Meganeurites and The Man

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According to the entry in the lab notebook the date was April 11, 1975. Dom Purpura was sitting at his microscope examining the latest set of Golgi sections prepared by his able technician, Marie Buschke. The tissue under study that day was from a small cortical biopsy taken from a 14 month-old child who exhibited developmental delay and other features of a progressive neurological disease of unknown etiology. The study was part of a systematic assessment of cases of undiagnosed and progressive forms of psychomotor regression being carried out at the Rose F. Kennedy Center for purposes of diagnosis and family counseling. The presence of chromosomal abnormalities, known inborn errors of metabolism, and other conditions had been ruled out in these children by previous diagnostic tests. In each case the collected tissue samples were subject to careful scrutiny by a team of investigators using a variety of biochemical and morphological techniques. Dom's portion of the biopsy was subject to staining with the Golgi method—a favorite of his as it revealed neurons in their entirety, including detailed views of neuronal dendrites and dendritic spines. What Dom likely expected to see as he examined the Golgi-impregnated neurons in these sections was further evidence of the phenomenon of dendritic spine "dysgenesis" – the loss of integrity of dendrites and dendritic spines which he had discovered and documented a year earlier in other cases of profound mental retardation of unknown etiology (Purpura, 1976).

The particular neuron that captivated his attention on this day, however, did not display a loss of dendritic spines like he had seen in the previous cases. Rather, this neuron was odd, indeed strikingly odd, because it possessed what looked like a "large banana" (as dutifully recorded in the lab notebook) inserted between the cell body and the point of departure of the axon. No less than 22 photographs were taken of this bizarre-looking cell, capturing not only its large banana but also all aspects of the cell's perikaryon, its apical and basilar dendrites, and its axon and axonal branches. Then, looking around in this section, and in others taken from the same case, Dom found many additional Golgi-impregnated pyramidal neurons that possessed similar bananas and, occasionally, little dendritic spine-like protrusions budding at their banana surface. That is, in spite of the fact that these enlargements were placed near the axon hillock and initial segment of the axon, they seemed to be comprised of dendritic-like membrane. Dom was on the verge of making a serendipitous and astonishing discovery.

The serendipity at work here was that for the first time, the century-old rapid Golgi technique (used by S. Ramon y Cajal to establish the Neuron Doctrine) had been successfully applied to a family of disorders that also had been known for a century—Tay-Sachs disease or GM2 gangliosidosis. The reason the diagnostic tests on this child had not detected the presence of this disorder was because the child was normal for the catabolic lysosomal enzyme (β-hexosamidase) that is typically defective in this disease. Instead the child was missing a small previously unknown lysosomal protein now known as the GM2-activator, which is critical for the function of β-hexosamindase. The biochemical and ultrastructural studies that accompanied Dom's study readily showed the presence of GM2 ganglioside and its storage in neurons and eventually provided clear evidence that this disorder was indeed the AB variant form of GM2 gangliosidosis (deBaecque et al., 1975).

The next entry in Dom's lab notebook was made 25 days later on May 7th and is a description of Golgi-impregnated neurons derived from archival formalin-fixed brain tissue from a child dying with typical Tay-Sachs disease. Since the turnaround time for impregnating and embedding tissues for Golgi staining is at a minimum three weeks, it seems clear that the word went out immediately upon the sighting of the first banana-possessing neurons that more Tay-Sachs tissue should be found and processed immediately. In the weeks and months that followed, even more archival cases of storage diseases were found, stained by the Golgi method, and analyzed. As with the initial biopsy, banana-possessing pyramidal neurons were everywhere and in the autopsy cases the bananas were often huge, exceeding the adjacent cell body in volume. Remarkably, some neurons exhibited not only dendritic spine-like protrusions on their bananas but also entirely new dendrites, themselves complete with dendritic spines. Furthermore, electron microscopy revealed that the ectopic dendritic spines possessed normal-appearing synaptic contacts, arguing that these aberrant connections were functional.

What Dom clearly recognized here was that in spite of the fact that these were essentially morphologically mature neurons, they were nonetheless producing new dendritic-like membrane complete with dendritic spines and synapses, similar to immature neurons. This renewal of dendritogenesis had never before been reported in normal or diseased neurons from postnatal brain. What these observations indicated was that there was

something very special occurring in ganglioside storage diseases that had the ability to trigger a recapitulation of dendrite growth well after the normal period of dendritogenesis in the third trimester. Dom knew this well, for he had spent years as both physiologist and Golgi-morphologist coming to understand the importance of normal dendrite maturation in the development of the human cerebral cortex and in cognition. Indeed, it is likely that no one in the world at that time would have been better prepared to immediately understand the importance of "ectopic" dendrite growth in a disease state like Tay-Sachs. If there was ever a case of serendipity meeting the prepared mind, this was it.

Publication of these important findings came later in 1976, followed by a second paper in 1977. Here, the bananas officially became known as meganeurites (Purpura and Suzuki, 1976), the accompanying new dendritic spines and neurites as ectopic dendrites, and the phenomenon of abnormal re-growth of dendrites on mature neurons as ectopic dendritogenesis (Purpura, 1977). By 1978 the AB variant-affected child subject to the original biopsy also died and autopsy studies revealed that the slender bananas of the original study had enlarged considerably and further ectopic dendrites had emerged (Figure 2) (Purpura, 1978). As Dom concluded in the latter Nature paper, "The unprecedented findings here of aberrant dendritic growth induction in otherwise mature human cortical neurons suggests that the pursuit of factors associated with abnormal ganglioside metabolism will shed light on the developmental mechanisms of neurone shape regulation and dendritic membrane distribution."

Realizing the difficulties of getting good quality Golgi stains on old formalin-fixed human tissue as well as the need for an exploitable model system for study, in 1976 Dom initiated a collaboration with Henry Baker who had discovered an inherited ganglioside storage disease (GM1 gangliosidosis) in Siamese cats and had established a research colony of these animals at the University of Alabama in Birmingham. On August 10, 1976, the first lab notebook entries documenting photographs taken of Golgi-impregnated pyramidal neurons from this model were made. Importantly, pyramidal neurons in the neocortex of cats with GM1 gangliosidosis generated ectopic dendrite growth in a manner essentially identical to that in human Tay-Sachs disease (Purpura and Baker, 1977) and therefore provided the first experimental model in which to explore the causes and physiological consequences of this remarkable phenomenon.

While it was later shown that meganeurites could form in a variety of conditions in which materials accumulated within pyramidal neurons (e.g., lipofuscin storage with old age), the presence of dendritic-spine covered meganeurites and ectopic dendrites has remained a finding unique to lysosomal diseases. And while ectopic dendritogenesis was later discovered in cases of lysosomal storage diseases like α-mannosidosis (Purpura and Walkley, 1981) that should not include ganglioside storage, even here subsequent studies revealed the involvement of metabolic errors in ganglioside metabolism and the accumulation of GM2 ganglioside specifically in ectopic dendrite-bearing pyramidal neurons (Goodman et al., 1991).

In the ensuing quarter century, Dom's prescient discovery involving dendritic spine dysgenesis linked to mental retardation has been rediscovered in the new light of modern molecular neurobiology (Ramakers, 2002). While understanding the biological function of gangliosides has proved far more enigmatic than imagined in those early days, research work on the neurobiology of lysosomal diseases has itself expanded dramatically and today includes greater appreciation that pathogenic events like ectopic dendritogenesis hold the key to fully understanding and treating these disorders (Platt and Walkley, 2004).

As for the chronicler of this historical vignette, my contact with Dom began in the summer of 1976 when the Purpura-Baker collaboration was initiated. I had recently completed my studies in veterinary medicine and had enrolled in the doctoral program at the University of Alabama in Birmingham to pursue interests in genetic brain diseases in the Department of Comparative Medicine, chaired at the time by Henry Baker. A year's interlude developed when I was accepted into a graduate study program in neurophysiology at the University of Edinburgh. Upon returning to Birmingham in 1978, the stay again was brief as I soon packed my bags, this time to spend a year in New York working with Dom and his colleagues in the Kennedy Center on this intriguing discovery of meganeurites and ectopic dendritogenesis. Somehow one year turned to two, two to four and on and on, now 25 years hence. As Dom moved from Chairman to Dean I inherited not only his technician and his lab space—but also his Golgi slide collection and (as apparent from the above) his laboratory notebooks. But Dom also passed along to me something far more valuable—the intriguing observation of mature pyramidal neurons in lysosomal diseases sprouting new dendrites. This marvelous and unexplained phenomenon became *my* window to the world of neuroscience and brain disease—a world in which I have been gleefully immersed ever since. I cannot imagine any better gift from mentor to student, and it is one for which I am eternally grateful.

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