行政院國家科學委員會專題研究計畫　成果報告

水腦對感覺運動皮質的影響：細胞結構之研究

計畫類別：個別型計畫
計畫編號：NSC93-2314-B-002-242-
執行期間：93 年 08 月 01 日至 94 年 10 月 31 日
執行單位：國立臺灣大學醫學院外科

計畫主持人：郭夢菲
共同主持人：曾國藩

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 95 年 1 月 31 日

國科會專題研究計畫成果報告撰寫格式

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本格式說明僅為統一成果報告之格式，以供撰寫之參考，並非限制研究成果之呈現方式。精簡報告之篇幅（不含封面之頁數）以 4 至 10 頁為原則，完整報告之篇幅則不限制頁數。

成果報告繳交之期限及種類（精簡報告、完整報告或期中報告等），應依本會補助專題研究計畫作業要點及專題研究計畫經費核定清單之規定辦理。

二、內容格式：依序為封面、中英文摘要、目錄（精簡報告得省略）、報告內容、參考文獻、計畫成果自評、可供推廣之研發成果資料表、附錄。

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(四)頁碼編寫：請對摘要及目錄部分用羅馬字 Ⅰ 、II、 III……標在每頁下方中央；報告內容至附錄部分請以阿拉伯數字 1.2.3.……順序標在每頁下方中央。

(五)附表及附圖可列在文中或參考文獻之後，各表、圖請說明內容。

(六)計畫成果自評部份，請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

(七)可供推廣之研發成果資料表：凡研究性質屬***應用研究***及***技術發展***之計畫，請依本會提供之表格（如附件二），每項研發成果填寫一份。

三、計畫中獲補助國外或大陸地區差旅費、出席國際學術會議差旅費或國際合作研究計畫差旅費者，須依規定撰寫心得報告（出席國際學術會議者須另附發表之論文），以附件方式併同成果報告繳交，並請於成果報告封面註記。

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使用 A4 紙，即長 29.7 公分，寬 21 公分。

2. 格式

中文打字規格為每行繕打（行間不另留間距），英文打字規格為 Single Space。

3. 字體

報告之正文以中英文撰寫均可。在字體之使用方面，英文使用 Times New Roman Font，中文使用標楷體，字體大小請以 12 號為主。

附件一

行政院國家科學委員會補助專題研究計畫成果報告

（計畫名稱）

水腦對感覺運動皮質的影響：細胞結構之研究

計畫類別：☑ 個別型計畫　　□ 整合型計畫
計畫編號：NSC　93－2314－B－002－242－
執行期間：2004 年 08 月 01 日至 2005 年 10 月 31 日

計畫主持人：郭夢菲
共同主持人：曾國藩
計畫參與人員：李育龍

成果報告類型(依經費核定清單規定繳交)：☑精簡報告　□完整報告

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□赴大陸地區出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年□二年後可公開查詢

執行單位：國立臺灣大學醫學院外科

中　華　民　國　94 年　11 月　24　日

（一）中、英文摘要及關鍵詞 (keywords)：

關鍵詞：水腦症、星狀細胞、大腦皮質、細胞內染料注射、微膠細胞

Keywords: Hydrocephalus, astrocytes, cerebral cortex, intracellular dye injection, Lucifer yellow, microglia

中文摘要

水腦是由於腦脊髓液分泌過多、流暢失常或吸收不良造成腦室膨大的一種病症，不論在成人或兒童都可能引發動作、行為及認知上的異常。動物及人的研究顯示，此症常伴有腦室周邊組織（如：白質）及大腦皮質的破壞。然而這些受影響皮質的結構及個別神經元的狀況卻少有探討與瞭解。本計畫以 Nissl 染色以及 PGP9.5, GAD, NOS 等抗體標示神經元，而以 GSA I-B4 凝集素以及 GFAP, CD11b, ED-1 等抗體標示神經膠細胞來瞭解在側腦室上方，受壓迫的感覺運動皮質結構上的變化。此外，我們應用“已固定組織細胞內染料注入法”及隨後的免疫組織轉變反應及單一神經細胞重組技術來探討皮質的傳出神經元—第三及第五層錐狀細胞的整體胞體及樹突型態在水腦時的改變，並探討其整體樹突叢、樹突分支密度、樹突長度及其棘突密度等的改變。透過這些資料能了解這些受壓迫細胞在構造外功能上可能產生的改變。此外，我們也以“活腦薄片神經聯繫追蹤技術”探討這兩種錐狀細胞的近距離軸突投射，藉此瞭解這兩種細胞是否仍控制著其標的構造，以推論在水腦時，受壓迫的皮質的整體功能狀態。

本實驗的結果增進我們瞭解水腦症的病理生理變化，同時也可瞭解受水腦壓迫的大腦皮質的功能狀態。

英文摘要

Hydrocephalus, a pathological enlargement of the cerebral ventricles resulted from oversecretion, abnormality of flow, or malabsorption of cerebrospinal fluid is known to cause motor, behavioral and cognitive deficits in children and adults. Animal and human studies thus far indicate damages to structures along the periventricular area, in particular nerve fibers of the white matter. However, despite the dramatic thinning of the cortex above the lateral ventricle, no studies have dedicated to understand the cytoarchitecture and individual neuron status of the affected cortical area. In this study, we use Nissl and anti-PGP9.5, GAD, and NOS as neuron markers and the lectin GSA I-B4 and anti-GFAP, CD11b, ED-1 as glial markers to study the changes of the cytoarchitecture of the affected cortex, the sensorimotor cortex. In addition, we use fixed-tissue intracellular dye injection technique to study the soma-dendritic arbors of the layer III and V pyramidal neurons, the cortical output neurons, of the affected sensorimotor cortex. The technique and its subsequent immunoconversion and reconstruction reveals the complete soma-dendritic morphology of neurons selected for injection and allows detailed analysis of the extent of their dendritic arbors, branching profuseness, dendritic length and dendritic spine densities. These detailed morphological data can reveal the functional status of these neurons under compression. In addition, we performed neuronal tracing in live slices in a custom-designed incubation chamber to reveal the short-distance projections of these two types of pyramidal neurons to find out whether they still control their targets. These data are crucial to determine how the compressed cortex may function during hydrocephalus.

（二）前言

Hydrocephalus is a pathological dilatation enlargement of the cerebral ventricles that results from oversecretion, obstruction of pathway, or malabsorption of cerebrospinal fluid (CSF). During hydrocephalus the numbers and activities of cholinergic, GABAergic, and dopaminergic neurons were found to be reduced (Del Bigio et al., 1998; Miyake et al., 1992; Tashiro et al., 1997). In previous studies on neonatal in hydrocephalus, we demonstrated that the tissue level of DA, DOPAC and HVA in the striatum increased significantly in acute hydrocephalus. The expression of mRNA of MAO-A and MAO–Bo, a major enzyme metabolizing dopamine, was also significantly upregulated (Kuo, 2005). It was reversed by CSF diversion. On the contrary, the expression of MAO-A mRNA decreased during chronic hydrocephalus. The expression of MAO-B mRNA did not show much significant change. We speculated that during hydrocephalus the increase of dopamine, an autooxidant, will damage the neuron and/or glial cells. MAO may play a role in neuroprotection by decreasing the tissue concentration of dopamine. These metabolic disturbances of in the brain are believed to contribute to the functional impairment elicited by hydrocephalus. Morphologically, atrophy of the periventricular white matter and damaged axons are consistent findings in human brains with severe hydrocephalus (Gadsdon et al., 1978). Animal studies clearly demonstrate decreased blood flow with anaerobic metabolism in the white matter of hydrocephalic brains (Chumas et al., 1994). The periventricular axons and brain tissue may be damaged by a combination of mechanical injury (stretch), impaired cerebral blood flow, and metabolic disturbance (change of neurotransmitters, accumulation of waste products in the CSF, and etc). (Del Bigio, 1993; McAllister and Chovan, 1998). In kittens with chronic severe hydrocephalus, karyorrhectic cells or focal leukomalacia was found in the white matter, suggesting that primary glial cell death may also contribute to white matter atrophy in hydrocephalus (Del Bigio et al., 1994). It has also been suggested, on the basis of minimal evidence, that there may be primary demyelination of periventricular axons (Leech, 1991). Despite these suggested damages in especially the lower layers, the fate of the cortical neurons in the different layers of the affected cortex remains unclear.

In the adult rats, kaolin-induced hydrocephalus has been described to begin with an acute phase in the first 4 weeks of high basal intracranial pressure and considerably increased CSF outflow resistance and a rise in the brains water content. This is followed by chronic phase which was characterized by normal basal pressure, declined outflow resistance, progression of ventriculomegaly and decreases of taurine and alanine plus an increase in glutamine in the cerebrum (Kondziella et al., 2002). Overall, fewer changes were detected in the metabolic levels of various potential neurotransmitters in these adult cases (Kondziella et al., 2002). In contrast, when rats were induced hydrocephalus at the age of 3 weeks, Del Bigio and Vriend (1998) found up to 100% increased amounts of glutamine, glutamate, aspartate and GABA in the parietal cortex analyzed by HPLC 1, 2 and 4 weeks after kaolin instillation while Jones et al. (1997) and Harris et al. (1997) reported, on the contrary, significant decrease in the neurotransmitters and amino acids. Although this could result from the different strains of rats used, the discrepancy nevertheless points out that the nature of neurons in the affected cortical area needs remains to be investigated. Another important unresolved issue is that the severity of the behavioral and

cognitive deficits associated with chronic hydrocephalus in children and adults does not correlate well with ventricular size (Cardoso and Del Bigio, 1989; Jackson and Lorber, 1984; Del Bigio et al. 2003).

Based on the above review, the neuropathological changes associated with the acute and chronic hydrocephalus are no doubt under-explored. To find out how cortical function is affected by hydrocephalus, the status of the cortical neurons, especially layer III and V pyramidal neurons (Chen et al., 2003a,b). This shows that mechanical force alone can alter cortical output neurons, presumably cortical function too. Although epidural mechanical compression affected structures of all cortical layers, it nevertheless compromised those in the superficial layers the most (Chen et al., 2003a). It is of great interests to find out how the morphology of the cortical output neurons is altered following hydrocephalus and is the effect graded from the ventricular wall to the pial surface.

In this proposal, we study the basic cytoarchitecture of the affected cortex to determine the general patterns of the short- and long-term effects of hydrocephalus on cortex, including neuronal densities, capillary densities, reactive glial densities, and axonal damages in rats with kaolin-induced hydrocephalus at juvenile age. We fixed intracellular dye injection technique to reveal the whole soma-dendritic arbors of the layer III and V pyramidal neurons of the sensorimotor cortex. Visually guided intracellular dye injection were preformed with glass micropipettes on semi-fixed cortical slices (300- μ m thick) on an upright fixed-staged fluorescence microscope with the assistance of a 3-axial hydraulic micromanipulator (Wang et al., 1996; Wang et al., 2002). In addition, we performed anterograde tracing on live cortical slices (Tseng et al., 1996) to label the axons of these pyramidal neuron to find out whether the damage to periventricular axons reported in this model affects the outputs of these cortical neurons. Results obtained advance our understanding of the effect of hydrocephalus on cerebral cortex. In addition, understanding the effect of the compression by hydrocephalus on cortical neurons also provide insight to understanding what may happen to cortical neurons under the expansion of brain or meningeal tumors which in many cases will no doubt compress on cortical tissue, too.

（三）研究目的

The aim of the present study is to find out the short- and long-term effects of hydrocephalus on cerebral cortex at the cytoarchitectural and individual cell levels.

At the cytoarchitectural level:

1. To find out how the affected sensorimotor cortex and each of its layers change.
2. To determine is there any changes in the capillary densities of the affected cortex. This will be an indicator for any signs of ischemia during hydrocephalus.
3. To determine is there any change in the neuronal densities of each cortical layer and the densities and distribution of NOS-producing cells and GABAergic cells in the cortex. This will reveal signs of cell loss, changes in excitatory-inhibitory balance, and free radical environment.
4. To determine the responses of the astrocytes and microglial cells of the affected cortex to hydrocephalus. Our preliminary data indicate that there is gradation in the severity of the

responses from the ventricular side to the pial surface. Regionally specific glial reactions suggest the possibility that certain areas are affected more than others.

At the individual cell level:

1. To determine how hydrocephalus affects the soma-dendritic morphology, including dendritic arbor, dendritic length, and dendritic spine densities, of layer III and V cortical pyramidal neurons. These data give clues to the function of these neurons and consequently the output of the affected cortex.
2. To determine the projection (output) of the layer III and V cortical pyramidal neurons of the affected cortex. Short distance-projection axons will be studied in this proposal since serious damage to the periventricular structures close to the expanded ventricle, such as white matter, has been repeatedly observed in previous studies. Data from anterograde slice tracing in the present proposal will help to define whether the output neurons of the affected cortex are still capable of sending out their output.

These data will provide better understanding on how cortical functions are affected following the development of hydrocephalus. These information are crucial to studying how hydrocephalus shall be treated and how cortical functions may recover after surgical treatmentward in coming years.

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（五）研究方法

Young male Wistar rats (Charles River), 3-week-old were be used for inducing hydrocephalus. Animals were anesthetized with 4% chloral hydrate 8% (400mg/ kg body wt, i.p.). To induce hydrocephalus, we used 25-gauge needle to inject 0.06 ml of sterile Kaolin suspension (25%, Sigma, St. Louis, MO) into the cisterna magna of the animal under microscopic view. Control rats at different ages received a sham injection with same amount of 0.9% saline solution brief needle insertion. Animals were divided into groups and examined 1, 2 and 4 weeks after intracisternal kaolin injection to study the effects of acute (1 week after), chronic hydrocephalus (4 weeks after) and subacute stage on cerebral cortex adjacent to the lateral ventricles. The mortality rate of inducing hydrocephalus, measuring intracranial pressure and other processes in neonatal rats was about 50%. Each group contained 10 animals.

1. The measurement of intracranial ventricular pressure (ICP)

In each survival group, the ICP (intraventricular pressure, intracranial pressure) were measured at the end of the survival using a 23-gauge scalp needle filled with 0.9% normal saline. Under i.p. anesthesia with 4% choral hydrate 400 mg/Kg, the rat was mounted on a stereotaxic device. Following the exposure of the skull, a burr hole was made 1 mm anterior and 2 mm lateral to the bregma were burred. The scalp needle was inserted into the lateral ventricle. The phenomenon of CSF pulsation will be used as an additional confirmation of the location of the tip. The height of the CSF column was measured and translated into pressure. The experimental rats were further grouped according to the ICP into normal (or compensated) and high pressure hydrocephalus.

2. Behavioral evaluation

Following the induction of hydrocephalus, animals were closely monitored for signs of neurological deficits, seizure-related or abnormal behavior. The behavioral manifestation help in the interpretation of the results.

3. Tissue processing and histochemical procedures

At the end of the survival, rats received an overdose of pentobarbital (200mg/Kg, i.p.) (0.63 g/ kg body wt, i. p.) and perfused transcardially with 50 ml of saline followed by 500 ml of a fixative containing 4% paraformaldehyde in 0. 1 M phosphate buffer (PB), pH 7.3 for 30 minutes. The brains were carefully dissected and postfixed in the same fixative at room temperature for 3 days. In the experimental group, only animals with obvious hydrocephalus were processed further. Following postfixation, the whole brain was be blocked and placed in 0. 1 M PB containing 30% sucrose until it sunk. Cryosections, 20 μm thick, in the coronal plane was prepared and collected in 0. 1 M PB. Sections were sorted into series and processed as follows.

3-A: To identify the cytoarchitecture and layers of the cortex, a series of the sections were stained with the Nissl's method. The rest were processes histochemically as described below.

3-B: An antibody to the neuron-specific Protein Gene Product (PGP) 9.5 (1:1000, Chemicon, Temecula, CA) to identify neuronal elements. This help us to determine is there any change in neuronal densities or layer thickness. This staining can also be used to evaluate is there any

distortion of the apical dendrites of layer II/III and V pyramidal neurons labels the large apical dendritic trunks of pyramidal neurons prominently (Chen et al., 2003a and b).

3-C: A series of sections will be processed with an antibody to GAD (1:1500, Chemicon), a marker of GABAergic neurons, to determine is there any change in the distribution of inhibitory neurons. Histochemical methods were similar to that described in Liu et al. (2002).

3-D: Another series of sections were reacted with an antibody to the glial fibrillary acidic protein, GFAP (1:400, Chemicon), a marker of astrocytes, to find out is there any reactive astrocytic response. Histochemical procedures followed that of the Chen et al. (2003a).

3-E: Another series of sections were reacted with the lectin GSA I-B_4 (1:8, Sigma, St. Louis, MO) that recognized microglial cells to reveal is there any reactive microglial change. In addition, this lectin also stains blood vessels, thus can be used to determine is there any change in the blood perfusion of the affected area. Histochemical methods will follow that of Chen et al. (2003a). Alternatively, some additional sections were reacted with anti-rat macrophage CD11b (1:10, OX-42, Setotec, UK) or Anti-ED-1 (Chemicon; Liu et al., 2003). These two antibodies also identify reactive microglial cells.

3-F: Another series of sections will be reacted with an antibody specific to the neuronal Nitric Oxide Synthase (nNOS; 1:1000, Chemicon) to determine is there any change in the density and the distribution of NO producing neurons. Alterations in the number and/or distribution of these neurons may be related to changes in free radical environment and bear relationship with the degree of neuronal death if observed any.

After processing through the primary antibodies, sections were rinsed in buffer and through secondary antibodies and then standard avidin-biotin-horseradish peroxidase (HRP) reactions (Vector, Burlingame, CA). Sections were then reacted with DAB (Sigma) as chromagen and mounted in Permount (Fisher, Fair Lawn, NJ) as described below (Wang et al., 2002; Chen et al., 2003 a, b).

4. Fixed tissue intracellular dye injection

This method and the setup had been established in the Department of Anatomy and Cell Biology in the Medical School of National Taiwan University for many years (Wang et al., 1996). Briefly, at the end of the survival, rats received an overdose of pentobarbital (0.63g/ kg body wt, i.p.) and perfused transcardially with 50 ml of saline followed by 500ml of a fixative containing 2% paraformaldehyde in 0.1 M PB, pH 7.3 for 30 minutes. Cortical tissue of the affected cortical area were removed immediately and sectioned with a vibratome (TPI, St. Louis, MO) into 300-μm-thick coronal slices. The fixed tissue intracellular dye injection was performed under the guidance of fluorescence, cortical neurons in slices were revealed by soaking slices in a solution containing 10 nM 4', 6-diamidino-2-phenyl-indole (DAPI; Sigma) for 30 minutes to make their nuclei fluoresce before conducting intracellular injection. Lucifer Yellow (LY, Sigma) was the intracellular dye, since DAPI and LY could be visualized simultaneously under the same fluorescence filter combination 18.

For injection, a slice was placed in a dish on the stage of the fixed-staged, upright microscope equipped epifluorescence (Zeiss Axioskop) and covered with a thin layer of 0.1 M PB. With the help of a long-working distance lens, selected DAPI-labeled neurons in each cortical layer

especially layer II/III and layer V which contain mainly pyramidal neurons were impaled with an intracellular micropipette, containing 4% LY (Sigma) in water, mounted on a 3-axial hydraulic micromanipulator (Narishige, Japan). Each cell was filled with LY using constant negative current till its terminal dendrites brightly fluoresce. Several neurons could be filled in isolation in each slice. Slice were then removed, postfixed in 4% paraformaldehyde in 0.1 M PB for 3 days, rinsed thoroughly in 0.1 M PB, cryoprotected, and sectioned into 60 μm-thick sections with a sliding microtome (Tseng et al., 19'91). Sections were collected in 0.1 M PB.

5. Immunoconversion of the injected dye

Sections will be treated with 10% normal goat serum and 1% Triton X-100 in 0O.1 M phosphate-buffered saline (PBS) for 1 hour. After washing three times in 0O.1 M PBS, sections will be incubated with 1:200 anti-LY (peroxidase conjugated; Molecular Probes, Eugene, OR) for 18 hours at 4°C. Finally, sections will be reacted with 0.05% DAB and 0.003% H_2O_2 in 0.05 M Tris Buffer. Sections will then be mounted on slide, dehydrated, cleared, and coverslipped with Permount.

6. Anterograde tracing with dextran in live cortical slices

We follow the protocol described by Tseng et al. (1996). The protocol was designed to study the neural connection of closely positioned structures in the central nervous system in vitro. Briefly, rats were deeply anesthetized. Following decapitation, the cerebral cortex was blocked and placed into precooled (approximately 4℃) Ringer solution gassed with 95% O_2 and 5% CO_2 (Chen and Tseng, 1997). Live slices of the cerebral cortex, 300 μm-thick were prepared with a vibratome. The slices were then incubated in a custom-designed chamber (Tseng et al., 1996) with continuous Ringer solution, saturated with 95% O_2 and 5% CO_2, perfusion and maintained at 35℃ (Chen and Tseng, 1997). After an hour of incubation, the anterograde tracer Dextran (10Kd, biotin and tetramethylrhodamine-labeled, Molecular Probes) was applied. The tracer was touched to the tip of a glass micropipette and applied to the area of interest, e.g., selected area of the layer III and V of the studied cortex with the help of a mechanical micromanipulator (Leitz) under a dissecting microscope. The slices were incubated for 6 hours additionally before it was floated onto a small slip of filter paper and fixed in 4% paraformaldehyde in 0.1 M PB for 3 days. At the end of the fixation, the slices were cryoprotected and resected into 60 μm-thick sections with a sliding microtome as those that had been through fixed-tissue dye injection. Sections were reacted to the biotin of the tracer using standard avidin-biotin-HRP technique and DAB as chromagen.

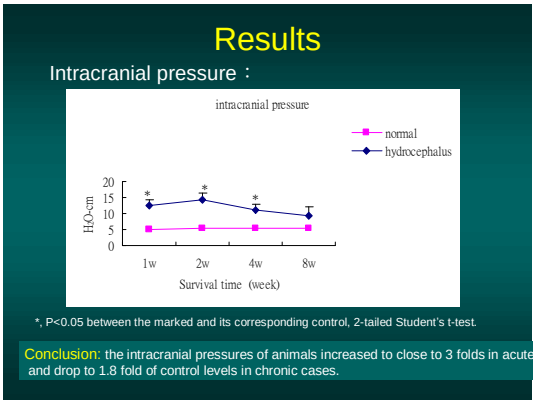
7. Data analysis

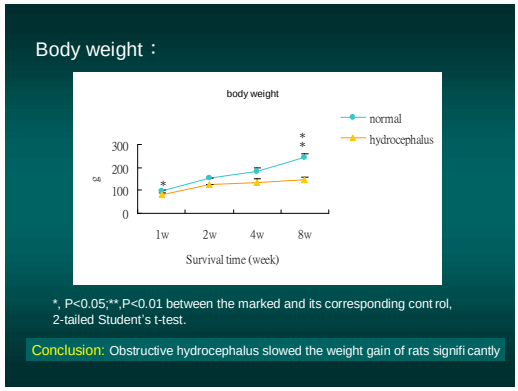
In each animal, the area of the lateral ventricle were plotted and quantified with a PC-based program (Freemax Image-Pro, Media Cybernetics, Silver Spring, MD) and used as an index to classify the severity of the hydrocephalus. Experimental animals were grouped into different groups depending on the sizes of their lateral ventricles, the stages of hydrocephalus, and the intracranial pressures.

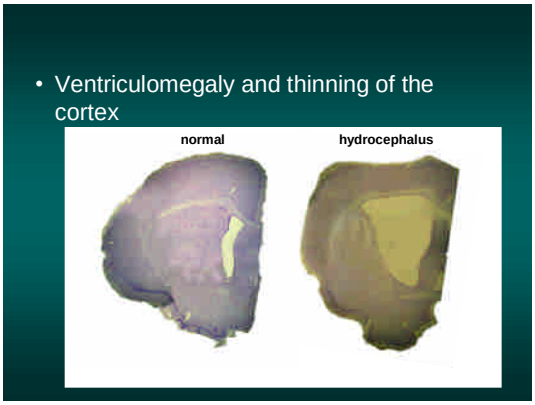
Data from the compressed cortical area were compared to their correspondent sham-operated group. Morphological data collected and compared includes: the total cortical thickness, thickness of individual layers, densities of neurons in each cortical layer, numbers and

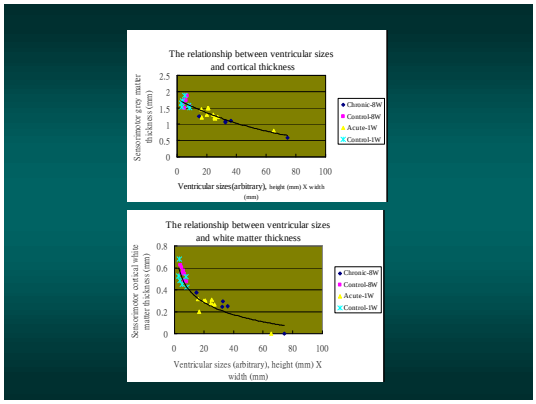
distributions of GABAergic neurons, number and distribution of NOS-positive neurons, densities of astrocytes and microglial cells, and densities of capillaries. We reconstructed the soma-dendritic trees of layer III and V pyramidal neurons through serial sections using a Camera Lucida drawing tube and a 40X objective lens in two-dimensional plane. The demarcation between soma and dendrites were taken as the point where the convex curvature of the soma became concave (Tseng and Royce, 1986). Dendrites were divided into basal and apical dendrites following the method described for rat cortical pyramidal neurons (Tseng and Prince, 1993). Their length were determined from the two-dimensional reconstructions using a PC-based software (Freemax Image-Pro). Selective segments of their dendrites were reconstructed using a 100X oil-immersion objective lens for details of the dendritic spines. Parameters of the dye-injected cells analyzed included the apparent shape of individual cell, the total dendritic length of each category of injected cells in each cortical layer, the shape and total length of the apical dendrites of layer III and V pyramidal neurons, the dendritic spine densities, the cell body sizes, the total dendritic domain (area covered by the dendrites of each cell), and any other peculiar phenomena that might have been revealed. A PC-based program, Freemax Image-Pro were used for most of the analysis (Wang et al., 2002; Chen et al., 2003a,b).

（六）結果與討論（含結論與建議）









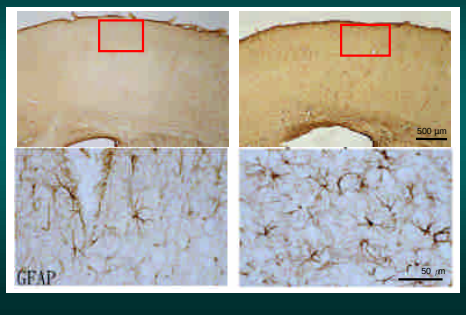
Density of neurons as revealed by PGP9.5 immunohistochemistry (neuron marker)

		Layer Ⅱ/Ⅲ	Layer Ⅴ	Layer Ⅵ
1 week	Control (n=3)	33.00 ± 1.00	20.00 ± 2.00	32.33 ± 2.87
	Hydrocephalus (n=4)	31.25 ± 3.03	25.75 ± 3.83	34.50 ± 2.69
8 week	Control (n=4)	30.25 ± 6.38	20.50 ± 2.60	30.00 ± 2.55
	Hydrocephalus (n=4)	25.00 ± 4.85	25.50 ± 3.28	31.75 ± 8.41

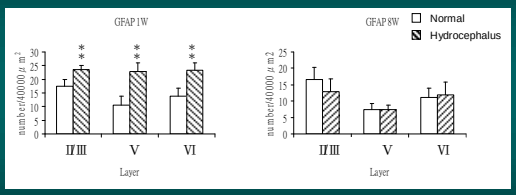
Values are means ± S.E. for the number of animals (n) analyzed
*, P<0.05 between the marked and its corresponding control, 2-tailed Student's t-test.

- Astrocyte (1W)

normal **hydrocephalus**



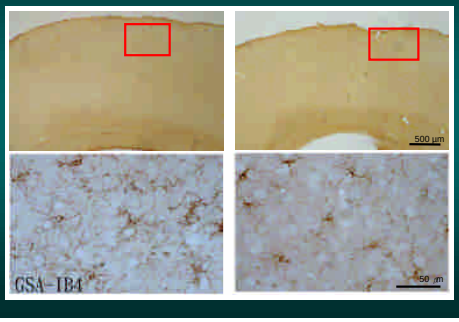
- Densities of astrocytes in different cortical laminae



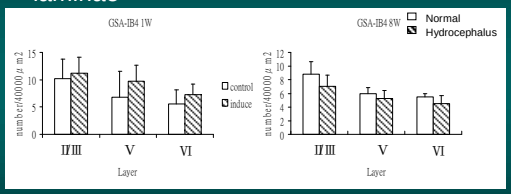
**, P<0.01 between the marked and its corresponding control, 2-tailed Student's t-test.

- Microglia (1w)

normal **hydrocephalus**

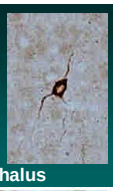


- Densities of microglia in different cortical laminae

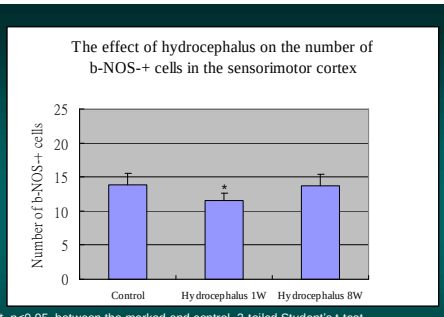


*, P<0.05 between the marked and its corresponding control, 2-tailed Student's t-test.

- brain NOS (1w)



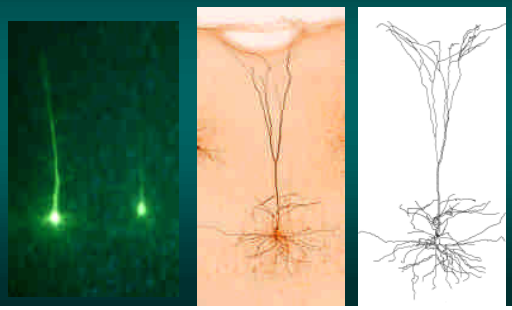
normal **hydrocephalus**

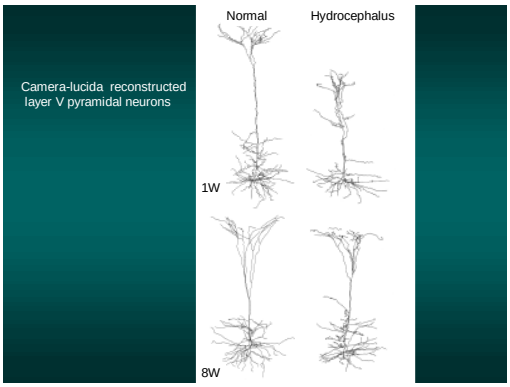


*, p<0.05, between the marked and control, 2-tailed Student's t-test

Conclusion: There were no changes obvious changes in the total number of neurons in each cortical layers; reactive changes of astrocytes, but not microglia, were observed in acute animals; there is a small but significant decrease of bNOS-positive neurons.

- Intracellular dye injection and immunoconversion
  - slices soaked in 10^{-7} M DAPI in 0.1M PB for 30 min
  - intracellular injection of Lucifer Yellow 4% in ddH_2O
  - Immunohistochemical conversion of LY
  - Camera lucida reconstruction of injected neurons in 2-D plane.





Dendritic length

		Total	Apical	Basal
1 wk	Control (n=5)	10311 ± 739	6537.33 ± 472	3774 ± 905
	Hydrocephalus (n=9)	7952 ± 1724*	4878.81 ± 1314*	3073 ± 546
8 wk	Control (n=7)	9725 ± 1416	6126.98 ± 1256	3598 ± 742
	Hydrocephalus (n=8)	8939 ± 1610	5051.83 ± 1528	3888 ± 266

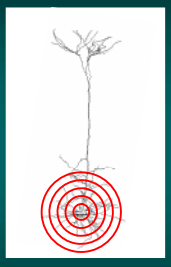
Values are mean ± S.E. for the number of neurons (n) studied
*, P<0.05 between the marked and its corresponding control, 2-tailed Student's t-test.

Dendritic spine density

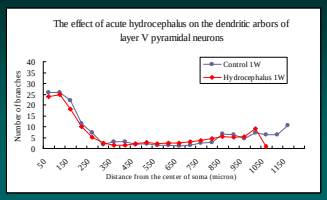
		Basal dendrite		Apical dendrite	
		Proximal	Distal	Proximal	Distal
1 wk	Control	10.15 ± 2.03 n=25	9.73 ± 1.73 n=29	9.50 ± 1.93 n=20	6.83 ± 0.90 n=21
	Hydrocephalus	7.00 ± 1.73** n=35	7.59 ± 1.32** n=31	6.40 ± 1.60** n=24	4.81 ± 2.01** n=30
8 wk	Control (n=7)	8.09 ± 1.31 n=34	8.72 ± 1.34 n=29	8.63 ± 1.41 n=20	7.63 ± 1.22 n=20
	Hydrocephalus (n=8)	7.26 ± 1.01* n=31	7.05 ± 1.00** n=21	7.35 ± 1.13* n=23	5.35 ± 0.84** n=24

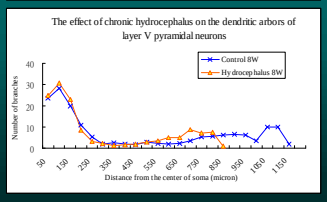
Values are means ± S.E. for the number of dendritic segments studied (n)
*, P<0.05 and **, P<0.01 between the marked and its corresponding control, 2-tailed Student's t-test.

● *Sholl's* analysis



Analysing the number of dendritic branches intersected by concentric circles of equidistance from the center of soma.





我們整理出以下之結果：

一、 急性水腦之腦壓約為正常鼠之 3 倍；慢性水腦之腦壓約為正常鼠之 1.8 倍。

二、 水腦鼠體重增加不良。

三、 腦皮質厚度隨腦室大小增加呈線性減少；而腦白質厚度隨腦室大小增加呈曲線下降（初期急劇下降、後期減緩）。顯示白質厚度在腦壓增加初期即受到影響。

四、 PGP 9.5 免疫組織化學研究（一種 neuron marker）顯示：皮質在急性與慢性水腦與對照組沒有差異。皮質 b-NOS 陽性神經元在急性水腦則有顯著減少。

五、 星狀細胞密度研究顯示：

i. 急性水腦之 layer II/III、V、VI之密度都增加。

ii. 慢性水腦則沒有差異。

六、 微膠質細胞之密度研究顯示：不論是急性或慢性水腦，皮質 layer II/III、V、VI各層與對照組都沒有差異。

七、 神經樹突之長度在慢性水腦沒有變化；但急性水腦時，樹突頂端長度變短。

八、 神經樹突棘之密度研究顯示：在急性水腦時，不論是樹突基底或樹突頂端之密度，都明顯減少；在慢性水腦亦然。

九、 Sholl's 分析顯示：慢性水腦時，樹突在距離神經細胞體遠處，分枝數下降許多。

（七）計畫成果自評部份，請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

本研究成功造成新生水腦(鼠體重增加不良)。急性水腦之腦壓約為正常鼠之 3 倍；慢性水腦之腦壓約為正常鼠之 1.8 倍，與人類之 normal pressure hydrocephalus 相仿，在慢性水腦時會因代償作用，造成腦室雖大，腦壓卻不高。而此代償作用造成的機轉，可用腦皮質

與腦白質厚度之改變速度來解釋。腦白質厚度在水腦產生初期即受到影響，但腦皮質厚度則隨腦室大小增加呈線性減少。這可說明在急性水腦時，早期臨床上便出現腦壓高（頭痛、嘔吐）的症狀，卻不太會有神經學缺損；而慢性水腦時，雖然沒有明顯腦壓高的症狀，卻反而會有神經學缺損，推論可能與累積之皮質厚度變薄有關。雖然構造上（PGP 9.5 免疫組織化學研究）顯示：皮質神經元密度在急性與慢性水腦與對照組沒有差異，我們推論，由於皮質厚度減少，總神經元數是減低的，可能因此造成功能障礙。

星狀細胞密度研究顯示：急性水腦之 layer II/III、V、VI之密度都增加。慢性水腦則沒有差異。這可能說明急性水腦時，腦皮質變薄加上星狀細胞反應造成密度增加；但慢性水腦時，則由於星狀細胞總數降低，密度反而沒有變化。

急性水腦時，樹突頂端長度變短，且不論是樹突頂端或樹突基底神經樹突棘之密度，都明顯減少；在慢性水腦時，神經樹突之長度沒有變化，但不論是樹突基底或樹突頂端之神經樹突棘之密度，都明顯減少，且慢性水腦時，樹突在距離神經細胞體遠處，分枝數下降許多。

本實驗由於研究時間不足（僅一年）無法加作 CSF diversion 對這些細胞構造有無治療效果，希望未來研究可進一步探討之。