moderate in the centrum ovale white matter, and mild in the cortices. The "background gliosis" was nearly always associated with capillaries and the vasculature. In comparison, the regional patterns of gliosis in the hippocampi were related to the glial and neuronal architecture. Characteristically, a moderately dense fibrillary and in some cases in the CA2 region, gemistocytic astrogliosis was noted. In the one exception, Patient 11 with early type 3 GD without overt clinical myoclonus, there was no "background gliosis" detectable, but hippocampi CA4, CA3, and likely CA2, were astrogliotic (Fig. 1C). These findings can be compared with the GFAP and H&E staining of the same brain regions in a normal subject (Fig. 2).

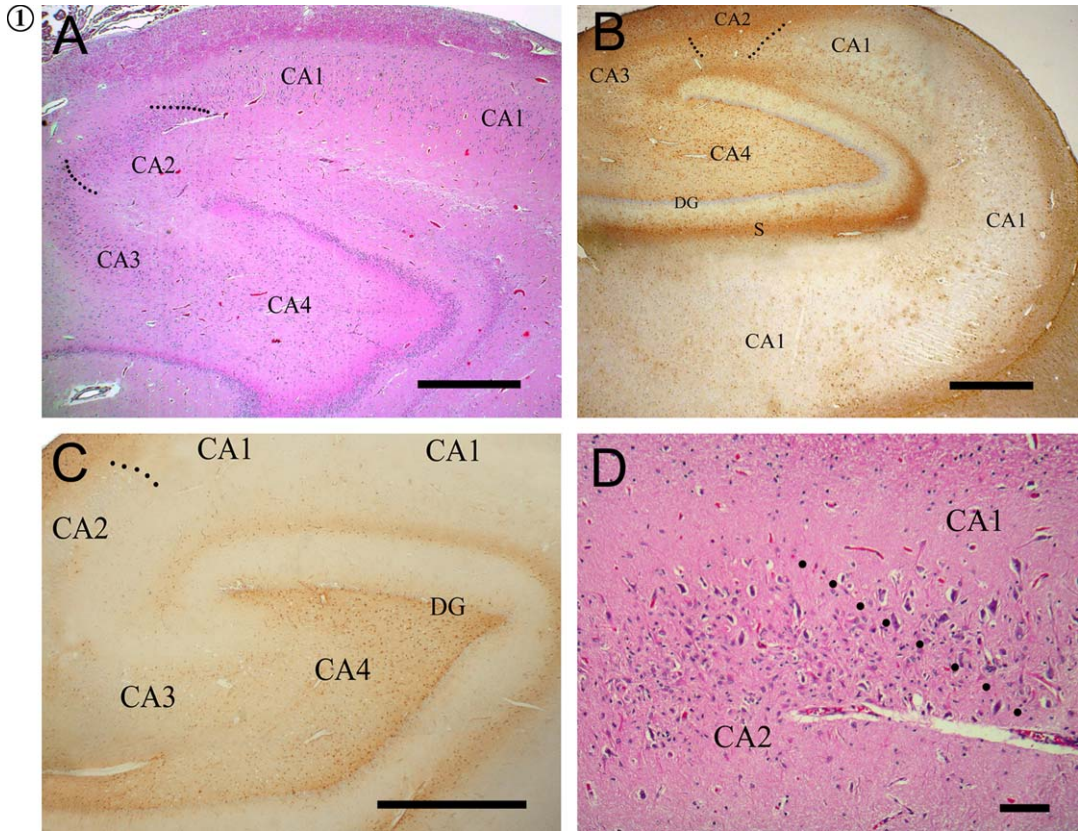
Hippocampal anti-glucocerebrosidase immunohistochemistry

We attempted to identify a possible functional relationship between the pattern of neuronal damage and level of glucocerebrosidase expression. Glucocerebrosidase-specific immunohistochemistry in a normal control (Fig. 3A) and in a patient with type 1 GD (Fig. 3C) demonstrated strong, dense 8E4 reactivity in hippocampal CA2-4 region pyramidal neurons (Figs. 3A and C), whereas CA1 pyramidal cell neurons had a less dense, sparser, scattered immunoreactivity (Figs. 3B and D). The 8E4-antibody immunostaining in cerebral cortical layer 5, and to a lesser extent in layer 3, was discernibly darker than in the other cortical layers, but the difference was not as marked as seen in the hippocampus (not shown). 8E4-antibody immunoreactivity in other brain sections from patients with GD did not demonstrate a distinct pattern (not shown).

Hippocampal pathology in GD patients with parkinsonism and dementia

Four patients had type 1 GD and parkinsonism with dementia. Two of these patients (Patients 5 and 7) [27] had numerous, hippocampal CA2-4, intraneuronal, synuclein-positive inclusions (Figs. 4A, B, D–F), reminiscent of the brainstem-type Lewy bodies seen in the substantia nigra in idiopathic Parkinson disease. The Lewy body-like synuclein inclusions were absent in CA1 (Fig. 4C). A number of hippocampal pyramidal cell neurons imbedded in the stratum lacunosum moleculare near the most proximal CA1 region also had Lewy body-like synuclein inclusions.

Two of these patients lacked hippocampal CA2-4, Lewy body-like synuclein inclusions. Patient 6 had marked neuronal loss of substantia nigra neurons accompanied by brainstem-type Lewy bodies. Patient 4 had, in addition to brainstem-type Lewy bodies, numerous cortical-type Lewy bodies in the temporal lobe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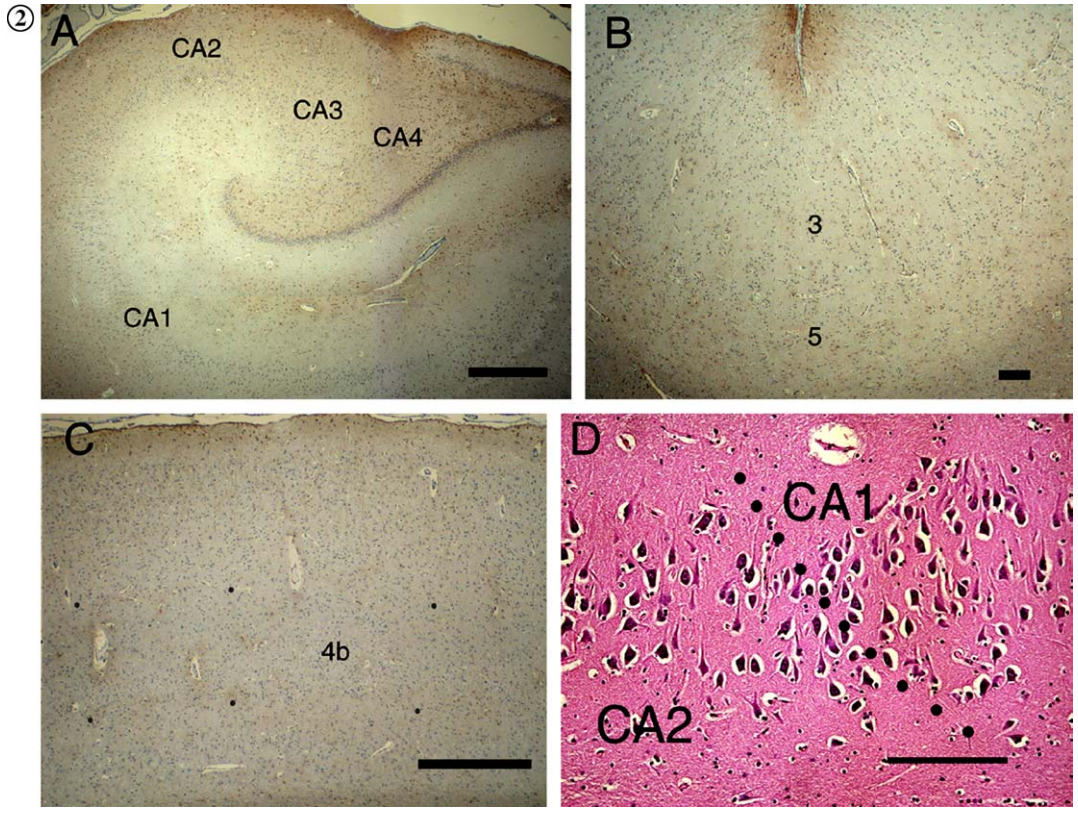
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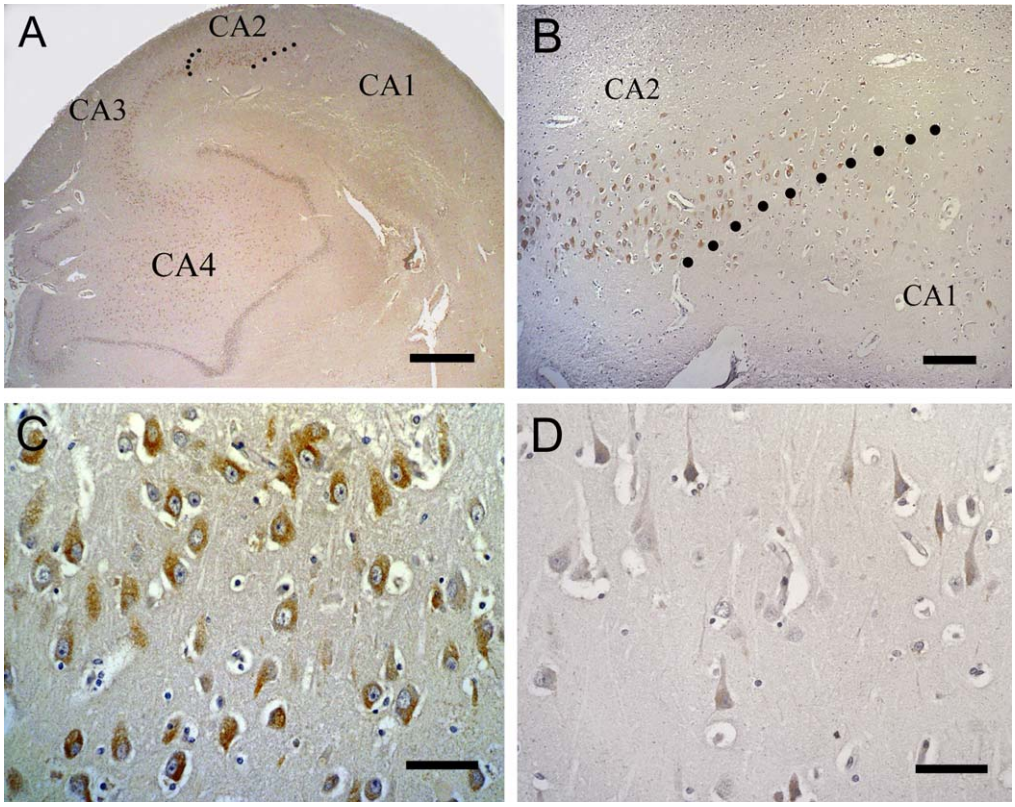

Fig. 3. Strong 8E4 (anti-glucocerebrosidase) immunoreactivity in CA2-4 region. (A) Normal human control, hippocampal formation. Immunoreactivity to 8E4 anti-glucocerebrosidase antibody is strong in the pyramidal cell neurons of the CA2, CA3, and CA4 regions. CA1 has much weaker immunoreactivity. (8E4 immunostain, 40× original magnification). (B) Normal human control, hippocampal, CA2–CA1 interface. Strong immunoreactivity to 8E4 anti-glucocerebrosidase antibody is present in the pyramidal cell neurons of the CA2 region, whereas the CA1 pyramidal cell neuron staining dense is sparse (8E4 immunostain, 100× original magnification). (C) Type 1 Gaucher disease (Patient 2). Hippocampal CA2 region. CA2 pyramidal cell neurons have dense, granular, cytoplasmic 8E4 immunoreactivity (8E4 immunostain, 200× original magnification). (D) Type 1 Gaucher disease (Patient 2). Hippocampal CA1 region. Some CA1 pyramidal cell neurons have mild to moderate 8E4-glucocerebrosidase immunoreactivity, but other neurons lack significant immunoreactivity (8E4 immunostain, 200× original magnification). Scale bars: A, 1 mm; B, 150 μm; C, 50 μm; D, 50 μm.

Fig. 1. Hippocampal CA2–CA4 pyramidal cell neuronal loss and astrogliosis in Gaucher disease. CA1 region is spared. (A) Type 2 Gaucher disease (Patient 10). Hippocampal pyramidal neuron cell loss and astrogliosis are most severe in CA2 (region bracketed between rows of dots), moderate to severe in CA3 and moderate in CA4. The CA1 region of the hippocampus has minimal background gliosis and is largely spared of significant pathology (hematoxylin and eosin, H&E, 40× original magnification). (B) Type 1 Gaucher disease (Patient 2). Hippocampal pyramidal neuron cell loss is undetectable and astrogliosis may not be readily detectable, except with glial fibrillary acidic protein staining (GFAP). Hippocampal CA2 region is strongly and densely GFAP immunoreactive. CA3 and CA4 also have moderate GFAP immunoreactivity whereas the CA1 region only has background scattered perivascular immunoreactivity. DG denotes the dentate gyrus. S denotes the stratum lacunosum, a region that almost always stains highly GFAP immunoreactive, whether in normal and disease states. (GFAP immunoperoxidase, 40× original magnification). (C) Type 3 Gaucher disease (Patient 11). This patient's symptoms included developmental delay, supranuclear gaze palsy and mild dysphagia, but no myoclonus or seizures. The patient died shortly after a bone marrow transplantation. CA4 region has mild gliosis and GFAP immunoreactivity that extends into CA3 and proximal CA2. CA1 does not have an appreciable increase in GFAP immunoreactivity. No neuronal loss was visible (GFAP immunoperoxidase, 40× original magnification). (D) Type 2 Gaucher disease (Patient 10). Close-up of hippocampal CA2–CA1 interface. The few remaining hippocampal pyramidal cell CA2 neurons are basophilic and shrunken. Astrogliosis with eosinophilic astrocytes and glial processes is prominent. The CA1 region at the interface has mildly affected pyramidal cell neurons (slightly basophilic) and mild astrogliosis. One hundred and fifty micrometers from the CA2–CA1 interface, the CA1 region has no significant changes above background (not shown). (H&E, 100 original magnification). Scale bars: A, B, and C, 1 mm; D, 100 μm.

Fig. 2. Normal control, GFAP immunostaining of hippocampus, cerebral cortex, calcarine cortex. (A) The hippocampal CA2-4 region in a non-disease state has very little GFAP immunoreactive astrogliosis. (B) The normal cerebral cortex and cortical layers 3 and 5 lack significant GFAP detectable astrogliosis. (C) Calcarine cortex layer 4, outlined by the region between the dot markings and layer 4b have no significant astrogliosis by GFAP immunostaining. (D) Normal control of CA2/CA1 junction with abundant hippocampal pyramidal neurons (H&E, 100× original magnification). Scale bars: A, 1 mm; B, 250 μm; C, 1 mm; D, 200 μm.

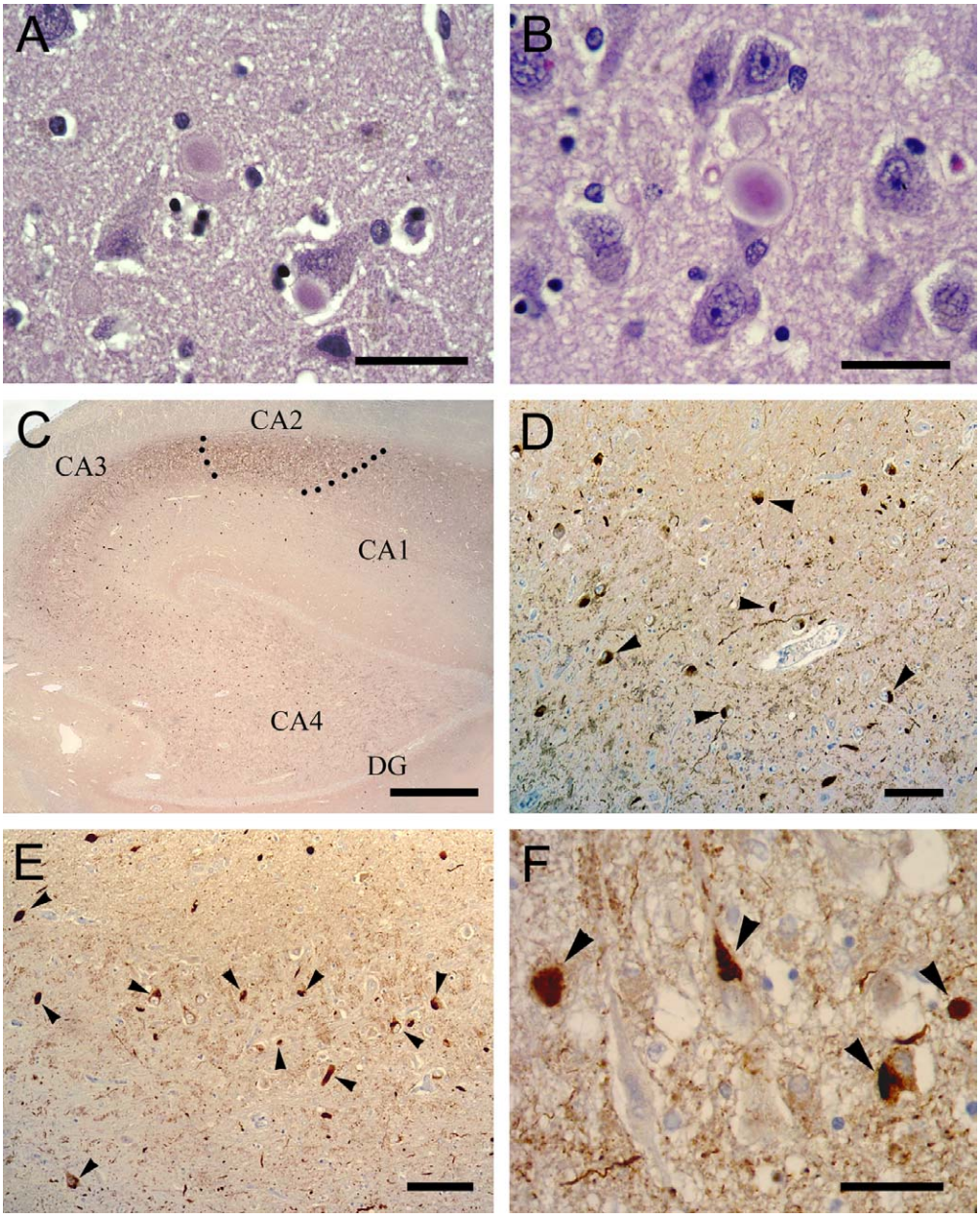

Fig. 4. Neuronal intracytoplasmic inclusions similar to brainstem-type Lewy bodies in hippocampal pyramidal cell neurons of the CA2-4 sector in Patient 5 with GD and parkinsonism. (A) Hippocampal CA2 region. Many large neuronal intracytoplasmic inclusions morphologically resembling brainstem-type Lewy bodies are present in CA2 pyramidal cell neurons (H&E, 400× original magnification). (B) Hippocampal CA3 region. The same inclusions are present in CA3 pyramidal cell neurons. (H&E, 400× original magnification). (C) α-Synuclein immunoreactive, intracytoplasmic, pyramidal cell neuron inclusions are abundant in the hippocampal CA2-4 regions. Except for a few inclusions at the CA2–CA1 interface and pyramidal cells in the stratum lacunosum, CA1 is devoid of α-synuclein immunoreactive Lewy body-like inclusions (synuclein immunoperoxidase, 40× original magnification). (D) α-Synuclein immunoreactive, intracytoplasmic, pyramidal cell neuron, and inclusions (arrow heads) are abundant in the hippocampal CA4 region (synuclein immunoperoxidase, 200× original magnification). (E) Synuclein immunoreactive, intracytoplasmic, pyramidal cell neuron, and inclusions (arrow heads) are also plentiful in the hippocampal CA2 region (synuclein immunoperoxidase, 200× original magnification). (F) Hippocampal CA2 region, close-up. α-Synuclein immunoreactive inclusions fill the cytoplasm of pyramidal cell neurons. Some of the inclusions (far left arrowhead) have a immunoreactive morphology similar to a brainstem-type Lewy body (synuclein immunoperoxidase, 400× original magnification). Scale bars: A, 38 μm; B, 35 μm; C, 1 mm; D, 80 μm; E, 100 μm; F, 45 μm.

entorhinal cortex and cingulate gyrus, consistent with Parkinson disease and diffuse Lewy body dementia. Thus, two neuropathologic patterns can be discerned. One pattern has CA2-4 Lewy body-like synuclein inclusions, while the second pattern lacks CA2-4 Lewy body-like synuclein inclusions, but resembles diffuse Lewy body dementia.

All four patients with parkinsonism and dementia had hippocampal CA2-4 gliosis, depletion of substantia nigra (SN) neurons, SN gliosis and SN brainstem-type

Lewy bodies. Patient 4 with diffuse Lewy body disease had, in addition, mild CA2 neuronal loss and dystrophic neurites in the CA2-3 region. Patient 7 had a combination of the two patterns with the presence of diffuse Lewy body dementia, cortical Lewy bodies, brainstem-type Lewy bodies in the substantia nigra and CA2-4 Lewy body-like synuclein inclusions in the hippocampus.

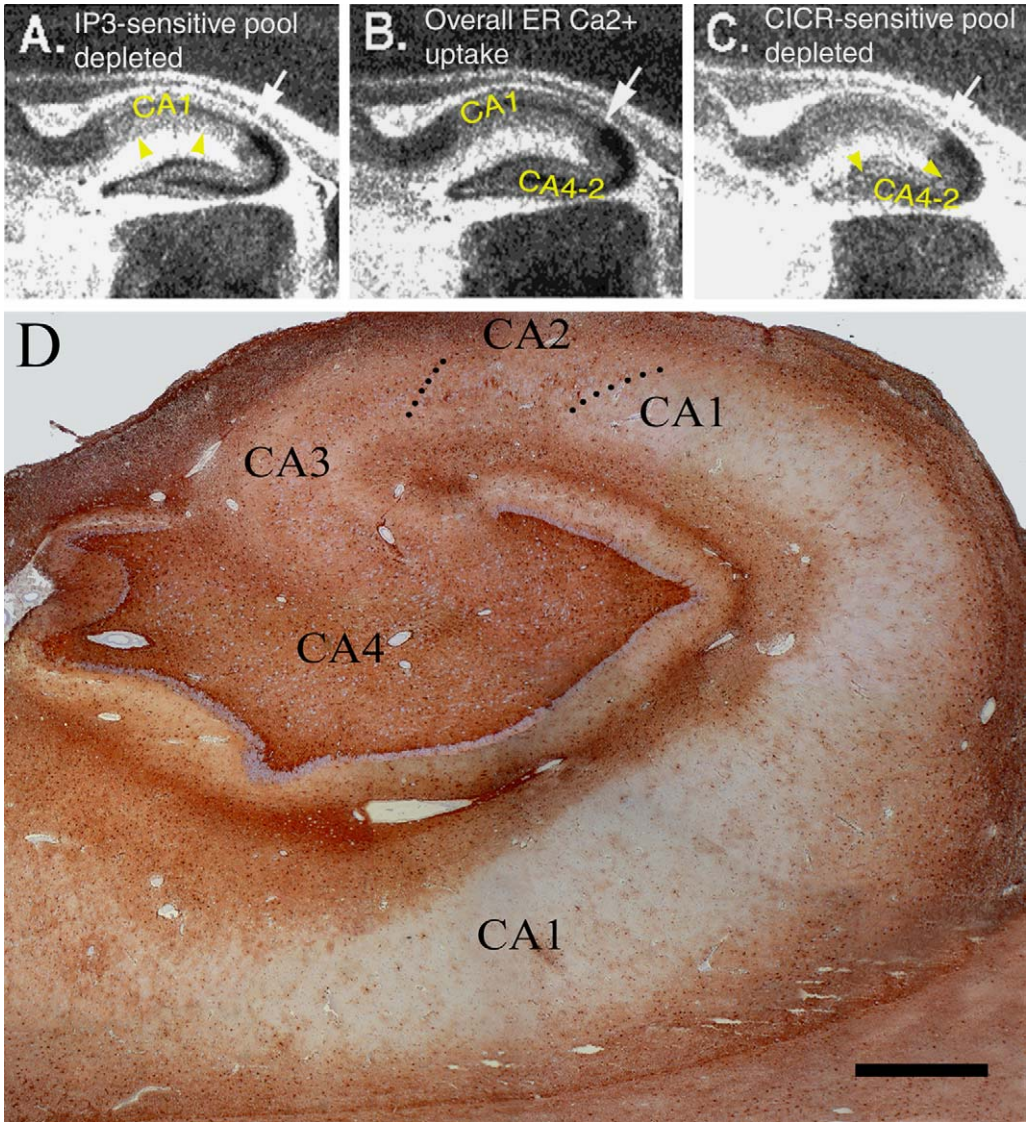

Fig. 5. CICR-sensitive calcium pools in rat hippocampus CA2-4 region match CA2-4 region-human pathology. Selective enrichment of CICR in rat hippocampal sub-regions. CA2 belongs to the CICR-sensitive pool. Endoplasmic reticulum (ER) calcium pools were localized in sagittal rat brain sections using the $^{45}Ca^{2+}$ autoradiographic technique of Verma et al. [32]. Darker areas reflect higher amounts of ER sequestered $^{45}Ca^{2+}$. The interface between CA1 and CA2 regions is indicated by the white arrows. The selective enrichment of CICR and IP3 sensitive pools in the hippocampus has been demonstrated previously by Verma et al. [32]. The present study refines the previous studies by placing the hippocampus CA2 region (interface at white arrow) as belonging to the CICR-sensitive pool with CA4 and CA3. (A) The $^{45}Ca^{2+}$ uptake performed in the presence of 10 μM IP3, selectively depletes the IP3-sensitive pools. The CA1 region in (A) (just above the yellow arrowheads) has lighter density than CA1 in (B), overall ER $^{45}Ca^{2+}$, indicating IP3-sensitive calcium depletion is primarily in CA1. (B) The overall ER $^{45}Ca^{2+}$ uptake showing non-selective ER $^{45}Ca^{2+}$ uptake. (C) $^{45}Ca^{2+}$ uptake performed in the presence of 10 mM caffeine to selectively deplete the CICR-sensitive pools. CA2-4 region (borders the yellow arrowheads) has lighter density than CA2-4 region in (B) indicating caffeine-sensitive calcium depletion of CICR regions to be primarily in CA2-4. Note that CA1 region in (C) is significantly less bright than in (A). Although the IP3 and CICR regions do overlap, (A)–(C) indicate that the CICR pools in the CA2-4 regions and the dentate gyrus region are characterized by their relative resistance to IP3, but sensitivity to caffeine. (D) Hippocampus of a patient with type 1 GD with no neurological abnormalities (Patient 3). GFAP immunoreactivity demonstrates astrogliosis in CA2-4 regions (CICR-sensitive region) contrasting with relatively spared CA1 region (IP3-sensitive region). Dense GFAP immunoreactive astrogliosis abuts directly against the dentate granular cells neurons. (GFAP, 400× original magnification). Scale bar: D, 1 mm.

Hippocampal $^{45}Ca^{2+}$ uptake autoradiography suggests a functional link between CA2-4 neuropathology and increased risk of CA2-4 neuronal excitatory cytotoxicity induced by excess glucocerebroside

Endoplasmic reticulum (ER) calcium pools were localized in sagittal rat brain sections with darker regions corresponding to greater amounts of ER sequestered $^{45}Ca^{2+}$ (Fig. 5B). Exposure to inositol triphosphate (IP3), depleted IP_3-sensitive pools, resulting in decreased ER sequestered $^{45}Ca^{2+}$ that was lighter in coloration in CA1 (Fig. 5A). In a similar manner, the same experiment performed in the presence of caffeine selectively depleted CICR-sensitive pools in CA2-4, that were lighter in color (Fig. 5C) than CA2-4 region of controls (Fig. 5B). The localization of CICR-sensitive pools in mammalian brain is correlated with the pattern of gliosis seen in the hippocampus of patients with GD (Fig. 5D).

These sets of experiments were performed to more clearly define the precise location of the CICR-sensitive hippocampal neurons. Earlier studies did not define the CA2 region as belonging to either the IP_3-sensitive pool or CICR-sensitive pool [31–33]. Corroboration of the CA2-4 neurons as CICR-sensitive allows functional correlation of CA2-4 neurons known to be glutaminergic, excitatory, that have recurrent, feedback loops and susceptible to cytotoxic injury (see discussion), with recent findings documenting sensitization of marked potentiation of ryanodine receptors induced by increased physiologic levels of GlcCer [34].

Cortical pathology

The cerebral cortex of the temporal lobe, entorhinal cortex, parietal lobe, cingulate gyrus, posterior parietal lobule, and occipital lobe had neuropathological findings

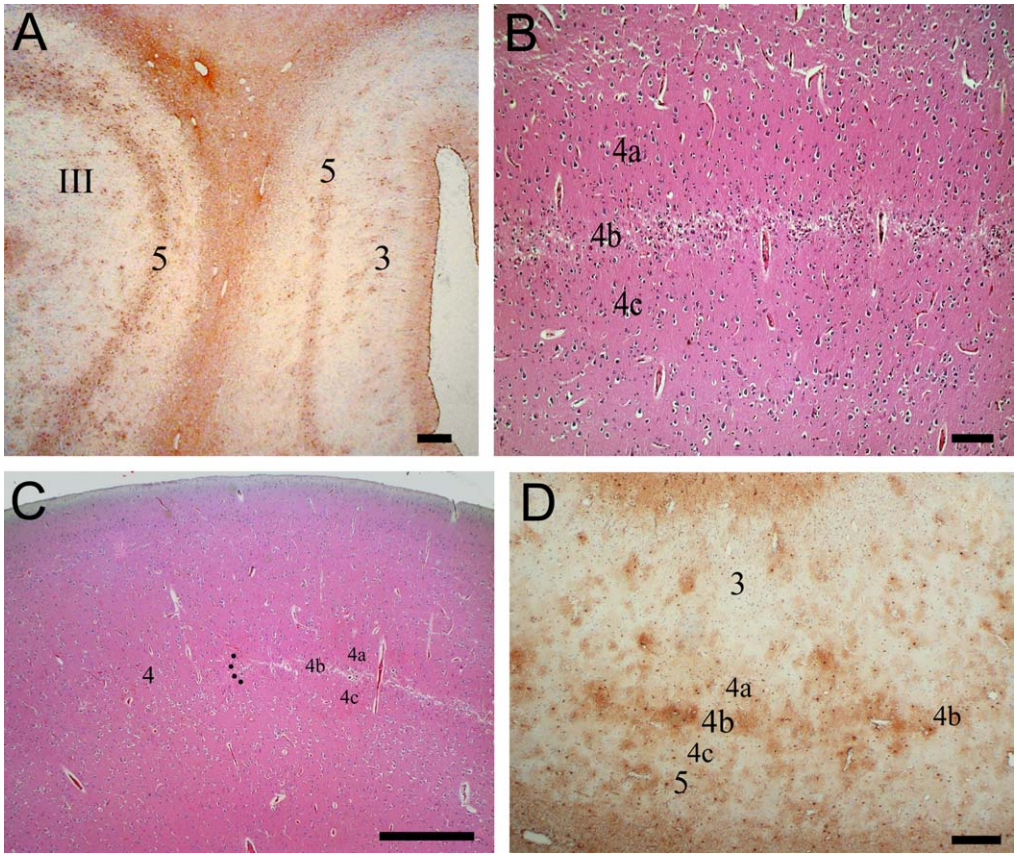

Fig. 6. Cortical layer-specific neuropathology. (A) Type 1 Gaucher disease (Patient 4), cerebral cortex in parietal lobe: laminar astrogliosis involves cortical layer 5 most consistently. Laminar astrogliosis accentuates the background perivascular astrogliosis along cortical layer 3, but may be diffuse and less organized in other areas (III) (GFAP, 40× original magnification). (B) Type 2 Gaucher disease (Patient 9), calcarine cortex, stria of Gennari: a precise, demarcated line of neuronal loss and astrogliosis involves layer 4b, but spares layer 4a and 4c. Pathology involving of all of layer 4 in the calcarine cortex was not observed. (H&E, 40× original magnification). (C) Type 2 Gaucher disease (Patient 9), calcarine cortex, termination of V1, stria of Gennari, interface where 4a, 4b, and 4c merge (curved row of dots) into a single layer 4: layer 4b (which corresponds to the stria of Gennari) has severe neuronal loss and astrogliosis that abruptly terminates at the point where layer 4b ends (H&E, 20× original magnification). (D) Type 1 Gaucher disease (Patient 4), calcarine cortex, V1, stria of Gennari: in type 1 GD, neuronal cell loss is not identified. A laminar region of astrogliosis along layer 4b, and to a lesser extent, cortical layers 3 and 5 is present (GFAP, 40× original magnification). Scale bars: A, 250 μm; B, 100 μm; C, 1 mm; D, 150 μm.

in cortical layers 3 and 5 (Fig. 6A, C). Cortical layer 5 was most consistently and prominently affected, whereas the pathology in layer 3 was more variable (Fig. 6A). Type 2 GD patients had astrogliosis and scattered, focal, mild to moderate neuron cell loss while those with type 1 GD disease had astrogliosis without identifiable neuronal loss. Cerebral cortical astrogliosis in type 1 disease was often mild, and only detected by GFAP immunoreactivity in most of the regions examined (Fig. 6A). The posterior parietal lobule, temporal lobe, entorhinal cortex, cingulate gyrus, and occipital lobe were most consistently affected. Normal cortical GFAP staining can be seen in Fig. 2B.

Calcarine cortical layer 4b neurons were affected in a specific laminar pattern. In brain samples from type 2 GD patients, layer 4b neurons had severe neuronal cell loss and astrogliosis (Figs. 6B and C). In type 1 GD, astrogliosis in layer 4b was present, in addition to the mild layer 3 and 5 cerebral cortical astrogliosis (Fig. 6D). Layer 4b pathology in type 2 and 3 GD was highly specific and did not encroach upon layer 4a or 4c, nor layer 4 (outside of V1), beyond the limitation of the line of Gennari, which corresponds to layer 4b (Fig. 6C). These findings can be compared with normal calcarine cortex GFAP staining in Fig. 2C.

General Gaucher disease neuropathology

Perivascular Gaucher cells (Fig. 6B and 7B) and non-specific white matter gliosis (not shown) were seen in all 14 patients. Non-specific grey matter and perivascular gliosis was present in 12 of 14 patients (all except Patients 11 and 12). Laminar cortical pathology of layers 3 and 5 were identified in 13 of 14 patients (excepting the sensorimotor cortex of Patient 12). Pathology in layer 4 of the calcarine cortex was noted in 12 of 14 patients (except in Patients 11 and 12). Midbrain, red nucleus gliosis was present in 12 of 14 patients (except Patients 12 and 13). In general, patients with type 2 and 3 GD had more prominent gliosis and larger and more frequent collections of perivascular Gaucher cells than type 1 patients. The brainstem was evaluated in 11 of 14 patients (except Patients 6, 12, and 13) and was significantly and diffusely gliotic in 10. The one exception was Patient 11 who died of complications of bone marrow transplantation [35]. His brainstem had only gliosis of the red nucleus and the white matter tracts located in close proximity to the red nucleus, including the oculomotor tracts. Patients 2 and 14 had prominent, diffuse gliosis of the brainstem, which included the rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF) and the paramedian pontine reticular formation (PPRF). Parenchymal Gaucher cells were present in the hippocampi and striatum of the three patients with type 2 GD (Patients 8–10). Rare intraneuronal tubular structures that stained blue with AB8GX/PAS (Gaucher cells stain blue on AB8GX/PAS) were identified in the striatum along the margins of the pallidum and the subthalamic nucleus in Patient 8 (Fig. 7).

Discussion

In this paper, we describe specific and unifying neuropathological features across the spectrum of clinical types and subsets of Gaucher disease. The most consistent and characteristic regions of pathologyA2-4, and calcarine layer 4b, were also the most unusual. The specificity of layer 4b neuronal loss and astrogliosis and the finding of CA2-4, Lewy body-like synuclein inclusions in hippocampal pyramidal cell neurons are unique in pathology. Only a few diseases, dementia with diffuse Lewy body disease, and neuronal ceroid lipofuscinosis, specifically target the hippocampal CA2-4 [36,37]. Equally remarkable is the sparingof adjacent regions such as CA1 in the hippocampus and lamina 4a and 4c on either side of 4b. The neuronal selectivity and precise regional borders delimiting injury match the functional borders of neuronal groups, and suggest that the functional characteristics of neuronal lamina and regions play a role in the selectivity. For instance, layer 4b which has large, recurrent, excitatory glutaminergic feedback suggests a possible selective vulnerability to this type of injury, but a generalized injury to the entire calcarine 4 layer would suggests injury unrelated to functional characteristics of neuronal lamina. Lastly, the matching distribution of glucocerebrosidase immunoreactivity, gliosis, neuronal loss and CA2-4 Lewy body-like synuclein inclusions in the same hippocampal region, raises the possibility of a link between the cytotoxic mechanisms in GD and the formation of Lewy bodies in Parkinson disease and diffuse Lewy body disease. We discuss these considerations in detail below.

CA2-4 is susceptible to hyperexcitability, and mitigated by strong GABAergic interneurons that maintain a delicate equilibrium

The differences between the CA1 and CA2-4 regions have been noted since the time of Ramon and Cajal who designated CA1 as the "regio superior" small cell region and the CA3 as "regio inferior" large cell region [38]. Hippocampal CA1 pyramidal cell neurons differ from CA2-4 neurons in a number of ways. Hippocampal CA1 pyramidal cell neurons receive input from layer III (rather than layer II) of the entorhinal cortex, return axonal projections back to the entorhinal cortex [39], have a far weaker and sparser array of recurrent projections [40,41], and receive no mossy fiber input [41]. CA1 hippocampal pyramidal cell neurons do not branch collateral projections distributed within CA1 [42,43], and the massive association network present in CA3 is

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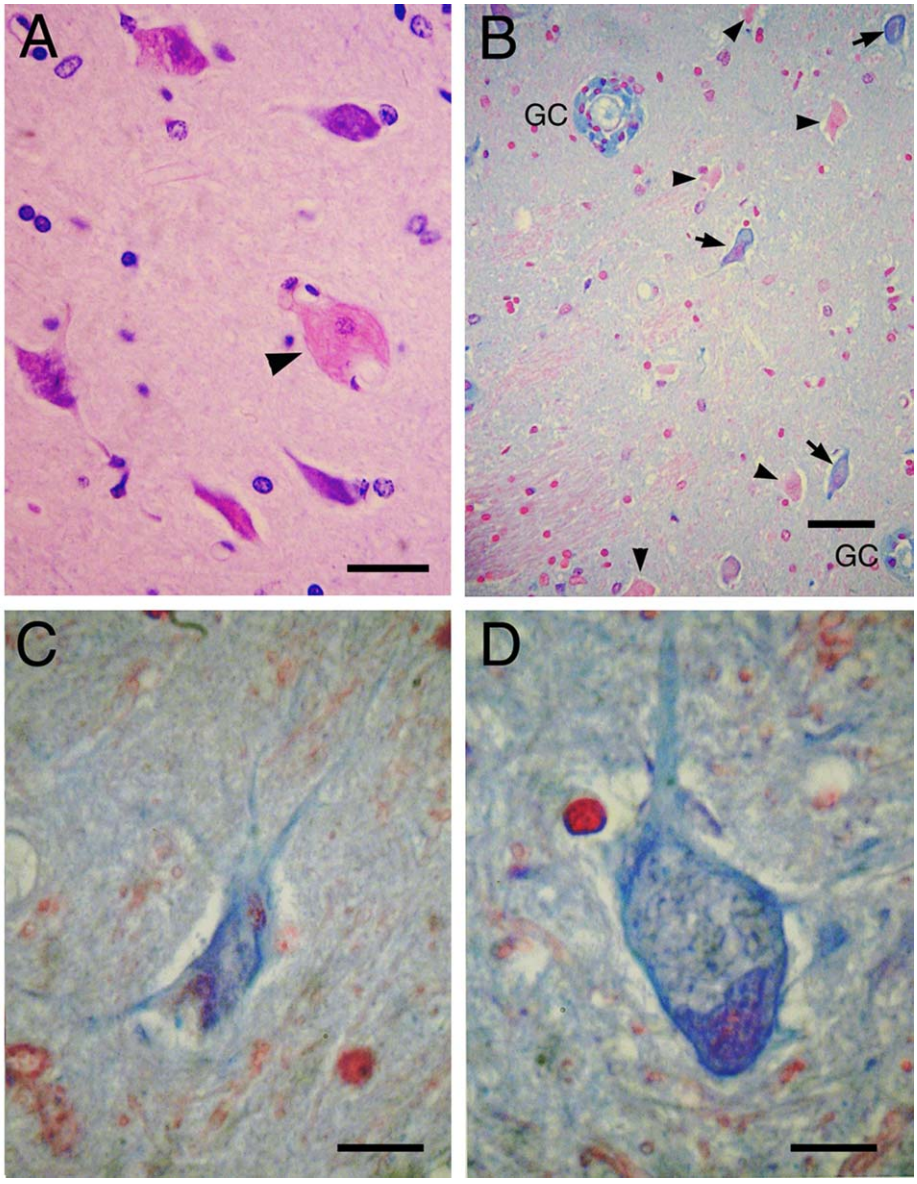
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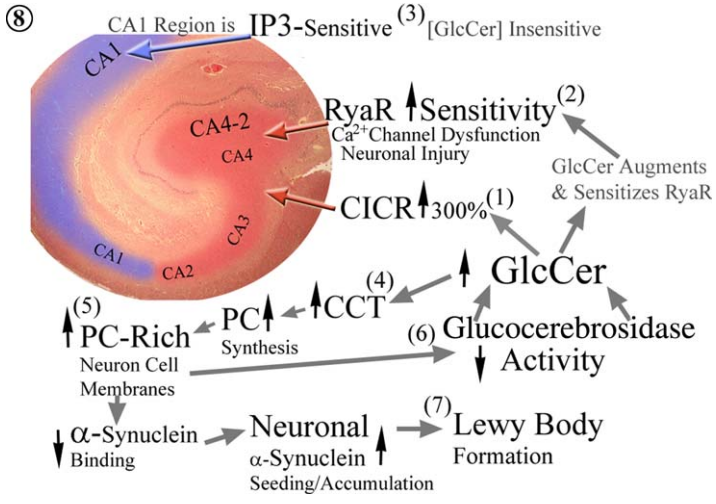
CA1 Region is IP3-Sensitive (3) [GlcCer] Insensitive
CA1
CA4-2
CA4
CA3
CA1
CA2
RyaR ↑Sensitivity (2)
Ca2+ Channel Dysfunction
Neuronal Injury
GlcCer Augments
& Sensitizes RyaR
CICR ↑300% (1)
GlcCer
(4)
CCT
PC
Synthesis
(5)
PC-Rich
Neuron Cell
Membranes
(6) Glucocerebrosidase
Activity
α-Synuclein
Binding
Neuronal
α-Synuclein
Seeding/Accumulation
(7)
Lewy Body
Formation

largely absent in CA1 [41]. In contrast, the CA2-4 hippocampal pyramidal cell neurons receive cortical input from layer 2 of the entorhinal cortex, receive significant recurrent excitatory glutaminergic projections, and except for CA2, receive mossy fiber input from the dentate gyrus granule cells [44,45]. Each hippocampal CA3 pyramidal cell neuron, on the average, contacts 30,000–60,000 neurons in the ipsilateral hippocampus and receives 25,000 excitatory glutaminergic synapses [46,47]. Mossy fibers, which have the second largest synaptic structures in the mammalian CNS, are excitatory, glutaminergic, and have a very large input into CA3, as well as CA4. CA2 receives recurrent, excitatory feedback from CA3 neurons. In concert with the intrinsic bursting properties of hippocampal CA3 pyramidal cell neurons, there is a pronounced positive feedback characteristic that predominates in the CA2-4 regions [41,48], and produces a hyperexcitable state that predisposes the CA2-4 region to large synchronous discharges, initiated within the CA2 or CA3 regions [39,49–51]. The hyperexcitable state is moderated by the strong inhibitory GABAergic interneurons, but subtle alterations in the firing properties of neurons or in the delicate equilibrium between excitation and inhibition can breach this level of inhibitory control and result in seizures that spread into CA1 [41]. Thus, a small change in neuronal sensitivity or excitability can result in aberrant, pathologic hyperexcitability states.

Increased levels of GlcCer increases sensitivity of CA2-4 specific RyaR receptors and potentiates spontaneous neuronal discharges (calcium-induced calcium release)

Astrogliosis is a response to a variety of insults to the brain [52]. In hippocampal sclerosis associated with hypoxia or ischemia, the entire hippocampus can be gliotic with degenerative changes. In mild to moderate cases, the mid to proximal portion of CA1 is gliotic, and affected by seizure activity that originates in the CA3-2 excitatory, glutaminergic feedback loops [41]. In GD, functional biochemical modulating factors, superimposed upon the functional anatomic susceptibility to aberrant excitatory disease states, may affect the localization of pathology by targeting the CICR-susceptible neuronal population and augmenting the sensitivity of selected calcium channel receptors in CA2-4. Biochemically, IP_3-sensitive Ca^{2+} pools localize predominantly to the CA1 region [31–33,53] and co-localize with the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) [53,54], while the CICR Ca^{2+} pools co-localize with ryanodine receptor binding sites and ryanodine receptors (RyaR) in CA2-4 [31–33,53] (Fig. 8(**2**)). Increased cellular GlcCer augments, modulates and sensitizes the RyaR probably through a redox receptor mechanism, and potentiates CICR in the CA2-4 regions [34,55] (Fig. 8(**2**)). In contrast, CA1 has IP_3 and SERCA-sensitive Ca^{2+} pools that are insensitive to GlcCer (Fig. 8 (**3**)) and lack CICR-susceptible, RyaR sensitive Ca^{2+} pools, subject to GlcCer perturbations. Thus, selective targeting of CA2-4 may occur through the integration of the functional anatomy and biochemical characteristics of hippocampal CA2-4 neurons in the context of GD.

Another potential modulating factor in GD is decreased CA2-4 glucocerebrosidase activity. Dense 8E4-antiglucocerebrosidase labeling in CA2-4 indicates high enzyme levels in this region. High CA2-4 glucocerebrosidase enzyme levels may be necessary to maintain low cytosolic GlcCer levels in the CA2-4 pyramidal cell neurons thereby avoiding continuous augmentation of RyaR. This is consistent with the high regionally specific expression of the CICR mechanism in CA2-4 (Fig. 5). In GD, a sufficient decrease in glucocerebrosidase activity would result in elevated GlcCer that increases spontaneous CICR by up to 300%, and causes ryanodine calcium channel dysfunction in patients with type 2 Gaucher disease [34].

Fig. 7. Cytoplasmic and membrane pathology in neurons from type 2 Gaucher disease patient (Patient 8). (A) One apparent globus pallidus neuron (arrowhead) is filled and distended by tubular–filamentous structures. The cell is favored to be neuronal rather than monocytic because of the centrally located nucleus that is not displaced to the side, spacing of other neurons, the large size of the cell without an accompanying entourage of perivascular Gaucher cells (H&E 200× original magnification). (B) Low power view of globus pallidus with blue perivascular Gaucher cells (GC), the usual pink (periodic acid–Schiff staining) neurons, and blue (alcian blue staining) neurons that tinctorially stain the same as Gaucher cells. Alcian blue 8GX/PAS at pH 2.5 normally stains neurons pink. But in Gaucher disease, isolated neurons, in rare instances, may exhibit a blue staining characteristics identical to Gaucher cells that normally stain blue (alcian blue 8GX/PAS, pH 2.5, 100× original magnification). (C) High power view of an alcian blue stained neuron with blue cytoplasm, blue neuronal membrane and neuronal processes (alcian blue 8GX/PAS at pH 2.5, 400× original magnification). (D) Close-up view of a neuron with proximal axonal process. Alcian blue stains the membrane and the internal cytoplasmic contents of the neuron. The internal cytoplasmic structures appear to have a convoluted tubular–filamentous structure (alcian blue 8GX/PAS at pH 2.5, 400× original magnification). Scale bars: A, 30 μm; B, 50 μm; C, 15 μm; D, 15 μm.

Fig. 8. Possible downstream effects of glucocerebrosidase dysfunction. Decreased functional glucocerebrosidase activity resulting in elevated intraneuronal glucocerebroside (GlcCer) concentrations could induce a 300% increase (**1**) [34] in calcium-induced calcium release (CICR) and sensitize, modulate and augment the RyaR calcium channel through its redox sensor in hippocampal regions CA2-4 resulting in predisposition to neuronal injury (**2**) [34,55]. The IP3-sensitive calcium pools in CA1 are relatively insensitive to elevations in GlcCer (**3**) [34]. GlcCer also perturbs lipid metabolism by directly stimulating CCT activity (**4**) [85] favoring phosphocholine (PC) synthesis and increased PC content in neuron cell membranes (**5**) [85]. One possible result of increased PC content in cell membranes is the accumulation of α-synuclein protein through decreased α-synuclein binding to transport vesicles [36,62,76,84,86], which can seed the rate limiting step to synuclein inclusion or Lewy body formation (**7**) [74,75,88]. Glucocerebrosidase activity is enhanced by negatively charged phospholipids, but PC rich membranes fail to similarly enhance glucocerebrosidase activity (**6**) [81–83].

Calcarine cortex layer 4b in GD has similar connectivity to hippocampal CA2-4 regions and exhibits neuronal loss and astrogliosis

Layer 4b is a dense fiber plexus lying in between 4a and 4c lamina corresponding to the stria of Gennari in the calcarine cortex [56,57]. Calcarine layer 4b receives excitatory glutaminergic projections from the magnocellular layer of the lateral geniculate via calcarine layer 4c, and sends excitatory glutaminergic projections to the middle temporal visual area (MT) [58–60]. The layer 4b connections to area MT differ from the usual pattern of visual cortical feedback loops and interconnections in that the projections from layer 4b (and deeper part of layer 5) to MT directly feed back from MT returning to the same layer(s) and constitute the major direct recurrent excitatory loop between MT and the visual cortex [61,62]. In addition, some authors consider Brodmann's layer 4b to actually be part of layer III [59,60,63–66]. In the MT projection, a strong point to point and direct reciprocal projection from 4b and 2/3a has also been described [58]. Pathology that is specific to layer 4b suggests that functional neuroanatomic characteristics that differentiate layer 4b from the other lamina 4 layers in the calcarine cortex, may predispose layer 4b to injury. One major difference in layer 4b is the large recurrent, excitatory glutaminergic, feedback loops with area MT, a feature that layer 4b has in common with the hippocampal CA2-4 region.

GD, Parkinson disease, and diffuse Lewy body dementia have in common cytotoxic pathogenic mechanisms and intraneuronal synuclein inclusions

Cytotoxicity is thought to be the major pathogenic factor in neuronopathic Gaucher disease [34,55,67–69], idiopathic Parkinson's disease [70,71], and diffuse Lewy body dementia [72–76]. Pathology of CA2-3 is shared by GD and diffuse Lewy body dementia [36,77–79], four-repeat taupathies [80], and neuronal ceroid lipofuscinosis [37]. Thus, type 1 GD by itself, without Parkinson disease or diffuse Lewy body dementia, type 1 GD with dementia + Parkinson disease with or without diffuse Lewy body dementia, or diffuse Lewy body dementia by itself, have in common CA2-3 region pathology.

In GD, the pathological changes of astrogliosis, neuronal loss, and CA2-4 and cortical Lewy body-like synuclein inclusions, affect the CA4 region as well as the CA2-3 region. To our knowledge, the presence of Lewy body-like synuclein inclusions within pyramidal cell neurons of CA2-4 with sparing of CA1 have been reported only in our current studies. The presence of Lewy body-like synuclein inclusions in CA2-4 matches exactly the distribution pattern of neuronal loss and gliosis in GD that in turn matches the pattern of glucocerebrosidase enzyme immunolabeling. This raises the possibility of a common pathophysiologic mechanism.

Increased membrane PC content induced by increased GlcCer may further reduce glucocerebrosidase activity

Perturbations of lipid synthesis and metabolism may contribute to cytotoxic effects, GlcCer accumulation, and Lewy body formation (Fig. 8). Membrane phospholipid content affects the activity of glucocerebrosidase. Negatively charged phospholipids (e.g., phosphatidylserine, phosphatidylinositol) enhance glucocerebrosidase activity while phosphatidylcholine (PC) lacks the ability to enhance this activity [81–83]. Thus, increased cellular PC may reduce glucocerebrosidase activity (Figs. 8(**5**)–(**6**)). Decreased glucocerebrosidase activity in GD, in turn, could cause a further reduction in glucocerebrosidase activity through the activation of CTP:phosphocholine cytidylyltransferase (CCT) which has been shown to favor PC synthesis [84]. The finding that GlcCer induces activation of CCT, suggests a possible pathogenic mechanism in GD, involving an overproduction of PC with resultant PC-rich neuronal cytoplasmic, vesicular and plasma membranes, effectively causing a further reduction in glucocerebrosidase activity [85] (Figs. 8(**4**)–(**6**)). Thus, a perturbation in phospholipid metabolism in GD favoring PC synthesis may further exacerbate the consequences of glucocerebrosidase deficiency and may be a possible modulating factor among the different GD phenotypes.

Increased membrane PC content induced by increased GlcCer may favor α-synuclein aggregation

Coincidentally, wild-type α-synuclein binds acidic phospholipids well, but binds neutral phospholipid (phosphotidylcholine) vesicles poorly [76,86]. Vesicles in the fast-moving component of axonal transport carry α-synuclein. However, mutant α-synuclein is devoid of vesicle binding activity, resulting in its accumulation in the neuronal cytoplasm [74,87]. The aggregation and nucleation of mutant and wild-type α-synuclein appears to be the rate-dependent step in the formation of Lewy bodies in Parkinson disease and diffuse Lewy body dementia [75,88]. Thus, elevated GlcCer that increases PC content in neuronal membranes may also hinder axonal transport of α-synuclein, favoring its aggregation–nucleation and facilitating Lewy body formation (Figs. 8(**5**)-(**7**)).

References

[1] R.O. Brady, J.N. Kanfer, R.M. Bradley, D. Shapiro, Demonstration of a deficiency of glucocerebroside-cleaving enzyme in Gaucher disease, J. Clin. Invest. 45 (1966) 1112–1115.

[2] E. Beutler, G.A. Grabowski, Gaucher disease, in: C.R. Scriver et al. (Eds.), Metabolic and Molecular Basis of Inherited Disease, McGraw-Hill, New York, 2001, pp. 3635–3668.

[3] R.O. Brady, J.A. Barranger, Glucosylceramide lipidosis: Gaucher disease, in: J.B. Stanbury et al. (Eds.), The Metabolic

Basis of Inherited Disease, McGraw-Hill, New York, 1983, pp. 842–856.
[4] E.I. Ginns, R.O. Brady, S. Pirruccello, C. Moore, S. Sorrell, F.S. Furbish, G.J. Murray, J. Tager, J.A. Barranger, Mutations of glucocerebrosidase: discrimination of neurologic and non-neurologic phenotypes of Gaucher disease, Proc. Natl. Acad. Sci. USA 79 (1982) 5607–5610.
[5] E.I. Ginns, F.P. Tegelaers, R. Barneveld, H. Galjaard, A.J. Reuser, R.O. Brady, J.M. Tager, J.A. Barranger, Determination of Gaucher disease phenotypes with monoclonal antibody, Clin. Chim. Acta 131 (1983) 283–287.
[6] R.O. Brady, Sphingolipidoses, Annu. Rev. Biochem. 47 (1978) 687–713.
[7] J.D. Miller, R. McCluer, J.N. Kanfer, Gaucher disease: neurologic disorder in adult siblings, Ann. Intern. Med. 78 (1973) 883–887.
[8] A. Vellodi, B. Bembi, T.B. de Villemeur, T. Collin-Histed, A. Erikson, E. Mengel, A. Rolfs, A. Tylki-Szymanska, Management of neuronopathic Gaucher disease: a European consensus, J. Inherit. Metab. Dis. 24 (2001) 319–327.
[9] A. Niemann, Ein unbekanntes Krankheitsbild, Jahrb f Kinderheilk (1914) 1–10.
[10] P.C.E. Gaucher, De L'epithelioma primitif de la rate hypertrophie idiopathique de la rate sans leucemie Medicine, University of Paris, Paris, 1882.
[11] M. Adachi, B.J. Wallace, L. Schneck, B.W. Volk, Fine structure of central nervous system in early infantile Gaucher disease, Arch. Pathol. 83 (1967) 513–526.
[12] B.Q.M.J. Banker, The Cerebral Pathology of Infantile Gaucher Disease, Academic Press, New York, 1962, pp. 73–79.
[13] R.M. Norman, O.C. Lloyd, The neuropathology of infantile Gaucher disease, J. Pathol. Bacteriol. 72 (1956).
[14] R.E. Lee, The Pathology of Gaucher Disease, Alan R. Liss, New York, 1982, pp. 177–217.
[15] E.M. Kaye, M.D. Ullman, E.R. Wilson, J.A. Barranger, Type 2 and type 3 Gaucher disease: a morphological and biochemical study, Ann. Neurol. 20 (1986) 223–230.
[16] O.E. Espinas, A.A. Faris, Acute infantile Gaucher disease in identical twins. An account of clinical and neuropathologic observations, Neurology 19 (1969) 133–140.
[17] N. Conradi, M. Kyllerman, J.E. Mansson, A.K. Percy, L. Svennerholm, Late-infantile Gaucher disease in a child with myoclonus and bulbar signs: neuropathological and neurochemical findings, Acta Neuropathol. (Berl.) 82 (1991) 152–157.
[18] N.G. Conradi, P. Sourander, O. Nilsson, L. Svennerholm, A. Erikson, Neuropathology of the Norrbottnian type of Gaucher disease. Morphological and biochemical studies, Acta Neuropathol. (Berl.) 65 (1984) 99–109.
[19] N.G. Conradi, H. Kalimo, P. Sourander, Reactions of vessel walls and brain parenchyma to the accumulation of Gaucher cells in the Norrbottnian type (type III) of Gaucher disease, Acta Neuropathol. (Berl.) 75 (1988) 385–390.
[20] J.F. Neil, R.H. Glew, S.P. Peters, Familial psychosis and diverse neurologic abnormalities in adult-onset Gaucher disease, Arch. Neurol. 36 (1979) 95–99.
[21] D. Soffer, T. Yamanaka, D.A. Wenger, K. Suzuki, Central nervous system involvement in adult-onset Gaucher disease, Acta Neuropathol. (Berl.) 49 (1980) 1–6.
[22] L.F. Van Bogaert, Un cas de maladie de Gaucher de l'adulte avec syndrome de Raynaud, pigmentation, et rigidite du type extrapyrajidal aux membres inferieurs, Ann. Med. 45 (1939) 57–70.
[23] R.O. McKeran, P. Bradbury, D. Taylor, G. Stern, Neurological involvement in type 1 (adult) Gaucher disease, J. Neurol. Neurosurg. Psychiatry 48 (1985) 172–175.
[24] O. Neudorfer, N. Giladi, D. Elstein, A. Abrahamov, T. Turezkite, E. Aghai, A. Reches, B. Bembi, A. Zimran, Occurrence of Parkinson's syndrome in type I Gaucher disease, QJM 89 (1996) 691–694.
[25] M. Machaczka, M. Rucinska, A.B. Skotnicki, W. Jurczak, Parkinson's syndrome preceding clinical manifestation of Gaucher disease, Am. J. Hematol. 61 (1999) 216–217.
[26] J. Varkonyi, H. Rosenbaum, N. Baumann, J.J. MacKenzie, Z. Simon, J. Aharon-Peretz, J.M. Walker, N. Tayebi, E. Sidransky, Gaucher disease associated with parkinsonism: four further case reports, Am. J. Med. Genet. 116A (2003) 348–351.
[27] N. Tayebi, M. Callahan, V. Madike, B.K. Stubblefield, E. Orvisky, D. Krasnewich, J.J. Fillano, E. Sidransky, Gaucher disease and parkinsonism: a phenotypic and genotypic characterization, Mol. Genet. Metab. 73 (2001) 313–321.
[28] N. Tayebi, J. Walker, B. Stubblefield, E. Orvisky, M.E. LaMarca, K. Wong, H. Rosenbaum, R. Schiffmann, B. Bembi, E. Sidransky, Gaucher disease with parkinsonian manifestations: does glucocerebrosidase deficiency contribute to a vulnerability to parkinsonism?, Mol. Genet. Metab. 79 (2003) 104–109.
[29] A. Fogli, K. Wong, E. Eymard-Pierre, J. Wenger, J.P. Bouffard, E. Goldin, D.N. Black, O. Boespflug-Tanguy, R. Schiffmann, Cree leukoencephalopathy and CACH/VWM disease are allelic at the EIF2B5 locus, Ann. Neurol. 52 (2002) 506–510.
[30] R.A. Barneveld, F.P. Tegelaers, E.I. Ginns, P. Visser, E.A. Laanen, R.O. Brady, H. Galjaard, J.A. Barranger, A.J. Reuser, J.M. Tager, Monoclonal antibodies against human beta-glucocerebrosidase, Eur. J. Biochem. 134 (1983) 585–589.
[31] A. Verma, D.J. Hirsch, S.H. Snyder, Calcium pools mobilized by calcium or inositol 1,4,5-trisphosphate are differentially localized in rat heart and brain, Mol. Biol. Cell 3 (1992) 621–631.
[32] A. Verma, D.J. Hirsch, M.R. Hanley, O. Thastrup, S.B. Christensen, S.H. Snyder, Inositol trisphosphate and thapsigargin discriminate endoplasmic reticulum stores of calcium in rat brain, Biochem. Biophys. Res. Commun. 172 (1990) 811–816.
[33] A. Verma, C.A. Ross, D. Verma, S. Supattapone, S.H. Snyder, Rat brain endoplasmic reticulum calcium pools are anatomically and functionally segregated, Cell Regul. 1 (1990) 781–790.
[34] E. Lloyd-Evans, D. Pelled, C. Riebeling, J. Bodennec, A. de-Morgan, H. Waller, R. Schiffmann, A.H. Futerman, Glucosylceramide and glucosylsphingosine modulate calcium mobilization from brain microsomes via different mechanisms, J. Biol. Chem. 278 (2003) 23594–23599.
[35] M.A. Garvey, C. Toro, S. Goldstein, G. Altarescu, E.A. Wiggs, M. Hallett, R. Schiffmann, Somatosensory evoked potentials as a marker of disease burden in type 3 Gaucher disease, Neurology 56 (2001) 391–394.
[36] K. Gwinn-Hardy, N.D. Mehta, M. Farrer, D. Maraganore, M. Muenter, S.H. Yen, J. Hardy, D.W. Dickson, Distinctive neuropathology revealed by alpha-synuclein antibodies in hereditary parkinsonism and dementia linked to chromosome 4p, Acta Neuropathol. (Berl.) 99 (2000) 663–672.
[37] M. Haltia, R. Herva, J. Suopanki, M. Baumann, J. Tyynela, Hippocampal lesions in the neuronal ceroid lipofuscinoses, Eur. J. Paediatr. Neurol. 5 (Suppl. A) (2001) 209–211.
[38] R.Y. Cajal, Histologie du Systeme Nerveux de l'Homme et des VertebresVolume II, Maloine, Paris, 1911.
[39] D. Johnston, T.H. Brown, Giant synaptic potential hypothesis for epileptiform activity, Science 211 (1981) 294–297.
[40] S. Radpour, A.M. Thomson, Coactivation of local circuit NMDA receptor mediated epsps induces lasting enhancement of minimal schaffer collateral epsps in slices of rat hippocampus, Eur. J. Neurosci. 3 (1991) 602–613.
[41] D. Johnston, D.G. Amaral, Hippocampus, in: G.M. Shepard (Ed.), The Synaptic Organization of the Brain, Oxford University Press, New York, 1998, pp. 429–457.
[42] N. Tamamaki, K. Abe, Y. Nojyo, Columnar organization in the subiculum formed by axon branches originating from single CA1 pyramidal neurons in the rat hippocampus, Brain Res. 412 (1987) 156–160.

[43] D.G. Amaral, C. Dolorfo, P. Alvarez-Royo, Organization of CA1 projections to the subiculum: a PHA-L analysis in the rat, Hippocampus 1 (1991) 415–435.
[44] R. Miles, R.K. Wong, Excitatory synaptic interactions between CA3 neurones in the guinea-pig hippocampus, J. Physiol. 373 (1986) 397–418.
[45] B.A. MacVicar, F.E. Dudek, Local synaptic circuits in rat hippocampus: interactions between pyramidal cells, Brain Res. 184 (1980) 220–223.
[46] N. Ishizuka, W.M. Cowan, D.G. Amaral, A quantitative analysis of the dendritic organization of pyramidal cells in the rat hippocampus, J. Comp. Neurol. 362 (1995) 17–45.
[47] X.G. Li, P. Somogyi, A. Ylinen, G. Buzsaki, The hippocampal CA3 network: an in vivo intracellular labeling study, J. Comp. Neurol. 339 (1994) 181–208.
[48] R.D. Traub, R. Miles, Multiple modes of neuronal population activity emerge after modifying specific synapses in a model of the CA3 region of the hippocampus, Ann. N. Y. Acad. Sci. 627 (1991) 277–290.
[49] D. Johnston, T.H. Brown, The synaptic nature of the paroxysmal depolarizing shift in hippocampal neurons, Ann. Neurol. 16 (Suppl.) (1984) S65–S71.
[50] D. Johnston, T.H. Brown, Control Theory Applied to Neural Networks Illuminates Synaptic Basis of Interictal Epileptiform Activity, Raven Press, New York, 1986, pp. 263-274.
[51] D. Johnston, T.H. Brown, Mechanisms of Neuronal Burst Generation, Academic Press, London, 1984, pp. 277–301.
[52] L.F. Eng, A.C. Yu, Y.L. Lee, Astrocytic response to injury, Prog. Brain Res. 94 (1992) 353–365.
[53] K.K. Miller, A. Verma, S.H. Snyder, C.A. Ross, Localization of an endoplasmic reticulum calcium ATPase mRNA in rat brain by in situ hybridization, Neuroscience 43 (1991) 1–9.
[54] A.M. Gunteski-Hamblin, J. Greeb, G.E. Shull, A novel Ca^{2+} pump expressed in brain, kidney, and stomach is encoded by an alternative transcript of the slow-twitch muscle sarcoplasmic reticulum Ca-ATPase gene. Identification of cDNAs encoding Ca^{2+} and other cation-transporting ATPases using an oligonucleotide probe derived from the ATP-binding site, J. Biol. Chem. 263 (1988) 15032–15040.
[55] E. Korkotian, A. Schwarz, D. Pelled, G. Schwarzmann, M. Segal, A.H. Futerman, Elevation of intracellular glucosylceramide levels results in an increase in endoplasmic reticulum density and in functional calcium stores in cultured neurons, J. Biol. Chem. 274 (1999) 21673–21678.
[56] S.L. Polyak, The Vertebrate Visual System, University of Chicago Press, Chicago, 1957.
[57] K. Brodemann, Vergleichende Lokalisation-slehre der Grosshirnrinde, Barth Press, Leipzig, 1909.
[58] D. Fitzpatrick, J.S. Lund, G.G. Blasdel, Intrinsic connections of macaque striate cortex: afferent and efferent connections of lamina 4C, J. Neurosci. 5 (1985) 3329–3349.
[59] W.B. Spatz, Thalamic and other subcortical projections to area MT (visual area of superior temporal sulcus) in the marmoset *Callithrix jacchus*, Brain Res. 99 (1975) 129–134.
[60] J. Tigges, M. Tigges, S. Anschel, N.A. Cross, W.D. Letbetter, R.L. McBride, Areal and laminar distribution of neurons interconnecting the central visual cortical areas 17, 18, 19, and MT in squirrel monkey (Saimiri), J. Comp. Neurol. 202 (1981) 539–560.
[61] J.M. Allman, E.R. McGuinness, in: Visual Cortex in Primates, Alan R. Liss, Los Angeles, 1988, pp. 279–326.
[62] K.S. Rockland, T. Knutson, Feedback connections from area MT of the squirrel monkey to areas V1 and V2, J. Comp. Neurol. 425 (2000) 345–368.
[63] R. Hassler, Comparative Anatomy of the Central Visual Systems in Day-and Night-Active Primates, Plenum Press, New York, 1967, pp. 410–434.
[64] W.B. Spatz, Topographically organized reciprocal connections between areas 17 and MT (visual area of superior temporal sulcus) in the marmoset *Callithrix jacchus*, Exp. Brain Res. 27 (1977) 559–572.
[65] R.E. Weller, J.H. Kaas, Cortical and Subcortical Connections of Visual Cortex in Primates, Humana Press, Englewood Cliffs, NJ, 1981, pp. 121–155.
[66] I.T. Diamond, M. Conley, K. Itoh, D. Fitzpatrick, Laminar organization of geniculocortical projections in *Galago senegalensis* and *Aotus trivirgatus*, J. Comp. Neurol. 242 (1985) 584–610.
[67] Y.A. Hannun, R.M. Bell, Lysosphingolipids inhibit protein kinase C: implications for the sphingolipidoses, Science 235 (1987) 670–674.
[68] K. Suzuki, Twenty five years of the "psychosine hypothesis": a personal perspective of its history and present status, Neurochem. Res. 23 (1998) 251–259.
[69] S. Tapasi, P. Padma, O.H. Setty, Effect of psychosine on mitochondrial function, Ind. J. Biochem. Biophys. 35 (1998) 161–165.
[70] Y. Mizuno, N. Sone, K. Suzuki, T. Saitoh, Studies on the toxicity of 1-methyl-4-phenylpyridinium ion (MPP+) against mitochondria of mouse brain, J. Neurol. Sci. 86 (1988) 97–110.
[71] G.T. Mandybur, Y. Miyagi, W. Yin, E. Perkins, J.H. Zhang, Cytotoxicity of ventricular cerebrospinal fluid from Parkinson patients: correlation with clinical profiles and neurochemistry, Neurol. Res. 25 (2003) 104–111.
[72] H.A. Lashuel, B.M. Petre, J. Wall, M. Simon, R.J. Nowak, T. Walz, P.T. Lansbury Jr., Alpha-synuclein, especially the Parkinson's disease-associated mutants, forms pore-like annular and tubular protofibrils, J. Mol. Biol. 322 (2002) 1089–1102.
[73] K.S. McNaught, P. Shashidharan, D.P. Perl, P. Jenner, C.W. Olanow, Aggresome-related biogenesis of Lewy bodies, Eur. J. Neurosci. 16 (2002) 2136–2148.
[74] V. Narayanan, S. Scarlata, Membrane binding and self-association of alpha-synucleins, Biochemistry 40 (2001) 9927–9934.
[75] L. Narhi, S.J. Wood, S. Steavenson, Y. Jiang, G.M. Wu, D. Anafi, S.A. Kaufman, F. Martin, K. Sitney, P. Denis, J.C. Louis, J. Wypych, A.L. Biere, M. Citron, Both familial Parkinson's disease mutations accelerate alpha-synuclein aggregation, J. Biol. Chem. 274 (1999) 9843–9846.
[76] E. Jo, J. McLaurin, C.M. Yip, P. St. George-Hyslop, P.E. Fraser, Alpha-synuclein membrane interactions and lipid specificity, J. Biol. Chem. 275 (2000) 34328–34334.
[77] J.E. Galvin, K. Uryu, V.M. Lee, J.Q. Trojanowski, Axon pathology in Parkinson's disease and Lewy body dementia hippocampus contains alpha-, beta-, and gamma-synuclein, Proc. Natl. Acad. Sci. USA 96 (1999) 13450–13455.
[78] A.J. Harding, B. Lakay, G.M. Halliday, Selective hippocampal neuron loss in dementia with Lewy bodies, Ann. Neurol. 51 (2002) 125–128.
[79] H. Uchikado, E. Iseki, K. Tsuchiya, T. Togo, O. Katsuse, K. Ueda, M. Kato, K. Kosaka, Dementia with Lewy bodies showing advanced Lewy pathology but minimal Alzheimer pathology–Lewy pathology causes neuronal loss inducing progressive dementia, Clin. Neuropathol. 21 (2002) 269–277.
[80] T. Ishizawa, L.W. Ko, N. Cookson, P. Davias, M. Espinoza, D.W. Dickson, Selective neurofibrillary degeneration of the hippocampal CA2 sector is associated with four-repeat tauopathies, J. Neuropathol. Exp. Neurol. 61 (2002) 1040–1047.
[81] F. Sarmientos, G. Schwarzmann, K. Sandhoff, Specificity of human glucosylceramide beta-glucosidase towards synthetic glucosylsphingolipids inserted into liposomes. Kinetic studies in a detergent-free assay system, Eur. J. Biochem. 160 (1986) 527–535.
[82] A.M. Vaccaro, M. Tatti, F. Ciaffoni, R. Salvioli, Factors affecting the binding of glucosylceramidase to its natural substrate dispersion, Enzyme 42 (1989) 87–97.
[83] G.L. Dale, D.G. Villacorte, E. Beutler, Solubilization of glucocerebrosidase from human placenta and demonstration of a phospho-

lipid requirement for its catalytic activity, Biochem. Biophys. Res. Commun. 71 (1976) 1048–1053.

[84] S. Jackowski, J. Wang, I. Baburina, Activity of the phosphatidylcholine biosynthetic pathway modulates the distribution of fatty acids into glycerolipids in proliferating cells, Biochim. Biophys. Acta 1483 (2000) 301–315.

[85] J. Bodennec, D. Pelled, C. Riebeling, S. Trajkovic, A.H. Futerman, Phosphatidylcholine synthesis is elevated in neuronal models of Gaucher disease due to direct activation of CTP:phosphocholine cytidylyltransferase by glucosylceramide, FASEB J. 16 (2002) 1814–1816.

[86] W.S. Davidson, A. Jonas, D.F. Clayton, J.M. George, Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes, J. Biol. Chem. 273 (1998) 9443–9449.

[87] P.H. Jensen, M.S. Nielsen, R. Jakes, C.G. Dotti, M. Goedert, Binding of alpha-synuclein to brain vesicles is abolished by familial Parkinson's disease mutation, J. Biol. Chem. 273 (1998) 26292–26294.

[88] S.J. Wood, J. Wypych, S. Steavenson, J.C. Louis, M. Citron, A.L. Biere, Alpha-synuclein fibrillogenesis is nucleation-dependent. Implications for the pathogenesis of Parkinson's disease, J. Biol. Chem. 274 (1999) 19509–19512.