Cotton rats (*Sigmodon hispidus*) with a high prevalence of hydrocephalus without clinical symptoms

Short running title: Hydrocephalus in cotton rats

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ABSTRACT

Normal-pressure hydrocephalus (NPH) is a condition in which the ventricle is enlarged without elevated cerebrospinal fluid pressure, and it generally develops in later life and progresses slowly. A complete animal model that mimics human idiopathic NPH has not yet been established, and the onset mechanisms and detailed pathology of NPH are not fully understood. Here, we revealed a high spontaneous prevalence (34.6%) of hydrocephalus without clinical symptoms in inbred cotton rats (*Sigmodon hispidus*). In all 46 hydrocephalic cotton rats, the severity was mild or moderate and not severe. The dilation was limited to the lateral ventricles, and neither hemorrhage, ventriculitis, meningitis, or tumor formation was found in hydrocephalic cotton rats. These findings indicate that the type of hydrocephalus in cotton rats is similar to that of communicating idiopathic NPH. Histopathological examinations revealed that the inner granular and pyramidal layers (layers IV and V) of the neocortex became thinner in hydrocephalic brains. A small number of pyramidal cells were positive for Fluoro-Jade C (a degenerating neuron marker) and Iba1+ microglia were in contact with the degenerating neurons in the hydrocephalic neocortex, suggesting the possibility that hydrocephalic cotton rats are more or less impaired projections from the neocortex. This study highlights cotton rats as a candidate for novel models to elucidate the pathology of idiopathic NPH. Additionally, cotton rats have some noticeable systemic pathological phenotypes, such as chronic kidney disease and metabolic disorders; thus, they might also be useful for researching the comorbidities of NPH to other diseases.

Key words: brain ventricle, ependymal cells, neocortex, pyramidal cells, rodents

INTRODUCTION

Hydrocephalus is a clinical condition characterized by increased cerebrospinal fluid (CSF) within the cranial vault, which lowers the quality of life and can even be life-threatening. Iassacs et al. (2019) summarized 52 published studies from more than 170 million population; the incidence of hydrocephalus is 8.12/10,000 at birth and the overall global prevalence is 8.47/10,000.[1] The prevalence of hydrocephalus in children (≤ 18 years), adults (19–64 years), and elderly (≥ 65 years) is 8.78/10,000, 1.09/10,000, and 17.48/10,000, respectively,[1] indicating that both congenital and acquired are critical. The onset mechanisms of hydrocephalus can be divided into obstructive and communicating. Ventricular obstruction is the most common mechanism of congenital hydrocephalus,[2] whereas acquired hydrocephalus is caused by both obstructive factors, namely, the mass effect of tumors, and communicating factors, such as dysfunctional subarachnoid space, CSF overproduction, CSF absorption failure, and decreased venous compliance.[2]

Adults, rather than children, are more susceptible to chronic hydrocephalus in which the ventricle is enlarged with normal or low-grade elevated CSF pressure, and it generally develops in later life and progresses slowly.[3] Most chronic cases are recognized as normal-pressure hydrocephalus (NPH). NPH cases are subdivided into those with a known cause, such as meningitis, subarachnoid hemorrhage, and head trauma (secondary NPH), and those with no obvious precipitating factors (idiopathic NPH).[3,4] The latter may be associated with impaired CSF absorption, vascular diseases, and compensated congenital hydrocephalus.[4] The common clinical manifestations of NPH are progressive gait dysfunction, urinary incontinence, and cognitive impairment.[3,4] However, the onset mechanisms of idiopathic NPH, detailed NPH pathology, and the association of NPH with other symptoms are not completely understood.

Animal models of hydrocephalus can help resolve these problems. However, the spontaneous prevalence of hydrocephalus in common laboratory mice (*Mus musculus*) is less than 0.4% in the highest strain,[5] and that in common laboratory rats (*Rattus norvegicus*) is approximately 0.3%.[6] Hydrocephalus develops in some colonies, mutants, and transgenes; however, it is mostly congenital, obstructive, or fatal in these animal models,[7] except in L1CAM-deficient mice, which develop relatively mild and severe hydrocephalus in many and a few cases, respectively.[8] Gebhardt-Henrich et al. (2008) reported that about half of their stock colonies of golden hamsters (*Mesocricetus auratus*) also develop mild to severe hydrocephalus without obvious symptoms.[9] Although several laboratory models of hydrocephalus are present, a complete animal model that mimics human idiopathic NPH has not yet been established, and the accumulation of the candidates and their pathophysiological knowledge is still insufficient.

Cotton rats (*Sigmodon hispidus*) that belong to the family Cricetidae, such as hamsters, are widely distributed in the southern United States, and have been selected as laboratory animal models in research on disorders. We have reported that cotton rats have noticeable systemic pathological phenotypes, such as chronic kidney disease with anemia, metabolic disorders, and false caudal autotomy.[10-14] Additionally, these species possess unique phenotypes in endocrine, reproductive, and digestive organs,[15-18] indicating that cotton rats might be useful experimental animal models. However, the central nervous system of cotton rats has not yet been investigated. In this study, we diagnosed hydrocephalus at a high rate during routine dissections, and revealed the prevalence of non-fatal hydrocephalus and their histopathological findings in cotton rats.

MATERIALS AND METHODS

Animals

A total of 133 inbred cotton rats, maintained at the Hokkaido Institute of Public Health (HIS/Hiph), were used in the present study. Male (n = 71) and female (n = 62) cotton rats were divided into young- (0.1–6.0 months old), middle- (6.1–12.0 months old), and old-age (>12.1 months old) groups. Animal experiments were performed as per the guidelines issued by the Hokkaido Institute of Public Health (approval no. K27-03 and K30-01).

Diagnosis of hydrocephalus

The animals were euthanized by cutting the abdominal aorta under deep anesthesia using isoflurane. The heads, after the removal of the skin, eyes, and Harderian glands, were fixed using 10% neutral buffered formalin, and the brain was then removed from the cranium. The brains were cut coronally in a plane passing through the hippocampus and the arcuate hypothalamic nucleus, and hydrocephalus was diagnosed if a minimum of one lateral ventricle was visible to the naked eye. The severity of hydrocephalus was evaluated according to a previous study on hamsters,[9] and the brains were diagnosed as being normal, mild hydrocephalus (if only the temporal region of the lateral ventricle was dilated), moderate hydrocephalus (if parietal and temporal regions of the lateral ventricle were dilated), or severe hydrocephalus (if edematous dilatation of the brain was detected from the appearance).

Histopathology

After diagnosis, a total of 60 brains (37 normal brains, and 14 mild and nine brains with mild and moderate hydrocephalus, respectively) were embedded in paraffin using

standard procedures and sliced coronally into 5-μm thick sections. The sections containing the hippocampus and arcuate nucleus were deparaffinized and stained using hematoxylin and eosin to perform a preliminary evaluation of the histopathology of hydrocephalus. Sections from six hydrocephalus-affected brains that exhibited the most representative pathological findings, in addition to six normal brains, were further analyzed in detail. Based on the preliminary results, the primary somatosensory cortex was evaluated to confirm the effects of hydrocephalus on the neocortex. Degenerating neurons were detected using Fluoro-Jade C staining according to the manufacturer's instructions (Biosensis, Thebarton, Australia). Briefly, the deparaffinized sections were incubated in a potassium permanganate solution for 10 min, and a Fluoro-Jade C solution containing DAPI for 10 min under dark conditions. The slides were dried at 45°C and directly coverslipped with non-aqueous mounting media.

Immunohistochemistry and immunofluorescence

The antibodies used for immunohistochemistry are shown in Table 1. For immunohistochemistry, the deparaffinized sections were boiled in 0.01 M citrate buffer (pH 6.0) using a microwave, for four times at 5 min intervals, treated using 0.3% hydrogen peroxidase/methanol solution for 30 min to eliminate endogenous peroxidase, blocked using 10% normal rabbit serum (Nichirei, Tokyo, Japan) or 10% normal goat serum (Nichirei) for 30 min, followed by incubation with primary antibodies overnight at 4°C. Next, the sections were treated with appropriate secondary antibodies for 30 min at approximately 25°C, followed by treatment with streptavidin-peroxidase (Nichirei) for 30 min at approximately 25°C. The immunopositive reactions were developed using a 3,3'-diaminobenzidine tetrahydrochloride-H_2O_2 solution. The sections were

155 counterstained using hematoxylin.

156 For immunofluorescence, the deparaffinized sections were boiled in 0.01 M citrate
157 buffer (pH 6.0) as aforementioned, blocked with 2.5% normal horse serum (Vector
158 Laboratories, CA, USA) for 30 min, and incubated using anti-Iba1 antibody (rabbit
159 polyclonal; Fujifilm Wako, Osaka, Japan; 1:3000) and anti-neurofilament heavy chain
160 antibody (mouse monoclonal, clone RMdO 20; Cell Signaling, MA USA; 1:2000)
161 overnight at 4 °C. Next, the sections were treated with DyLight 594 labeled anti-rabbit
162 IgG and DyLight 488 labeled anti-mouse IgG cocktail (prediluted, Vector Laboratories)
163 for 30 min at approximately 25°C, and coverslipped with DAPI Fluoromount-G
164 (SouthernBiotech, AL, USA). The sections were observed using an all-in-one
165 fluorescence microscope (BZ-X800, Keyence, Osaka, Japan).

166

167 **Statistical analysis**

168 The prevalence of hydrocephalus was analyzed using the likelihood ratio test.

169

170 **RESULTS**

171 **Prevalence of hydrocephalus**

172 Of the 133 cotton rats, 46 were diagnosed with hydrocephalus (prevalence: 34.6%).
173 The prevalence of hydrocephalus was not different between males and females (Fig. 1A
174 and B) and among young-, middle-, and old-aged groups (Fig. 1C). Tables 2 and 3 show
175 the severity of hydrocephalus. Neither sex differences nor age-dependent changes were
176 found in the severity of hydrocephalus in cotton rats (Tables 2 and 3).

177

178 **Gross findings**

No cotton rats were diagnosed with severe hydrocephalus, unlike the report of Gebhardt-Henrich et al. (2008).[9] The size of the telencephalon with moderate hydrocephalus was larger than that of the same sex and age group (Fig. 2A). Most of the bilateral hydrocephalus had moderate dilation of lateral ventricles, and unilateral hydrocephalus often had mild dilation (Fig. 2B). In contrast, no clear dilation of the third and fourth ventricles and cerebral aqueduct was found in hydrocephalus (Fig. 2B-D).

Histopathological findings

In normal cotton rats, histological components on the anterior-posterior axis of the brain were similar to those in the rat brain.[19] The neocortex, hippocampus, piriform cortex, thalamus, and hypothalamus were identified in coronal sections in plane containing the arcuate nucleus in the cotton rat brain (Fig. 3A). The shape of the hippocampus may have been slightly affected by hydrocephalus (Fig. 3B); however, obvious lesions due to hydrocephalus were observed in the neocortex of cotton rats (Fig. 3B), which was remarkably thinner, and had an increased cell density depending on the severity (Fig. 3B, 4A). The primary somatosensory cortex of cotton rats can be divided into five layers: the molecular (I), outer granular and pyramidal (II/III), inner granular (IV), inner pyramidal (V), and polymorphous cell (VI) layers. In all 23 cases of hydrocephalus in 60 neocortices, the thickness of layers I, II/III, and VI remained relatively normal, whereas the inner granular and pyramidal layers (layers IV and V) were thinner (Fig. 4A). The white matter tended to be diffusely vacuolized in the areas adjacent to dilated ventricles in hydrocephalic brains (Fig. 4A). The morphology of pyramidal cells was distorted in layer V of the moderate hydrocephalic neocortex (Fig. 4B). Fluoro-Jade C staining revealed no apparent differences between normal and hydrocephalic neocortex at low magnification

(Fig. 5A). However, at higher magnification, cotton rats with moderate hydrocephalus exhibited a small number of positive reactions for both Fluoro-Jade C and DAPI in the degenerating neurons of the neocortex (Fig. 5B). In particular, several Fluoro-Jade C-positive neurons were accompanied by other cells with small nuclei in the vacuolar structures (Fig. 5C), and the type of the cells attached to degenerating neurons was evaluated. Double immunofluorescence revealed Iba1+ microglia in contact with the neurofilament heavy chain+ degenerating neurons (Fig. 5C).

The MBP+ myelin sheath was dense in animals with moderate hydrocephalus due to thinning of the neocortex (Fig. 6A). Conversely, GFAP+ astrocytes in the neocortex revealed no differences between normal and moderate hydrocephalic cotton rats (Fig. 6B). Iba1+ microglia had larger cell bodies in animals with moderate hydrocephalus than in normal animals, and some microglia infiltrated into the vacuolar structures of the neocortex (Fig. 6B).

Hemorrhage, ventriculitis, meningitis, and tumor formation were not observed in the brain of any of the 60 cotton rats used for histopathological examination (Figs. 3 and 4). In the normal brain of cotton rats, ependymal cells were aligned in a single layer and expressed vimentin (Fig. 7A). At the apex, the ependymal cells had relatively low densities and longer cilia (Fig. 7A). Cotton rats with moderate hydrocephalus had thinner ependymal cells in a single layer than did the normal animals, while vimentin expression and cilia did not differ between normal and hydrocephalic brains (Fig. 7A). In the normal brain of cotton rats, the epithelial cells of the choroid plexus were aligned in a single layer and expressed cytokeratin 8 (Fig. 7B). In cotton rats with moderate hydrocephalus, the size of the epithelial cells was slightly more variable than in the normal animals; however, the expression of cytoskeleton 8 did not differ between normal and hydrocephalic brains

(Fig. 7B).

DISCUSSION

The present study revealed a high spontaneous prevalence of hydrocephalus without clinical symptoms in inbred cotton rats. The prevalence is much higher in cotton rats (34.6%) than in mice (highest strain, less than 0.4%) and common laboratory rats (approximately 0.3%).[5,6] Cotton rats belong to the family Cricetidae, not Muridae, such as laboratory mice or common rats. A colony of golden hamsters, which also belong to Cricetidae, developed a simple recessive fatal hydrocephalus.[20] In another golden hamster colony, a high prevalence (52.0%) of mild to severe hydrocephalus with no apparent symptoms has been reported.[4,21] Additionally, hydrocephalus was experimentally induced with several pathogens in golden hamsters,[22-24] indicating these species are prone to spontaneously-occurring hydrocephalus. These findings support the proposal that cotton rats belonging to the same Cricetidae family as hamsters are suitable for hydrocephalus models.

The severity of hydrocephalus in normally born cotton rats was limited to mild or moderate, but not severe, and obstruction, hemorrhages, ventriculitis, meningitis, or tumor formation were not found in the hydrocephalic brain. Considering the absence of major abnormalities, the type of hydrocephalus in cotton rats is similar to that in communicating idiopathic NPH.[3,4] The spontaneous prevalence of hydrocephalus was similar for all sex and age groups, indicating that cotton rats are a species with a predisposition to idiopathic NPH. Although hydrocephalus develops at a high rate in some mice and rat colonies, mutants, and transgenes, such as Aqp4-null or ciliary motility-associated protein-deficient, it is mostly congenital, obstructive, and fatal.[7,25-27] Although

mild hydrocephalus may develop in an L1CAM-deficient mouse strain,[28] this also may not completely reproduce the pathology of idiopathic NPH, because L1CAM is known as a key factor of X-linked hydrocephalus that is congenital, hereditary, and obstructive in humans.[7,29] Collectively, complete animal models mimicking human NPH have not yet been established; however, this study proposes cotton rats as a candidate for a new model of idiopathic NPH.

The present study revealed the histopathological features of the hydrocephalic brain in cotton rats. Mild and moderate hydrocephalus mostly affected the inner granular and pyramidal layers (IV and V) of the neocortex (these layers were thinner depending on the severity), in addition to the potential for edematous changes in the white matter. Although MBP+ myelin sheaths appeared to be rich in hydrocephalic cotton rats, it might be caused simply by the cortical compression due to the ventricular enlargement. However, the cell bodies of some pyramidal cells, which are the main projection neurons in the neocortex, were morphologically abnormal, and a small number of them were positive for Fluoro-Jade C in cotton rats with moderate hydrocephalus, indicating neurodegeneration. Additionally, a number of Iba1+ microglia surrounded the nerve fibers in the hydrocephalic neocortex, although we could not determine whether the role of these microglia was damaging or repairing in nature. Microglial changes are caused by various neuroinflammatory diseases, and in the case of multiple sclerosis, several types of microglia (or infiltrating monocytes) abnormally surround the axon, wrapping the axon initial segment of neurons and appearing to be in contact with the neuronal cell bodies.[30] The present findings indicating that some microglia contact the degenerating neurons in the vacuolar structures might also reflect a similar change in microglial-neuronal contact in the hydrocephalic neocortex. This suggests the possibility of the brain of hydrocephalic

cotton rats having more or less impaired projections from the neocortex. In hydrocephalus induced by kaolin injection in the rat, the alteration of pyramidal cells in the somatosensory cortex, as well as the hippocampus, is associated with learning and memory deficits.[31] In future studies, behavioral tests are expected to compare the learning and memory capabilities, as well as detailed gait behavior of normal and hydrocephalic cotton rats.

As described above, obstruction was not found in the ventricles or aqueduct of hydrocephalic cotton rats. In addition, no abnormalities leading to ventricular obstruction, such as hemorrhage, ventriculitis, meningitis, and tumor formation, were found in cotton rats, indicating the communicating type of hydrocephalus. To verify the direct cause of hydrocephalus in cotton rats, we evaluated the ependymal cells contributing to the movement and absorption of CFS and the choroid plexus producing CFS. It is well known that the functional abnormality of either of the ependymal cells or choroid plexus causes communicating hydrocephalus.[2] The choroid plexus was well developed in all animals studied, and there was no difference between normal and hydrocephalic cotton rats. The ependymal cells in moderate hydrocephalic brains were flatter than those in normal brains, and thus the function of ependymal cells might be affected by hydrocephalus. However, the cilia of ependymal cells, which are critical structure for moving the CFS,[32] were normal in hydrocephalic cotton rats. Thus, factors that cause hydrocephalus in cotton rats seem communicated rather than obstructive; however, further studies on the expressed molecule, like aquaporins, in normal and hydrocephalic brains are needed to determine the exact causes of this type of hydrocephalus.

Although the common clinical symptoms of NPH are progressive gait dysfunction, urinary incontinence, and cognitive impairment,[3,4] the onset mechanisms and association

of NPH with other symptoms are not fully understood. A recent cohort study has revealed that hypertension and type 2 diabetes mellitus are common comorbidities in idiopathic NPH, and type 2 diabetes mellitus increases mortality in idiopathic patients with NPH,[33] urging the need for a model of comorbidities. In addition to NPH, cotton rats have noticeable unique phenotypes in urinary, circulatory, reproductive, digestive, and integumentary systems, including systemic pathological phenotypes, such as chronic kidney disease and metabolic disorders.[10-14] Thus, cotton rats might also be useful for researching the comorbidities of NPH in other diseases. In conclusion, cotton rats may serve as a novel and useful model for elucidating the pathology of hydrocephalus, particularly idiopathic NPH.

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DISCLOSURE

Authors declare no Conflict of Interests for this article.

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398

Figure Legends

Figure 1. Prevalence of hydrocephalus in male (A) and female (B) cotton rats and comparison among different age groups (C).

Figure 2. Gross findings in hydrocephalus in cotton rats.

(A) Dorsal view of moderate hydrocephalic brain (right; 11.5-month-old female) and normal brain (left; 14.5-month-old female). (B) Coronal slice of moderate (upper) and mild (middle) hydrocephalic brains and normal brain (lower) in a plane passing through the hippocampus and the arcuate hypothalamic nucleus. *Dilated lateral ventricle; arrows, third ventricle. (C and D) Coronal slices of moderate hydrocephalic brain (uppers) and normal brain (lowers) in a plane containing the cerebral aqueduct (C; arrows) and the fourth ventricle (D; arrows). Scale bars: 10 mm.

Figure 3. Coronal image of the cotton rat brain at a level of the arcuate nucleus.

(A) Whole image in the normal brain. *Lateral ventricle. Arrows and arrowheads indicate the third ventricle and choroid plexus, respectively. Hi, hippocampus; HT, hypothalamus; NC, neocortex; PC, piriform cortex; Th, thalamus. (B) High magnified images of the neocortex and hippocampus in normal and moderate hydrocephalic brains. Hematoxylin-eosin stain. Scale bars: 2 mm (A), 1 mm (B).

Figure 4. Histopathological findings in the neocortex of hydrocephalic cotton rats.

(A) Layer structures of the primary somatosensory cortex in normal brain and mild and moderate hydrocephalic brains. Layers I, molecular; II/III, outer granule and pyramidal; IV, inner granule; V, inner pyramidal; VI, polymorphous cell layers. *Lateral ventricle.

(B) High magnified images of the layer V. Hematoxylin-eosin stain. Scale bars: 100 μm (A), 20 μm (B).

Figure 5. Degenerating neurons in the neocortex of hydrocephalic cotton rats.

(A) Lower magnification of the primary somatosensory cortex in a normal brain and a moderate hydrocephalic brain. Fluoro-Jade C stain. (B) Higher magnification image of boxed area in panel (A). Arrows indicate positive reactions for both Fluoro-Jade C and DAPI. (C) Microglia in contact with degenerating neurons in hydrocephalic brains. Left, a Fluoro-Jade C-positive degenerating neuron (arrow) and a small cell attached to it (arrowhead). Right, colocalization (arrow) of neurofilament heavy chain (NF-H) and Iba1 in the vacuole. Scale bars: 100 μm (A), 20 μm (B, C).

Figure 6. Immunohistochemistry for brain-specific cells in normal and hydrocephalic cotton rat brains.

(A) The primary somatosensory cortex with immunohistochemistry for myelin basic protein (MBP). Boxes in upper correspond to regions indicated in lower. (B) Immunoreaction for glial fibrillary acidic protein (GFAP) and ionized calcium-binding adaptor molecule 1 (Iba1). Iba1+ microglia appear larger in moderate hydrocephalic brain (arrowheads) and localize adjacent to the vacuole (arrows). Scale bars: 100 μm (uppers in A), 20 μm (lowers in A, B).

Figure 7. Ependymal cells and choroid plexus in normal and hydrocephalic cotton rat brains.

(A) Histological findings (uppers; hematoxylin-eosin stain) and immunoreactivity for

447 vimentin and α-tubulin of the ependymal cells. (B) Histological findings (uppers;
448 hematoxylin-eosin stain) and immunoreactivity for cytokeratin 8 of the choroid plexus.
449 Bars = 20 μm (A, lowers in B) and 100 μm (uppers in B).

Table 1. Summary of immunohistochemistry conditions

Antigen	Primary antibody	Secondary antibody
Myelin basic protein (MBP)	Mouse monoclonal (clone SMI94; BioLegend, CA, USA; 1:100)	Rabbit anti-mouse IgG+IgA+IgM antibody (Nichirei)
Glial fibrillary acidic protein (GFAP)	Mouse monoclonal (clone 6F2; Diagnostic BioSystems, CA, USA; 1:100)	Rabbit anti-mouse IgG+IgA+IgM antibody (Nichirei)
Ionized calcium binding adaptor molecule 1 (Iba1)	Rabbit polyclonal (cat. # 013-27691; Fujifilm Wako, Osaka, Japan; 1:3000)	Goat anti-rabbit IgG antibody (cat. #: 426012, prediluted, Nichirei),
Vimentin	Rabbit monoclonal (clone D21H3; Cell Signaling, MA USA; 1:1000)	Goat anti-rabbit IgG antibody (Nichirei)
α-tubulin	Mouse monoclonal (clone DM1A; Thermo Fisher Scientific, MA, USA; prediluted)	Rabbit anti-mouse IgG+IgA+IgM antibody (Nichirei)
Cytokeratin 8	Mouse monoclonal (clone 1E8, BioLegend; 1:300)	Rabbit anti-mouse IgG+IgA+IgM antibody (Nichirei)

Table 2. Prevalence of hydrocephalus according to sex

	Total (n=133)	Female (n=62)	Male (n=71)
Normal	65.4% (87)	61.3% (38)	69.0% (49)
Hydrocephalus			
Mild	15.0% (20)	14.5% (9)	15.5% (11)
Moderate	19.5% (26)	24.2% (15)	15.5% (11)
Severe	0% (0)	0% (0)	0% (0)
P-value[†]		0.2749	

[†]*P*-values were obtained from the likelihood ratio test for sex differences in the severity of hydrocephalus.

Table 3. Age-related changes regarding prevalence of hydrocephalus

	Total (n=133)	0.1–6.0 months (n=45)	6.1–12.0 months (n=56)	>12.1 months (n=32)
Normal	65.4% (87)	62.2% (28)	66.1% (37)	68.7% (22)
Hydrocephalus				
Mild	15.0% (20)	15.6% (7)	10.7% (6)	21.9% (7)
Moderate	19.5% (26)	22.2% (10)	23.2% (13)	9.4% (3)
Severe	0% (0)	0% (0)	0% (0)	0% (0)
P-value[†]			0.6833	

[†]*P*-values were obtained from the likelihood ratio test for age-related changes in the severity of hydrocephalus.

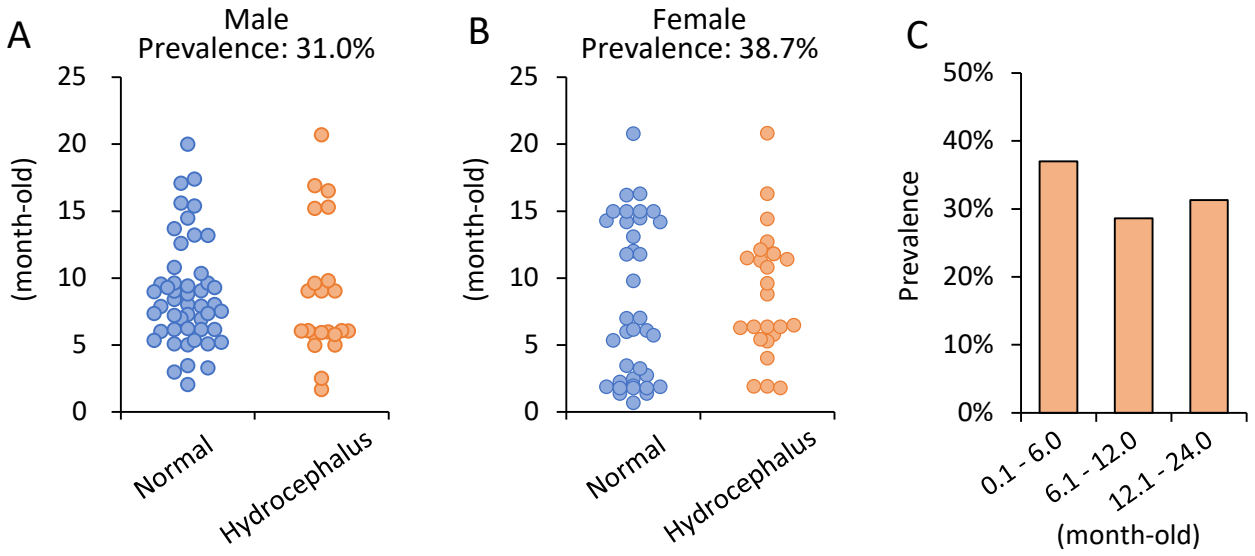
A
Male
Prevalence: 31.0%
(month-old)
25
20
15
10
5
0
Normal
Hydrocephalus
B
Female
Prevalence: 38.7%
(month-old)
25
20
15
10
5
0
Normal
Hydrocephalus
C
Prevalence
50%
40%
30%
20%
10%
0%
0.1 - 6.0
6.1 - 12.0
12.1 - 24.0
(month-old)

Fig. 1

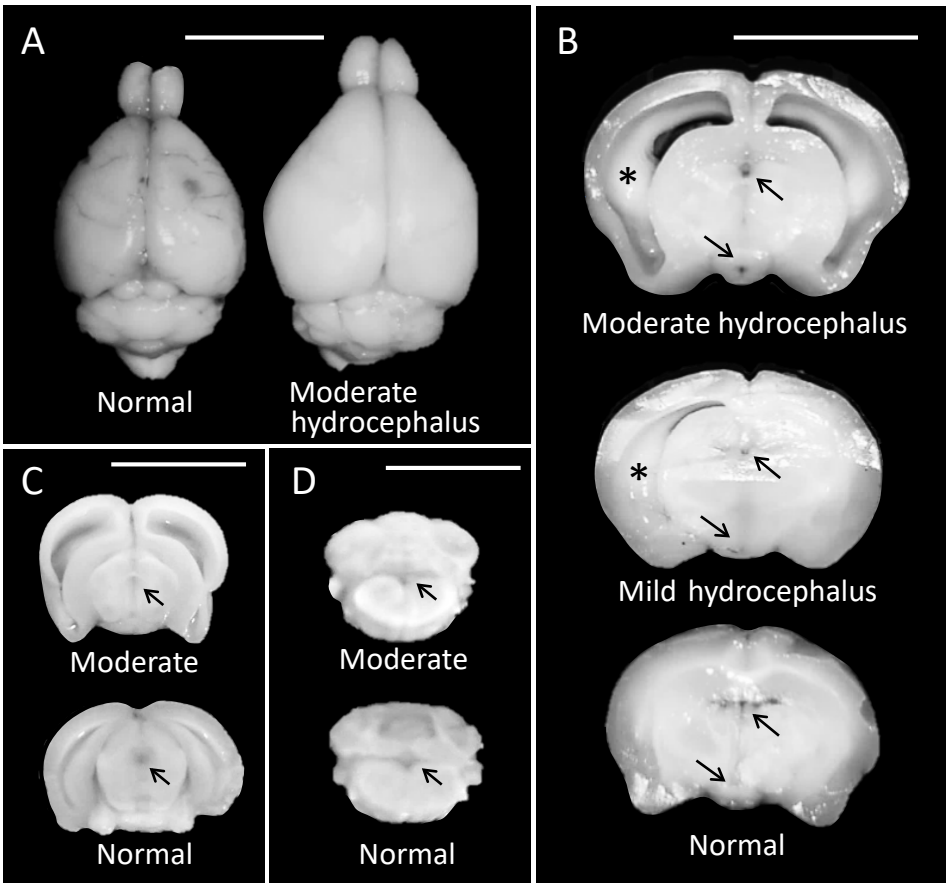
A
Normal
Moderate hydrocephalus
B
Moderate hydrocephalus
Mild hydrocephalus
Normal
C
Moderate
Normal
D
Moderate
Normal

Fig. 2

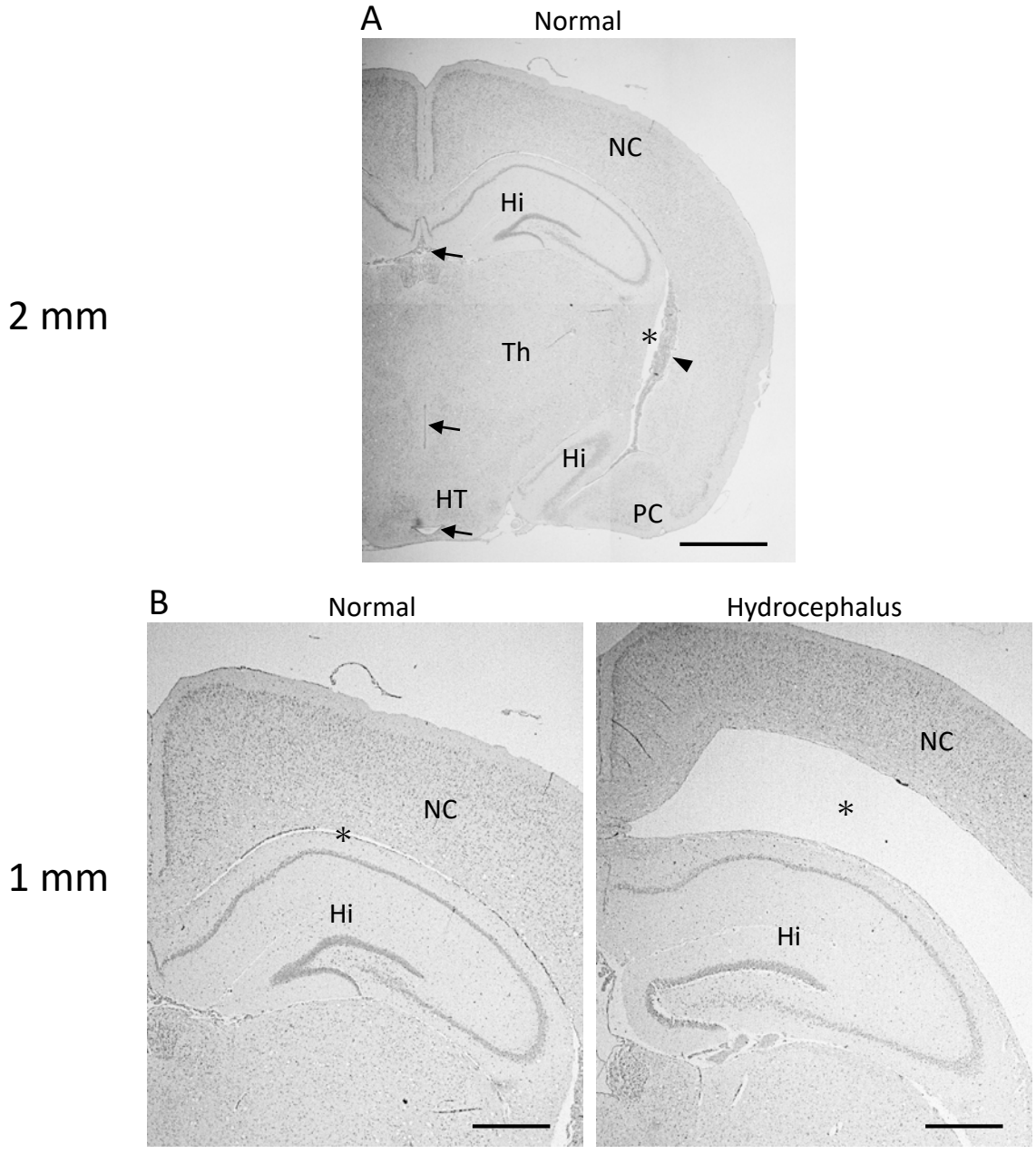
A
Normal
NC
Hi
Th
Hi
HT
PC
2 mm
B
Normal
Hydrocephalus
NC
Hi
NC
Hi
1 mm

Fig. 3

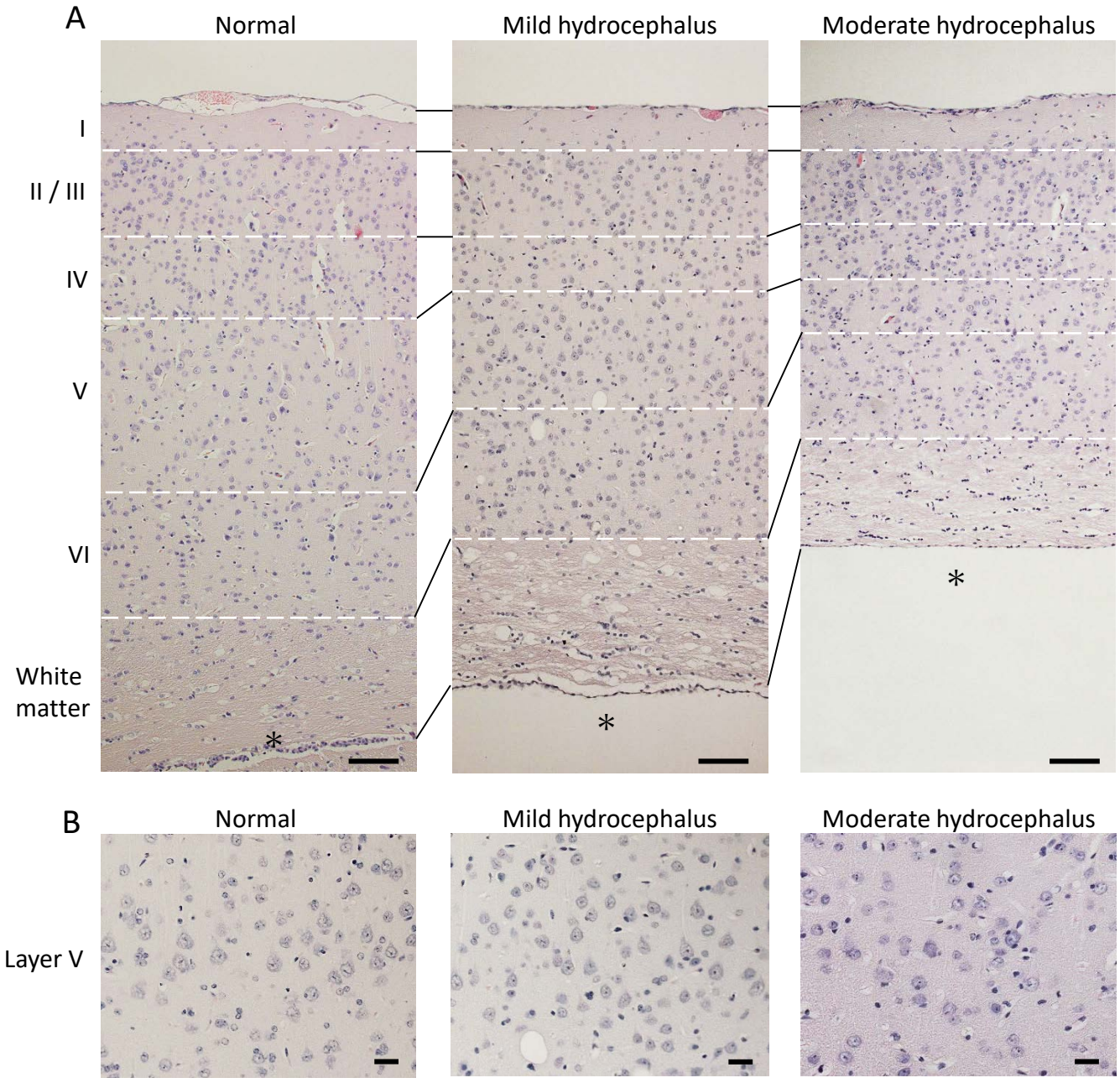
A
Normal
Mild hydrocephalus
Moderate hydrocephalus
I
II / III
IV
V
VI
White matter
*
*
*
B
Normal
Mild hydrocephalus
Moderate hydrocephalus
Layer V

Fig. 4

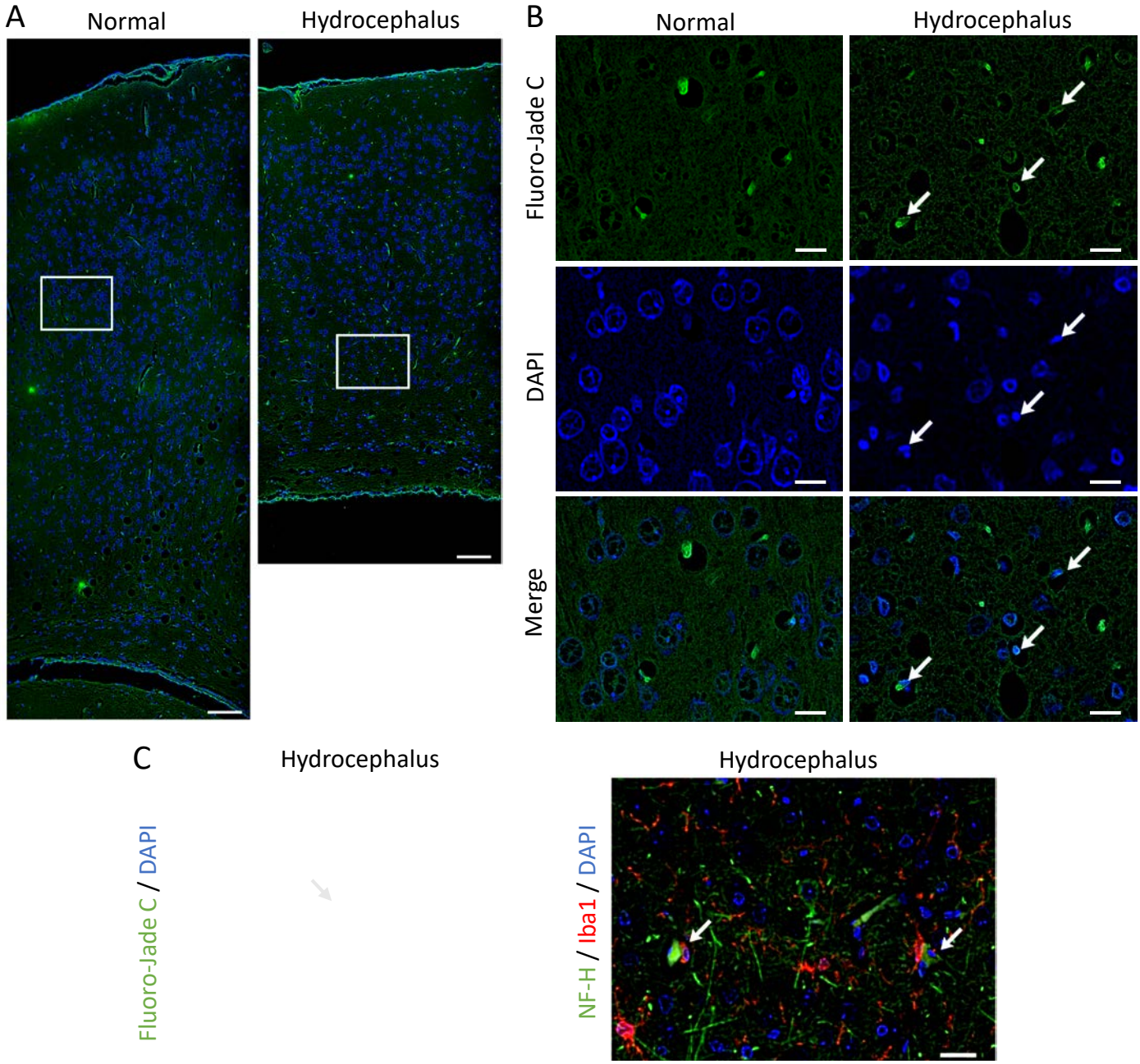
A
Normal
Hydrocephalus
B
Normal
Hydrocephalus
Fluoro-Jade C
DAPI
Merge
C
Hydrocephalus
Fluoro-Jade C / DAPI
Hydrocephalus
NF-H / Iba1 / DAPI

Fig. 5

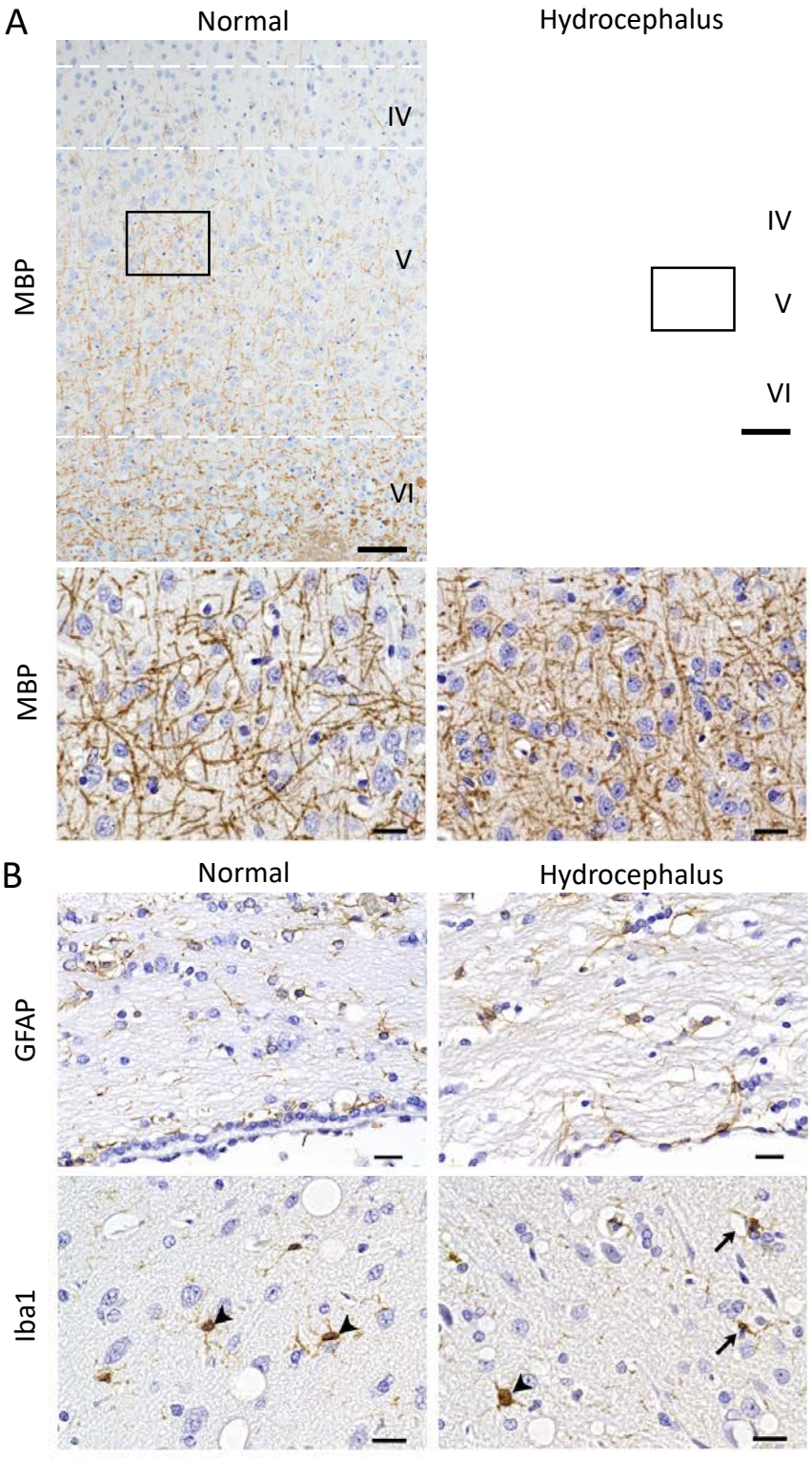
A
Normal
Hydrocephalus
IV
V
VI
MBP
MBP
B
Normal
Hydrocephalus
GFAP
Iba1

Fig. 6

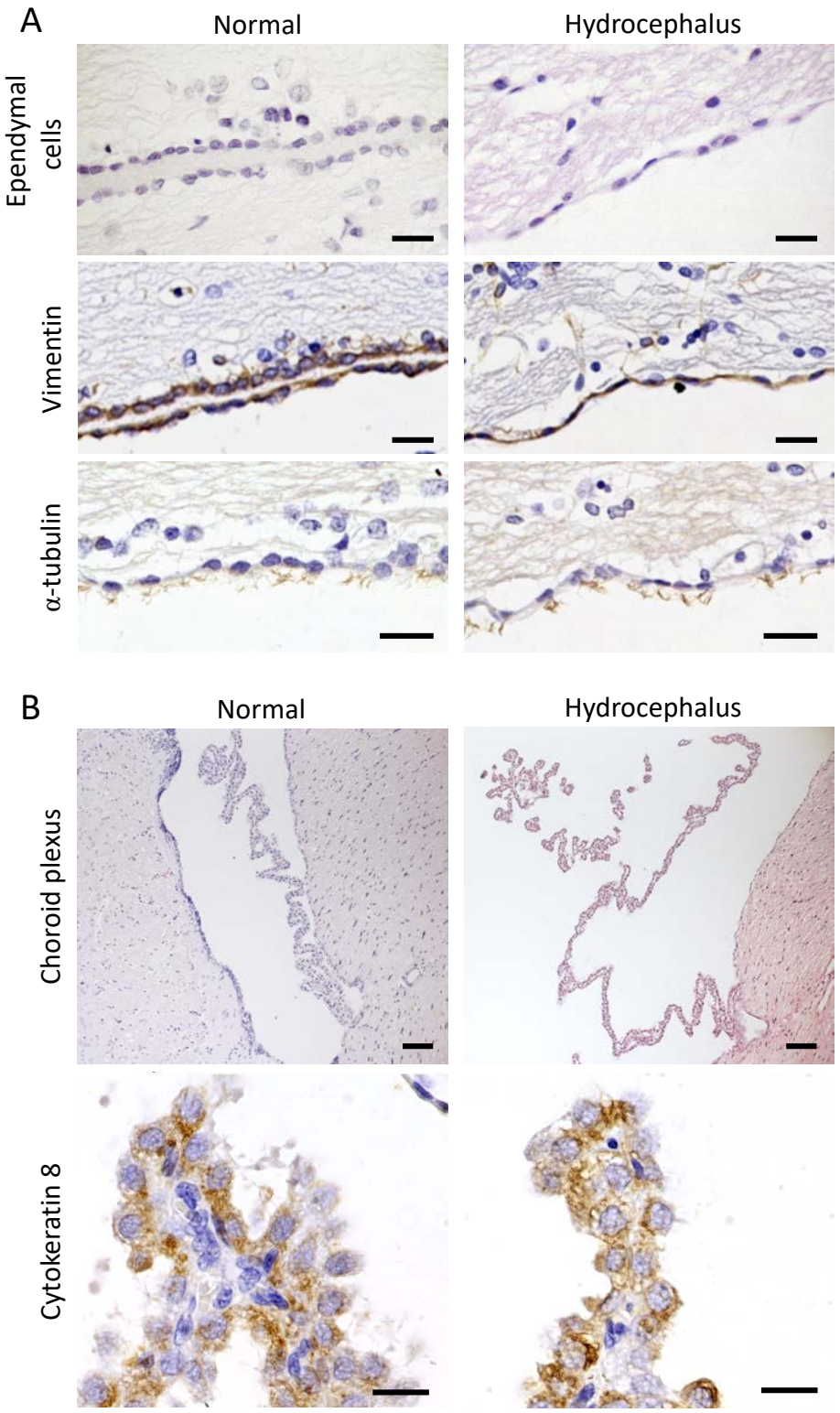
A
Normal
Hydrocephalus
Ependymal cells
Vimentin
α-tubulin
B
Normal
Hydrocephalus
Choroid plexus
Cytokeratin 8

Fig. 7