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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.

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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 ventricular space 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 bait when placed into the Y-maze. In each trial the lamp of one of the goal boxes was lit and a piece of solid bait (approximately 3 g) was placed directly under the lit lamp, but not under the unlit lamp in the other box. A rat was placed in the start box and if, after the door was opened, it entered the lit box and took the bait at the first attempt, this was regarded as a correct response.

The time required for each rat to leave the start box, pass through the Y-maze, and finally reach the end of the goal box was measured and designated as the response latency time. If the response latency time exceeded 30 s, the trial was stopped and evaluated as an incorrect response, and the response latency time was recorded as being 30 s.

Ten trials were counted as one session; three sessions at intervals of one every 2 or 3 days were counted every week. The test consisted of up to 24 sessions. The lighting of either goal box in each session was determined in accordance with Gellermann's method [8]. A mean correct response rate and a mean response latency time for individual animals in each session (ten trials) were calculated and statistically analyzed by three-way analysis of variance (ANOVA). In each session, comparisons between the two groups were analyzed by Welch's *t*-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 *t*-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 commisure, namely, ventricular dilation was

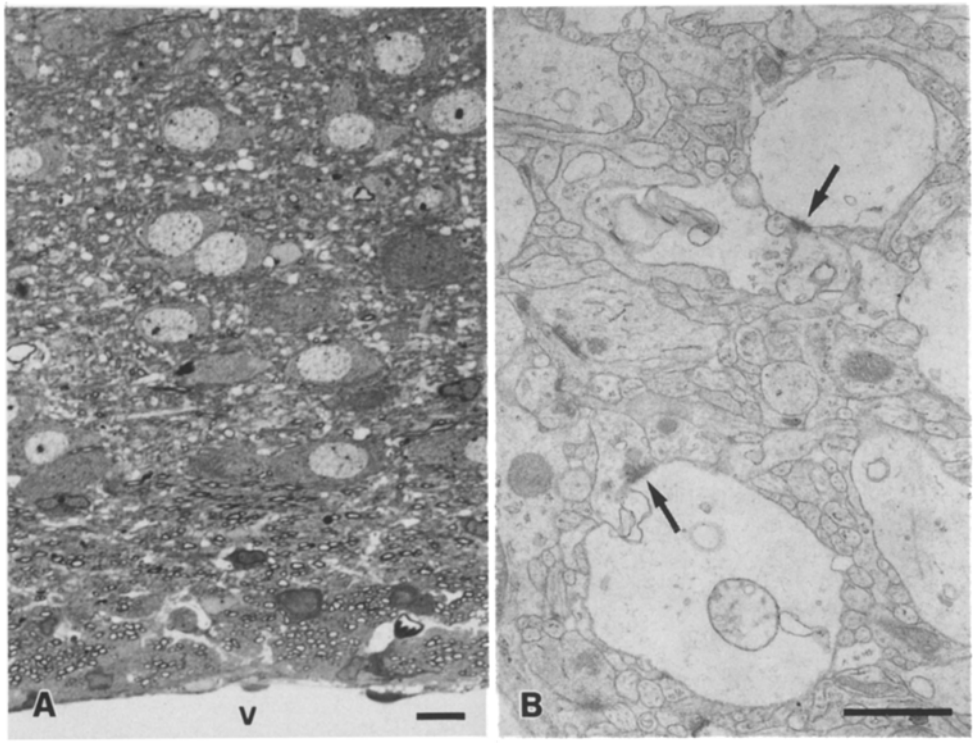


Fig. 1. **A** Photomicrograph of a 1-μm plastic section obtained from a 2-week-old hydrocephalic rat, showing layer VI and periventricular white matter with marked thinning. Neuropils appear as irregularly scattered spotty areas. *V*, Ventricular cavity. *Bar* 10 μm. **B** Electron microscope observation of layer VI neuropil revealed edema of dendrites accompanied by immature synapses (*arrows*). *Bar* 1 μm

expressed as the ratio of *a*/*b* with *a* representing the outermost span of the bilateral frontal horns and *b* the maximum width of the brain. This *a*/*b* ratio was calculated for 13 rats of the control group and 11 rats of the shunt group. The differences between the two groups were statistically analyzed by Welch's *t*-test.

Results

Investigation of the brains of 2-week-old HTX-rats

Electron microscope findings of the cerebral cortex. Regardless of the manifestation of hydrocephalus, six layers of cortical lamination were formed in the cerebral cortex within 7 days of birth. Thereafter, however, the cortex of hydrocephalic rats progressively became thinner with exacerbation of hydrocephalus. The density of the cortex neurons of hydrocephalic rats increases by 2 weeks of age, making it difficult to distinguish the six layers [34]. A thick section of layer VI demonstrated edema of the periventricular white matter and the adjacent gray matter of layer VI (Fig. 1 A). Electron microscope observation of the neuropil of layer VI revealed edema of the dendrites accompanied by immature synapses (Fig. 1 B).

Findings in qualitative and quantitative Golgi study of the cerebral cortex. Golgi-impregnated pyramidal neurons in layer VI of hydrocephalic rats demonstrated bending and winding of apical dendrites. The stretching and disappearance of basal dendrites of these neurons were also observed (Fig. 2). Furthermore, varicosity formation separated by thin constrictions were noted in some basal dendrites. Further magnified views of these morphologically abnormal apical and basal dendrites revealed a significant decrease in their spines as compared to non-hydrocephalic rats, as analyzed by Student's *t*-test (Figs. 2, 3). Morphological abnormalities of the dendrites such as bending, winding, stretching and varicosity formations were not observed in layers II and III of hydrocephalic rats. Nevertheless, a decrease in dendritic spines was obvious, and the difference was statistically significant again in comparison with non-hydrocephalic rats (Fig. 3).

Neurobehavioral evaluation of rats with arrested shunt-dependent hydrocephalus

Light-darkness discrimination test using a Y-maze. Eleven rats of the shunt group and 14 rats of the control group were subjected to a light-darkness discrimination test using a Y-maze. In both groups, the correct response rates progressively increased with advancing sessions, as demonstrated by the significant session effect of analysis of variance [$F(23,529)=29.16$, $P<0.01$ ANOVA]. However, the shunt group showed significantly lower correct response rates than the control group, as confirmed by the group effect of analysis of variance [$F(1,23)=10.16$; $P<0.01$ ANOVA]. There was no interaction between the two factors of group and session. Comparisons of mean correct response rates in each session by means of Welch's *t*-test demonstrated significant differences between the two groups in 9 out of 24 sessions (Fig. 4 A).

The response latency time of both groups shortened progressively with advancing sessions, as demonstrated by the significant session effect of analysis of variance [$F(23,529)=13.99$; $P<0.01$ ANOVA]. The shunt group, however, showed a significantly longer response latency time than the control group, as demonstrated by the group effect of the analysis of variance [$F(1,23)=10.35$; $P<0.01$ ANOVA]. There was no interaction between the

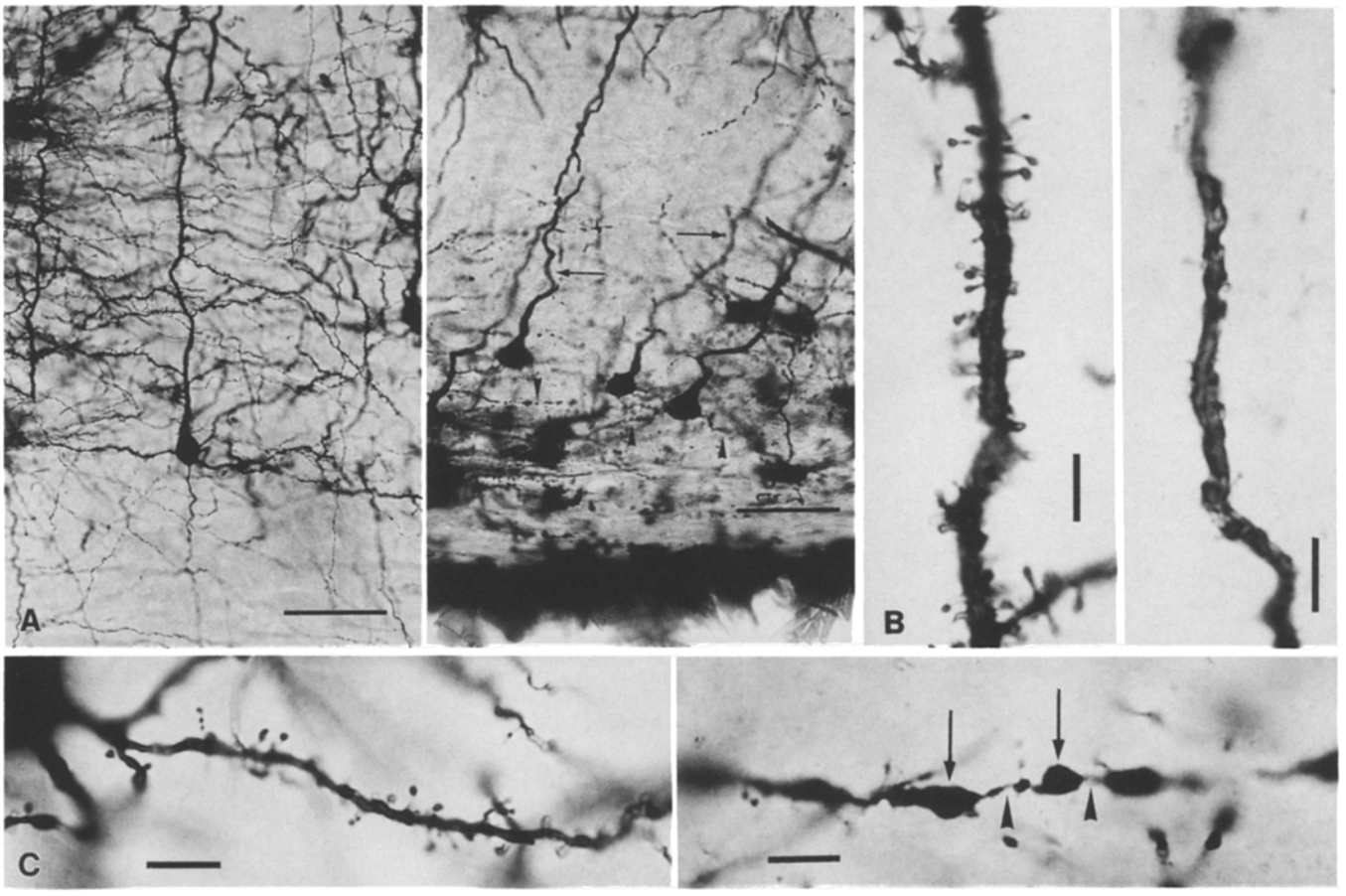


Fig. 2A–C. Photomicrographs of Golgi-impregnated pyramidal neurons in layer VI of 2-week-old rats without (*left*) and with (*right*) hydrocephalus. **A** Bending and winding of apical dendrites (*arrows*) and stretching and disappearance of basal dendrites are observed in hydrocephalic rat (*right*). *Bars* 50 μm. **B** Apical dendrite of a hydrocephalic rat (*right*) has very few spines. *Bars* 5 μm. **C** Basal dendrite of a hydrocephalic rat (*right*)has very few spines. Additionally, large varicosity formations (*arrows*) separated by thin constrictions (*arrow heads*) are observed. *Bars* 5 μm

two factors of group and session. The mean response latency time in each session of the two groups was compared by means of Welch's *t*-test. The mean response latency time of the shunt group was significantly prolonged in 8 of the 24 sessions (Fig. 4B).

Analysis of locomotor activity measured with Automex II. The nocturnal activity of the shunt group ($n=11$) changed more remarkably with time than did that of the control group ($n=7$). As analyzed by Student's *t*-test, the difference in activity between the two groups counts (measured every 30 min) was statistically significant for four measurements (Fig. 5A). The mean 12 h cumulative activity counts of the shunt group were higher than those of the control group, as analyzed by means of Welch's *t*-test (Fig. 5B).

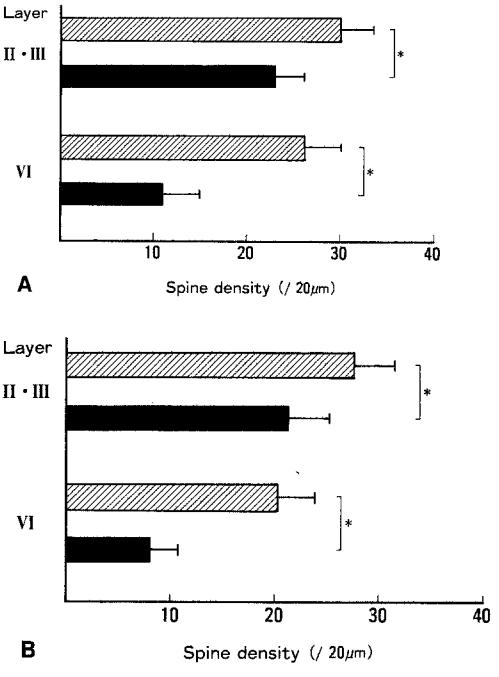
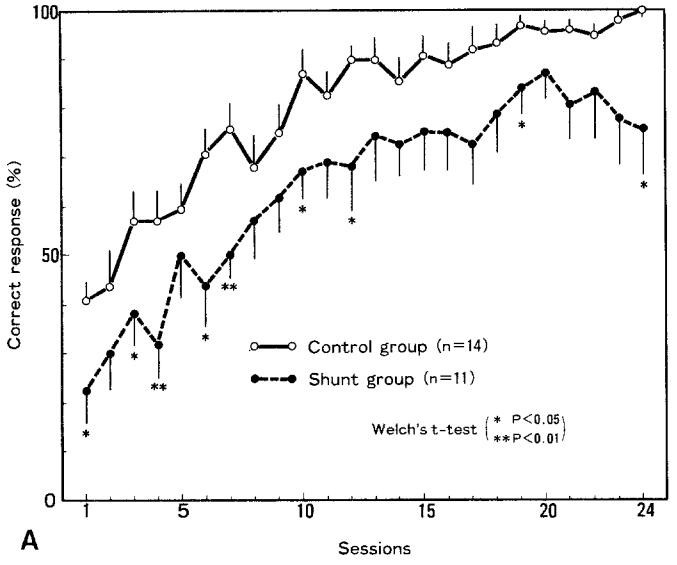


Fig. 3. **A** Mean values of spine density per 20 μm lengths of apical dendrites and **B** basal dendrites of pyramidal neurons in layer II · III and layer VI of rats with and without hydrocephalus. Significant differences were confirmed by Student's *t*-test. *Horizontal bars* indicate the standard deviations from the mean. ▨ Control, ■ hydrocephalus. * $P<0.001$



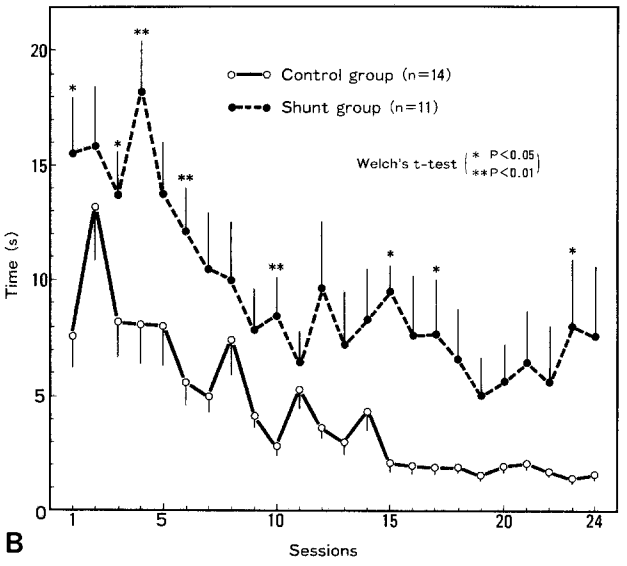


Fig. 4A, B. Light-darkness discrimination test using a Y-maze. *Vertical bars* indicate standard errors of the mean. **A** Correct response rates in both groups increased progressively with advancing sessions, as demonstrated by the significant session effect of analysis of variance [$F(23,529)=29.16$, $P<0.01$ ANOVA]. However, rats of shunt group showed significantly lower correct response rates than rats of the control group, as confirmed by the group effect of analysis of variance [$F(1,23)=10.16$, $P<0.01$ ANOVA]. Comparisons by Welch's t-test of mean correct response rates in each session demonstrated significant differences between two groups in 9 of 24 sessions. **B** In both groups, response latency time progressively shortened with advancing sessions, as demonstrated by the significant session effect of analysis of variance [$F(23,529)=13.99$, $P<0.01$ ANOVA]. However, rats of the shunt group recorded a significantly longer response latency time than rats of control group, as confirmed by the group effect of analysis of variance [$F(1,23)=10.35$, $P<0.01$ ANOVA]. Comparisons, with Welch's t-test, of mean response latency time in each session demonstrated significant differences between the two groups in 8 of the 24 sessions

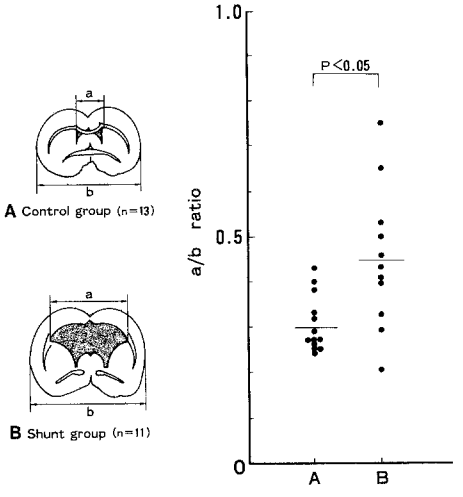


Fig. 6. Ventricular dilation rate (a/b ratio). Variability in a/b ratios differed between A control group and B shunt group, as analyzed by the F-test [$F(10,12)=6.11$, $p<0.01$]. Analysis of the differences by Welch's t-test showed a/b ratios of the shunt group to be significantly greater. *Horizontal bars* reveal the mean a/b ratio value in each group

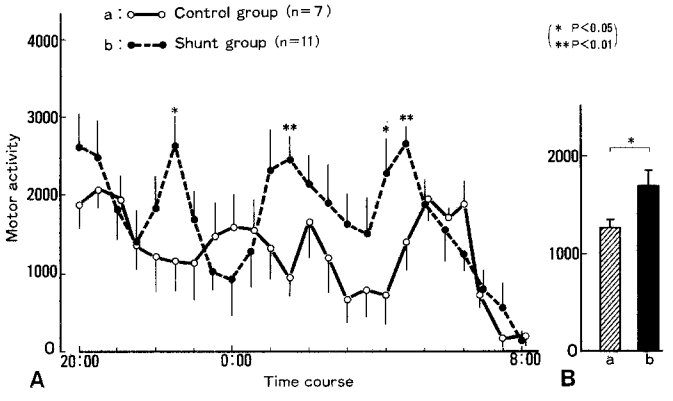


Fig. 5A, B. Analysis of nocturnal activity measured with Automex II. The activity counts of each animal were recorded every 30 min and expressed as an arbitrary unit. *Vertical bars* indicate standard errors of the mean. **A** Nocturnal activity of the shunt group changed more remarkably with time than that of the control group. As analyzed by Student's t-test, the differences in activity between the two groups were statistically significant at four measurements. **B** Mean 12 h cumulative activity counts of the shunt group b were significantly higher than those of the control group a, as analyzed by Welch's t-test

Investigation of the brains of rats with arrested shunt-dependent hydrocephalus

Ventricular dilation rate (a/b ratio). The variability in the a/b ratio was different between the two groups as was analyzed by the F-test [$F(10,12]=6.11$; $P<0.01$]. An analysis of the differences by means of Welch's t-test showed a significantly greater a/b ratio for the shunt group (Fig. 6).

Reconstitution of cortical lamination of the cerebral cortex. The cerebral cortex of all members of the shunt group revealed a six-layered structure as seen in normal rats. Prior to the shunt procedure, thinning of the cerebral cortex in 4-week-old hydrocephalic rats made it impossible to distinguish the six layers of cortical lamination. It would appear, therefore, that the cortical lamina-

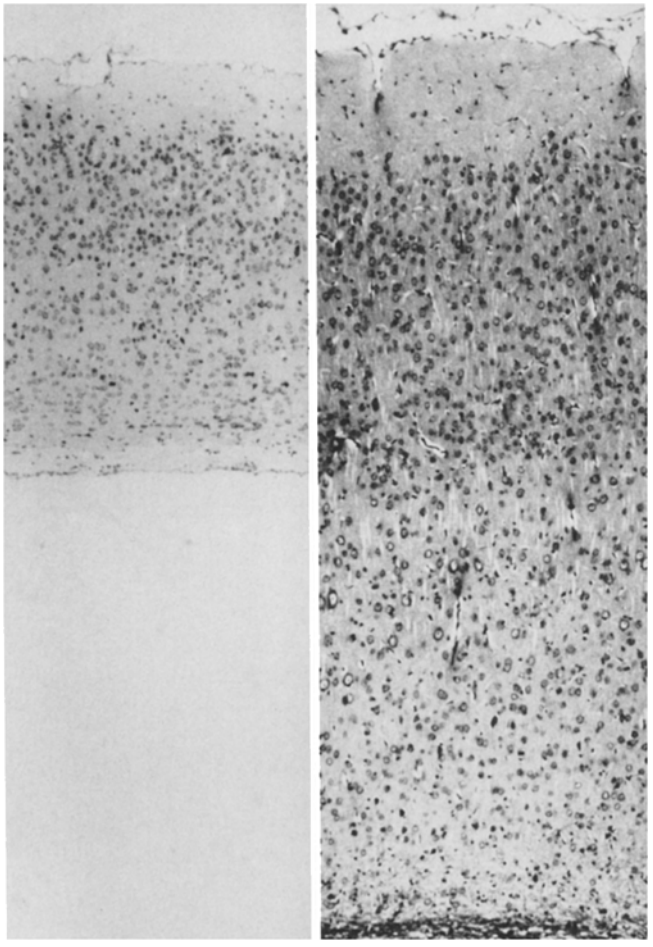


Fig. 7. In comparing the cerebral cortex of 4-week-old hydrocephalic rats (*left*), in which thinning of the cortical mantle made it impossible to distinguish the six layers, the cortical lamination of rats of the shunt group (*right*) seems to have been reconstituted after the shunt procedure

tion of these animals was reconstituted after the shunt procedure (Fig. 7).

Qualitative and quantitative Golgi studies. Light-microscope observation of apical dendrites of Golgi-impregnated pyamidal neurons in layers II and III revealed no differences in diameter, length, and branching pattern between the shunt group and the control group. Spine density of apical dendrites and basal dendrites of the two groups were compared quantitatively between the two groups. This clearly indicated that the spine density in the shunt group was significantly lower than that in the control group, as was confirmed by Student's *t*-test (Fig. 8).

Discussion

According to the neuropathological investigation on the hydrocephalic brain by Nakayama et al. [22], Weller and Shulman [35], and Rubin et al. [26–28], principally the paraventricular white matter was damaged in concert with dilation of the ventricles. These authors further demonstrated that the gray matter of the cerebral cortex was somehow spared in spite of marked thinning of the

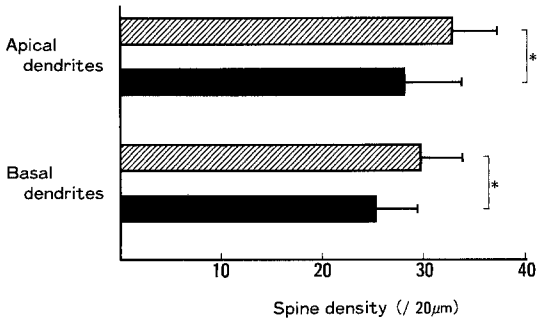


Fig. 8. Spine density of Golgi-impregnated pyramidal neurons in layer II·III. As analyzed by Student's *t*-test, spine density of the shunt group was significantly lower than that of the control group. *Horizontal bars* indicate standard deviations of the mean. ▨ Control group, ■ shunt group. * $P<0.001$

cortical mantle. However, they dealt simply with the hydrocephalic brain through routine histopathological methods alone, with no adequate reference to morphological changes involving dendrites, axons and synapses.

On the other hand, Borit and Sidman [2] and McAllister et al. [18] impregnated the brains of congenitally hydrocephalic oh-mice [2] and the brains of young rats with kaolin-induced hydrocephalus [18], which served as the respective animal models using the Golgi method. These authors stated that marked morphological changes existed in the dendrites and dendritic spines of cortical neurons and stressed that neuronal damage occurs at the cellular level in the thin cerebral cortex of the hydrocephalic brain.

In the present study, damage to cortical pyramidal neurons was investigated principally by qualitative and quantitative Golgi studies in congenitally hydrocephalic HTX-rats [13, 21, 34]. While the thickness of the cortical mantle tended to decrease in proportion to dilation of the ventricles in 2-week-old HTX-rats, bending and winding of the apical dendrites, and stretching, disappearance, and/or varicosity formations of the basal dendrites of pyramidal neurons were observed in layer VI. Although these morphological changes of the dendrites were similar to those presented in the reports of Borit and Sidman [2] and by McAllister et al. [18], it would appear that these changes were more pronounced in congenitally hydrocephalic HTX-rats. Electron microscope observation of the cortical layer VI in these rats revealed the presence of edema in dendrites accompanied by immature synapses. This finding suggests that edema of dendrites may play an important role in the pathogenesis of the varicosity formations in basal dendrites. As anticipated, a significant decrease in the number of spines of the pyramidal neurons in layer VI was observed. It was concurrently noted, however, that there was a significant reduction in dendritic spines of the pyramidal neurons in layers II and III, that there was virtually no edema effect, and that there was mild morphological change in the dendrites. This finding suggests the possibility that there is no close relationship between a decrease in spines and edema.

Dendritic spines now are recognized as representing "specific postsynaptic receptive structures on the dendrites" [11, 23, 29, 30]. Therefore, the detected decrease in spines is assumed to reflect a disturbance in synaptogenesis [24]. What conceivable mechanism could then ex-

plain a decrease in spines? It is well known that disturbance of the cerebral blood flow and the cerebral metabolism occur as hydrocephalus progresses [4, 20]. A first hypothesis is the possibility that these pathological conditions affect the formation of spines (i.e., synaptogenesis) as a primary effect. On the other hand, attention should also be directed toward an experimental study reported by Schapiro and Vukovich [29]. They showed that external sensory stimulation during the period of spine growth may accelerate synaptogenesis. In addition, visual deprivation [10] and interruption of the visual pathway [9] during periods of spine development result in a decrease in the number of spines in adult animals. Recently, Chovanes et al. [3] quantified the monoamine level of a hydrocephalic brain, and suggested that infantile hydrocephalus may impair axonal transport of monoamine or damage projections from the brain stem. Considering a second mechanism on the basis of these reports, it is noteworthy that impairment of synaptogenesis may occur as a result of the decline in afferent input to the cerebral cortex. Further investigations are warranted before the mechanism of synaptogenetic impairment can be elucidated.

We assessed how a ventriculoperitoneal shunt could modify the morphological and functional disorders of neurons. Previously, such large animals as cats [7, 28] and rabbits [4] were used as hydrocephalic animal models for experimental shunt operations. It has, however, become increasingly difficult in recent years to use such large animals in experiments. This being the case, the use of HTX-rats with arrested shunt-dependent hydrocephalus as experimental models is significant in the study of hydrocephalus [21, 34]. Although one may believe that HTX-rats with arrested hydrocephalus should be utilized as control animals in an investigation of shunt-dependent HTX-rats, this is difficult because most HTX-rats with rapidly progressive hydrocephalus cannot survive for more than a few weeks after birth [34]. For this reason, nonhydrocephalic HTX-rats, subjected to sham operations, were used as the control group in this study.

The learning ability of these rats was evaluated in a light-darkness discrimination test using a Y-maze. The results showed that they had lower correct response rates and longer response latency times than rats from the control group. Also, evaluation of nocturnal activity indicated that the shunt group showed greater activity than the control group. In other words, the possibility of decreased activity being responsible for their learning disability can be denied. Although the size of their ventricles was found to be statistically larger than those of the control animals subjected to a sham operation, there was variability in the a/b ratio of the animals in the treated group. This fact indicates that thickening of the cortical mantle could occur to some extent after a ventriculoperitoneal shunt procedure. Cortical lamination of rats with arrested shunt-dependent hydrocephalus appeared as six layers, as seen in normal mature rats. Also, observation of Golgi-impregnated neurons revealed no differences between the shunt group and control group in regard to the branching properties of dendrites. However, the spine density of the apical and basal dendrites of the pyramidal neurons in layers II and III of the shunt group was significantly lower than of the control group. On the other hand, despite the shunt procedure, it was obvious that there had been a reduction in the number of spines in the pyramidal neurons of the cerebral cortex, and that the animals under such conditions had suffered deterioration of their learning abilities. This result appears to be similar to the pathological conditions found in children with so-called intractable hydrocephalus, in whom a high order of mental retardation persists despite the use of the shunt procedure for the human congenital hydrocephalic conditions [15, 25, 38].

The light-darkness discrimination test, using either a Y-maze [31] or T-maze [5, 33], has been used in assessing the learning abilities in aged rats [31], rats with fornix-fimbria lesions [5], and rats with ischemic hippocampal injuries [33]. There has been increasing interest in investigations regarding the relationship between learning ability and morphological conditions and functions of hippocampal neurons. Matthies et al. [17], for example, reported that there had been a sixfold increase in free acetylcholine levels in the hippocampus of rats that had just undergone a light-darkness discrimination test. Wenzel et al. [36, 37] observed significant increases in and morphological changes of the synapses of the CA3 sector of hippocampus in rats, which had been subjected to a Y-maze learning test. These experimental studies prompted us to believe that changes could also be detected in the hippocampal pyramidal neurons in the rats with arrested shunt-dependent hydrocephalus. However, the authors failed to obtain good staining of the hippocampal pyramidal neurons, and adequate evaluation of dendrites and spines was not possible. This report, therefore, has been confined to a description of the changes in the neurons of the cerebral cortex. Whether or not morphological changes occurring in the synapse upon learning is a phenomenon specific to the hippocampus, or a phenomenon that may be found in other parts of the brain as well, remains unknown at this time. Elucidation of the question awaits future investigations. Although the present investigation was not sufficiently successful to make clear a direct correlation between impairment of synaptogenesis and learning disability, we are confident that there is a close relationship between the two.

In general, in the rat brain, the migration of the cerebrocortical neurons comes to completion within a week after birth and cortical lamination is completed [1, 6, 14, 19]. Hydrocephalus in HTX-rats is already manifest at birth, and rapidly progresses in the subsequent 4 weeks [13, 34]. This period coincides with the peak of synaptogenesis in the cerebral cortex of rats [16]. Therefore, the reduced spine density in the rats with arrested shunt-dependent hydrocephalus indicates that since the shunt procedure was performed at a late stage, it failed to play an adequate role in avoiding impairment of synaptogenesis due to hydrocephalus.

On the other hand, cortical lamination of the cerebral cortex in a human is completed by the fifth month of fetal life, and there is rapid acceleration of subsequent synaptogenesis [12, 23]. In humans, therefore, hydrocephalus that has developed during fetal life may impair synaptogenesis of the cerebral cortex. Such speculation may lend support in explaining the ineffectiveness of shunt surgery, after which normal psychomotor development of children with congenital hydrocephalus can not be generated

[15, 25, 38]. Accordingly, it seems reasonable to reconsider the intrauterine treatment of congenital hydrocephalus, although some investigators doubt its usefulness [32]. A novel surgical technique for intrauterine treatment of congenital hydrocephalus must be developed at some future date.

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