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Neurobiology and cellular pathogenesis of glycolipid storage diseases

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Disorders of lysosomal metabolism often involve the accumulation of specific types of glycolipid, particularly gangliosides, because of either degradative failure or other currently unknown mechanisms. Although the precise role of gangliosides in cells remains enigmatic, the presence of specific abnormalities secondary to ganglioside accumulation in lysosomal diseases has suggested important biological functions. Chief among these is the growth of new dendrites on particular classes of mature neurons secondary to an increase in GM2 ganglioside. That GM2 has also been shown to be elevated in normal immature neurons coincident with dendritic sprouting provides a compelling argument that this ganglioside plays a role in dendritic initiation. This discovery has led to the search for other regulators of dendritic differentiation that may in some way be linked to the expression and/or function of GM2 ganglioside. Principal candidates that have emerged include tyrosine kinase receptors, small GTPases and calcium/calmodulin-dependent protein kinase II. Understanding the mechanism underlying ectopic dendritogenesis in lysosomal diseases can be expected to generate significant insight into the control of dendritic plasticity in normal brain. The detrimental aspects of ganglioside accumulation in storage diseases as well as the potential link between gangliosides and dendritogenesis also provide a strong rationale for developing pharmacological means to manipulate ganglioside expression in neurons.

Keywords: ganglioside; dendrite; endosome; lysosome

1. INTRODUCTION

> We may feel sure that, in the future as in the past, there will be many who will try to solve the problems of the commoner diseases, the control of which is of such vital interest to the community at large. Let us hope that there will always be some who will seek to guess the riddles and to learn the lessons of the rarer maladies.
>
> (Garrod 1928, p. 1059)

Remarkable advances have occurred in understanding the molecular genetics of lysosomal diseases over the past quarter of a century and recent discoveries continue to demonstrate that these rare diseases hold considerable potential for revealing insights into normal cell biology (Walkley 2001). In spite of advances in determining the causes of lysosomal diseases, until recently fewer laboratories have focused on the issue of pathogenic cascades in cell and organ dysfunction and research advances in these areas have, therefore, been limited. In organs like brain, the goal of understanding each step in the cascade of events ensuing from primary protein defect to abnormal neuron function and clinical neurological disease is formidable. For example, the causes of, and relationship between, even the two most conspicuous features of cellular pathology of most GSDs affecting brain—ectopic dendritogenesis and neuroaxonal dystrophy—remain largely unknown. Although, as described in detail in § 3, the growth of ectopic dendrites appears associated with elevations in the expression of a particular glycolipid (GM2 ganglioside) within affected neurons, a similar direct association with ganglioside storage is not evident for the axonal pathology (Walkley 1998). The two phenomena also largely appear to occur in different populations of neurons, with neuroaxonal dystrophy predominating in GABAergic neurons, and ectopic dendritogenesis occurring in select populations of excitatory neurons.

Numerous other pathogenic features also occur in brain in lysosomal disorders, including demyelination, neuronal cell death and gliosis. In storage disorders like the NCLs, the massive death of neurons and regional brain atrophy are characteristic findings in early disease, although in these diseases neuronal glycolipid storage is generally absent. In most GSDs, by contrast, conspicuous neuron death in early disease is less common and is restricted primarily to select populations of neurons such as cerebellar Purkinje cells. In neither the GSDs nor the NCLs, however, is there a clear understanding of the molecular mechanisms leading to neuron death. A recently discovered component of neuron loss in GSDs is microglial activation (Wada *et al.* 2000). Not only do activated microglia appear early in these diseases, but slowing their activation may contribute to slowing disease progression overall. These and related findings provide compelling evidence that the more we know about the details and relationships of disease cascades, the more likely we can

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use this knowledge to directly intervene in the mechanism of neurodegeneration to the benefit of patients with these disorders. Knowledge gained from studies of the cellular and molecular pathology of storage disease also generates insight into novel pathways and mechanisms at work in normal neurons. A significant goal in the study of rarer forms of disease is surely to make discoveries that, when elaborated, might prove useful in the understanding or treatment of other conditions, including commoner ones like Alzheimer's or cerebrovascular disease. Indeed, one impetus for the current studies, reviewed here, is that understanding the mechanism responsible for ectopic dendritogenesis in Tay–Sachs and related GSDs may lead eventually to treatments that could stimulate dendritogenesis in mature neurons with dendrites damaged by stroke or traumatic injury.

2. ECTOPIC DENDRITOGENESIS WAS DISCOVERED IN GM2 GANGLIOSIDOSIS

In 1975 during a Golgi analysis of dendrites in a cortical biopsy from a child with an undiagnosed and progressive form of mental retardation it was noticed that many neurons had elongated swellings at their axon hillocks and from these projected long dendritic spine-covered processes (Purpura & Suzuki 1976; Purpura 1978). The swelling at the axon hillock was referred to as a 'meganeurite', and the associated abnormal dendritic membrane as 'ectopic', owing to its peculiar and inappropriate location on the cell, and also, to its emergence at an inappropriate time on these otherwise mature neurons (figure 1). Thus, it appeared that these postnatal, mature neurons were undergoing a recapitulation of dendritogenesis normally seen only in early development, and as a result an entirely new basilar dendritic system was being generated below the normal basilar dendrites of the perikaryon. The child in this case was subsequently diagnosed with a rare form of GM2 gangliosidosis (AB variant) in which the degradative enzyme (β-hexosaminidase) for GM2 ganglioside was normal, but failed to function as a consequence of a deficiency in its activator protein (Conzelmann & Sandhoff 1978; Sandhoff & Kolter 2003). The discovery of ectopic dendritogenesis in a ganglioside storage disease was believed to indicate that gangliosides contributed in some manner to dendritic growth regulatory mechanisms and that the resulting altered synaptic connectivity was a basis for brain dysfunction, most notably mental retardation. This remarkable and serendipitous discovery led to many subsequent studies some of which were focused on the application of GM1 (an abundant and therefore readily obtainable ganglioside) to *in vitro* or *in vivo* model systems (reviewed in Ledeen (1985)) whereas others were directed at better defining the phenomenon of ectopic dendritogenesis and its relationship to mechanisms of dendritogenesis in normal neurons. It is this latter series of studies that will be reviewed here.

(a) *New membrane is dendritic and possesses numerous synapses*

The availability and use of animal models, particularly of spontaneous genetic conditions in companion animals and livestock (see Jolly & Walkley 1997), considerably expanded knowledge of ectopic dendritogenesis by

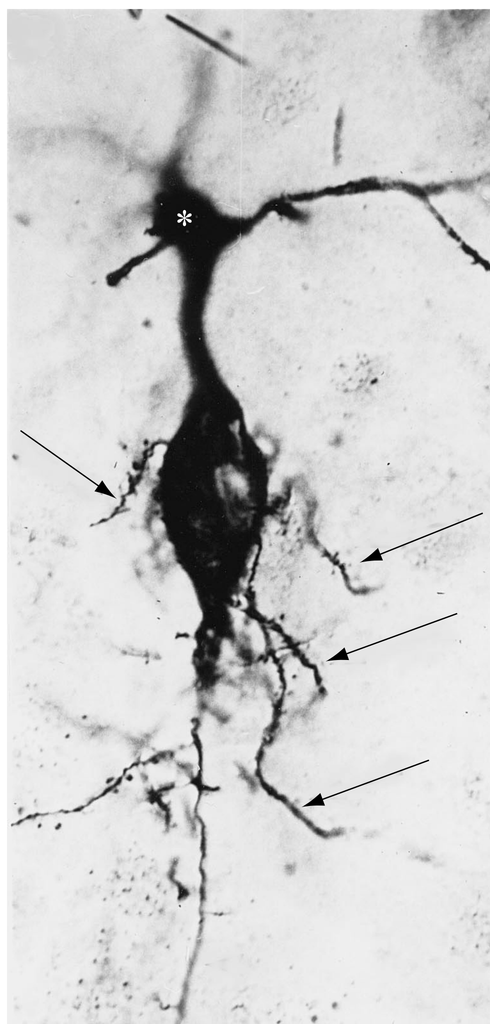

Figure 1. Ectopic dendritogenesis as observed in lysosomal storage disorders. Golgi impregnated pyramidal neuron from a 3.5-year-old child diagnosed with NPC. Arrows identify ectopic spine-covered dendrites emanating from a large meganeurite located below the cell body as indicated by the asterisk; magnification, ×650.

revealing its occurrence in many types of storage disease (table 1). Meganeurites were documented to occur specifically at the axon hillock area of pyramidal neurons and to push the sodium channel-rich initial segment of the axon more distal with their continued expansion (Walkley & Pierok 1986). In most storage diseases, meganeurite surfaces were found to be covered with synapses, or with postsynaptic densities lacking visible axonal contacts (Purpura & Suzuki 1976; Walkley *et al.* 1981*a*). Neurons in some storage diseases, for example the NCLs, exhibited meganeurites that lacked spines, synapses and neuritic outgrowth. In such circumstances these axon hillock enlargements were viewed as volume accommodators and the membrane as non-dendritic. That is, the stimulus for production of dendritic-like membrane (identified by postsynaptic densities and spines) appeared to be independent of that for membrane expansion to allow space to accommodate accumulating storage bodies (Walkley 1998). Often, as documented in feline models of GM1 and GM2 gangliosidosis, ectopic dendritic sprouts occurred at the axon hillock in the absence of meganeurite formation. Over time these dendritic neurites, whether

Table 1. The occurrence of ectopic dendritogenesis in animal models of neuronal storage disorders.

animal model	reference
ectopic dendrite growth is present	
GM2 gangliosidosis (feline, canine)	Walkley *et al.* (1990); Cummings *et al.* (1985)
GM1 gangliosidosis (feline, canine, ovine)	Walkley (1995); J. F. Cummings and S. U. Walkley (unpublished data); Ahern-Rindell *et al.* (1988)
Niemann–Pick type A (feline)	Walkley & Baker (1984)
NPC (feline)	March *et al.* (1997)
MPS type I (feline, canine)	Walkley *et al.* (1988*b*)
MPS type VI (feline)	S. U. Walkley, M. E. Haskins and M. A. Thrall (unpublished data)
α-mannosidosis (feline)	Walkley *et al.* (1981*a*,*b*)
ectopic dendrite growth is absent	
NCLs (canine, ovine, murine)	Walkley *et al.* (1995, 1996)
GM2 gangliosidoses (murine)	Walkley *et al.* (2000)
GM1 gangliosidosis (murine)	S. U. Walkley and A. D'Azzo (unpublished data)
MPS I, III A, VII (murine)	S. U. Walkley, M. S. Sands, P. Stanley and E. F. Neufeld (unpublished data)
Niemann–Pick type A (murine)	Walkley *et al.* (2000)
NPC (murine)	Zervas & Walkley (2001)
α-mannosidosis (guinea-pig)	S. U. Walkley, J. J. Hopwood and A. C. Crawley (unpublished data)
fucosidosis (canine)	Walkley (1995)

meganeurite associated or not, exhibited a capacity to mature into normal appearing dendrites complete with spines and synapses. Indeed, even as short processes during early disease, ectopic dendrites were found to be richly vested with asymmetrical synaptic contacts (Walkley *et al.* 1981*b*, 1990). Although the axon hillock was the most prominent location for ectopic dendrites, occasional sprouting was also observed at the base of apical dendrites of pyramidal neurons or from perikaryal surfaces.

The phenomenon of ectopic dendritogenesis, although limited to genetic conditions affecting the endosomal–lysosomal system, has been replicated in one non-genetic experimental model. In this case a lysosomal storage disease (α-mannosidosis) was induced in cats through the administration of a reversible inhibitor of lysosomal α-mannosidase (an indolizidine alkaloid, swainsonine) isolated from plants known as locoweed (*Astragalus* spp.) (Walkley *et al.* 1988*a*). The resulting disease was a remarkable phenocopy of the genetic form of α-mannosidosis that also occurs in cats. In this series of experiments new dendrite growth occurred after the disease was several weeks in duration, with this being the case whether the disease induction began just after birth or as late as 1 year of age. These studies thus revealed that neurons that are normal during early development can none the less be induced to sprout new dendrites. These studies also allowed for the evaluation of the reversibility of the cellular pathology of a storage disease (Walkley *et al.* 1987). Although cessation of inhibitor administration led to the rapid reversal of lysosomal storage and the normalization of the perikaryal structure (including the shrinkage and disappearance of meganeurites), many ectopic dendrites were found to persist even after long periods of time (years). One interpretation of these results is that contacts made by adjacent axons caused the new dendrites to become stabilized and to function as an integral part of the normal basilar dendritic arbor (Walkley 1998).

(b) *Ectopic dendritogenesis is limited by disease, species and neuron type*

Ectopic dendrites have never been reported to occur in non-lysosomal brain diseases and even some neuronal storage diseases lack this phenomenon. As shown in table 1, seven types of storage disease have been found to exhibit this phenomenon in animal models and they include both primary gangliosidoses as well as mucopolysaccharide and glycoprotein storage disorders. Although the overall incidence of (for example) cortical pyramidal neurons with ectopic dendrites can vary by disease, the morphological appearance of the new dendrites on individual neurons generally is remarkably similar for all such diseases. In addition to differences in incidence based on disease type, important species differences have also been noted (table 1). In human storage diseases, as shown for NPC (figure 1), spiny meganeurite formation often accompanies the growth of ectopic dendrites and the overall extent of the new dendritic membrane can be remarkable. In carnivores, dendritic spouts also occur at this region of the pyramidal neuron but axon hillocks may not enlarge into meganeurites presumably owing to there being insufficient storage to create this structure. Most notable, however, in species differences, is the finding that rodent models of storage diseases exhibit little or no ectopic dendrite growth even though meganeurites may occur (Walkley *et al.* 2000). Although the exact reason for this absence remains to be determined, one possibility is that the metabolic derangements that are responsible for new dendrite growth occur too late in mice relative to postnatal critical periods for dendritic plasticity. This view is based on the rapid maturation of the rodent brain compared with that of carnivores and humans. In the last two species, intra-neuronal storage typically has sufficient time to accrue during the early postnatal months (cats) and years (humans) before full brain maturation. In rodents this may not happen because adulthood (as measured by sexual maturity) is

reached only a few weeks after weaning. Understanding those factors limiting ectopic dendrite growth in mice may provide important insight into the mechanisms of post-natal dendritic plasticity in higher species.

Another interesting feature of ectopic dendritogenesis relates to neuron-type differences. This was evident even in the biopsy from the case of GM2 gangliosidosis AB variant mentioned in § 2, in that ectopic dendrites were observed on cortical pyramidal neurons but not on non-pyramidal-type cells (Purpura 1978). This finding was considerably elaborated in later studies of animal models where it was found that pyramidal neurons of the cerebral cortex and hippocampal formation (at times including granule cells of the fascia dentata) and multipolar cells of the amygdala and claustrum were principally affected with ectopic dendritogenesis (Walkley 1998). Interestingly, ectopic dendrites as seen in various types of storage disease (table 1) always occurred on these same types of neuron. Thus, it appeared that ectopic dendritogenesis in storage diseases was revealing something special about dendritic plasticity in particular classes of neuron, rather than about the primary metabolic disturbance involving the lysosome. A search ensued for a single type of metabolic derangement common to *all* ectopic dendrite-bearing neurons, but absent in these same types of neuron in storage diseases lacking this phenomenon.

3. ECTOPIC DENDRITOGENESIS CORRELATES WITH AN ABNORMAL INCREASE IN GM2 GANGLIOSIDE

As noted in § 2, far from being limited to primary GM2 gangliosidoses in which they were discovered, ectopic dendrites were found in animals from those with MPS storage diseases to those with the rare glycoprotein storage disease known as α-mannosidosis. Initially this was believed to indicate the importance of the lysosomal defect and storage, rather than ganglioside storage *per se*, in the generation of ectopic dendrites. However, several types of lysosomal disease were found that did not exhibit ectopic dendrite growth despite significant intraneuronal storage. In such cases meganeurites often occurred but they routinely lacked the presence of dendritic spines and new synapses, consistent with a function solely related to storage. In storage diseases with ectopic dendrites the incidence varied widely, ranging from nearly all pyramidal neurons exhibiting the phenomenon in the Sandhoff form of GM2 gangliosidosis in the cat, to only 15–20% with ectopic dendrites in diseases such as α-mannosidosis (Siegel & Walkley 1994). This limited occurrence in the latter disease proved to be particularly useful in revealing a single metabolic derangement common to all neurons with ectopic dendrite growth.

(a) *α-Mannosidosis provides evidence for a link between storage of GM2 ganglioside and ectopic dendritic sprouting*

In a feline model of α-mannosidosis only a small proportion of cortical pyramidal neurons exhibit ectopic dendrite sprouting, although qualitatively the neurites themselves closely resemble those seen in other storage diseases (Walkley *et al.* 1981*a*). During studies evaluating the ultrastructure of ectopic dendrite-bearing neurons in this disease it was found that neurons with ectopic dendrites consistently exhibited storage vacuoles containing membranous leaflets indicative of lipid or glycolipid accumulation. Immunohistochemical studies using an antibody to GM2 ganglioside demonstrated increased staining for GM2 in a select population of cortical neurons, including a subset of pyramidal neurons in layers II, III and V (Goodman *et al.* 1991). Cross correlation studies using combinations of Golgi, histochemical and immunohistochemical staining and electron microscopy established that pyramidal neurons with ectopic dendrites consistently were the same ones that exhibited heightened expression of GM2, whereas most pyramidal neurons without ectopic dendrites contained little or no GM2. Studies in the induced model also showed that the GM2 elevations preceded ectopic dendrite growth, consistent with a role in causing the phenomenon of new dendrite growth rather than simply being a consequence of it (Goodman *et al.* 1991).

Subsequent studies showed that GM2 elevations occurred in the cerebral cortex of all of the storage diseases in which pyramidal neurons exhibited ectopic dendrite growth (Siegel & Walkley 1994; Walkley 1995). These included GM2 gangliosidosis in which essentially all neurons exhibited GM2 storage and almost all pyramidal neurons possessed ectopic dendrites, to MPS type I and Niemann–Pick diseases types A and C, all of which exhibited intermediate levels of GM2 accumulation and ectopic dendrites on 20–40% of pyramidal neurons. The GM2 ganglioside, although not widely studied outside the context of Tay–Sachs disease, is well known as the immediate precursor for the synthesis of GM1 ganglioside. The latter is one of the major gangliosides in normal, mature brain, whereas GM2 is essentially undetectable (Suzuki 1965). Why elevations of the latter occur in widely different storage diseases lacking primary defects in GM2 degradation is unknown, although several plausible mechanisms have been suggested for NPC and MPS diseases.

(b) *GM2 ganglioside storage in Niemann–Pick disease type C may indicate a role for NPC1 in ganglioside trafficking*

The neuronal storage disorder known as NPC was originally classified with other Niemann–Pick disorders (A and B types) caused by clinical and pathological similarities. Subsequently it was shown that most cases of NPC disease were caused by a defect in NPC1, a transmembrane protein, rather than by the absence of a lysosomal enzyme (Carstea *et al.* 1997). The presence of normal lysosomal sphingomyelinase function in NPC disease (as opposed to types A and B) and the discovery that LDL-cholesterol challenge to cultured NPC fibroblasts provided an important diagnostic tool, were supportive of the view that cholesterol was a principal storage product in this disease (Pentchev *et al.* 1994) and that glycolipid accumulation was probably secondary. Recent studies, however, have established that GM2 and GM3 ganglioside storage is more centrally involved in the pathogenic cascade of this disease, at least as far as neurons are concerned (Gondré-Lewis *et al.* 2003). When mice with a genetic deficiency of NPC1 were crossed with others lacking the synthetic enzyme responsible for the production of GM2 and higher-order gangliosides, most neurons in the double

mutants were reported to lack cholesterol storage (Liu *et al.* 2000). This absence of cholesterol storage in many neurons was confirmed in subsequent studies, which also showed that neurons in double mutants that did store cholesterol also stored the one other disease-associated ganglioside (GM3) that could be synthesized in the double mutant mice (Gondré-Lewis *et al.* 2003). The latter findings were compatible with the hypothesis that NPC1 functions as a permease (Davies *et al.* 2000) responsible for shuttling specified degradative products (e.g. simple GSLs) from the endosomal–lysosomal system to the Golgi for re-utilization, possibly as part of a feedback mechanism for control of GSL homeostasis (Zervas *et al.* 2001). Cholesterol, by contrast, appears to accumulate in NPC neurons only in conjunction with the storage of GM2 or GM3 gangliosides. Given that ectopic dendrites sprout from pyramidal neurons in feline and human NPC disease (just as in Niemann–Pick type A disease and other storage disorders), it appears that the cause of the GM2 elevation—whether owing to an absence of a primary degradative enzyme or to defects in proteins involved in substrate shuttling or other functions—may not be critical in this phenomenon. Rather, what appears important to stimulate ectopic dendritogenesis is simply the abnormal elevation, by whatever means, of GM2 ganglioside during a period of postnatal brain maturation after normal dendritogenesis is complete.

(c) *GM2 ganglioside storage in mucopolysaccharidoses is linked to cholesterol accumulation and ectopic dendritogenesis*

Abnormal accumulation of GM2 and GM3 gangliosides has also been found to occur in several types of MPS storage disease (Constantopoulos *et al.* 1980) and as described in § 3a, the abnormal elevation of GM2 correlates with the presence of ectopic dendritogenesis in feline MPS type I (Walkley *et al.* 1988*b*) and MPS VI (S. U. Walkley, M. E. Haskins and M. A. Thrall, unpublished data) diseases. Neurons in MPS diseases accumulate specific types of GAG secondary to defects in specific lysosomal hydrolases, but as suggested by accompanying ganglioside accumulation, the storage process is complex. For example in MPS type III A (Sanfilippo A) disease neurons accumulate not only heparan sulphate secondary to an inherited deficiency of lysosomal heparan *N*-sulphatase but also GM2 and GM3 gangliosides, cholesterol and autofluorescent material (McGlynn *et al.* 2003). Ganglioside storage in MPS disease is reportedly caused by the inhibition of neuraminidase or other lysosomal enzymes by accumulating GAGs (Kint *et al.* 1973; Avila & Convit 1975; Baumkotter & Cantz 1983). An explanation for the storage of cholesterol and autofluorescent materials is less clear and may indicate major defects in intracellular vesicular trafficking (Pagano *et al.* 2000; McGlynn *et al.* 2003). Studies analysing substrate accumulation in murine models of MPS disease have furthermore revealed that individual storage materials, including gangliosides, may be found in separate vacuoles, raising significant questions about mechanisms underlying such secondary storage (McGlynn *et al.* 2003).

As was the case for NPC disease, the presence of ectopic dendrites on GM2-storing cortical neurons in MPS I and VI diseases in cats again indicates that primary defects in ganglioside degradation are not crucial for the abnormal accumulation of GM2 and its association with re-growth of dendrites.

4. MULTIPLE FACTORS CONTRIBUTE TO THE REGULATION OF NORMAL DENDRITOGENESIS

An explanation of why ectopic dendrite growth occurs in storage diseases could conceivably be found in known mechanisms of dendritic differentiation in normal neurons. However, although knowledge of such regulatory processes is expanding rapidly, an understanding of key components of such mechanisms and of differences between neuron types and brain regions, stages of development, species, and so forth, remains remarkably incomplete. The underlying mechanism controlling dendritic differentiation in mammalian cortical pyramidal neurons is a case in point. It is well known that during formation of the cerebral cortex postmitotic neurons from the ventricular zone migrate as bipolar cells along radial glial guides to their final location in the maturing cortical plate. It is only upon cessation of migration that they undergo a burst of dendritic outgrowth. Dendritic neurites sprout both from the leading process, now recognizable as the apical dendrite, and from the perikaryon. The trailing process, by contrast, becomes the axon. Over the following days to weeks, depending on the species, individual neurons form characteristic dendritic arbors that persist for the lifetime of the cell. During this time there is evidence that an initial overgrowth of some dendritic processes is followed by sculpting and loss of redundant processes before stabilization of the characteristic arbor. In normal, mature mammalian brain, distal branches of established dendrites on pyramidal neurons may slowly elongate (Buell & Coleman 1979), and dendritic spines themselves may exhibit remarkable plasticity (Matus 2000), but further initiation of new primary dendrites on normal mature neurons has not been observed. Based on these findings, dendritogenesis is often viewed as occurring in three somewhat overlapping stages consisting of initiation of sprouting, elongation and branching of processes forming secondary and tertiary dendrites, and finally sculpting/pruning and stabilization leading to the formation and maintenance of characteristic dendritic arbors of mature neurons.

Although the precise control mechanisms responsible for dendritogenesis in pyramidal and other neuron types in mammalian brain remains incomplete, a variety of studies provide ample evidence for key roles played by both extrinsic and intrinsic factors, as has been recently reviewed (Cline 1999; McAllister 2001; Whitford *et al.* 2002).

(a) *Extrinsic factors exhibit influence over dendritogenesis*

Numerous recent studies point to critical roles for neurotransmitters and other exogenous proteins in the growth and development of neuronal dendritic arbors. Indeed, the role of synaptic activity in the dynamic modulation of dendrites has been evident since the seminal studies on the effects of environmental enrichment on dendritic arbor and spine morphology of cortical pyramidal neurons in rats (Volkmar & Greenough 1972). Sub-

sequent studies have reinforced the view that electrical activity can have significant influence over synaptic connectivity of many types of neuron (Katz & Shatz 1996), but most of these findings have focused on the influence over axonal rather than dendritic arbors. The role of neuronal activity in the development and sculpting of dendritic arbors is more controversial (see discussion in Cline (1999)), with studies suggesting both positive and negative influences depending on the experimental paradigm used. A common theme in many of these studies is that electrical activity-dependent mechanisms show differences based on the type of neuron (e.g. pyramidal versus non-pyramidal, simple versus complex neurons, etc.) being analysed. Differences in the response of different types of dendrite (apical versus basilar of pyramidal neurons) have also recently been noted (Groc *et al.* 2002). The role of glutamate and its influence through NMDA and AMPA receptors is central in many such studies, with the evolving ratio of these two receptor types during postnatal maturation possibly underlying differences in experimental findings (Cline 1999).

In addition to neurotransmitters like glutamate as extrinsic factors influencing dendritic differentiation in neurons, another class of compounds with demonstrated effects is the neurotrophins. These include several types of closely related protein, including NGF, BDNF and NT-3 and NT-4. The application of BDNF and NT-4 have been shown to cause exuberant dendritic sprouting when applied to immature ferret cortical neurons in culture (McAllister *et al.* 1995, 1996), possibly with full length and truncated TrkB receptors playing differing roles in this process (Yacuobian & Lo 2000). Increases in BDNF *in vivo* secondary to transgene expression similarly showed altered dendrite growth in some brain regions in mice (Tolwani *et al.* 2002). As was the case for electrical activity-related events in the control of dendrite elaboration, cell-type differences in response to neurotrophin stimulation have also been noted, as were differences in apical versus basilar dendritic domains in the case of pyramidal neurons. Osteogenic proteins, also known as BMPs, represent another family of growth factors that have been implicated in regulating dendritogenesis in a variety of neuron types in both the CNS and the PNS (Guo *et al.* 2001). Other extrinsic protein signals with demonstrated effects on specialized portions of the dendritic arbor include semaphorin 3A, a chemorepellant for cortical axons that also functions as a chemoattractant for apical dendrites (Polleux *et al.* 2000).

(b) *Numerous intrinsic factors, including GM2 ganglioside, are implicated in normal dendritogenesis*

The discovery that an elevated expression of GM2 ganglioside within specific types of neuron in storage diseases consistently correlated with the renewal of dendritogenesis prompted examination of developing cortical neurons for the presence of this and other gangliosides. Interestingly, in the same species (cat) in which the GM2-ectopic dendrite correlation was developed, immuno-histochemical analysis of developing cerebral cortex revealed that GM2 expression was robust in neurons immediately after migration into the cortical plate and at the time of normal dendritic sprouting (Goodman & Walkley 1996). As the cortical neurons matured and dendritic arbors became established during the first three postnatal weeks, GM2 expression diminished to adult levels. Similar correlations between GM2 and normal dendritic sprouting were subsequently established in ferret (Zervas & Walkley 1999), human (Walkley *et al.* 2000) and murine (Gondré-Lewis *et al.* 2001) cortices. In each species, GM2 expression was seen primarily as vesicular, with GM2-positive punctae being scattered throughout the neuronal perikarya and extending into proximal dendrites. When other gangliosides were analysed none showed a similar correlation with dendritic sprouting, even though some (e.g. GD2) showed prominent vesicular staining similar to GM2 but persisting into adulthood (Zervas & Walkley 1999). Thus, in both known circumstances in which cortical pyramidal neurons initiate new dendritic sprouts—during normal development and in several types of neuronal storage disease—heightened expression of GM2 ganglioside within these neurons was a common factor. Furthermore, GM2 appeared to be acting endogenously because in diseases such as α-mannosidosis only pyramidal neurons with intracellular GM2 elevations also exhibited ectopic dendrites. These findings, taken as a whole, led to the development of the hypothesis that GM2 ganglioside is a component of a regulatory mechanism controlling dendritic sprouting in mammalian cortical pyramidal neurons (Walkley *et al.* 2000).

Other diverse, endogenously expressed proteins have also been implicated in dendritic growth regulation. Altered expression of Rho-related GTPases, most notably Rho, Rac and Cdc42 have been linked to increases or decreases in dendritogenesis in several model systems (Threadgill *et al.* 1997; Lee *et al.* 2000; Li *et al.* 2000). Newly discovered GPI-anchored proteins such as CPG15 may influence dendrite outgrowth through possible presynaptic effects (Corriveau *et al.* 1999) or as an intrinsic signal (Nedivi *et al.* 1998). Notch1, a putative cell surface receptor protein, has been implicated in promotion of dendritic branching and as an inhibitor of dendrite outgrowth as neurons mature (Redmond *et al.* 2000). As described in § 4a, glutamate stimulation of NMDA and AMPA receptors, as an externally derived activity-dependent mechanism, has also been shown to influence dendritogenesis (reviewed in Cline (1999)). Glutamate receptor activation is known to cause changes in synaptic Ca^{2+} levels that in turn lead to activation of CaMKII, PKC, PKA and other Ca^{2+}-dependent pathways, and several studies have linked CaMKII to dendritogenesis and dendritic plasticity (Glazewski *et al.* 1996, 2000; Soderling 2000). The α-isoform of CaMKII is a major component of this kinase in excitatory cortical neurons, occurring in perikaryal, dendritic and synaptic locations. CaMKII has been implicated in mechanisms of learning and memory and in development of critical periods in postnatal brain (Lisman *et al.* 2002). Mice lacking the gene for CaMKIIα exhibit not only limbic system seizures (Butler *et al.* 1995) but also defects in a variety of brain maturational processes (Chen *et al.* 1994; Gordon *et al.* 1996). Expression of the α-subunit of CaMKII in neurons is developmentally regulated, with its peak in expression correlating with synapse and dendritic arbor maturation (Cline 1999). The discovery that inhibitors of CaMKII could cause a renewal of dendritogenesis led to the proposal that this kinase is a

critical factor for the final stage of dendritogenesis—i.e. stabilization of the arbor (Wu & Cline 1998; Lisman *et al.* 2002).

The use of swainsonine to create an animal model with both GM2 elevation and ectopic dendritogenesis, as mentioned in § 2a, is one of the few *in vivo* models in which dendrite growth manipulations have been successfully accomplished. As described in § 4a, *in vitro* application of growth factors like BDNF or NT-4, or genetic overexpression of BDNF, also resulted in more elaborate dendrite growth. The dendritic stimulation resulting from exposure to growth factors may be regulated through downstream signal cascades involving Rho GTPases (Rho, Rac and Cdc42) as their over-expression in immature neurons also was found to cause an increase in dendritic outgrowth (Threadgill *et al.* 1997; Li *et al.* 2000). Interestingly, each of these cited studies, except swainsonine-induced dendritogenesis, was done on young postnatal neurons at a time when GM2 expression would have been normally occurring. Whether more mature neurons would be as sensitive to the stimulatory effects of growth factors or GTPase overexpression (in the presence of reduced GM2) is currently unknown.

5. GANGLIOSIDES HAVE BEEN LINKED TO PROTEINS INVOLVED IN DENDRITIC PLASTICITY

Gangliosides are found in most types of mammalian cell but occur in highest concentrations in brain. Although their functions remain elusive, important advances in delineating possible roles for individual gangliosides have occurred in recent years (Louch *et al.* 2002; Miljan & Bremer 2002; Vyas *et al.* 2001, 2002). The primary focus for understanding ganglioside function has centred on their structure. Gangliosides are sialic-acid-containing glycolipids with variable polar carbohydrate head groups that extend from the plasmalemma while the rest of the molecule is anchored in the membrane by hydrophobic tails (Ledeen & Yu 1982). These molecules are thus poised to interact (selectively) with other membrane constituents or with exogenous molecules. A critical question for the role of GM2 ganglioside in dendritic initiation is whether there are known ganglioside–protein interactions that could provide clues to how such a mechanism may be configured.

Substantial published data support the view that gangliosides are synthesized in the Golgi by a series of transferases and from here they are transported to the plasmalemma in exocytic vesicles (Schwarzmann & Sandhoff 1990; Yu 1994). There is evidence that these vesicles may contain GPI-anchored proteins because inhibitors of GSL synthesis have been reported to alter transport of such proteins (Futerman 1995). As mentioned in § 4b, at least one GPI-anchored protein (CPG15) has been implicated in the control of dendritogenesis (Nedivi *et al.* 1998). Gangliosides, after insertion in the plasmalemma, eventually re-enter the neuron through the endosomal system and when in late endosomes and lysosomes are degraded to simpler components and recycled. There is evidence that GM2 can be transported from late endosomes/lysosomes to the Golgi, possibly by an NPC1-mediated mechanism (Zervas *et al.* 2001) where it would be reused in subsequent synthesis of higher-order gangliosides (Trinchera & Ghidoni 1990; Trinchera *et al.* 1990). Lysosomes also contain β-hexosamindase and GM2 activator protein essential for the complete degradation of GM2 ganglioside (Sandhoff & Klein 1994).

In the past few years evidence has mounted that gangliosides and other GSLs at the cell surface are co-localized with cholesterol in specialized patches of membrane rather than being dispersed randomly (Brown & London 1998; Prinetti *et al.* 1999; Hakomori 2000; Hoessli *et al.* 2000; Kasahara & Sanai 2000). These specialized microdomains have been variously referred to as ‘rafts’, detergent-insoluble glycolipid-enriched complexes, GSL-enriched microdomains and GSL signalling domains (Harder 2003). In addition to gangliosides, a variety of proteins have been found to be associated with rafts. These include GPI-anchored proteins, protein receptors (including receptor tyrosine kinases), and a variety of signal transduction elements including Rho GTPases, c-src and Src family kinases. The concept that has emerged is that rafts represent signalling platforms that link receptor–ligand interactions at the cell surface with signal transduction events inside the cell. There is also evidence that some rafts may be in association with actin, thus providing a connection to the cytoskeleton (Brdickova *et al.* 2001). Studies have shown the presence of rafts within endosomes (Kobayashi & Hirabayashi 2000), and signal transduction events may occur in this compartment (Ceresa & Schmid 2000). A recent, provocative speculation is that GSL storage diseases like NPC actually represent ‘log jams’ of rafts within the endosomal–lysosomal system (Simons & Gruenberg 2000; Lusa *et al.* 2001).

As discussed in § 4, many of the proteins believed present in rafts have been implicated in the control of dendritogenesis, including Trk receptors and Rho GTPases. There is evidence from several studies that gangliosides may modulate the function of such proteins, particularly growth factor receptors. Numerous types of ganglioside have been shown to have graded, or at times opposite, modulatory effects on specific types of growth factor receptor, for example, those for epidermal growth factor and platelet-derived growth factor (Weis & Davis 1990; Bremer 1994; Hynds *et al.* 1995; Yates *et al.* 1995; Sachinidis *et al.* 1996; Zurita *et al.* 2001; Miljan & Bremer 2002). The potential influence on receptor function may occur through multiple mechanisms, including modulation of ligand binding, influence of receptor dimerization as well as with receptor activation and subcellular localization (Miljan & Bremer 2002; Proia 2003). For example, recent studies have implicated the endogenous expression of GM1 ganglioside in rat cerebellar granule cells in the phosphorylation of TrkB receptors treated with BDNF (Pitto *et al.* 1998) and in NG108-15 cells for Trk receptors treated with NGF (Mutoh *et al.* 1995). GM1 stimulation of normal fibroblasts has been reported to cause enhanced release of NT-3, with the result of increased TrkC autophosphorylation (Rabin *et al.* 2002). The ganglioside GT1b, which is a binding partner for myelin-associated glycoprotein, has been found to form a specific association with the neurotrophin receptor, $p75^{NTR}$, possibly as part of a receptor complex to allow MAG signalling in adult murine dorsal root ganglion cells (Yamashita *et al.* 2002). Whereas few studies have focused on GM2 and its possible relation with neurotrophin receptors, one study has implicated this ganglioside in the acti-

vation of ciliary neurotrophic factor receptor in immortalized motor-neuron-like cells (NSC-34) (Usuki *et al.* 2001).

Another protein with possible links to both dendritogenesis and gangliosides is CaMKII. Complex gangliosides residing at the plasmalemma after transport from the Golgi are eventually endocytosed and degraded into simpler gangliosides while transiting through the endosomal–lysosomal pathway. As described earlier, there is evidence that GM2 ganglioside (which in mature neurons would be generated by enzyme degradation of more complex gangliosides) can leave late endosomes by an NPC1-mediated mechanism and transit to the Golgi (see Zervas *et al.* 2001). Dysfunction of NPC1 leads to accumulation of GM2 ganglioside and cholesterol in the endosomal–lysosomal system of neurons. Interestingly, application of an inhibitor (KN93) specific for CaMKII to normal cultured fibroblasts (Sato *et al.* 1998) and to neurons (Gondré-Lewis *et al.* 2002) has resulted in creation of an NPC cellular phenotype, i.e., accumulation of GM2 ganglioside and cholesterol within vesicular compartments. One possible explanation of this change is that activation of an NPC1-mediated trafficking mechanism for ganglioside/cholesterol recycling is linked directly or indirectly to CaMKII function. The possible association between CaMKII and GM2 ganglioside expression deserves attention because this kinase has been suggested to have as one of its functions the stabilization of dendrite growth in several types of neuron (see discussion in § 4b), an action opposite to that proposed for GM2 ganglioside. Furthermore, the prominent α-subunit of CaMKII is known to have a distribution in mammalian brain restricted to certain classes of neuron, including cortical pyramidal neurons and multipolar cells of the amygdala, but absent in GABAergic intrinsic neurons in these areas (Liu & Jones 1996; Tighilet *et al.* 1998; McDonald *et al.* 2002), a distribution that mirrors that of ectopic dendritogenesis in storage disorders. Finally, expression of the α-subunit in pyramidal neurons during development is also known to occur in the early postnatal period essentially coincident with dendritic maturation (Bayer *et al.* 1999). Although the latter finding is consistent with this kinase serving as a dendritic growth stabilizer, it is also consistent with a role in a regulated decrease in GM2 expression that occurs at this time (Walkley *et al.* 2000). Interestingly, there are two independent *in vitro* studies suggesting that some gangliosides (e.g. GT1b) may be able to alter CaMKII activity, and possibly that of other calmodulin-dependent enzymes, by mimicking the effect of Ca^{2+}/calmodulin (Fukunaga *et al.* 1990; Higashi *et al.* 1996). To be relevant *in vivo*, however, the ganglioside and the kinase would have to be in the same cytoplasmic compartment and such associations have, so far, not been reported.

Significant fluctuations in ganglioside expression have long been known to occur during mammalian brain development (Tettamanti 1971) but factors controlling ganglioside expression, and the significance of the changes, remain poorly defined. As gangliosides are produced through a synthetic cascade consisting of several parallel pathways with limited interactions between the key pathways (Yu 1994), production of one type of ganglioside would be dependent on availability of its immediate precursor. Likewise, altering the expression of key synthetic enzymes in the early branch points in the pathway could substantially alter the types of ganglioside later synthesized. Differential regulation of individual synthetic enzymes could conceivably fine-tune expression of different gangliosides in individual neurons. A variety of studies have shown that effectors of protein kinases can induce differentiation in neuronal cells and that this change is accompanied by alterations in ganglioside composition (Yu & Bieberich 2001). For example, it has been shown that the activities of several of the ganglioside synthetic enzymes can be downregulated by the action of PKC, with this effect being reversible in some instances by membrane-bound phosphatases (reviewed in Yu & Bieberich (2001)). PKA has also been suggested to play a role through upregulation of the enzyme responsible for GM2 and GD2 production (GM2/GD2 synthase). Presumably, such phosphorylation/dephosphorylation events would either affect the synthetic enzymes directly or influence their translocation and/or retention in specific subcellular sites. Regardless, such a mechanism could provide an explanation for the differential production of gangliosides in individual types of neuron during brain development.

6. GANGLIOSIDE EXPRESSION IN NORMAL AND STORAGE DISEASE-AFFECTED NEURONS IS SUSCEPTIBLE TO PHARMACOLOGICAL MANIPULATION

If ganglioside accumulation within neurons in storage diseases is detrimental to their normal structural integrity and function, reducing their synthesis could conceivably reduce the storage burden and thereby reduce or delay clinical disease (Abe *et al.* 1992). This could be particularly advantageous in late-onset ganglioside storage diseases in which residual ganglioside catabolic enzyme activity would be predicted to occur (Platt & Butters 1998). It may also be useful in diseases in which ganglioside storage is secondary to altered homeostatic mechanisms and/or to increased synthesis, and in which no primary defects in ganglioside degradative pathways have been shown to occur. Alternatively, if gangliosides like GM2 are an important part of a dendritic growth and differentiation mechanism in normal, developing neurons, drugs that alter GM2 expression pre- or postnatally would be predicted to have the capacity to alter dendritogenesis.

Several experimental studies now support the concept that reducing ganglioside synthesis in lysosomal diseases with ganglioside storage will ameliorate clinical disease progression. The class of compounds most often used in these studies has been the iminosugars NB-DNJ (or OGT 918) and *N*-butyldeoxygalactonorjirimycin (OGT 923), which exhibit partial inhibition of glucosylceramide synthase, a pivotal early enzyme in the GSL synthetic pathway (Platt & Butters 1998; Butters *et al.* 2003). Studies have shown that these agents reduce ganglioside storage in both the Tay–Sachs and Sandhoff models of GM2 gangliosidosis in mice (Platt *et al.* 2001, 2003), as well as in feline and murine models of NPC disease. For the latter study, oral administration of the drug reduced CNS levels of GM2 and GM3 gangliosides, delayed the onset of neurological dysfunction and prolonged the life of the animals by *ca.* 30% (Zervas *et al.* 2001). More recently, simi-

lar studies have been done on mice with MPS III A disease, in which ganglioside storage is a downstream consequence of GAG storage. Here, not only were GM2 and GM3 levels reduced in immunostained sections of brain, but cholesterol and autofluorescent materials were also reduced (S. U. Walkley, unpublished data). Taken as a whole, these data suggest that inhibitors of ganglioside synthesis have the ability to favourably impact ganglioside accumulation in GSDs, regardless of the reason for the initial storage, and that in some cases disease outcomes are improved as a result.

The iminosugar compounds are also useful for analysis of the role of ganglioside expression in normal, developing brain. To further evaluate the role of GM2 ganglioside in normal dendritogenesis, primary dissociated cultures of ferret cortical neurons have been treated with NB-DNJ to partly inhibit ganglioside synthesis. Early treatment of cultures with NB-DNJ (before significant dendrite outgrowth) led to a dramatic reduction in both GM2-immunoreactivity and in the number of basilar dendrites produced by the neurons. Adding GM2 to cultures in which the inhibitor was applied prevented the effect on dendrite initiation. These findings are consistent with the hypothesis that GSL synthesis and GM2 ganglioside are part of a mechanism responsible for the control of dendritogenesis of neocortical pyramidal neurons (Zervas *et al.* 2003).

7. WHERE TO GO FROM HERE?

The re-initiation of dendritic sprouting as occurs on cortical pyramidal neurons in neuronal storage diseases remains an unprecedented finding even a quarter of a century after its discovery. Although we have learned a great deal about the features of this remarkable and unusual phenomenon, some key issues remain unresolved, including knowing precisely how it is triggered and how glycolipids are involved. Understanding why ectopic dendrites sprout, why the phenomenon is limited to cortical pyramidal cells and a few other select types of neurons, and why it occurs in humans and other higher mammalian species but not rodents probably holds key insights into many aspects of dendritic plasticity in normal brain. Studies reviewed herein indicate that it is possible to pharmacologically manipulate (i.e. reduce) ganglioside expression in neurons affected by storage diseases. Determining whether this treatment could block the formation of ectopic dendritic sprouting, however, has been limited principally by the species available for these studies. Studies reviewed here in which ganglioside levels were increased secondary to induction of a storage disease reveal that neurons are responsive to new dendritogenesis even very late in postnatal development, if not longer, and that the new dendrites can become integral parts of the basilar dendritic tree. Whether pharmacological induction of dendritogenesis could be used for repair of neurons damaged in other types of disorder such as stroke remains to be determined. However, the discovery of ectopic dendritogenesis reminds us that many types of neuron possess far more structural plasticity than once believed. The challenge that remains is to understand, and ultimately to harness, this remarkable plasticity.

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GLOSSARY

AMPA: α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
BDNF: brain-derived growth factor
BMP: bone morphogenic protein
CaMKII: calcium calmodulin-dependent protein kinase II
CNS: central nervous system
GABA: γ-aminobutyric acid
GAG: glycosaminoglycan
GM1: II^3NeuAc-GgOse$_4$Cer
GM2: II^3NeuAc-GgOse$_3$Cer
GPI: glycosylphosphatidylinositol
GSD: glycolipid storage disease
GSL: glycosphingolipid
GTP: guanosine 5′-triphosphate
MPS: mucopolysaccharidose
NB-DNJ: *N*-butyldeoxynojirimycin
NCL: neuronal ceroid lipofuscinosis
NGF: nerve growth factor
NMDA: *N*-methyl-D-aspartate
NPC: Niemann–Pick type C
NT-3: neurotrophin 3
NT-4: neurotrophin 4
PKA: protein kinase A
PKC: protein kinase C
PNS: peripheral nervous system