Learning disability and impairment of synaptogenesis in HTX-rats with arrested shunt-dependent hydrocephalus

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Abstract. Using HTX-rats with congenital hereditary hydrocephalus, we used neuropathological methods, including quantitative Golgi study and neurobehavioral evaluation, to investigate the following problems. (1) What kind of damage does congenital hydrocephalus cause to developing brain tissue? (2) How much can the damage be repaired by ventriculoperitoneal shunting if performed at 4 weeks of age, enabling 4-week-old hydrocephalic rats to survive beyond sexual maturation? (3) What is the status of learning ability of long-term surviving rats with arrested shunt-dependent hydrocephalus? The findings of our study suggest that congenital hydrocephalus impairs the development and formation of the dendrites and spines of the cerebrocortical neurons. Following ventriculoperitoneal shunting, we confirmed that rats with arrested shunt-dependent hydrocephalus demonstrated learning disability in a light-darkness discrimination test using a Y-maze. The development of the dendrites and spines of the cerebrocortical neurons seemed to take place to some degree after shunting, but normal spine density could not be restored. Also suggested was a possible relationship between learning disability and a decrease in spine density, i.e., impairment of synaptogenesis.

Key words: Congenital hydrocephalus - Rat - Synaptogenesis - Golgi study - Ventriculoperitoneal shunt - Learning disability

Although there have been numerous histopathological studies of the congenitally hydrocephalic brain, attention has been focused primarily on alterations of the cerebral white matter and ependyma induced by hydrocephalus [22, 26, 27, 35]. On the other hand, the kind of damage to the development of neurons caused by congenital hydrocephalus and the manner in which ventriculoperitoneal shunting modifies impairment of morphological and functional development of neurons are questions that remain unanswered.

To assess qualitatively hydrocephalus-induced morphological alterations of neurons, Golgi studies on the brain of the oh-mouse, a congenital hydrocephalic strain, were reported by Borit and Sidman [2] in 1972, and on the brains of kaolin-induced hydrocephalic rats by McAllister et al. [18] in 1985. In 1983, we received congenitally hydrocephalic HTX-rats from Kohn [13], of the University of Texas, and successfully bred them. In comparison with various congenital hydrocephalic animal strains previously reported, Wada [34] reported that HTX-rats have a high incidence of hydrocephalus in each generation and develop a pathological state at a steady rate; also, HTX-rats can survive for prolonged periods. Because of these favorable qualities, we were convinced that they were the most suitable for various studies on the development and impairment of congenitally hydrocephalic brains.

Using these rats, and neuropathological techniques, including the rapid Golgi method, and neurobehavioral evaluation, we investigated the following problems: (1) What kind of tissue damage does congenital hydrocephalus cause to the developing brain? (2) How extensively can brain-tissue damage be repaired if ventriculoperitoneal shunting is performed when the animals are 4 weeks of age, enabling them to survive beyond sexual maturation? (3) What is the status of learning ability of rats with arrested, shunt-dependent hydrocephalus?

Materials and methods

Experimental animals

Congenitally hydrocephalic male HTX-rats [13, 34] were used. Nonhydrocephalic rats served as control animals. Female HTX-rats were not used because their motor activity is easily influenced during the estrous cycle.
Preparation of HTX-rats with arrested shunt-dependent hydrocephalus

Hydrocephalic rats experience progressive expansion of the head circumference after birth. Progressive exacerbation of hydrocephalus causes spastic paraparesis in hydrocephalic rats at the age of 4 weeks, which coincides with marked decreases in body weight and locomotor activity. Such animals are diagnosed as having rapidly progressive hydrocephalus [34].

In this experiment, these rats were subjected to a ventriculoperitoneal shunting performed aseptically by the following procedure: hydrocephalic rats were anesthetized by the inhalation of 1.0% halothane while they were in a prone position. The cranial skin was incised, the calvaria exposed, and a hole approximately 1 mm in diameter was bored in the skull 3 mm posterior to the right coronal suture and 3 mm to the right of the sagittal suture. After the dura mater was exposed, the animal was placed in a supine position and a right flank laparotomy was performed to expose the peritoneal space. A valveless tube (Dow Corning, New York, N.Y., Silastic catheter, 0.25 mm I.D.) approximately 16 cm long was implanted in the subcutaneous tissue from the skull to the right flank. The end on the cranial side was inserted into the peritoneal cavity 4–5 mm from the surface of the calvaria. After the flow of cerebrospinal fluid in the abdominal tube was confirmed, the cranial tube was fixed to the skull using Aron Alpha. The abdominal tube was inserted into the peritoneal space, and the skin was sutured.

Eleven rats survived more than 2 months after the shunt procedure and demonstrated recovery of body weight and locomotor activity comparable to that of nonhydrocephalic rats. They were selected from the rats that underwent the operation and were regarded as rats with arrested shunt-dependent hydrocephalus (shunt group). They were used for the light-darkness discrimination test using a Y-maze. The same procedure was also applied to 14 nonhydrocephalic rats at 4 weeks of age. These were regarded as the control rats (control group) and also used for the light-darkness discrimination test.

Light-darkness discrimination test using a Y-maze

Both groups were given restricted diets (10 g/day), but were allowed free access to water. They were maintained under standard lighting conditions with a daily 12 h:12 h light:dark cycle. After 10 days under these conditions, they were used for a light-darkness discrimination test.

The test was carried out in a Y-shaped maze consisting of one start box and two goal boxes (each box: 40 cm long × 22 cm wide × 30 cm high). The start box was separated from the other boxes by a door, and a small lamp was placed at the end of each goal box for light-darkness discrimination testing. Brightness at the center of the Y-maze was controlled at about 40 lx when the lamp in either goal box was lit, and at 1 lx when it was not lit.

The light-darkness discrimination test was carried out in accordance with the following schedule. Prior to testing, the rats underwent conditioning for 10 days to prepare them to be easily lured to the goal boxes. Brightness at the center of the anterior commissure, namely, ventricular dilation was statistically analyzed by three-way analysis of variance (ANOVA). In each session, comparisons between the two groups were analyzed by Welch's r-test.

Measurement of locomotor activity with Automex II

Automex II (Tokai, Tokyo) allows for measurement, over time, of locomotor activity of individual animals. After completion of the light-darkness discrimination test, the 11 rats from the shunt group and the 7 rats from the control group were individually placed on Automex II from 8:00 p.m. to 8:00 a.m. the following morning (a 12-h period). The activity counts of each animal were recorded every 30 min for a total of 24 times. The statistical significance of the difference in activity counts between the two groups at each measurement was evaluated. Furthermore, the difference between the two groups of 12-h mean cumulative counts was statistically analyzed by Welch’s r-test.

Neuropathological study

All 11 rats of the shunt group and 14 rats of the control group that had completed the light-darkness discrimination test were anesthetized with an intraperitoneal injection of pentobarbital (4 mg/100 g body weight). In accordance with the rapid Golgi method [30], the brain was fixed by transcardiac perfusion and then treated by the usual method. It was embedded in celloidin and prepared in 100 μm sections for light microscopic observation. Also, after fixation, thin brain slices were prepared from coronal sections passing through the anterior commissure. After being photographed, the slices were embedded in paraffin, impregnated by the Klüver-Barrera method, and used for determination of the cerebrocortical lamination. The layers of cortical neurons were determined in accordance with the criteria of Zilles and Wree [39]. Five 2-week-old hydrocephalic rats and five 4-week-old hydrocephalic rats were also treated in a similar manner.

2-week-old HTX-rats. Part of the specimens from 2-week-old hydrocephalic rats were postfixed in phosphate buffered 2% glutaraldehyde solution, embedded in Epon, and then prepared in ultrathin and thick sections. The former sections were used for electron microscopic examination, and the latter sections were stained with toluidine blue and used for light microscopic examination. The pyramidal neurons used for the Golgi study were selected from layers II, III, and VI in the frontoparietal cortex in accordance with the following criteria: the cell body was situated as nearly as possible in the center of each section; there was clear staining of apical and basal dendrites; there was no overlapping of a pyramidal cell and a blood vessel. In each group, the dendritic spines of 30 pyramidal cells were quantitatively analyzed. The number of spines obtained in 20 μm lengths on the apical and basal dendrites at a distance of approximately 150 μm from the cell body was recorded, and then spine density of each group was calculated. The statistical significance of the differences between two groups was assessed by means of Student's t-test.

Rats with arrested shunt-dependent hydrocephalus. Golgi-impregnated pyramidal neurons in layers II and III were selected from the shunt group and control group in accordance with the criteria outlined above. After observation of the dendritic branching pattern of the pyramidal neurons, the spine densities of apical and basal dendrites of 60 cells in each group were compared by means of Student's t-test.

Evaluation of ventricular size

The degree of ventricular dilation was determined by the size of the ventricles observed on the coronal sections passing through the center of the anterior commissure, namely, ventricular dilation was