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Long-term hydrocephalus alters the cytoarchitecture of the adult subventricular zone

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Abstract

Hydrocephalus can develop secondarily to a disturbance in production, flow and/or absorption of cerebrospinal fluid. Experimental models of hydrocephalus, especially subacute and chronic hydrocephalus, are few and limited, and the effects of hydrocephalus on the subventricular zone are unclear. The aim of this study was to analyze the effects of long-term obstructive hydrocephalus on the subventricular zone, which is the neurogenic niche lining the lateral ventricles. We developed a new method to induce hydrocephalus by obstructing the aqueduct of Sylvius in the mouse brain, thus simulating aqueductal stenosis in humans. In 120-day-old rodents ($n = 18$ per group), the degree of ventricular dilatation and cellular composition of the subventricular zone were studied by immunofluorescence and transmission electron microscopy. In adult patients (age > 18 years), the sizes of the subventricular zone, corpus callosum, and internal capsule were analyzed by magnetic resonance images obtained from patients with and without aqueductal stenosis ($n=25$ per group). Mice with 60-day hydrocephalus had a reduced number of Ki67+ and doublecortin+ cells on immunofluorescence, as well as decreased number of neural progenitors and neuroblasts in the subventricular zone on electron microscopy analysis as compared to non-hydrocephalic mice. Remarkably, a number of extracellular matrix structures (fractones) contacting the ventricular lumen and blood vessels were also observed around the subventricular zone in mice with hydrocephalus. In humans, the widths of the subventricular zone, corpus callosum, and internal capsule in patients with aqueductal stenosis were significantly

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smaller than age and gender-matched patients without aqueductal stenosis. In summary, supratentorial hydrocephalus reduces the proliferation rate of neural progenitors and modifies the cytoarchitecture and extracellular matrix compounds of the subventricular zone. In humans, this similar process reduces the subventricular niche as well as the width of corpus callosum and internal capsule.

Keywords

Subventricular zone; neural stem cells; hydrocephalus; aqueductal stenosis; neurogenesis; fractones; human brain

1. Introduction

Hydrocephalus is a neurological condition produced by disturbances in the production, flow, and/or absorption of cerebrospinal fluid (**CSF**), which results in dilation of the ventricular system. According to its time course, hydrocephalus in humans can be classified as acute, sub-acute, or chronic. Acute hydrocephalus occurs over days, subacute hydrocephalus over weeks, and chronic hydrocephalus over months to years. Hydrocephalus has an estimated incidence of 1 in 1500 births and is associated with multifocal motor deteriorations and cognitive decline (Fernandez-Carrocer and Gonzalez-Mora, 2004; Gallia et al., 2006; Zhang et al., 2006). The management of hydrocephalus in both young and older patients remains important because CSF hydrodynamics play a key role in supporting neuronal function via a number of growth factors and setting the extracellular matrix milieu (Gonzalez-Perez et al., 2012; Tarnaris et al., 2006; Zhang et al., 2013). Disruption of these normal functions leads to various neurological disorders, which can impact one's cognitive function, motor abilities, and mental faculties (Moghekar et al., 2012; Tarnaris et al., 2009).

The subventricular zone (**SVZ**) is a neurogenic niche adjacent to the lateral wall of the lateral ventricles (Fuentelba et al., 2012). The ependymal cell layer, adjacent to the SVZ, propels CSF in the ventricular system (Mirzadeh et al., 2008; Sawamoto et al., 2006) and regulates brain development (Han and Alvarez-Buylla, 2010; Mirzadeh et al., 2010). In the adult brain, ependymal cells regulate cell lineage (Lim et al., 2000), as well as the proliferation and migration of precursor cells within the SVZ (Del Carmen Gomez-Roldan et al., 2008; Sawamoto et al., 2006). Recently, branched fractal structures (referred to as *fractones*) that resemble basement membranes but have a distinctive morphology have been described in the SVZ (Mercier et al., 2002, 2003). Fractones are extracellular matrix structures that are in direct contact with the adult neurogenic niche, and found to regulate the proliferation of neural stem cells by facilitating the infiltration of cytokines into the SVZ niche (Kerever et al., 2007).

Experimental models of hydrocephalus are not easy to establish because sudden obstruction of the ventricular system is often catastrophic (Gonzalez-Perez, 2012; Townsend et al., 2012). As a result, most models rely on genetic manipulation. Some studies utilize mutant animals lacking either cilia (Banizs et al., 2005; Chen et al., 1998; Sapiro et al., 2002) or have cilia with reduced motility (Ibanez-Tallon et al., 2004; Torikata et al., 1991). A study evaluating Tg737Orpk mice, which have a hypomorphic allele of Polaris that is an essential

protein for ciliogenesis, showed that ciliary movement not only helps maintain CSF flow, but also directs tangential migration of neuroblasts born in the SVZ toward the olfactory bulb in mice with congenital hydrocephalus (Sawamoto et al., 2006). The chronic effects of obstructive hydrocephalus on the brain cytoarchitecture and neural progenitors are therefore difficult to establish because of a lack of non-genetic animal models. Herein, we describe a novel mouse model where we induce long-lasting hydrocephalus by pre-aqueductal obstruction. Our findings indicate that chronic hydrocephalus reduces neural progenitors in the rostral SVZ and alters fractone organization in the absence of severe reactive gliosis, tissue injury, and damage to epithelial cells. Hence, this experimental technique induces a primarily supratentorial hydrocephalus that is useful for studying the effects of increased hydrostatic pressure on the brain cytoarchitecture, extracellular matrix components, cellular apoptosis, cell migration, and the efficacy of pharmacologic and non-pharmacologic interventions on hydrocephalus.

2. Materials and methods

Animals

A total of 36 Balb/C male mice (P60) were used. Mice were assembled into two groups: sham-operated (control) and 60-d hydrocephalus groups. All animals were housed under standard biotery conditions in standard rearing cages (28 × 12 × 15 cm). The relative humidity in the mouse facility was between 40% and 60%. Temperature was maintained in an air-conditioned room at 22 ± 1 °C (the extreme room temperature was in a range of ±2 °C). This housing condition is within the norm dictated by international regulatory agencies. Nonetheless, it should be noted that a recent report suggests that this temperature range produces mild stress, which may be important when cancer or metabolic disorders are modeled in mice (Kokolus et al., 2013). Water and food were delivered *ad libitum*. All experimental procedures described herein were approved by the Institutional Animal Care and Use Committee of the University of Colima.

Surgical procedure in rodents

Obstructive hydrocephalus was induced by placing a piece of cellulose acetate film into the atrium of the aqueduct of Sylvius. Mice were intraperitoneally anesthetized with 2 % Avertin (2,2,2-tribromoethanol plus 2-methyl-2-butanol) pre-warmed to 37 °C. Then, animals were placed in a stereotaxic apparatus (RWD life Science Co., Ltd Model 68003) with a heater pad to keep the animal's body temperature at 37 °C. The mouse's head was shaved and cleaned with 0.1% chlorhexidine gluconate solution, followed by 70% ethanol and 0.1% chlorhexidine gluconate. The skin was incised in the midline with a sterile surgical blade N 15 from just anterior to Bregma to just posterior to Lambda. The pericranium was removed with a cotton swab. The coordinates used to access the atrium of the aqueduct were −2.6 mm (anterior-posterior) and 0 mm (medial-lateral) coordinates relative to Bregma. At that set point, a hole was drilled with pre-sterilized microdrills and fine forceps. In all cases, the deepest layer of bone was carefully removed with forceps to expose the dura mater. Before introducing the occlusive film, the dura mater was opened with fine forceps. A piece of cellulose acetate film (3 × 0.5 × 0.1 mm; length, width and thickness, respectively) was then implanted into the ventricular system (Figure 1A). To reduce bleeding during this

process, absorbable gelatin sponges (Surgifoam, Ethicon™) were gently placed on the interhemispheric vein before introducing the cellulose acetate lamina. After introducing this film, one-minute compression was immediately applied to the blood vessel. The surgical wound was glued with tissue adhesive agent (3M™ Vetbond™ 1469SB). The sham procedure consisted of drilling a hole in the skull (2.6 mm and 0 mm (coordinates relative to Bregma) and removing bone fragments without opening the dura matter. For anesthesia recovery, animals were placed in a pre-warmed cage containing a clean bed. Analgesia was provided with subcutaneous injections of ketorolac (Dolo-Vet™) (5 mg/kg) every 12 hours for 1 day following surgery.

Morris Water Maze (MWM) task

MWM is a widely used device to evaluate the presence of cognitive impairments. This experimental apparatus consisted of a circular water tank (120 cm wide and 40 cm high) surrounded by extra-maze cues. An escape platform (10 cm wide and 30 cm high) was placed in the water. To assess for gross physical, sensory, motor, or motivational impairments, we used the visible-platform version of the MWM (Gulinello et al., 2009; Vorhees and Williams, 2006). 53 days after surgery, 8 mice in the hydrocephalic group and 6 mice in the control group were used. The water temperature was 27 ± 1 °C that is sufficient to motivate the animals to escape, but insufficient to inhibit learning (Morris, 1984). All animals were tested in the morning between 9:00 and 11:00. Every day, all mice were randomly released into the water from one of the four quadrants. The location of the platform changed and mice were allowed to swim for 60 s to find the platform. After the animal found the platform, it was allowed to remain there for 60 s and was then moved to a pre-warmed cage. The time and distance needed to find the hidden platform were collected and analyzed. Data collection was automated by a video image motion analyzer (Ethovision, Noldus Information Technology, USA).

Tissue processing

Mice were anesthetized with sodium pentobarbital (50 mg/kg body weight) before being sacrificed. Transcardial perfusion was done with 0.9% NaCl solution at 37 °C followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB). The brains were post-fixed overnight at 4 °C in the same fixative. To analyze the complete organization of SVZ cells and the cytoplasmic transformation of astrocytes, 40-µm thick coronal sections were cut with a vibratome from 1.18 mm to -3.40 mm in the anterior to posterior direction relative to Bregma (Paxinos and Franklin, 2001). Fluorescent immunostainings were performed as described below.

Immunohistochemistry

Samples were rinsed (10 min $\times$ 3) in 0.1 M buffer phosphate buffer saline (PBS) to rehydrate the tissue. The sections were then blocked in 0.1 M PBS + 0.1% Triton + 10% goat serum for 1 h at room temperature in agitation, and the samples were then incubated with primary antibodies overnight at 4 °C in blocking solution + 0.1% Triton-X. To label proliferating cells, a polyclonal rabbit IgG anti-Ki67 (1:500; Novocastra code:NCL-Ki67p) was used. To label neuroblasts, a polyclonal guinea pig anti-doublecortin (DCX) IgG (1:1000; Millipore, Cat. No. AB2253) was used. The following day, the tissue was left for

15 min at room temperature. The tissue sections were then rinsed three times with 0.1 M PBS, and then incubated with the appropriate secondary antibodies [AlexaFluor® 488 goat anti-rabbit IgG (1:1000; Invitrogen, Cat. No. A11008) and AlexaFluor® 594 goat anti-guinea pig IgG (1:1000; Invitrogen, Cat. No. A11076)] dissolved in blocking solution for 60 min at room temperature in darkness. After washing three times in 0.1 M PBS, tissues were mounted and covered with fluoroshield mounting medium (Abcam; Cat. No. Ab104139) with diamidino-2-phenylindole (DAPI; ab104139).

For immunohistochemistry, the tissue sections were immersed in 3% hydrogen peroxide (H₂O₂) dissolved in distilled water for 20 min and then washed three times with 0.1 M PBS. The sections were blocked in 0.1 PBS + 0.1% Triton + 10% goat serum for 1 h at room temperature. Samples were then incubated either with rabbit anti-glial fibrillary acidic protein IgG (GFAP) (1:500 dilution, Dako Cytomation Cat. Z0334) or rabbit anti Iba-1 IgG, dilution (1:150 dilution, Wako Pure Chemical Industries, Cat. 019-19741). All primary antibodies were incubated overnight at 4° C in blocking solution + 0.1% Triton-X. The following day, the tissue sections were rinsed 3 times with 0.1 M PBS, incubated with the biotinylated secondary antibody (1: 400 dilution, goat anti-rabbit IgG+IgM Biotin, AbD Serotec Cat. 401008) and dissolved in blocking solution for 60 min at room temperature in darkness. The sections were then rinsed three times in 0.1 M PBS and incubated in darkness with avidin biotin complex (Vectastain ABC Kit, Elite Cat. No. PK-6100 Standard) for 45 minutes at room temperature. After three rinses in 0.1 M PBS, the sections were revealed for 5 minutes at room temperature using 0.03% 3,3'-diaminobenzidine tetrahydrochloride dihydrate (DAB, Aldrich, No. 261890). The sections were then washed three times in 0.1 M PBS, mounted and dehydrated using a series of ethanol solutions at crescent concentrations (70, 80, 90 and 95%). The slides were immediately sealed with 1–2 drops of resin (Entellan; Merck, Cat. No. 1079600500) and then air-dried before observation.

Transmission electron microscopy (TEM)

To further demonstrate the cellular composition of the SVZ, we did semi-thin and ultra-thin sections. For electron microscopy studies, two mice per group were perfused with 2% paraformaldehyde and 2.5% glutaraldehyde (EMS, Hatfield, PA) dissolved in 0.1M PB. Brains were then dissected and post-fixed in the same fixative solution. Coronal 200-μm sections were cut using a Leica VT-1000 vibratome (Leica, Heidelberg, Germany). Sections were post-fixed with 2% osmium for 2 h, rinsed, dehydrated and embedded in Durcupan resin (Fluka, Sigma-Aldrich, St. Louis, USA). Semithin sections (1.5 μm) were cut with an UC-6 Ultracut (Leica, Heidelberg, Germany) and stained lightly with 1% toluidine blue. Finally, ultra-thin sections (60–80 nm) were cut with a diamond knife, stained with lead citrate (Reynolds solution), and examined under a FEI Tecnai G2 Spirit transmission electron microscope (FEI Europe, Eindhoven, Netherlands) by using a digital camera (Morada, Olympus Soft Image Solutions GmbH, Münster, Germany).

Quantification

To quantify the number of Ki67- or DCX-expressing cells, ten 40-μm consecutive and randomly selected sections at 200-μm intervals (n = 5 animals per group) were analyzed from 1.2 mm to –1.7 mm (anterior-posterior coordinates from Bregma). Under the 40X

objective (field area = 0.64 mm²), the total number of labeled cells found between the ependyma and the brain parenchyma (250-µm from the ependymal surface) was quantified along the entire length of the lateral ventricle with the help of the X-Y positioning function of the computerized microscope. The double-labeling was quantified for those cells that clearly show discernible DAPI+ nuclei and that visibly match with cellular morphologies. Only the cells found in the same focal plane were quantified. The focal plane was set at the middle of the sections by checking the computerized Z-position of the microscope. This process was analyzed by overlapping pictures taken with a Zeiss Axio-Observer D1 microscope (Göttingen, Germany) and Axio-Vision 4.8.1 acquisition Software (Göttingen, Germany).

The cytoplasmic transformation of astrocytes was evaluated with a stereological grid, which provides a suitable estimation of reactive gliosis (Gonzalez-Perez et al., 2001). Briefly, the test grid consisted of 10 concentric circles with a separation between them of 10 µm. At least 120 astrocyte somas per group were then placed in the central circle and the number of points at which cytoplasmic processes crossed the test grid lines was recorded under 400X magnification. All GFAP+ processes seen in the same plane of focus were considered for quantification. For imaging, a Zeiss Axio-Cam MRc5 (Göttingen, Germany) was used and serial sections were made along the ventricles. Data were expressed as mean ± standard error. Furthermore, in order to obtain a visual representation and the exact distribution of data, the cell quantifications were plotted using box-and-whisker graphs. The Mann–Whitney “U” test was used to determine differences between groups. In all cases, the $P < 0.05$ was considered statistically significant. Nonparametric statistical analysis was chosen based on a kurtosis measure, which showed data distribution deviated from normality.

Humans

The human portion of this study was approved by the institutional review board prior to the start of this study. In order to compare the size of the SVZ, corpus callosum, and internal capsule in humans with and without aqueductal stenosis (AS), T2-weighted magnetic resonance images (MRI) were obtained and reviewed from patients with and without AS. The MRI were obtained from the last 25 adult patients (age > 18 years) who were operated on for AS (Supplemental Video) and compared to 25 adult patients who underwent MRI for other reasons excluding intracranial mass and/or vascular pathology (arteriovenous malformation, aneurysm, cavernoma, stroke, etc.) at a single tertiary care institution. The groups were matched by age (± 1 year) and gender. The size of the SVZ, corpus callosum, and internal capsule were measured by a clinician blinded to patient diagnoses using cropped images of the SVZ, corpus callosum, and internal capsule to facilitate blinding. All measurements were taken at the level of the foramen of Monro. The size of the SVZ was calculated by averaging the distance from the lateral ventricle to the midpoint of the caudate head on both the left and right sides. The size of the corpus callosum was measured in the midline at the genu of the corpus callosum. The size of the internal capsule was calculated by averaging the width of the genu of the internal capsule on both the left and right sides. The differences in the sizes of the SVZ, corpus callosum, and internal capsule between patients with and without AS were assessed using Mann-Whitney “U” test. $P < 0.05$ was considered statistically significant.

RESULTS

Pre-aqueductal obstruction produces long-term hydrocephalus

Sixty days after surgery, there were no obvious signs of local (redness, swelling or heat) or systemic complications (seizures, fever or weight loss) as a result of cellulose acetate lamina placement. At day 60 (P120), the animals were sacrificed and the walls of the lateral ventricles were analyzed. Hydrocephalic animals showed a three-fold ventricle enlargement throughout the ventricular system (Figure 1 B–C). The mean ventricular area between the lateral and the septal wall at + 0.98 mm to – 0.34 mm coordinates from Bregma was $0.63 \pm 0.15 \text{ mm}^2$ for the sham-operated group ($n = 5$ mice) and $1.87 \pm 0.14 \text{ mm}^2$ for the 60-d hydrocephalus group ($n = 5$ mice; $P < 0.05$, Mann Whitney's "U" test) (Figure 1D).

Cellulose acetate film produces limited glial reactivity and does not affect behavioral performance

Astrocytes actively respond to brain damage by increasing their number in injured areas (Estrada et al., 2009; Ramos-Remus et al., 2002). In order to analyze the glial scar in the parietal cortex in close contact to the cellulose acetate film, immunostainings were done for GFAP (astrocyte marker) and Iba-1 (microglia cell marker) (Ahmed et al., 2007). As expected, there was an increase in GFAP+ expression in the regions adjacent to the cellulose acetate film tract (Supplementary figure 1 A–B), but no significant GFAP expression was observed at more remote cortical areas. In average, the glial scar was limited at 250 to 550 μm from the interhemispheric brain line. Similar findings were observed with microglia cells (Supplementary figure 1 C–D).

In order to study whether experimental hydrocephalus produces a cognitive deficit, we used the visible-platform version of the MWM. This approach allowed us to assess gross physical, sensory or motor impairments in rodents (Gulinello et al., 2009; Vorhees and Williams, 2006). As shown by the escape latencies throughout the seven assays, our data indicates that the hydrocephalic group showed no significant differences as compared to controls (Figure 2A). Taken together this evidence suggests that the cellulose acetate film produces a persistent ventricular dilatation with limited effect on cerebral functions.

Chronic hydrocephalus increases astrocyte population in the SVZ

Astrocytic response has been implicated in injury and recovery patterns associated with hydrocephalus (Eskandari et al., 2011). To investigate changes in astrogliosis along the ventricular wall induced by hydrocephalus, the number of GFAP+ cells found at 500 μm from the SVZ niche along the ventricles was quantified (Figure 2B–C). The total number of astrocytes in the 60-d hydrocephalic group (515 ± 20 cells) was increased as compared to the controls (253 ± 18 cells); $P < 0.05$, Mann-Whitney's "U" test (Figure 2D). In response to neural lesions, reactive astrocytes increase their cytoplasmic complexity (Gonzalez-Perez et al., 2001). In order to determine the cytoplasmic transformation of astrocytes, the number of GFAP+ processes with a stereological grid was quantified. Interestingly, there were no statistically significant differences in the number of cytoplasmic intersections between the control group (12.4 ± 5 intersections; $n = 120$ cells) and the 60-d hydrocephalus group (12.3 ± 6 intersections, $n = 120$ cells; $P = 0.46$, Mann-Whitney "U" test). Astrogliosis is

ultrastructurally characterized by an astrocyte hypertrophy and an increase in the number of intermediate filaments. To further address astrocyte reactivity in our model, we confirmed by electron microscopy that these features were not present in the 60-d hydrocephalus group (Figure 2E–F). These findings indicate that chronic hydrocephalus increases the number of astrocytes along the ventricular wall without promoting their cytoplasmic complexity.

Hydrocephalus reduces the proliferation of SVZ neural precursors

The SVZ is a neurogenic niche lining the lateral wall of the lateral ventricles and its cell progenitors are in close contact with CSF. To determine whether long-lasting hydrocephalus produces changes on neural progenitor cells in the SVZ, the number of Ki67+ or DCX+ cells along the ventricular wall (+0.98 mm to –0.46 mm coordinates from Bregma) was calculated (Figure 3A–D). Based on Ki67 expression (Figure 3C), the 60-d hydrocephalus group showed a statistically significant reduction (79 ± 21 cells) in the number of proliferating cells as compared to controls (191 ± 15 cells; $P < 0.01$, Mann Whitney's "U" test). The number of neuroblasts (DCX-expressing cells) were also quantified (Figure 3C), and there was a ~50% reduction in the number of DCX+ cells in the hydrocephalic group (594 ± 32 cells) as compared to sham-operated controls (209 ± 26 cells; $P < 0.01$, Mann Whitney's "U" test).

Hydrocephalus modifies the cytoarchitecture of SVZ stem cell niche

To further determine whether long-lasting hydrocephalus produces changes in the cytoarchitecture of the SVZ, semi-thin sections from the sham-operated and the 60-d hydrocephalus groups were studied. The enlargement of lateral ventricle in the 60-d group led to a notable reduction in SVZ thickness when compared to the control group (Figure 4A–B). Despite this, there was still a continuous ependymal cell layer and, interestingly enough, the same cell types that are found in a normal SVZ (type A, B1, B, C, E and E2) (Doetsch *et al.*, 1997) were also present in the 60-d hydrocephalic mice even though the number of cell layers was reduced. More specifically, chains of migrating neuroblasts (type A cells) were smaller and less abundant than in the control group. Furthermore, fewer mitotic cells were identified in the hydrocephalic group, suggesting a decline in cell proliferation. To further investigate the possible effect of our model on the cellular organization of this structure, a transmission electron microscopy analysis was performed. Confirming our previous findings, the typical organization of the SVZ was indeed altered. TEM analysis confirmed not only a reduction in the SVZ thickness, but also the presence of altered SVZ ultrastructure (Figure 4C–D). While the typical cytoarchitecture was broadly conserved, there were some remarkable features in the 60-d hydrocephalus group. First, there was a reduction in the number of cell layers, from 5–6 in the control group to 2–3 layers in the 60-d group. The cellular composition in these animals is similar to that found in control animals, which included the presence of an ependymal layer (type E cells) separating the lateral ventricle from the rest of the SVZ, migrating neuroblasts (type A cells), astrocytes (type B cells) and dividing immature precursors (type C cells). Nevertheless, migrating chains were distinctly smaller (in terms of number of cells) and more dispersed along the ventricular wall in the 60-d hydrocephalus group as compared to the control group (Figure 4C–D). Mitotic cells were also less abundant in the hydrocephalus animals than in the control group. On the other hand, ependymal cells frequently showed narrowed expansions

at some levels (Figure 4E). In the control group, we found typical small fractones (2–5 μm) arranged along the ependymal cell layer (Figure 4F). Interestingly, in the 60-d hydrocephalus group, we observed large and highly branched networks of fractones that contacted the ventricular wall and SVZ blood vessels (Figure 4G). These structures were normally associated with unusually long cell-to-cell contacts with complex interdigitations in the ependymal layer (Figure 4H), which were only observed in the hydrocephalus group. These findings indicate that chronic hydrocephalus reduces the proliferation rate of adult neural precursors and produces significant changes in the cellular and extracellular matrix organization of the SVZ.

Effects of Aqueductal Stenosis on Subventricular Zone, Corpus Callosum, and Internal Capsule Size in Humans

In order to compare the SVZ size in humans with and without AS, T2-weighted magnetic resonance images (MRI) were obtained and reviewed from patients with and without AS (figure 5 A–E). The MRI were reviewed for 25 patients with AS and 25 patients without AS who underwent MRI for other reasons (10 headaches, 7 weakness and/or numbness, 6 memory difficulty, 2 blurry vision). There were 16 males and 9 females in each cohort. Symptoms were present for 36 (14.5–48) months prior to surgery [median (interquartile range)]. Of the 25 adult patients with AS, 19 (76%) were due to idiopathic narrowing of the aqueduct and 6 (24%) were due to tectal mass/cysts. Of the 19 patients with AS, 4 (21%) had evidence of a web overlying the aqueduct. The average age of patients with AS was 51.8 ± 16.8 years, while the average age of patients without AS was 51.8 ± 17.1 years ($p = 0.99$). After matching patients with and without AS by age (± 1 year) and gender, the width (in mm) of the SVZ (1.24 ± 0.51 vs. 2.20 ± 0.43 , $p < 0.0001$), corpus callosum (3.05 ± 1.76 vs. 8.13 ± 3.44 , $p < 0.0001$), and internal capsule (4.50 ± 1.90 vs. 5.43 ± 1.83 , $p = 0.006$) (Figure 5H) in patients with AS was significantly smaller as compared to age and gender-matched patients without AS (Figure 5 F–H). For patients with AS, postoperative MRI were obtained at a median [IQR] time of 1.4 (0.1–10.9) months following surgery. While the size of the corpus callosum was significantly larger following endoscopic third ventriculostomy (ETV), 3.05 ± 1.76 vs. 3.60 ± 1.842 , $p = 0.03$, the increase in sizes of the postoperative SVZ (1.24 ± 0.51 vs. 1.49 ± 0.70 , $p = 0.09$) and internal capsule (4.50 ± 1.90 vs. 4.83 ± 1.74 , $p = 0.34$) were not significantly different (Supplemental video). Taken together, our data indicate that chronic hydrocephalus produces important changes in the structure of the SVZ that appear to recover partially following ETV. This incomplete recovery may be secondary to either irreversible changes occurring due to the chronicity of the dysfunction or to the partial benefit provided by the ETV, in contrast to definitive shunting.

Discussion

There have been a few different animal models of hydrocephalus that aimed to test the efficacy of various therapeutic measures. In these models, CSF obstruction was accomplished by introducing irritating agents, such as kaolin, blood, silicone oil, or India ink into the CSF space (Dandy, 1919; Edwards et al., 1984; Hochwald, 1985; Milhorat et al., 1970). Among these, the most widely used method for inducing experimental hydrocephalus involves kaolin (aluminum silicate) injection into the cisterna magna, which produces

permanent fibrosis that obstructs CSF pathways (Di Curzio et al., 2013). However, the problem with these models, as well as others, is that it creates an inflammatory response, which leads to additional pathophysiological conditions that do not necessarily mimic hydrocephalus as it typically occurs in humans (Oreskovic and Klarica, 2011). In addition, methods that induce obstructive hydrocephalus such as those that involve placement of cotton, inflated Foley catheter or glass beads rarely lead to hydrocephalus and/or have significant morbidity/mortality (Baru et al., 2001; Hochwald, 1985). As a result, there is a lack of a hydrocephalus model that properly recapitulates hydrocephalus as it occurs in humans such as aqueductal stenosis. We report a simple and easy method to induce chronic hydrocephalus by pre-aqueductal obstruction in the mouse brain. This semi-obstructive method causes primarily supratentorial ventricular enlargement that has several fundamental advantages: 1) It generates CSF accumulation for 60 days that avoids the lethality observed by acute hydrocephalus methods; 2) The cellulose acetate film does not produce significant astroglial or microglial reactivity; 3) The ependymal cell layer is preserved unlike in most genetic mutant models; 4) The surgical approach is simple and has no significant morbidity/mortality; and 5) This model resembles the condition of aqueductal stenosis (AS) in humans by creating morphological changes similar to those found in the human brain, as demonstrated in this study.

To determine the effects of this approach on neural progenitor cells lining the ventricular wall, we quantified the number of neural progenitors along the anatomic limits of the SVZ niche (Doetsch et al., 1997; Gonzalez-Perez and Alvarez-Buylla, 2011; Mirzadeh et al., 2008). Our findings indicated that the increased CSF accumulation in the ventricular system associated with hydrocephalus reduces the proliferation rate of SVZ progenitors and the number of neuroblasts as shown by Ki67 and DCX expression, respectively. As shown by electron microscopy, present findings indicate that our hydrocephalus model does not affect the ependymal cells. The ependymal layer is a source of Noggin, an antagonist of bone morphogenetic protein 4 (BMP4) that regulates neuronal lineage in the SVZ (Lim et al., 2000). Therefore, we hypothesize that the changes in the number of neuroblasts could not be attributed to ependymal disruption. In contrast, our TEM analysis indicates that the SVZ stem cell niche undergoes significant changes secondary to a loss of cell layers, as well as a partial loss of proliferating cells. Interestingly, the hydrocephalic animals had an increase in the ramification of fractones that were contacting the SVZ astrocytes, the ventricular lumen, and the blood vessels close to the neurogenic niche. Fractones are extracellular structures in close contact with SVZ neural progenitors. Under confocal microscope, these appear as series of laminin-immunoreactive puncta. Under an electron microscope, each punctum corresponds to a branched fractal structure (Kerever et al. 2007). Fractones have been recently involved in the regulation of stem cell progenitor proliferation (Douet et al., 2012; Kerever et al., 2007), which is probably mediated by interactions between fibroblast growth factor-2 and heparan sulphates (Douet et al., 2013). A recent report indicates that vascular endothelial growth factor could play a role in the pathogenesis of hydrocephalus (Shim et al., 2013). However, we do not currently have enough evidence to elucidate the role of interactions between fractones and heparin-binding ligands on stem cell proliferation in our hydrocephalus model. MWM is a widely used device to evaluate the occurrence of cognitive impairments. Notably, all these structural changes did not modify the performance of

hydrocephalic mice in the MWM. These findings indicate that hydrocephalus does not induce physical, sensory, motor, or motivational impairments in our model. Taken together, this evidence suggest that the present hydrocephalus model is not as harmful as other models of hydrocephalus and/or cause cognitive deterioraiton. However, there is a possibility that cognitive deterioration may occur at very late stages as observed in humans.

The adult SVZ is a source of oligodendrocyte progenitor cells that partially help preserve the oligodendrocyte population in neighboring white matter regions (Galvez-Contreras et al., 2013; Gonzalez-Perez and Alvarez-Buylla, 2011; Menn et al., 2006). In our study, patients with chronic hydrocephalus by aqueductal stenosis showed a significant reduction in the width of the SVZ niche and the white matter regions (internal capsule and corpus callosum). These findings suggest that CSF accumulation-induces periventricular disturbances in the precursor cell population in the SVZ and local white matter precursors in humans similar to rodents. However, these findings may be tempered by the fact that hydrocephalus may induce stretching of these periventricular structures, which may be the reason for partial recovery following endoscopic third ventriculostomy. To the best of our knowledge, this is the first report showing the consequences of chronic hydrocephalus on the SVZ niche and white matter in the adult human brain. A moderate reduction in the number of SVZ precursor cells has also been observed in hydrocephalus induced by kaolin injection in ferrets (Di Curzio et al., 2013). This ferret study also found that kaolin injection produces white matter damage (Di Curzio et al., 2013). Conversely, an increase in the SVZ proliferation was described in a hydrocephalus model induced by neuraminidase injection from *Clostridium perfringens* (Del Carmen Gomez-Roldan et al., 2008). A possible explanation for this discrepancy between their findings and our study is that their study led to significant disruption of the ependyma, which creates a strong inflammatory response and subsequent necrosis. This inflammatory response can affect the proliferation of neural stem cells in the adult brain (Gonzalez-Perez et al., 2012). In fact, the reactive astrocytes that cover the denuded ventricular walls in this study appeared to function as a cellular barrier involved in water and solute transport, which helps to reestablish the interphase between CSF and the cerebral parenchyma (Roales-Bujan et al., 2012). On the other hand, it has been shown that cell proliferation appears to be partially modulated by extracellular hydrostatic pressure via protein kinase C/tyrosine signal transduction (Downey et al., 2006; Walsh et al., 2004). Taken together, our data suggest that altered CSF flow by itself can significantly disrupt the proliferation rate and the cytoarchitecture of the SVZ, both in rodents and humans.

The astrocyte response to injury is referred to as reactive gliosis and can be identified by immunohistochemical methods that label GFAP-expressing filaments (Bignami and Dahl, 1974; Eng, 1985). As shown by GFAP immunohistochemistry, the glial scar produced by the cellulose acetate was mainly restricted to the film tract with minimal effects elsewhere in the brain. In this study, we used Iba-1 immunostaining, which is a marker of active microglia (Ahmed et al., 2007). The morphological analysis of Iba-1 microglia cells suggested that microglial reactivity was mostly limited to the cellulose lamina tract. Additionally, hydrocephalus produced a thinning of the corpus callosum, but did not lead to an obvious loss of myelin staining as well as reactive astroglial changes in the white matter

(Johnston et al., 2013). To evaluate the effects of hydrocephalus along the ventricular walls, we quantified the number of GFAP+ astrocytes lining the striatal wall of the ventricle. Our findings indicate that hydrocephalus induces a moderate increase in the number of astrocytes in the striatum. These data indicate that hydrocephalus may not only increase the number of astrocytes in the white matter, but also may increase their numbers in the brain parenchyma.

In summary, we describe a simple method of inducing sub-acute obstructive hydrocephalus by pre-aqueductal obstruction of the CSF ventricular system. This model recapitulates the adult human condition and even mirrors the morphological changes. This method may therefore be useful in studying the neurological consequences induced by hydrocephalus.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

- Ahmed Z, Shaw G, Sharma VP, Yang C, McGowan E, Dickson DW. Actin-binding proteins coronin-1a and IBA-1 are effective microglial markers for immunohistochemistry. *J Histochem Cytochem.* 2007; 55:687–700. [PubMed: 17341475]
- Banizs B, Pike MM, Millican CL, Ferguson WB, Komlosi P, Sheetz J, Bell PD, Schwiebert EM, Yoder BK. Dysfunctional cilia lead to altered ependyma and choroid plexus function, and result in the formation of hydrocephalus. *Development.* 2005; 132:5329–5339. [PubMed: 16284123]
- Baru JS, Bloom DA, Muraszko K, Koop CE. John Holter's shunt. *Journal of the American College of Surgeons.* 2001; 192:79–85. [PubMed: 11192928]
- Bignami A, Dahl D. Astrocyte-specific protein and neuroglial differentiation. An immunofluorescence study with antibodies to the glial fibrillary acidic protein. *J Comp Neurol.* 1974; 153:27–38. [PubMed: 4593733]
- Chen J, Knowles HJ, Hebert JL, Hackett BP. Mutation of the mouse hepatocyte nuclear factor/forkhead homologue 4 gene results in an absence of cilia and random left-right asymmetry. *J Clin Invest.* 1998; 102:1077–1082. [PubMed: 9739041]
- Dandy WE. Experimental Hydrocephalus. *Annals of surgery.* 1919; 70:129–142. [PubMed: 17864139]
- Del Carmen Gomez-Roldan M, Perez-Martin M, Capilla-Gonzalez V, Cifuentes M, Perez J, Garcia-Verdugo JM, Fernandez-Llebrez P. Neuroblast proliferation on the surface of the adult rat striatal wall after focal ependymal loss by intracerebroventricular injection of neuraminidase. *J Comp Neurol.* 2008; 507:1571–1587. [PubMed: 18236450]
- Di Curzio DL, Buist RJ, Del Bigio MR. Reduced subventricular zone proliferation and white matter damage in juvenile ferrets with kaolin-induced hydrocephalus. *Exp Neurol.* 2013; 248C:112–128. [PubMed: 23769908]
- Doetsch F, Garcia-Verdugo JM, Alvarez-Buylla A. Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *J Neurosci.* 1997; 17:5046–5061. [PubMed: 9185542]
- Douet V, Arikawa-Hirasawa E, Mercier F. Fractone-heparan sulfates mediate BMP-7 inhibition of cell proliferation in the adult subventricular zone. *Neuroscience letters.* 2012; 528:120–125. [PubMed: 22985516]

- Douet V, Kerever A, Arikawa-Hirasawa E, Mercier F. Fractone-heparan sulphates mediate FGF-2 stimulation of cell proliferation in the adult subventricular zone. *Cell proliferation*. 2013; 46:137–145. [PubMed: 23510468]
- Downey C, Alwan K, Thamilselvan V, Zhang L, Jiang Y, Rishi AK, Basson MD. Pressure stimulates breast cancer cell adhesion independently of cell cycle and apoptosis regulatory protein (CARP)-1 regulation of focal adhesion kinase. *American journal of surgery*. 2006; 192:631–635. [PubMed: 17071197]
- Edwards MS, Harrison MR, Halks-Miller M, Nakayama DK, Berger MS, Glick PL, Chinn DH. Kaolin-induced congenital hydrocephalus in utero in fetal lambs and rhesus monkeys. *Journal of neurosurgery*. 1984; 60:115–122. [PubMed: 6537789]
- Eng LF. Glial fibrillary acidic protein (GFAP): the major protein of glial intermediate filaments in differentiated astrocytes. *Journal of neuroimmunology*. 1985; 8:203–214. [PubMed: 2409105]
- Eskandari R, Harris CA, McAllister JP 2nd. Reactive astrocytosis in feline neonatal hydrocephalus: acute, chronic, and shunt-induced changes. *Childs Nerv Syst*. 2011; 27:2067–2076. [PubMed: 21847645]
- Estrada FS, Hernandez VS, Medina MP, Corona-Morales AA, Gonzalez-Perez O, Vega-Gonzalez A, Zhang L. Astroglial is temporally correlated with enhanced neurogenesis in adult rat hippocampus following a glucoprivic insult. *Neurosci Lett*. 2009; 459:109–114. [PubMed: 19446003]
- Fernandez-Carrocera LA, Gonzalez-Mora E. Neurodevelopmental disorders in children with an antecedent of subependymal/intraventricular hemorrhage at 3 years of age. *Gaceta medica de Mexico*. 2004; 140:367–373. [PubMed: 15456146]
- Fuentealba LC, Obernier K, Alvarez-Buylla A. Adult neural stem cells bridge their niche. *Cell Stem Cell*. 2012; 10:698–708. [PubMed: 22704510]
- Gallia GL, Rigamonti D, Williams MA. The diagnosis and treatment of idiopathic normal pressure hydrocephalus. *Nature clinical practice. Neurology*. 2006; 2:375–381. [PubMed: 16932588]
- Galvez-Contreras AY, Quinones-Hinojosa A, Gonzalez-Perez O. The role of EGFR and ErbB family related proteins in the oligodendrocyte specification in germinal niches of the adult mammalian brain. *Frontiers in cellular neuroscience*. 2013; 7:258. [PubMed: 24381541]
- Gonzalez-Perez O. Hydrocephaly does not modify neuroblast chain migration in the subventricular zone (SVZ). *Gaceta medica de Mexico*. 2012; 148:130–136. [PubMed: 22622312]
- Gonzalez-Perez O, Alvarez-Buylla A. Oligodendrogenesis in the subventricular zone and the role of epidermal growth factor. *Brain Res Rev*. 2011; 67:147–156. [PubMed: 21236296]
- Gonzalez-Perez O, Gutierrez-Fernandez F, Lopez-Virgen V, Collas-Aguilar J, Quinones-Hinojosa A, Garcia-Verdugo JM. Immunological regulation of neurogenic niches in the adult brain. *Neuroscience*. 2012; 226:270–281. [PubMed: 22986164]
- Gonzalez-Perez O, Ramos-Remus C, Garcia-Estrada J, Luquin S. Prednisone induces anxiety and glial cerebral changes in rats. *J Rheumatol*. 2001; 28:2529–2534. [PubMed: 11708428]
- Gulinello M, Gertner M, Mendoza G, Schoenfeld BP, Oddo S, LaFerla F, Choi CH, McBride SM, Faber DS. Validation of a 2-day water maze protocol in mice. *Behavioural brain research*. 2009; 196:220–227. [PubMed: 18831990]
- Han YG, Alvarez-Buylla A. Role of primary cilia in brain development and cancer. *Current opinion in neurobiology*. 2010; 20:58–67. [PubMed: 20080044]
- Hochwald GM. Animal models of hydrocephalus: recent developments. *Proceedings of the Society for Experimental Biology and Medicine*. Society for Experimental Biology and Medicine (New York, N Y. 1985; 178:1–11.
- Ibanez-Tallon I, Pagenstecher A, Fliegauf M, Olbrich H, Kispert A, Ketelsen UP, North A, Heintz N, Omran H. Dysfunction of axonemal dynein heavy chain Mdnah5 inhibits ependymal flow and reveals a novel mechanism for hydrocephalus formation. *Hum Mol Genet*. 2004; 13:2133–2141. [PubMed: 15269178]
- Johnston MG, Del Bigio MR, Drake JM, Armstrong D, Di Curzio DL, Bertrand J. Pre- and post-shunting observations in adult sheep with kaolin-induced hydrocephalus. *Fluids and barriers of the CNS*. 2013; 10:24. [PubMed: 23845003]

- Kerever A, Schnack J, Vellinga D, Ichikawa N, Moon C, Arikawa-Hirasawa E, Efrid JT, Mercier F. Novel extracellular matrix structures in the neural stem cell niche capture the neurogenic factor fibroblast growth factor 2 from the extracellular milieu. *Stem Cells*. 2007; 25:2146–2157. [PubMed: 17569787]
- Kokulus KM, Capitano ML, Lee CT, Eng JW, Waight JD, Hylander BL, Sexton S, Hong CC, Gordon CJ, Abrams SI, Repasky EA. Baseline tumor growth and immune control in laboratory mice are significantly influenced by subthermoneutral housing temperature. *Proceedings of the National Academy of Sciences of the United States of America*. 2013; 110:20176–20181. [PubMed: 24248371]
- Lim DA, DTA, Trevejo JM, Herrera DG, García-Verdugo JM, Alvarez-Buylla A. Noggin Antagonizes BMP Signaling to Create a Niche for Adult Neurogenesis. *Neuron*. 2000; 28:713–726. [PubMed: 11163261]
- Menn B, Garcia-Verdugo JM, Yaschine C, Gonzalez-Perez O, Rowitch D, Alvarez-Buylla A. Origin of oligodendrocytes in the subventricular zone of the adult brain. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2006; 26:7907–7918. [PubMed: 16870736]
- Mercier F, Kitasako JT, Hatton GI. Anatomy of the brain neurogenic zones revisited: fractones and the fibroblast/macrophage network. *The Journal of comparative neurology*. 2002; 451:170–188. [PubMed: 12209835]
- Mercier F, Kitasako JT, Hatton GI. Fractones and other basal laminae in the hypothalamus. *The Journal of comparative neurology*. 2003; 455:324–340. [PubMed: 12483685]
- Milhorat TH, Clark RG, Hammock MK. Experimental hydrocephalus. 2. Gross pathological findings in acute and subacute obstructive hydrocephalus in the dog and monkey. *Journal of neurosurgery*. 1970; 32:390–399. [PubMed: 4984823]
- Mirzadeh Z, Han YG, Soriano-Navarro M, Garcia-Verdugo JM, Alvarez-Buylla A. Cilia organize ependymal planar polarity. *J Neurosci*. 2010; 30:2600–2610. [PubMed: 20164345]
- Mirzadeh Z, Merkle FT, Soriano-Navarro M, Garcia-Verdugo JM, Alvarez-Buylla A. Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain. *Cell Stem Cell*. 2008; 3:265–278. [PubMed: 18786414]
- Moghekar A, Goh J, Li M, Albert M, O'Brien RJ. Cerebrospinal fluid Abeta and tau level fluctuation in an older clinical cohort. *Archives of neurology*. 2012; 69:246–250. [PubMed: 22332192]
- Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of neuroscience methods*. 1984; 11:47–60. [PubMed: 6471907]
- Oreskovic D, Klarica M. Development of hydrocephalus and classical hypothesis of cerebrospinal fluid hydrodynamics: facts and illusions. *Progress in neurobiology*. 2011; 94:238–258. [PubMed: 21641963]
- Paxinos, G.; Franklin, KBJ. *The mouse brain in stereotaxic coordinates*. 2. Academic Press; San Diego, CA: 2001.
- Ramos-Remus C, Gonzalez-Castaneda RE, Gonzalez-Perez O, Luquin S, Garcia-Estrada J. Prednisone induces cognitive dysfunction, neuronal degeneration, and reactive gliosis in rats. *J Investig Med*. 2002; 50:458–464.
- Roales-Bujan R, Paez P, Guerra M, Rodriguez S, Vio K, Ho-Plagaro A, Garcia-Bonilla M, Rodriguez-Perez LM, Dominguez-Pinos MD, Rodriguez EM, Perez-Figares JM, Jimenez AJ. Astrocytes acquire morphological and functional characteristics of ependymal cells following disruption of ependyma in hydrocephalus. *Acta neuropathologica*. 2012; 124:531–546. [PubMed: 22576081]
- Sapiro R, Kostetskii I, Olds-Clarke P, Gerton GL, Radice GL, Strauss IJ. Male infertility, impaired sperm motility, and hydrocephalus in mice deficient in sperm-associated antigen 6. *Mol Cell Biol*. 2002; 22:6298–6305. [PubMed: 12167721]
- Sawamoto K, Wichterle H, Gonzalez-Perez O, Cholfen JA, Yamada M, Spassky N, Murcia NS, Garcia-Verdugo JM, Marin O, Rubenstein JL, Tessier-Lavigne M, Okano H, Alvarez-Buylla A. New neurons follow the flow of cerebrospinal fluid in the adult brain. *Science*. 2006; 311:629–632. [PubMed: 16410488]
- Shim JW, Sandlund J, Han CH, Hameed MQ, Connors S, Klagsbrun M, Madsen JR, Irwin N. VEGF, which is elevated in the CSF of patients with hydrocephalus, causes ventriculomegaly and ependymal changes in rats. *Experimental neurology*. 2013; 247:703–709. [PubMed: 23518418]

- Tarnaris A, Kitchen ND, Watkins LD. Noninvasive biomarkers in normal pressure hydrocephalus: evidence for the role of neuroimaging. *Journal of neurosurgery*. 2009; 110:837–851. [PubMed: 18991499]
- Tarnaris A, Watkins LD, Kitchen ND. Biomarkers in chronic adult hydrocephalus. *Cerebrospinal fluid research*. 2006; 3:11. [PubMed: 17020616]
- Torikata C, Kijimoto C, Koto M. Ultrastructure of respiratory cilia of WIC-Hyd male rats. An animal model for human immotile cilia syndrome. *Am J Pathol*. 1991; 138:341–347. [PubMed: 1992761]
- Townsend, M.; Beauchamp, D.; Evers, M.; Mattox, K. *Sabiston Textbook of Surgery: The Biological Basis of Modern Surgical Practice*. 19. Elsevier Saunders; Philadelphia, PA: 2012.
- Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nature protocols*. 2006; 1:848–858.
- Walsh MF, Woo RK, Gomez R, Basson MD. Extracellular pressure stimulates colon cancer cell proliferation via a mechanism requiring PKC and tyrosine kinase signals. *Cell proliferation*. 2004; 37:427–441. [PubMed: 15548175]
- Zhang J, Williams MA, Rigamonti D. Genetics of human hydrocephalus. *Journal of neurology*. 2006; 253:1255–1266. [PubMed: 16773266]
- Zhang S, Chen D, Huang C, Bao J, Wang Z. Expression of HGF, MMP-9 and TGF-beta1 in the CSF and cerebral tissue of adult rats with hydrocephalus. *The International journal of neuroscience*. 2013; 123:392–399. [PubMed: 23270462]

Highlights

- Preaqueductal obstruction effectively induces long-term hydrocephalus
- Long-term obstructive hydrocephalus (LTOH) reduces the proliferation rate of neural progenitors
- LTOH modifies the cytoarchitecture and fractones of the subventricular zone (SVZ)
- In humans, chronic obstructive hydrocephalus reduces the SVZ niche and the width of white matter

Figure 1.

Hydrocephalus model in the adult mice (A). On the top, schemes of axial, coronal and sagittal sections of the mouse brain are shown. Hydrocephalus was induced by placing a cellulose acetate film (3.0 mm length, 0.5 mm width and 0.1 mm thick) into the atrium of the aqueduct (−2.6 mm, 0 mm; anterior-posterior and lateral coordinates from Bregma, respectively) in mice. Phase-contrast imaging of the lateral ventricles of control (B) and hydrocephalus animals (C). Significant ventricular enlargement is observed 60 days after surgery (D). The average area of the ventricles was measured in >10 coronal sections taken every 200 μ m ($n = 5$ animals per group). The graph indicates that the hydrocephalic group showed statistically significant differences when compared to controls. * $P < 0.05$; Mann-Whitney “U” test. Bar = 200 μ m.

Figure 2.

Morris water maze was used to evaluate cognitive impairments. Throughout the study, no statistically significant differences were observed between the groups to find the escape platform (A). Schematic coronal sections used for quantification (B). GFAP immunostaining shows reactive astrocytes along the lateral ventricles (LV) in the control and the 60-d hydrocephalus group (C). The difference in the number of astrocytes along the ventricular wall was statistically significant between animals with long-lasting hydrocephalus (n = 5 animals per group) and control animals (D). Electron microscopy confirmed the absence of astrocyte hypertrophy after induced hydrocephalus (E). The inset (F) shows a detail of a fractone with ependyma to the right and astrocytic expansions to the left. Intermediate filaments (arrowhead) are not increased as compared to the control group. **Str**: Striatum; **LV**: Lateral ventricle; **bv**: blood vessel; **e**: ependymal cells; **b**: astrocytes. * $P < 0.05$; Mann-Whitney “U” test. Bars: C = 50 µm, E = 2 µm; F = 500 nm.

Figure 3.

SVZ niche immunostained with anti-Ki67 (green) and anti-DCX (red) antibodies in a control (A) and a 60-d hydrocephalic mouse (B). C: In the control group, the number of proliferative cells (Ki67+) and neuroblasts (DCX+ cells) was significantly higher than the 60-d hydrocephalic group (n = 5 animals per group). D: Schematic coronal sections used for quantification. Nuclei were stained with DAPI (blue). LV: Lateral ventricle; CC: Corpus callosum. * $P < 0.05$; Mann-Whitney “U” test. Bar = 30 µm.

Figure 4.

The SVZ of a control mouse shows its characteristic organization in several cell layers (**A**). Mitotic cells (arrowhead) are frequent. Conversely, in the 60-d hydrocephalus group, the SVZ had a significant reduction in both the width and number of proliferating cells (**B**). Consistent chains of migrating neuroblasts (A-type cells, arrows) are seen along the SVZ in the control group (**C**), while the SVZ of 60-d hydrocephalic mice primarily contain an ependymal cell layer and astrocytes. Nevertheless, all cell types (A-, B- and C-type cells) are conserved and small chains of migrating cells appear scattered throughout the ventricular wall in the hydrocephalus group (**D**). The ependymal cell layer is often narrowed in the hydrocephalus group (**E**, arrows) and there are numerous fractones, which are arranged along the cells lining the walls of the ventricle when compared to control animals (**F–G**, asterisks). Deep interdigitations (white arrowheads) connecting with fractones (asterisks) stand out in the ependymal layer in this hydrocephalus group (**H**). A and B are toluidine blue-stained semithin sections and C–H are transmission electron microscopy (TEM) images. LV: Lateral ventricle; cp: choroid plexus; bv: blood vessel; a: A-type cell; b: B-type cell; c: C-type cell; e: ependymal cell. Scale bar = A, B, 10 µm; C–E, 2 µm; F–H, 500 nm.

Figure 5.

Impact of hydrocephalus from aqueductal stenosis (AS) on the ventricular-subventricular zone (SVZ), corpus callosum, and internal capsule size in humans. Axial T2-weighted brain magnetic resonance image (MRI) and magnified T2 MRI of the SVZ (yellow line), corpus callosum (blue line), and internal capsule (green line) in a 44-year old female patient with AS due to idiopathic narrowing of the aqueduct that demonstrates the absence of pulsatile flow through the aqueduct as detected on MRI constructive interface in steady state (CISS). (A – B), a corresponding 43 year-old, female patient without AS who underwent an MRI for evaluation of headaches (D – E). Intraoperative photograph, as seen from a top view when going through the foramen of Monro, from the Supplementary Video, demonstrating stenosis of the cerebral aqueduct (white arrow), as well as the presence of mammillary bodies (yellow arrow), thalamus (blue arrow), and floor of the third ventricle (green arrow) from the patient featured in panels A and B. (C) The widths of the SVZ (1.24 ± 0.51 vs. 2.20 ± 0.43 , $p < 0.0001$) (F) corpus callosum (3.05 ± 1.76 vs. 8.13 ± 3.44 , $p < 0.0001$) (G), and internal capsule (4.50 ± 1.90 vs. 5.43 ± 1.83 , $p = 0.006$) (H) in patients with AS were significantly smaller as compared to age and gender-matched patients without AS.