In general, Aoyama et al. present an interesting study in dogs that has the potential to add important information about dendritic, axonal, and glial changes in juvenile hydrocephalus that is managed with and without shunt insertion. Based on immunostaining for the neuronal cytoskeleton in dendrites and axons, the astrocytic cytoskeleton, and synapses (using neurofilament, glial fibrillary acidic protein [GFAP], and synaptophysin, respectively), the authors suggest that cytoskeletal and axonal damage cannot be completely reversed within 4 weeks of shunt insertion and that reactive astrocytosis can persist in the periventricular white matter.

Unfortunately, almost all of the results are based on qualitative, subjective light microscopic observations, except for angle measurements of apical dendrites in the cerebral cortex. Nevertheless, Aoyama et al., frequently refer to changes in the “numbers” and “density” of cells and profiles, as in the following examples: “In the periventricular white matter, swollen and fragmented axons increased in numbers along with hydrocephalic progression,” “the numbers of GFAP-positive astrocytes were decreased compared with the preshunt group,” and “the most significant increase in the density of reactive astrocytes [was] seen after shunt insertion.” The subjectivity of these generalizations is compounded by the low magnification of the photomicrographs, which does not permit the reader to confirm the descriptive changes reported. Many well-intentioned authors of studies on the pathophysiology of hydrocephalus have struggled with the variability inherent in this disorder. For example, Jones and colleagues used stereological analyses to show that in H-Tx rats with advanced hydrocephalus, neuron and glial cell density in the cerebral cortex is significantly reduced by up to 30% but overall cell number throughout the cortical mantle is not affected. Most likely, these findings are due to the global distortion (compression and stretch) experienced by the entire cerebral hemisphere. Likewise, Del Bigio and Zhang painstakingly demonstrated that the mean number of terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end–labeled (presumably apoptotic) cells increases in a statistically significant manner (p < 0.03) in juvenile rats experiencing kaolin-induced hydrocephalus at 3 weeks postinduction but not at 1, 2, or 4 weeks. As with all studies of cellular damage associated with hydrocephalus, great care must be taken to collect data using minimal subjectivity so that reliable conclusions can be drawn.

Furthermore, the quantitative data presented on apical dendrite angles are not complete. Although the Kruskal–Wallis test did reveal a statistical difference (see Fig. 5 in their article) among multiple groups, most likely this result is attributable to differences between any of the experimental groups as compared with intact control animals. No appropriate post hoc tests were used to determine if statistically significant changes occurred between the pre-, post-, and nonshunt implantation groups, and the authors are correct in making these similarities known.

It is also important to recognize that this study focuses on a specific cortical region and includes a relatively short recovery period after shunt insertion. The tissue examined was obtained from the frontoparietal region at a coronal level through the optic chiasm, and apical dendrites were selected from neurons in layer V of the cerebral cortex. Thus, the findings reported may not be representative of more severely affected regions (for example, occipital cortex or less mature neurons in the superficial laminae), and a more protracted recovery period may be needed for full reversal of the pathology.

Perhaps one of the more intriguing points raised in the Discussion section of their article involves the role of reactive astrocytes in providing tolerance of and neuroprotection to neurons subjected to sublethal ischemia, especially because cerebral blood flow is known to be reduced in hydrocephalus. Preischemic/hypoxic conditioning has been widely reported in models of stroke and reperfusion, and recently Ding and associates demonstrated that this mechanism also occurs in adult-onset hydrocephalus. It is hoped that these types of endogenous protective mechanisms can be incorporated into traditional cerebrospinal fluid diversion procedures so that more effective treatments for hydrocephalus can be developed.

References
1. Del Bigio MR, Zhang YW: Cell death, axonal damage, and cell reorganization in hydrocephalus.
RESPONSE: We greatly appreciate the valuable comments of Dr. McAllister regarding our paper, and we would like to respond to his comments.

First, the major concern of this study was how to analyze neuronal changes quantitatively. Although our observations of histological changes were mainly qualitative in nature, as he has pointed out, we presented a statistical analysis of the curvature of the apical dendrites as a measure of quantitative analysis. To minimize subjectivity in the observation and interpretation of histological specimens, the analysis was conducted in a blinded fashion.

Although the actual numbers of neuronal cells and axons per unit area were not counted in this study, cortical neurons in the hydrocephalic groups were not reduced in number per microscopic field in comparison with those of the control group insofar as they were observed under the high-power view of a light microscope (our Fig. 3). In regard to axons in the periventricular white matter, the numbers of axons per microscopic field under high-power magnification decreased along with hydrocephalic progression (our Fig. 6). The number and density of reactive astrocytes were better seen in sections immunostained with GFAP as described in the text (figures not shown) than in the neurofilament and GFAP double-immunostained sections (our Fig. 6).

In regard to the second comment on quantitative data on apical dendrite angles, we performed a statistical analysis of the curvature of apical dendrites in each experimental group using the Kruskal–Wallis test for all groups as the first step. This test showed a significant difference detected by multiple comparisons between groups (p < 0.001). In the second step, we used the Steel–Dwass test as a post hoc test, because it is the most appropriate test for intergroup comparison to detect differences between specific interest groups. This test showed a statistically significant difference between the control group and each of the experimental groups, which means, at the very least, that the brains in which a shunt had been placed did not return to normal. No significant difference was detected between the pre- and postshunt implantation groups, however, probably because the number of samples was insufficient.

The third comment concerning the selection of cortical area and the time for recovery from shunt insertion in this experiment is important. We consider the area selected to be the most important for evaluation of neuronal changes along the pyramidal tract, from the cortex and the white matter in the frontal lobe and down to the internal capsule, because the purpose of our study was to investigate the neuronal mechanism of the impaired function in cognition and motor skills seen in children with shunts. More studies certainly are needed to focus on the other vulnerable areas in infants with hydrocephalus as well as to evaluate the reversibility of neurons in the longer period after shunt insertion.

We completely agree with the final comment referring to the neuroprotective role of reactive astrocytes in the treatment of hydrocephalus.

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