Neuronal effects of experimentally induced hydrocephalus in newborn rats

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To determine the effects of increased cerebrospinal fluid (CSF) pressure on neuronal morphology, obstructive hydrocephalus was induced by injecting kaolin into the fourth ventricle and cisterna magna of 1-day-old rats. The animals were sacrificed 10 to 12 days later, at which time severe ventriculomegaly and cortical thinning were apparent in the parieto-occipital region. Tissue from this area was processed by rapid Golgi methods. Well impregnated pyramidal neurons were examined by light microscopy, and their somatic and dendritic features compared to those of age-matched littermate controls. The somata of medium pyramidal neurons were unaffected, but their basilar dendrites had fewer branches and those that remained were shorter. A variable reduction in dendritic spines occurred, such that some branches were totally denuded while others exhibited spine densities similar to those seen in control animals. The most striking alteration was the occurrence of frequent dendritic varicosities. These enlargements of the dendritic shaft separated by extremely thin constrictions gave the affected segment a beaded appearance. Both dendritic spine loss and varicosity formation were most notable on distal portions of individual branches and within regions of the dendritic tree closest to the ventricular and meningeal surfaces. These alterations are consistent with other reports of dendritic changes associated with aging, mental retardation, and alcohol exposure. These observations suggest that hydrocephalus causes dendritic deterioration or retardation of dendritic maturation. The fact that neuronal morphology was not more severely affected may indicate that these effects are reversible.

Key Words • experimental hydrocephalus • dendrite • neuron • pyramidal neuron • cerebral cortex • rat

Infantile hydrocephalus is a common pathological entity with an incidence of three to four cases per 1000 live births. With the advent of ventricular shunting procedures, untreated cases demonstrating advanced signs and symptoms have become rare. Despite early intervention, however, the ultimate neurological outcome for any particular patient is unpredictable. While some patients develop normally, others manifest varying degrees of disability, generally related to spasticity and impaired cognitive functioning. Although impaired cognition is generally considered a result of damage to the cerebral cortex, attempts to correlate cortical mantle thickness with intellectual capacity have been inconclusive. This suggests that damage is occurring at the cellular level and therefore cannot be evaluated macroscopically.

The literature is replete with micropathological studies of hydrocephalus. Human cortical biopsies and necropsy material have been examined. Experimental models have also been designed, generally involving the use of inert substances such as kaolin or Silastic to mechanically occlude the cerebrospinal fluid (CSF) pathways in laboratory animals. The majority of studies published to date have focused on the ependyma and subependymal tissues. Using light and electron microscopy, investigators have reported flattening and loss of cilia from ependymal cells, disruption of ependymal cell junctions, and edema and astrocytosis of periventricular white matter. Cortical gray matter has been examined less thoroughly. Some authors studying the general pathological features of hydrocephalus have noted sparse axonal degeneration without changes in other neuronal structures, and have concluded that the gray matter is relatively unaffected by increased intracranial pressure. In a few reports, brief mention is made of potentially significant changes in neurons. Weller and
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Williams described cortical biopsies from two hydrocephalic infants showing focal neuronal loss. In an electron microscopic study of experimental hydrocephalus in young rabbits, Gopinath, et al., presented a photomicrograph of a neuron with disruption of the cell membrane and cytoplasm. However, neither group discussed these findings further.

Since dendrites comprise a major portion of a neuron's receptive surface, they are critical determinants of neuronal function. A complete assessment of neurological impairment following induction of hydrocephalus thus requires examination of the cerebral cortex to search for changes in these processes. Golgi methods were chosen for histological analysis because they permit the visualization of a neuron in its entirety. The methods employed in previous studies did not allow this visualization. The ultimate objective of this investigation is to advance our understanding of the cellular basis for clinical manifestations of infantile hydrocephalus.

Materials and Methods

Sprague-Dawley rats were taken from litters of 10 to 12 pups 6 to 24 hours after birth and anesthetized with ice. An incision was made and cervical muscles were reflected to expose the atlanto-occipital membrane. A sterile suspension of 25% kaolin was injected slowly through this membrane into the fourth ventricle and cisterna magna using a No. 27 needle mounted on a glass syringe. The injection procedure was monitored visually and stopped when the kaolin suspension was seen to be filling the fourth ventricle and spreading into the subarachnoid space on the posterior surface of the cerebellum. The wound was packed with antibiotic-soaked Gelfoam and sutured. Animals were allowed to revive slowly and were returned to the litter after normal movements returned. Control animals from half of the same litter were anesthetized with ice for the same period and surgical opening was performed, but no injections were made through the atlanto-occipital membrane.

Ten to 12 days postinjection, the animals were anesthetized with sodium pentobarbital (40 mg/kg) and sacrificed by cardiac perfusion of a mixture of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4). The brains were removed, stored in the same fixative for 1 to 5 days, and cut coronally into blocks 2 to 3 mm thick. Since the occipital region showed the greatest amount of this area were processed by rapid Golgi methods. This involved immersion of the tissue in a solution of 2.0% potassium dichromate and 0.2% osmium tetroxide, then in 0.75% silver nitrate. Impregnated blocks were then dehydrated, embedded in soft plastic, sectioned at 100 μm thickness, and mounted serially on glass slides.

Light microscopic analysis was limited to pyramidal neurons, as identified by a wedge-shaped soma and prominent apical dendrite. We selected only cells with somata located in the middle of the thick sections of which the dendrites were impregnated completely. These were segregated further into two groups based on their superficial or deep location within the cerebral cortex (Fig. 1). Although the Golgi methods do not reveal cortical laminae, the neurons examined seemed to be located in layers II to V, based on measurements taken perpendicular to the cortical surface. Representative neurons were drawn with a camera lucida attachment at × 500 or × 1250 magnification. Preliminary attempts were made to quantify some of the neuronal features observed qualitatively. From the drawings, dendritic branches were counted and their lengths determined with a Numonics digitizer and computer. By our convention, each dendritic segment between branch points was considered a branch, in addition to the terminal branches. Branches were also segregated by branch order. According to this scheme, the most proximal (primary) portion of a dendrite gives rise to second-order branches, which in turn give rise to third-order branches, and so forth. Dendritic spines were counted at × 1250 magnification and spine densities were calculated from the length measurements.

Results

Gross Morphology

In all experimental animals, kaolin was found in the subarachnoid space, on the ventral surface of the brain stem (pons), over the posterior surface of the cerebellum, and in the cisterna magna. Midsagittal
dissection revealed kaolin deposits on the floor and roof of the fourth ventricle, and in the cerebral aqueduct. Rodents have a prominent recess of the inferior colliculus which arises from the cerebral aqueduct, and that recess also contained kaolin. No deposits were found in the third or lateral ventricles.

The most conspicuous gross abnormalities were severe ventriculomegaly and thinning of the overlying brain tissue (Fig. 1). All portions of the lateral ventricles were dilated. This expansion reached its extreme in the parieto-occipital region where the cortex was so thin that it was translucent. The ventriculomegaly could be predicted from the dome-shaped skull caps of the experimental animals. Although the cerebral aqueduct was usually closed, the recess of the inferior colliculus was greatly enlarged and the overlying tectum thinned or obliterated. Likewise, anterior portions of the cerebellum were often destroyed.

FIG. 2. Camera lucida drawings of representative medium pyramidal neurons located deep within the parieto-occipital cortex (2 and 4 on Fig. 1). Arrowheads indicate dendrites illustrated in Fig. 4B and C. A: Neuron from an experimental animal exhibiting reduced numbers and lengths of basilar dendrites (b), as well as formation of varicosities (v). Varicosities are most pronounced on distal portions of basilar and apical terminal branches, where dendritic spines are also reduced. B: Control neuron demonstrating a pear-shaped soma, thick apical dendrite (a) with oblique side branches (sb) and few terminal branches (tb), and a rich plexus of basilar dendrites (b). Dendritic spines are uniformly distributed on each dendritic branch but absent from the soma and proximal parts of apical and basilar dendrites. Scale bars = 25 μm. Note scale difference for A and B.

FIG. 3. Camera lucida drawings of representative medium pyramidal neurons located superficially within the parieto-occipital cortex (1 and 3 on Fig. 1). A: Neuron from an experimental animal showing terminal branches (tb) denuded of dendritic spines but with many large varicosities (v). These features are present to a lesser degree on basilar dendrites (b), which also have fewer branches that are much shorter than in control specimens. The apical dendrite of this neuron is obliquely oriented in relation to the cortical surface (see Fig. 1A). Arrowheads indicate neuron illustrated in Fig. 4A. B: Control neuron with long richly branched basilar dendrites (b). Intermediate portions of the apical shaft exhibit fewer spines than the experimental neuron (asterisks). Scale bar = 25 μm.

General Dendritic Morphology

Tissue from the parieto-occipital cortex was examined, since this area demonstrated the most profound ventriculomegaly and gross cortical thinning. The most posterior parts of the occipital cortex were not included, because this tissue had thinned to less than 0.5 mm, making histological processing quite difficult. The regions selected for analysis corresponded roughly toCraigie’s neuroanatomical areas 17, 18, 39, and 41.24 Although all types of cortical neurons exhibited to a certain degree the changes described below, medium pyramidal cells were most consistently impregnated by the Golgi methods and, thus, were most available for analysis. Hereafter, neurons taken from experimental and control animals will often be referred to as experimental or control neurons, respectively.

Medium pyramidal neurons were defined as those cells with pyriform or pear-shaped somata with diameters of 15 to 30 μm.23,24,37 Each soma gave rise to a single apical dendrite and a series of basilar dendrites (Fig. 2B). The apical dendrites of control neurons generally coursed perpendicular to the cortical surface before dividing into terminal branches. Superficial neurons (Fig. 3B) had short apical dendrites that terminated
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Fig. 4. Photomicrographs of Golgi-impregnated medium pyramidal neurons. A: Experimental neuron seen in Fig. 3A showing spine reduction and varicosity formation on the basilar segment between the arrowheads. B: Segment of a basilar dendrite from the control neuron in Fig. 2B (arrowheads). Note the abundance of spines (arrows) and the lack of varicosities, although the contour of the dendrite is irregular. C: Segment of the basilar dendrite from the experimental neuron drawn in Fig. 2A (arrowheads). Compared to B, it has very few spines but large varicosities (arrows) separated by thin constrictions. Scale bars = 10 μm.

Medium pyramidal neurons from experimental animals demonstrated somatic features similar to the control cells (Figs. 2A and 3A). However, major differences were noted in the dendritic features of experimental neurons. Although the branching patterns of apical dendrites were similar to controls, the orientation of the apical shaft and terminal branches relative to the cortical surface was occasionally altered. Figure 3A demonstrates a bending apical dendrite which terminates parallel to the surface rather than perpendicular to it (Fig. 1A). The same neuron also exhibited an intertwining of terminal branches which was not seen in control neurons. Apical shafts of deeper neurons were markedly thinner in the experimental group (Fig. 5A and B).

Basilar dendrites from experimental cells also appeared markedly different from those of control cells. Although neurons from both groups gave rise to approximately four basilar dendrites, the branching patterns of these were less complex for experimental neurons (Figs. 2, 3, and 4A and C). A single control basilar dendrite contained three to 17 branches, whereas the range for experimental dendrites was one to five. The branch order, likewise, was reduced; control neurons exhibited a maximum of five branch orders, but experimental neurons never exceeded three branch orders. Measurements of the neurons illustrated indicate that basilar dendrites of experimental cells were as much as 40% shorter than those of control cells. This reduction in basilar dendrite length was most pronounced for neurons located deeper in the cortex.
Dendritic Spines

Specialized appendages, or spines, are characteristically found on the dendritic surface of medium pyramidal neurons. Three types of spines were identified on dendrites of both control and experimental neurons (Fig. 5). Thin spines, usually with bulbous expansions at their tips, were most prevalent. Stubby spines were thicker, lacked bulbous heads, and were usually quite short. Spines with thick necks and bulbous heads were termed “mushroom-shaped” and occurred infrequently.

 Compared to controls, experimental neurons exhibited some dendritic segments that were devoid of spines. Spine density was reduced considerably on other dendrites. These differences were most striking on distal portions of individual segments and terminal branches (Figs. 2A, 3A, 4, and 5). However, spine decreases were not observed on all dendritic branches of experimental cells. In fact, portions of experimental dendrites demonstrated increased numbers of spines (compare intermediate segments of apical shafts in Figs. 2 and 3). As a consequence, overall spine density was not appreciably different between experimental and control cells, but the range for individual branches was much larger in the experimental group.

Varicosities

A varicosity was defined as a local region of a dendritic segment that was either symmetrically enlarged to at least twice the normal diameter of the dendritic shaft on either side, or of normal diameter but situated between dendritic regions that were constricted to at least half the normal diameter. Thus, varicosities give the dendritic shaft a beaded appearance (Figs. 4 and 5). True varicosities were seldom present in control pyramidal neurons. Generally, each dendritic shaft was straight and of a uniform diameter along its entire length. Asymmetrical dilatations were thought to represent stubby spines or the normal contour of the dendritic shaft.

Numerous varicosities were noted on experimental neurons. In general, the greatest numbers were found on basilar dendrites (Fig. 4). Distal side branches of the apical dendrites were located close to the soma, and distal segments of terminal apical branches were identified near the cortical surface (Fig. 5). The main apical dendrites were relatively smooth, although irregular swellings were occasionally found, especially at branch points. The distal portions of these apical shafts were considerably thinner than in control specimens (Figs. 2 and 3). A definite pattern was noted, whereby the number of varicosities increased in more distal parts of the dendritic field; that is, closer to the ventricular and meningeal surfaces in reference to the soma. This was especially true for deeper neurons. Great variability existed, however, such that it was common to find a normal-appearing dendritic branch in a field of affected branches.

Likewise, there was much variation in the size and shape of varicosities observed on individual branches of experimental dendrites. More distally along branches a series of tight constrictions separating swellings were frequently encountered (Figs. 4 and 5). The swellings on a particular branch were usually similar in size and shape, often ovoid or spherical. Although spines were observed on both constrictions and swellings, the vast majority arose from swellings (Fig. 5B). These spines were usually quite long and thin. Many varicosities contained no spines at all.

Discussion

This study provides preliminary observations on the effects of acute experimental hydrocephalus on the morphology of neurons in the cortical gray matter of neonatal animals. Analyses of somatic and dendritic features of Golgi-stained medium pyramidal neurons from the parieto-occipital cortex have revealed differences between a group of hydrocephalic rats and their littermate controls. These differences can be summarized as follows: 1) a decrease in the number and length of dendritic branches, especially on basilar segments of pyramidal cells located deep in the cortex; 2) extreme variability in dendritic spine density, including total denudation of some segments; and 3) the appearance of frequent dendritic varicosities, especially at branch points and distal segments near the ventricular and meningeal surfaces.

It is unlikely that these alterations are caused by histological artifact or factors other than increased intraventricular pressure. Hydrocephalus was induced by injecting a suspension of kaolin into the fourth ventricle and cisterna magna. According to DeFeo, et al., kaolin causes an inflammatory reaction in the ependymal lining. Ependymal swelling leads to obstruction of the ventricular system, usually at the cerebral aqueduct, thereby increasing CSF pressure in the lateral ventricles. In our material the subarachnoid space over the parieto-occipital cortex, as well as the lateral ventricles, remained consistently free of kaolin crystals. Thus, the initial influence on the cortex is that of increased CSF pressure. The changes observed could be caused directly by increased pressure, or they could be mediated secondarily by vascular changes, axonal damage, or deafferentation. Likewise, histological artifact does not influence the results. Although Williams, et al., have found that dendritic alterations can occur if tissue is not fixed within 6 hours of death, all material in this study was taken from experimental and control animals that were perfused intracardially with fixative at the time of sacrifice.

It is now recognized that the majority of intrinsic and extrinsic cortical afferents terminate in dendritic spines of pyramidal neurons. Changes in dendritic branching patterns, caliber of the dendritic shaft, spine...
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![Diagram](image)

**Fig. 5.** High-magnification camera lucida drawings of apical shafts (A and B) and terminal branches (C and D) from the neurons illustrated in Figs. 2 and 3. A: This segment of a deep control neuron exhibits thin (th), stubby (st), and mushroom-shaped (ms) dendritic spines. Its apical shaft is much thicker and more uniform in diameter than its experimental counterpart shown in B. B: In the experimental neuron, spines appear somewhat longer (arrowheads) and varicosities (v) are present on the dendrites. C: This region of the apical dendrite of a superficial control cell has fewer spines on the apical shaft than its experimental counterpart shown in D. D: A terminal branch from this experimental neuron is completely denuded of spines (asterisks) and exhibits numerous large varicosities (v). Scale bar = 10 μm.

density, and spine morphology, therefore, have important functional significance. Indeed, the results of this study in many ways resemble those observed by other investigators who have analyzed cortical neurons in mentally retarded patients.

In an analysis of the neocortex of profoundly retarded children, Huttenlocher reported markedly simplified dendritic arborizations of pyramidal cells. He described stunting and loss of secondary branches from both apical and basilar dendrites. In the present study, only basilar dendrites appeared grossly stunted; however, examples were found of disoriented apical main stems and terminal branches near the meningeal surface. In all our cases, the middle cortical layers were least affected by these changes. These morphological results are similar to those in retardation, but our findings suggest a different causative mechanism. While idiopathic mental retardation is a diffuse process generally affecting all cortical layers, the pressure gradient of acute hydrocephalus may exert its maximal effects initially on periventricular and submeningeal cortical surfaces. Centrally located neurons might be protected by the diminution of centrifugal and centripetal pressure forces in the cerebral cortex.

Investigations relating changes in dendritic spine morphology and density to mental retardation have also been reported. Marin-Padilla was the first to use Golgi methods to demonstrate alterations in spines of infants with trisomic chromosomal defects. He found that dendritic spines become very long, thin, and tortuous or short, thin, and barely visible; variable spine loss was also noted. Studies of several infants with idiopathic neurobehavioral retardation and normal karyotypes revealed dendritic alterations similar to those described by Marin-Padilla. Furthermore, proximal dendritic segments were depleted of the more mature stubby and mushroom-shaped spines which normally covered their surfaces. Most recently, these same findings were reported in infantile rats that were exposed to ethanol in utero. These animals simulated the human fetal alcohol syndrome, which is accompanied by varying degrees of mental retardation. Our data indicate dendritic spine loss, although this effect was variable among individual branches of the same dendritic field. Some segments exhibited increased spine densities. The reduction of dendritic spines on the distal parts of basilar and apical branches supports the general concept of "spine dysgenesis" advanced by Purpura. The possibilities for spine remodeling (that is, changes in the proportions of spine types) await quantitative analysis of our material.

Dendritic varicosity formation is a phenomenon that has been studied in normal and pathological tissue. Immature neurons often demonstrate varicosities or beading. It appears that these may be important in both the growth of dendrites and the retraction of excessive branches. Immature dendrites possess filopodia or hair-like sprouts which usually arise from varicosities. These are much longer than dendritic spines and exhibit terminal growth cones, which suggest that they are newly forming branches. In contrast, vacuole formation and cavitation of varicosities may provide a mechanism for fragmenting certain dendritic branches, which are eventually eliminated from the developing neuron. Our analysis does not permit a determination of whether the pyramidal cells from the experimental animals represented degenerating neurons or neurons with retarded maturation. It is worth noting that our attempts to select only the best impregnated cells for analysis may have biased our sample toward the least affected neurons. Nevertheless, neurons in advanced stages of degeneration, such as those seen in the cortex of aged rats and severely demented patients, were not observed in our material. This raises the possibility that the initial effects of increased pressure may be reversible, at least at the cellular level, thereby providing a basis for reconstitution of the cerebral cortex that occurs following shunting procedures. Dendritic growth has been demonstrated in neurologically normal 60- to 90-year-old humans, as well as in animals subjected to environ-
ments "enriched" with other animals, larger cages, frequent handling, and various novel "toys." These results lend further support to the potential for repair.

The pattern of varicosity formation we observed is most like that described by Purpura in his analysis of cortical biopsies from young children with severe neurobehavioral retardation. Large irregular swellings at branching points and small regular beads and constrictions on distal branches were commonly observed in both studies. Purpura referred to data that indicated that varicose dendrites have prolonged conduction times. He speculated that these perturbations in central conduction systems could contribute to the neuronal dysfunction underlying neurobehavioral retardation, a theory that seems appropriate for hydrocephalic patients as well.

These preliminary results are now being expanded to elucidate further the consequences of hydrocephalus. In particular, quantitative analyses of dendritic length, spine density, and varicosity formation are underway to substantiate our qualitative observations. The temporal sequence of dendritic effects is being evaluated and is a critical prerequisite to determining if the changes are reversible following placement of a ventricular shunt. The morphology of neurons in other areas that appear less affected grossly, such as the frontoparietal cortex and neostriatum, is currently being examined as well. Electrophysiological and neurochemical analyses are required to determine the functional implications of these structural changes. Finally, human tissue must be analyzed so that we can correlate our findings with any changes that occur in infants. The information obtained from these studies suggests that cortical biopsies may become useful for evaluating the degree of damage produced by hydrocephalus and for directing physicians, surgeons, and rehabilitation specialists in optimizing treatment and outcome for patients.

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