The Behavioral Change of Anxiety Response in Kaolin-induced Hydrocephalus Rat Model

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The Behavioral Change of Anxiety Response in Kaolin-induced Hydrocephalus Rat Model

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<TABLE OF CONTENTS>

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

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Hydrocephalus is a pathological enlargement of the ventricle that results from various kinds of diseases in the central nervous system. Not only motor abnormalities such as abnormal gait and posture, but also intellectual/emotional impairment, are frequently seen in patients with hydrocephalus. The present study was designed to investigate anxiety response in the elevated plus maze in kaolin-induced hydrocephalus rats.

Cholecystokinin (CCK) and neuropeptide Y (NPY) which were known to play an important role in anxiety response were also evaluated in hydrocephalus by the immunohistochemical staining.

Hydrocephalus was induced in Sprague-Dawley rats by injection of 0.1ml volume of 20% kaolin solution into the cisterna magna (n=26).

Control rats received the same volume of saline (n=22). After kaolin injection the rats were sacrificed at 3 days, 4, 6 and 8 weeks following the elevated plus maze test, and evaluated CCK and NPY-immunoreactivity by immunohistological staining.

Hydrocephalus rats showed more entry into, and spent more time on the open arms of the elevated plus maze when compared to control rats. As well, hydrocephalus rats showed decreased motor activity for the close arms when compared to control rats. However, hydrocephalus rats did not show any decreased motor activity for the open arms compared to control animals. Compared to control rats, the numbers of CCK-immunoreactive neurons were significantly decreased and those of NPY-immunoreactive neurons were significantly increased. Also, the progress of hydrocephalus by time course in our hydrocephalus models has a negative correlation with the anxiety response.

These results suggest that decreased anxiety responses are also involved in the hydrocephalus rat models.

Key words: Anxiety response, Kaolin, Hydrocephalus, Elevated Plus Maze

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Ⅰ. INTRODUCTION

Hydrocephalus is a common neurological condition characterized by pathological dilatation of the cerebrospinal fluid (CSF)-containing spaces in the brain, usually due to obstruction of the normal flow or absorption of CSF. Hydrocephalus is a serious neurological problem and remains a common clinical problem in children. The pathophysiology of hydrocephalus-induced brain damage is multifactorial, with contributions by gradual physical stretching and compression of tissues, chronic ischemia, alterations in neurochemical function and possible

accumulation of metabolic waste products[1-3]. The large ventricles compress the surrounding parenchyma[4-6] leading, in addition to mechanical distortion, to change in the cerebral blood flow[7,8] and metabolism[9-11]. Changes in measured levels of various neurotransmitters and their metabolites in hydrocephalic brain parenchyma have been also reported[5,12-15].

Hydrocephalic children who survive without treatment usually have physical and mental disability[16]. The motor problems include gait instability[17] as well as arm and hand dysfunction[18]. More subtle memory and cognitive deficits are also observed in hydrocephalic children[19]. The basal ganglia is anatomically situated adjacent to the ventricular system and has been considered to play an important role in the initiation and control of voluntary movement[20,21]. Structural and/or functional injury in the neostriatum can read to motor functional disabilities, abnormal gait and posture and intellectual/emotional impairment, well documented in Parkinson's disease and other neurodegenerative disorders[22,23].

Although intellectual impairment is a common and important consequence of hydrocephalus[24], there is little information about its pathogenesis. Various studies have indicated that intellectual

impairment is mediated in part by alteration of neuronal innervation: the cholinergic system from the basal forebrain nuclei participates in learning and memory[25], the dopaminergic system from the ventral tegmental area and the medial part of the substantia nigra compacta participates in emotional control[26], and the noradrenergic system from the locus ceruleus and subceruleus is involved in cognition[27,28].

We previously demonstrated that learning and memory impairments in rats with kaolin-induced hydrocephalus are associated with the dysfunction of the septohippocampal cholinergic (SHC) system induced by ventricular dilatation[29].

Emotional states that would now be classified as anxiety were recognized as long ago as the classical Greek period but have undergone many phases of medical classification since then. Nowadays, 'anxiety' is a term used loosely to cover the cluster of physiological and emotional changes shared by several disorders in which anxiety is a major component[30]. The anxiety disorders are characterized by unrealistic, unfounded fear and anxiety[31].

The aim of this study is to examine the change of anxiety response in progressing of hydrocephalus using rats with kaolin-induced hydrocephalus. Therefore, the effects of anxiety response were

investigated in utilizing immunohistochemistry on CCK and NPY expression. We also measured ethologically elevated plus maze test as validity of anxiety response during hydrocephalus.

II. MATERIALS AND METHODS

1. Subjects

All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques. Male Sprague-Dawley (SD) rats (n=48), weighing 300-350 g, were used for the experiments. The animals were housed in groups of five per cage, with free access to rat chow and water. The cages were kept in a temperature and humidity-controlled room with a 12 h light-dark cycle.

The care and use of laboratory animals in this experiment were based on the Guidelines and Regulations for Use and Care of Animals in Yonsei University.

2. Kaolin-induced Hydrocephalus Model

Forty eight adult male Sprague-Dawley rats, each weighing between 300 and 350 g, were anethetized with a mixture of ketamine (75 mg/kg), acepromazine (0.75 mg/kg) and rompun (4 mg/kg). Each rat's neck was shaved and immobilized in a stereotaxic frame apparatus with neck flexed. 0.1 ml volume of 20% kaolin solution (Sigma, St. Louis, MO, USA) was injected, into the cisterna magna via the atlantooccipital dura. The same volume of sterile saline was similarly injected into the control rats. The kaolin-injected and control animals were killed at 3 day, 4, 6, and 8 week after injection.

3. Elevated Plus Maze

The elevated plus maze has been widely used to study anxiety states in rats (for example, Pellow, 1985; Lister, 1987; Gonzalez, 1998; File, 1999; Kampov-Polevoy, 2000). The apparatus was made of black-painted wood and consisted of two close arms and two open arms (50 cm long and 10 cm wide) opposite each other, with a central arena of 10

x 10 ㎠. The walls of the close arms were 49 cm high and the open arms had a 1 cm ridge along the outer 40 cm to prevent the rat from falling off. The maze was elevated 50 cm from the floor and lit by dim light. The rat was placed in the apparatus with its head in the central arena and the following measures were recorded by blind observer during a 5 min session: number of entries into the closed arms by the whole rat (measure of activity) and % time spent in the open arms by at least two forepaws (measure of anxiety)[32]. During the test, each rat was placed at the center of the maze facing one enclosed arm. All entries on open or closed arms were scored for 5min and total time spent on each arm was recorded. An entry was defined by placing at least two forepaws into an arm and no time was recorded when the animal was in the center of maze. Rats falling from the maze were excluded from the study.

4. Histology and Immunohistochemistry

Rats were deeply anaesthetized with 20% Urethane (Sigma, St Louis, MO, USA) and then transcardially perfused with 125 ml saline, followed by 250 ml of ice-cold 4% paraformaldehyde. The brain were removed from the skulls and post-fixed for 24 h at 4℃, and transferred to 30% sucrose until equilibrated. Sections were cut frozen into 20 ㎛ coronal sections and then immunoreacted with the primary rabbit anti-CCK-8 (Immunostar, Hudson, Wisconsin, USA) at a dilution of 1:200, or anti-NPY(Immunostar, Hudson, Wisconsin, USA) at a dilution of 1:1000, and then with a biotinylated goat anti-rabbit IgG (Vector Labs, Burlingame, CA, USA) secondary antibody. The signal was amplified using avidin and biotinylated horseradish peroxidase using Elite ABC Vectastatin Kit (Vector, Burlingame, CA, USA). 3, 3'-Diaminobenzidine tetrachloride dehydrate was used as a chromogen and cobalt chloride/nikel ammonium was used to intensify color changes.

The degree of ventricular dilation was studied in 20 ㎛ section stained with cresyl violet.

5. Statistical Analysis

Statistical analysis was performed with the SPSS version 11.0 statistical software package (SPSS Ins., Chicago, IL, USA). Independent or related measures t-tests were used when comparisons involved only two groups. When three or more groups were involved, analyses of variance (ANOVA) were carried out and, if significant, follow-up Scheffé's post hoc test was used to determine which pairs of groups were different from each other. The criterion for statistical significance was considered to be $P<0.05$ in all statistical evaluations.

III. RESULTS

1. Animal Model of Hydrocephalus

Hydrocephalus was induced in 26 SD rat by intracisternal injection of 0.1 ml of 20% kaolin solution. 22 control animals received the same volume of saline. The kaolin-injected and control animals were killed at 3 day (Group 1), 4 (Group 2), 6 (Group 3) and 8 week (Group 4) after injection (Table 1.).

Table 1. Experimental group

Group	Injection	
	Saline	Kaolin
Group 1(killed at 3 day)	5	5
Group 2(killed at 4 week)	5	6
Group 3(killed at 6 week)	6	7
Group 4(killed at 8 week)	6	8

Following kaolin injection, the animals showed clinical evidence of hydrocephalus; diminished activity and locomotion, incoordination, body tremor, abnormal posture with spastic extremities, and weight loss with poor appetite, compared with the control animals for first several days. All but the reduced activity were subsequently resolved in the most animals. A few with marked ventricular enlargement persistently demonstrated hind limb paraparesis with spasticity and weight loss even at group 3 or group 4 after kaolin injection. Overall, ventricular enlargement was characterized by an initial rapid enlargement until 4 weeks (Group 2), followed by a slower but steady progression at 6 (Group 3), 8 week (Group 4) (Figure 1.). The ventricles of the

hydrocephalic rats were expanded, compared with those of the control rats. The lateral ventricles were enlarged in the acute hydrocephalus (Group 1) and chronic hydrocephalus (Group 2, 3, 4) groups.

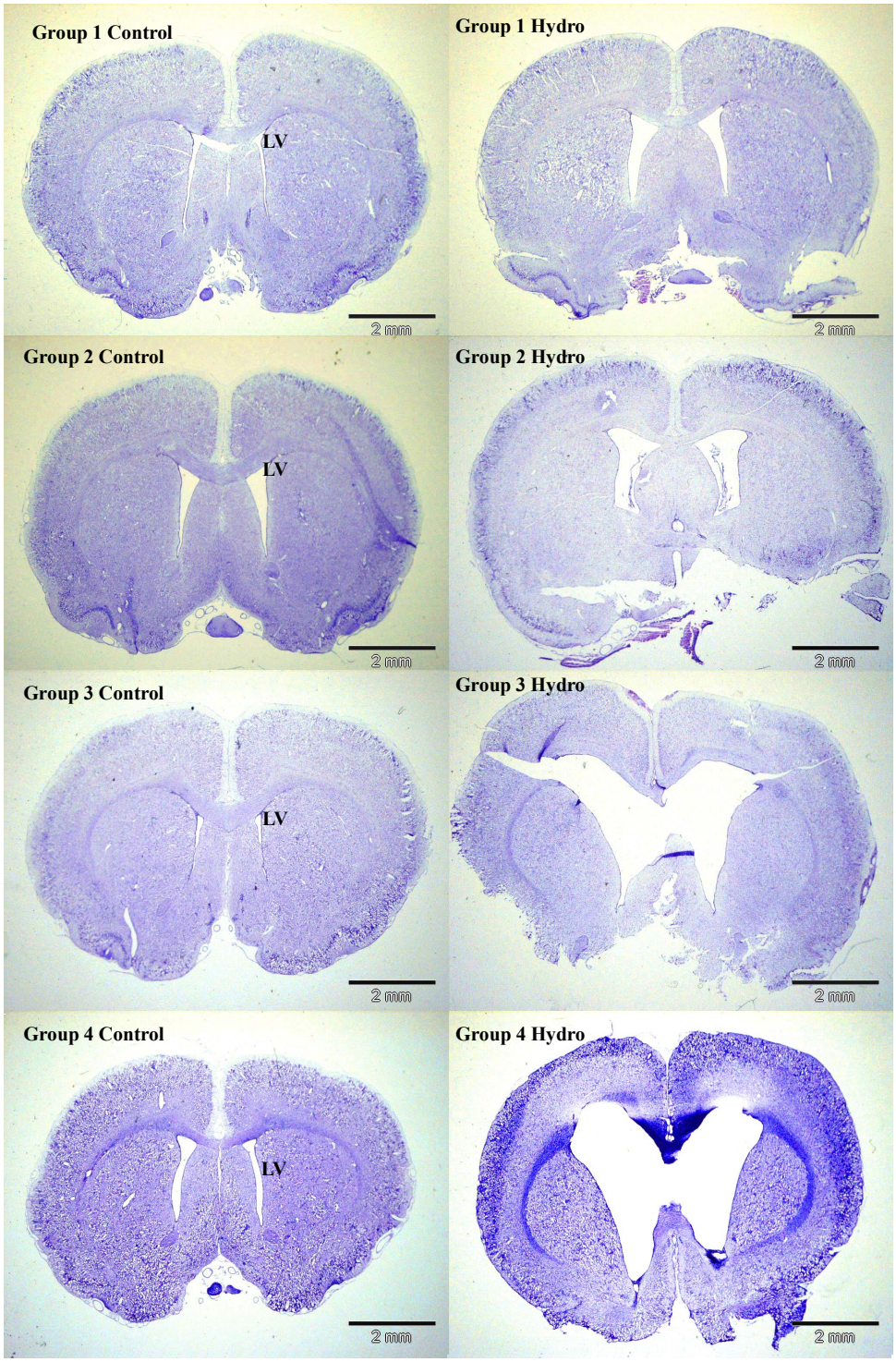
Group 1 Control
LV
2 mm
Group 1 Hydro
2 mm
Group 2 Control
LV
2 mm
Group 2 Hydro
2 mm
Group 3 Control
LV
2 mm
Group 3 Hydro
2 mm
Group 4 Control
LV
2 mm
Group 4 Hydro
2 mm

Figure 1. Nissl staining revealing the ventricular enlargement in the striatum. Left panels show the control group and right panels show the hydrocephalus (hydro) group. Ventricles of hydrocephalic rats demonstrated prominent dilation, compared with the control group, as indicated in low-resolution photographs. LV, lateral ventricle. Scale bar= 2 mm.

2. Elevated Plus Maze

The mean (± SEM) score for the behavioral parameter measured in the elevated plus maze are presented in the Table 2. The standard behavioral measures from the elevated plus maze show a typical spatial distribution with preference for the protected (closed arm and central squre) compared with the unprotected (open arms) areas of the plus maze. At the first, the hydrocephalic rat showed an increased level of anxiety on the performance of an elevated plus maze. Hydrocephalus models of group 1 and 2 made more entries into and spent more time on the open arms of the elevated plus maze when compared to their control group (Figure 2. A, B). But, in group 3 and 4, hydrocephalic rats displayed more entries and spent more time on the close arms compared to the control rats (Figure 2. C, D).

Hydrocephalic rats also showed more decreased locomotor activity than their control rats presented as measure of close arms entries (Figure 3.).

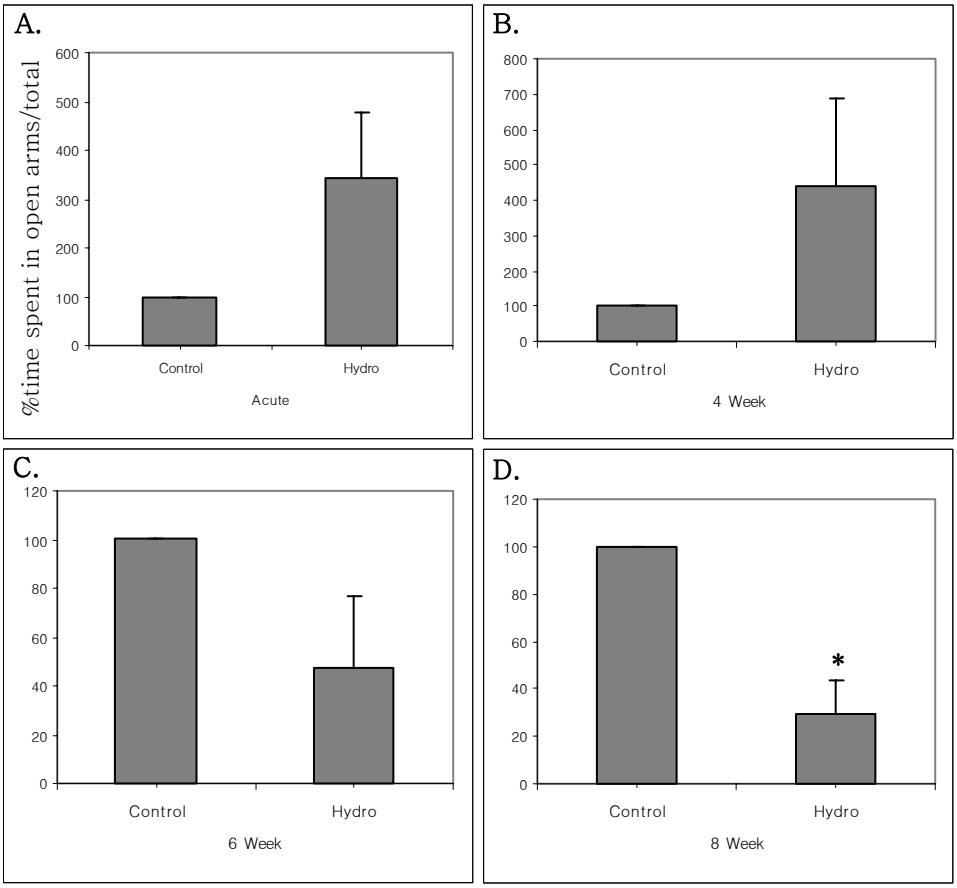

Figure 2. Time spent on open arms/total in elevated plus maze.

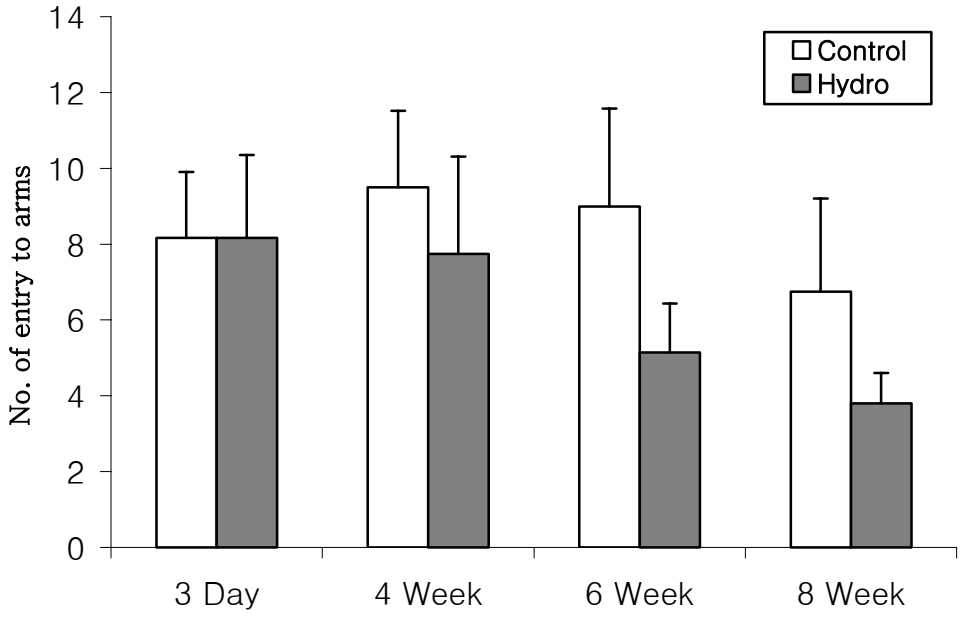

Figure 3. Number of entries in elevated plus maze.

Table 2.

Mean (± SEM) behavioral parameters measured in the elevated plus maze

	Group 1		Group 2		Group 3		Group 4	
	Control	Hydro	Control	Hydro	Control	Hydro	Control	Hydro
% Time in open arms	8.4 ± 3.7	29.1 ± 11.1	2.3 ± 1.2	10.2 ± 5.7	3.3 ± 1.1	1.5 ± 0.9	6.6 ± 5.4	1.9 ± 0.9
% Time in close arms	77.9 ± 4.9	51.1 ± 10.3 *	209.3 ± 39.4	82.2 ± 6.9 *	79.3 ± 6.3	91.2 ± 2.3	83.4 ± 7.4	91.5 ± 2.1
% time in open arms/total	100	344.7 ± 132.4	100	439.3 ± 246.7	100	47.1 ± 29.6	100	28.9 ± 14.6 *
% time in close arms/total	100	65.6 ± 13.26 *	100	39.3 ± 3.3 *	100	115.0 ± 2.9 *	100	109.7 ± 2.5 *
Total No. arm entries	8.2 ± 1.7	8.1 ± 2.2	9.5 ± 2.0	7.7 ± 2.5	9.0 ± 2.6	5.1 ± 1.3	6.0 ± 2.5	3.0 ± 0.8

Data represent the mean± SEM for five to eight rats. Group 1, killed at 3 day; Group 2, killed at 4 week; Group 3, killed at 6 week; Group 4, killed at 8 week. The values means±SEM. * P <0.05 in comparison with values from the control animals

3. CCK and NPY Immunohistochemistry

CCK-like immunoreactivity was mainly found in the periventricular hypothalamic nucleus (PE) (Figure 4. A and B), and NPY-like immunoreactivity was found in the hypothalamic areas including the arcutate nucleus (ACN) (Figure 4. C and D).

CCK-like immunoreactivity was gradually decreased in hypothalamic and thalamic areas, compared to the control in group 1, 2(Figure 5. A, B, A' and B'). It was also observed that CCK-immunoreactivity was gradually increased in group 3 and 4. and we represented the thing which CCK-immunoreactivity was increased in the group 3 and 4 against this (Figure 5. C, D, C' and D').

On the other hand, The NPY-immunoreactivity in hydrocephalic group 1 and 2 was significantly increased compared to the control group (Figure 6. A, B, A' and B'). The decrease of NPY-immunoreactivity was showed in hydrocephalic group 3, 4 (Figure 6. A, B, A' and B').

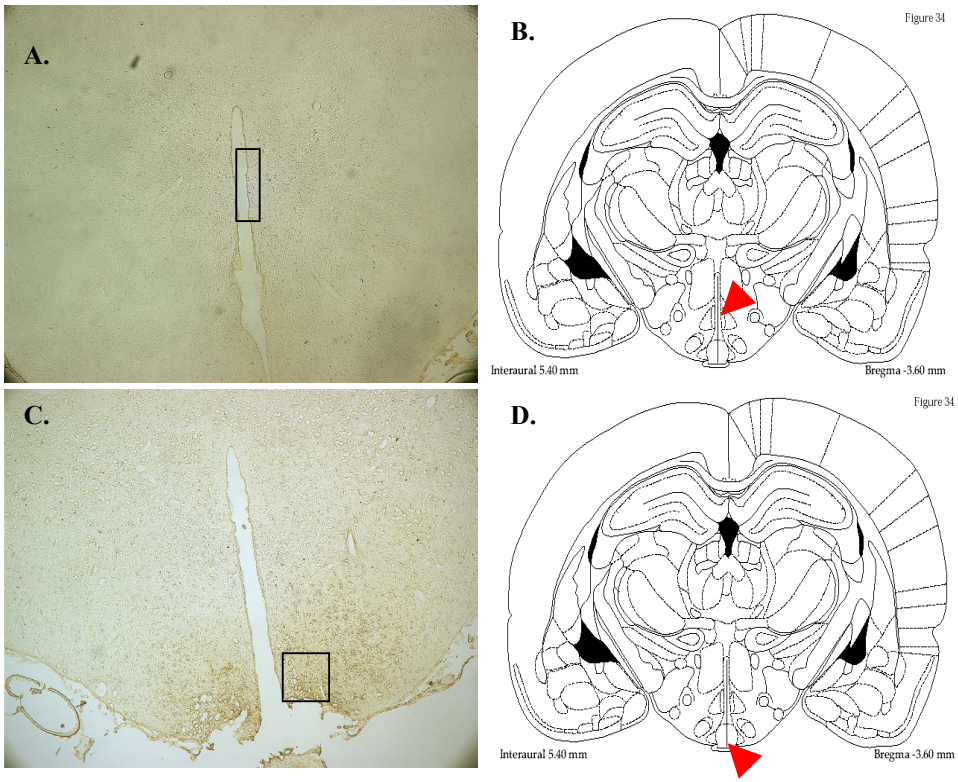

Figure 4. Immunohistochemistry of CCK and NPY. Representative photomicrographs of coronal sections showing CCK-like immunoreactivity in the PE (A and arrowhead in B), and NPY-like immunoreactivity in the ACN (C and arrowhead in D). Magnification X40. PE: the periventricular hypothalamic nucleus, ACN: the arcutate nucleus.

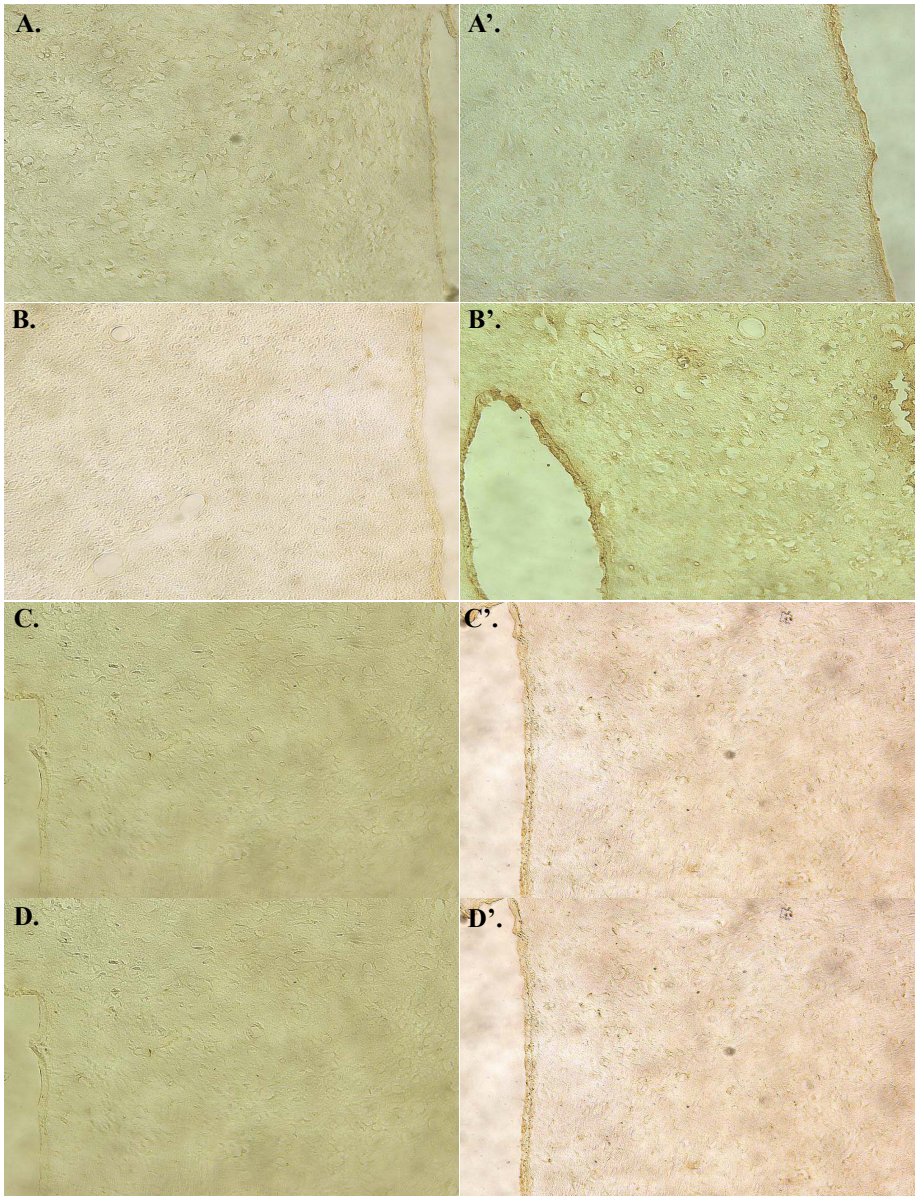

Figure 5. Representative photographs of CCK-like immunoreactivity. High-power photographs of CCK-like immunoreactivity of the hydrocephalus (A, B, C and D) and the control (A', B', C' and D') in PE of the same field illustrating the region shown in figure 4. Magnification X100. A, A': Group 1, B, B': Group 2, C, C': Group 3 and D, D': Group 4.

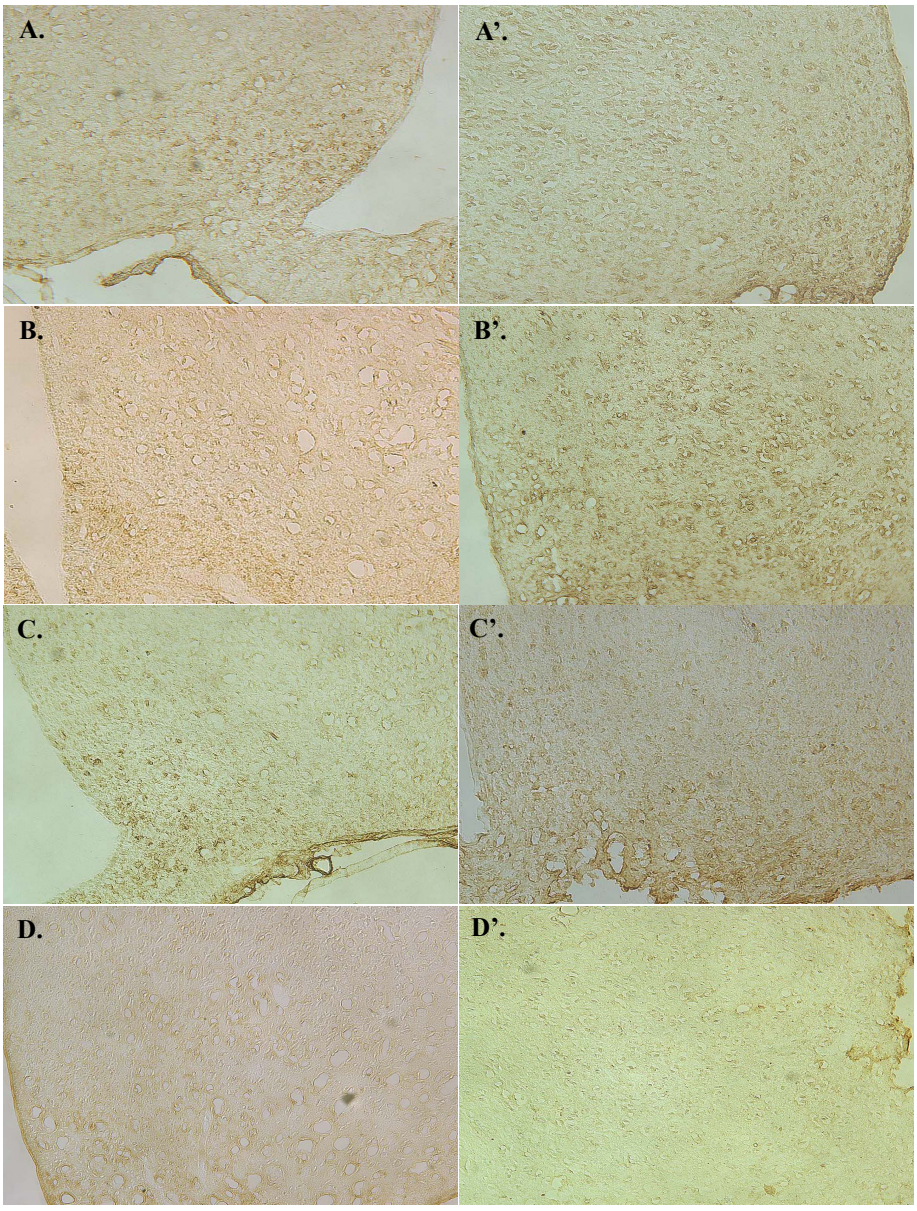

Figure 6. Representative photographs of NPY-like immunoreactivity. High-power photographs of NPY-like immunoreactivity of the hydrocephalus (A, B, C and D) and the control (A', B', C' and D') in ACN of the same field illustrating the region shown in figure 4. Magnification X100. A, A': Group 1, B, B': Group 2, C, C': Group 3 and D, D': Group 4.

IV. DISCUSSION

The goal of this study was to evaluate the effect of hydrocephalus on anxiety response, by using behavioral test and immunohistochemical techniques, and to determine whether the changes of ventricular enlargement were related to the degree of anxiety response.

Although not all the rats had a conspicuous ventricular enlargement at the first weeks after kaolin injection application[33], our results showed that the changes in ventricular size had begun in the early stages. It was observed that ventriculomegaly did not subside but continued to the late stages.

The present results demonstrate that hydrocephalic rat displayed reduced anxiety response in the elevated plus maze test at the early stages. Thus, the hydrocephalic rat made more entry into, and spent more time on the open arms/total elevated plus maze when compared to their controls. But, at the late chronic stages, hydrocephalic rat showed reversed phase in the elevated plus maze test.

In the elevated plus maze the 'total number of arm entry' is taken as the index of locomotor activity. Following ventricular enlargement total

number of arm entries was decreased in the hydrocephalic rats compared to the control rats.

The elevated plus maze is claimed to be an 'ethologically valid' animal model of anxiety because it uses 'natural stimuli' that can induce anxiety in humans. It is assumed that the open arms of the maze combine the fear of a novel, brightly-lit open space and the fear of balancing on a relatively narrow alley that afford good protection from potential predators. When a rat or mouse is allowed to explore freely the elevated plus maze for a fixed time, usually 5 min, it spend only 20-25% of its time exploring the open arms, suggesting that these assumptions are correct[34].

The recent studies have reported a progressive functional injury occurs in the cholinergic, dopaminergic, and noradrenergic systems as a result of hydrocephalus. This may contribute to the intellectual impairment. Because, the intellectual impairment has been related to alteration of neuronal innervation in the following regions: cholinergic basal fore brain nuclei(learning and memory), dopaminergic ventral tegmental area(cognition control), and noradrenergic locus ceruleus(cognition)[27].

The present results demonstrated that hydrocephalus in the early stages to 4 weeks significantly produced the decrease of CCK expression in the several hypothalamic and thalamic areas. Concomitantly, the hydrocephalic rats showed a substantial increase in NPY-like immunoreactivity throughout these areas when compared with the controls. Recent study has reported that there is an interaction between dopamine and CCK at the hypothalamic areas. For example, the injections of CCK into the paraventricular hypothalamic nucleus (PVN) significantly decreased DA release in the nucleus accumbens. It is possible that CCK in the hypothalamus inhibit the dopaminergic system, which may link its anxiogenic-like effect to a potential involvement in the development of stress-related depression through the antagonistic CCK-dopamine interaction[35].

Ⅴ. CONCLUSION

The Hydrocephalus rats showed more entries into, and spent more time on the open arms of the elevated plus maze when compared to control rats. In addition, compared to control rats, the number of CCK-immunoreactive neurons was significantly decreased and that of NPY-immunoreactive neurons was significantly increased at the early stage.

The present study clearly demonstrated that the anxiety response related with the hydrocephalus was reduced at the early stage, and increased following the late stages. These results suggest that the intellectual impairment of the hydrocephalus induces changes of the anxiety response. Further investigations are needed to clarify interaction of CCK-NPY and other neurotransmitter system.

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ABSTRACT(IN KOREAN)

카올린에 의해 유발된 뇌수종 백서 모형에서의 불안 반응의 행동학적 변화

<지도교수 박 용 구>

연세대학교 대학원 의과학과

황 용 섭

뇌수종은 중추신경계에서 여러 종류의 질병으로 인하여 병리학적으로 뇌실이 증가하는 것을 의미한다. 뇌수종 환자에서 보이는 병적 증상으로는 비정상적인 보행이나 자세와 같은 운동 이상 외에도 지력이나 감정의 손상 등이 나타난다.

본 연구는 카올린에 의해서 유발된 뇌수종 모델에서 상승덧셈미로를 이용하여 불안 반응을 행동학적으로 조사하고자 하였다. 뿐만 아니라 카올린에 의해서 유발된 뇌수종 모델에서 불안 생성 반응을 보이는 cholecystokinin (CCK)과 불안 용해 반응을 보이는 neuropeptide Y (NPY)에 대한 면역조직염색을 통해서도 밝히고자 하였다.

뇌수종은 Sprague-Dawley 쥐 모델 26 마리에서 0.1 ml 부피의 20% 카올린 현탁액을 대뇌 수조에 주입함으로써 유발되었다. 대조군 22 마리는 같은 부피의 식염수를 주입하였다. 쥐는 카올린 주사 후 3 일, 4 주, 6 주,

8 주 후에 상승덧셈미로 행동검사 실시한 후 면역조직염색을 통하여 면역 반응성을 검토해보았다.

뇌수종 쥐 모델은 대조군과 비교했을 때 상승덧셈미로에서 개방 가지로 더 많이 진입하였고, 더 많은 시간 동안 머물렀다($P<0.05$).

뿐만 아니라, 뇌수종 쥐 모델은 대조군과 비교했을 때 폐쇄 가지에서 감소된 운동 활성을 나타내었다($P<0.05$). 그러나, 뇌수종 쥐 모델은 대조군 모델과 비교하여 개방 가지에서 운동 활성의 감소는 보이진 않았다.

대조군과 비교하여서 뇌수종 쥐 모델에서의 CCK 면역 반응성을 가지는 신경세포의 수가 의미심장하게 감소하였으며, NPY 면역 반응성을 가지는 신경세포의 수는 증가되었다. 또한, 뇌수종이 진행되는 시간에 따라 불안 반응이 쥐 모델과 음성적인 관계를 가지고 있음을 보였다.

이로서 뇌수종 쥐 모델에서 초기에 불안 반응이 감소된다는 결과를 나타내었다. 이러한 결과를 근거로 뇌수종 유발 시에는 운동 기능의 손상뿐만 아니라 정신적 기능의 손상도 불러옴을 알 수가 있었다.

핵심되는 말: 불안 반응, 카올린, 뇌수종, 상승덧셈미로.