Structural and functional deficits of the hippocampus in hydrocephalic rats: the role of age at onset and duration of disease

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SUMMARY

We studied the pyramidal neurons of the hippocampus in neonatal, juvenile and adult rats with hydrocephalus of varying durations and related these changes to their learning and memory. Hydrocephalus was induced in 7-day-old neonates, 4-week-old juvenile and 16-week-old adult Albino rats by intra-cisternal injection 0.02-0.04 ml of 150mg/ml of kaolin in sterile water (150 mg/ml). We studied escape latency and platform crossings with the Morris water maze prior to animal sacrifice at 2 and 4 weeks post induction in neonates and at 4 and 8 weeks post induction in juveniles and adults. We examined pyramidal neurons with cresyl violet and modified Golgi stain and analyzed behavioural scores and pyknotic indices. Statistical significance was determined at $p<0.05$.

The basal dendrites of the pyramidal neurons were reduced in the hydrocephalic groups. In the CA1, the pyknotic index was significantly increased in both groups of hydrocephalic neonates but only in hydrocephalic juveniles and adults sacrificed at 4 weeks. In the CA3 it was increased in hydrocephalic neonates sacrificed at 2 weeks and hydrocephalic juveniles sacrificed at 4 weeks.

The escape latency was greater and the number of platform crossing was lower in the hydrocephalic rats than in their age matched controls. Pyramidal neurons were morphologically altered in hydrocephalus, in association with changes in spatial learning and memory. The CA1 region in young animals was particularly vulnerable. Functional recovery occurs with time and to a greater extent in older animals.

Keywords: Hydrocephalus – Hippocampus – Pyknotic index – Pyramidal neurons – Dendritic arbor

INTRODUCTION

Hydrocephalus is a neurological disorder characterized by enlargement of the brain ventricles due to inadequate passage of cerebrospinal fluid from the source of production within the cerebral ventricles to the area of absorption into the systemic circulation (Rekate, 2008). The pathophysiology of hydrocephalus is multifactorial in nature, with the primary consequences such as direct compression of brain tissue and blood vessels, white matter stretching, edema and reduced cerebral blood flow (McAllister and Chovan, 1998).

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The hippocampus is the anatomical substrate for learning and memory, functioning in the consolidation of short-term into long term memory. It is microscopically divided into 3 sub-regions (CA1, CA2, CA3), and distinct cellular layers with the pyramidal cells as the principal neurons. However, the CA2 is not well delineated compared to the well-outlined CA1 and CA3 sub-regions. Hippocampal pyramidal neurons connect to neural circuits controlling cognition, memory and motor functions. The expanding ventricular system in hydrocephalus has been shown to compress periventricular structures, and impairment of learning and memory has been reported in hydrocephalic patients and experimental animals (Shim et al., 2003). It is unclear how age at onset and chronicity determine the extent of injury to the hippocampus in hydrocephalus.

We therefore hypothesize that, in hydrocephalus, the alterations in the principal output neurons of the hippocampus and the neurological sequelae will vary with the age of onset and chronicity of the disease. In this study, we examined the population and dendritic architecture of pyramidal neurons of the hippocampus and neurobeviour in neonatal, juvenile and adult hydrocephalic rats following variable periods of hydrocephalus.

MATERIALS AND METHODS

We induced hydrocephalus in neonatal (7 days), juvenile (4 weeks) and adult (16 weeks) rats by intra-cisternal injection of 0.02ml, 0.04ml and 0.06 ml respectively of kaolin suspension (150mg/ml in sterile water). The juvenile and adult rats were induced after anaesthetizing them with intra-peritoneal injection of ketamine/xylazine at 90/10 mg/kg. The control groups received a sham injection into the cisterna magna. All procedures on animal handling conformed to the acceptable guidelines of EU Directive 2010/63/EU for animal experiments (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm) and the ethical use of animals in research according to the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC). The rats had free access to water and feed and were weighed twice weekly. They were assessed for the development of hydrocephalus by examination of the head, gait and general grooming with the presence of a dome-shaped head. We sacrificed the animals in batches at 2 (acute) and 4 (intermediate) weeks after induction in the neonates, and at 4 (intermediate) and 8 (chronic) weeks in the juvenile and adult rats.

Behavioural Test: Morris Water Maze Task (MWM)

We examined spatial learning and memory in the rats with the Morris Water Maze (MWM) task (Morris, 1984). Hydrocephalic rats (neonate, juvenile and adult) in their two different timelines and their age-matched controls were tested with a 4-day MWM task of multiple trials (R'Hooge and Deyn, 2001). Six to twelve animals per group were used for the study. Tests were performed in a circular pool (152 cm diameter, 40 cm depth) that was marked north, south, east and west dividing it into four quadrants (Northeast, Northwest, Southeast and Southwest). A hidden platform was fixed in the center of one of the quadrants and submerged 1.0 cm beneath water surface at room temperature. The water was made opaque by adding concentrated milk to prevent the animal from seeing the hidden platform while it is submerged in the water. Each animal underwent nine trials for the training period (three trials per day). For each trial, the rats were placed facing a different direction away from the location of the platform and allowed to swim until they found the underwater platform. If the rat failed to complete the task in 60 seconds, it was guided onto the platform for at least 15 seconds rest before the next trial. The mean of the escape times of the three trials for the three days of training was taken as the escape latency and was compared between control and hydrocephalic rats. On the fourth day, the rats which have undergone the training were tested for memory function by a probe trial in which the hidden platform was removed from the maze. Each animal was place in the maze from any of the four quadrants and allowed to explore the pool for 60 seconds only. The number of times the rats were able to cross the platform location was recorded as a test for memory function while

the duration of time spent in each quadrant was recorded (Nunez, 2008).

Tissue processing

Neonatal rats were perfused transcardially first with normal saline for three minutes to wash out the blood cells and then for 15-20 minutes with 10% neural buffered formalin (NBF) until well fixed, while juvenile and adult rats were anaesthetized with intra-peritoneal injection of ketamine at 90/10 mg/kg before transcardial perfusion with 10% NBF, using pallor of the liver and stiff muscles as an indication of good fixation. The brains were quickly dissected out and fixed in the same solution. After fixation, coronal sections were obtained at the level of the optic chiasma and examined to confirm ventriculomegaly. Hydrocephalus was categorized as mild, moderate or severe according to Olopade et al. (2012). The majority of the neonatal and juvenile rats developed severe hydrocephalus while the adult rats developed mild form of hydrocephalus in both timelines.

Cresyl staining

Sectioned brain tissues (5 µm) were stained with cresyl violet to assess the pyramidal neurons of the hippocampal sub-regions for pyknotic neurons and cell density. These were then viewed with Leica ICC50E light microscope (Switzerland).

Modified Golgi staining

Fixed brain tissues were immersed in potassium dichromate solution (3 g/100 ml of distilled water) stored at room temperature for 5 (neonates) days and 7 days (juvenile and adults respectively) in a dark chamber (solution was replaced every 24 hours). Tissues were then transferred into silver nitrate solution (2 g/100 ml) and stored for 3 days in the dark chamber. The tissues were then infiltrated at 60ºC for 30 minutes, embedded in molten paraffin wax and cooled overnight which was then sectioned at 60 µm thickness. Each section was then transferred in a cassette into a graded series of alcohol (70%, 90%, 100%, 100%) for 3 minutes each and cleared in xylene for 5 minutes. The sections were later mounted on a gelatinized slides, cover-slipped with DPX, air dried and viewed with Leica ICC50E light microscope (Switzerland).

Statistical analysis

We analyzed data by one-way ANOVA using Graphpad Prism Version 6.0 for windows (SanDiego, Califonia USA). Statistical significance was set at $p < 0.05$, for the null hypothesis being true by chance and the confidence interval calculated at 95% level. Photographed sections of the hippocampus were examined and analyzed for pathological changes and neuronal density (using Motic Image Plus 2.0 software).

RESULTS

General observation

The experimental rats (kaolin injection) showed signs of hydrocephalus which included scurfy fur indicating impaired grooming (common to all age groups), unsteady gait and enlarged dome-shaped head in all the age groups. They lost appetite for suckling (neonates) and for their food pellets (juveniles and adults). The rats in the control group were well groomed, with normal head shape and size and they fed well.

Body Weight

The experimental rats (6-14 per group) lost body weight within the first week of kaolin injection, although they gained weight over the following weeks but remained consistently lighter in weight compared to their age-matched controls (7-10 per group). They also had a significantly lower rate of weight gain throughout the study period when compared to their control counterparts (Fig. 1: blue circles and lines depict control groups, while red squares and lines depict hydrocephalic groups).

Morris Water Maze Test

The time taken by each animal to locate the underwater hidden platform, i.e., escape latency, was reduced progressively in all the groups, but not at the same rate. Only the juvenile hydrocephalic rats at 8 weeks post-induction (Hydro Juvenile Chronic) learnt at a significantly slower rate than the other

groups (Fig. 2A). During the probe trial however, the Hydro Neonate Acute group had a significantly fewer number of platform crossings than their age-matched controls (p=0.0421, Fig. 2B).

Gross examination of the brain

Gross examination of the brains in the control group revealed a compact structure with distinct corpus callosum, and the lateral ventricles were slit-like, which cannot be easily distinguished. The gross appearance of the hydrocephalic brains showed enlarged lateral ventricles, a visible aqueduct of Sylvius with thinning of the corpus callosum and cerebral cortex (Fig. 3).

Cresyl Violet

Pyknotic neurons were counted on the cresyl stained sections while the pyknotic index was calculated as the number of pyknotic neurons expressed as a percentage of the total number of pyramidal neurons in the sample frame (Taveira et al., 2012). The cresyl violet staining

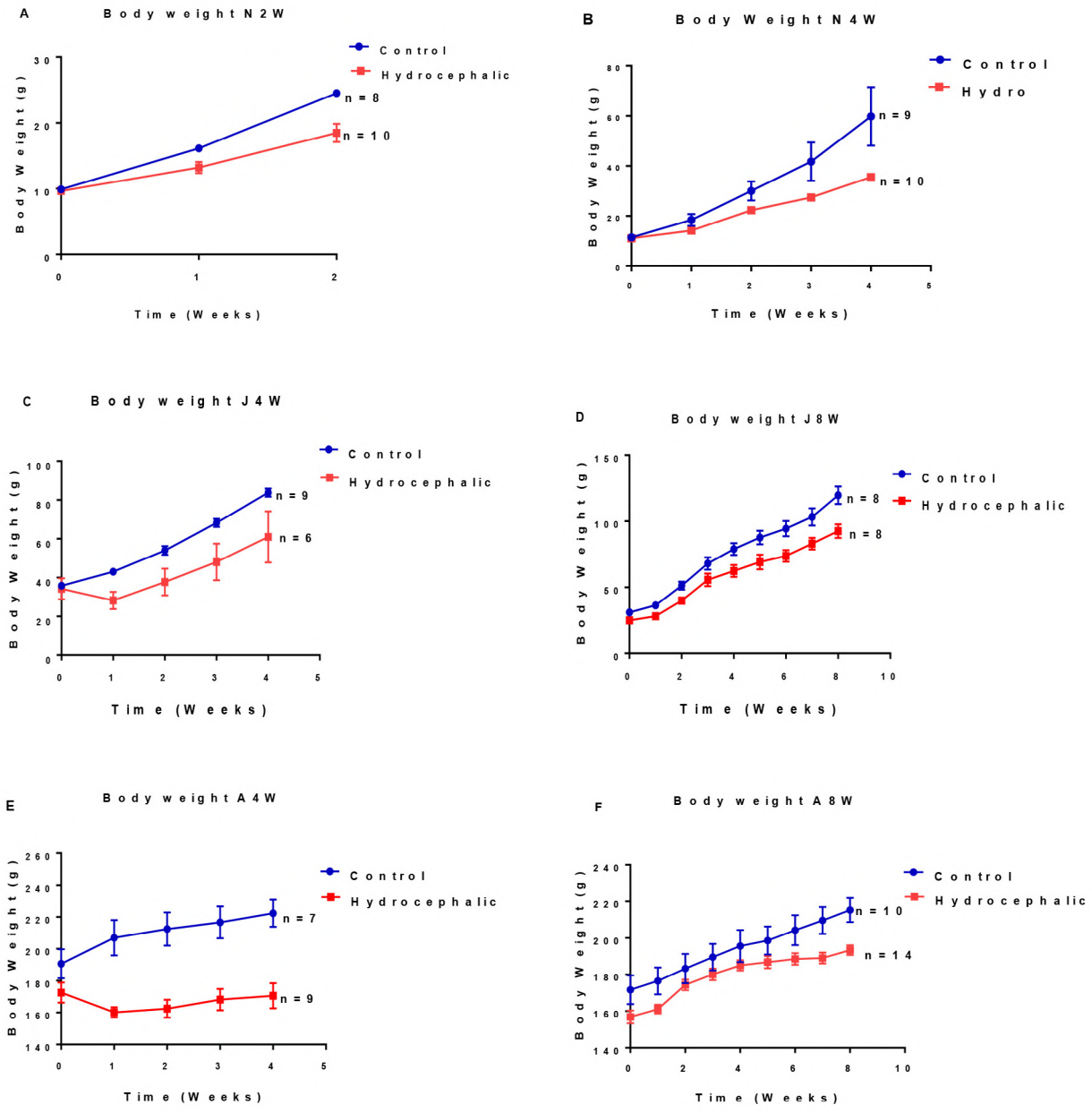

Fig. 1.- Body weight (g) gain of neonatal, juvenile and adult rats over time. **A**: neonates, acute. **B**: neonates, intermediate. **C**: juveniles, intermediate. **D**: juveniles, chronic. **E**: adults, intermediate. **F**: adults, chronic. *p<0.05, **p<0.01

of the hippocampal area of the control rat brains showed neuronal cells, with clearly defined cell bodies and nuclei while the experimental animals showed dark shrunken nuclei and abnormal clumping of heterochromatin, which were considered as pyknotic cells. In all the age groups, the wel-layered structure of the pyramidal layers of the control animals were confirmed with the cresyl violet stain, while the hydrocephalic animals showed varying degrees of disarray of the pyramidal layers (Figs. 4, 5 and 6).

Pyknotic Index

Comparison between the pyknotic index of hydrocephalic and control of each group was analyzed to determine the level of significance of their differences. In the CA1 sub-region, the pyknotic index was significantly higher in neonatal hydrocephalic animals (both acute and intermediate) than in age matched controls. In juveniles and adults, this difference was observed only in those with intermediate hydrocephalus. In the older rats with chronic (8

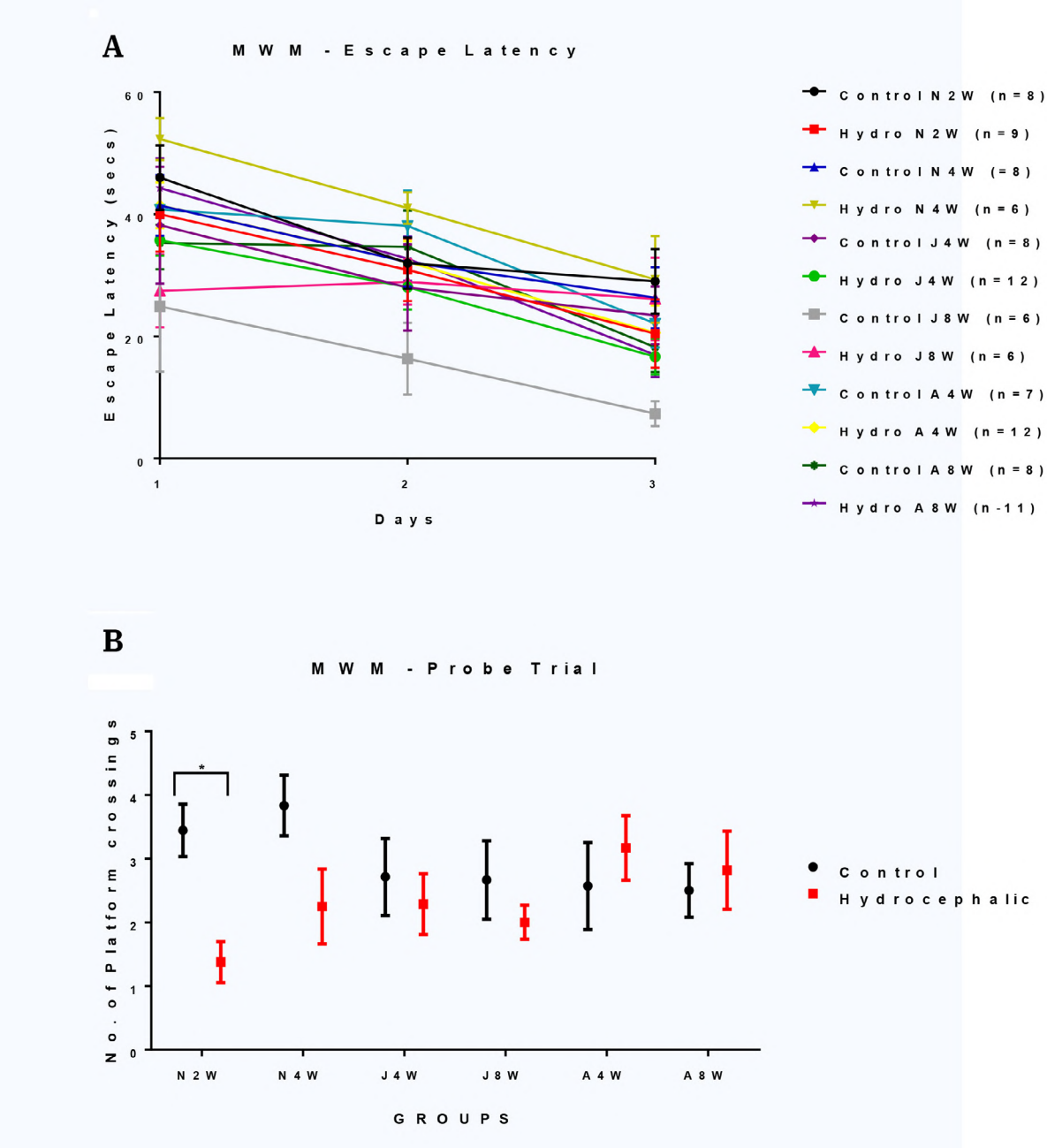

Fig. 2.- Bar chart showing (**A**) Escape latency from platform, (**B**) number of platform crossings during Morris Water Maze Test (N: neonate; J; juvenile; A: adult; W: weeks. *p<0.05

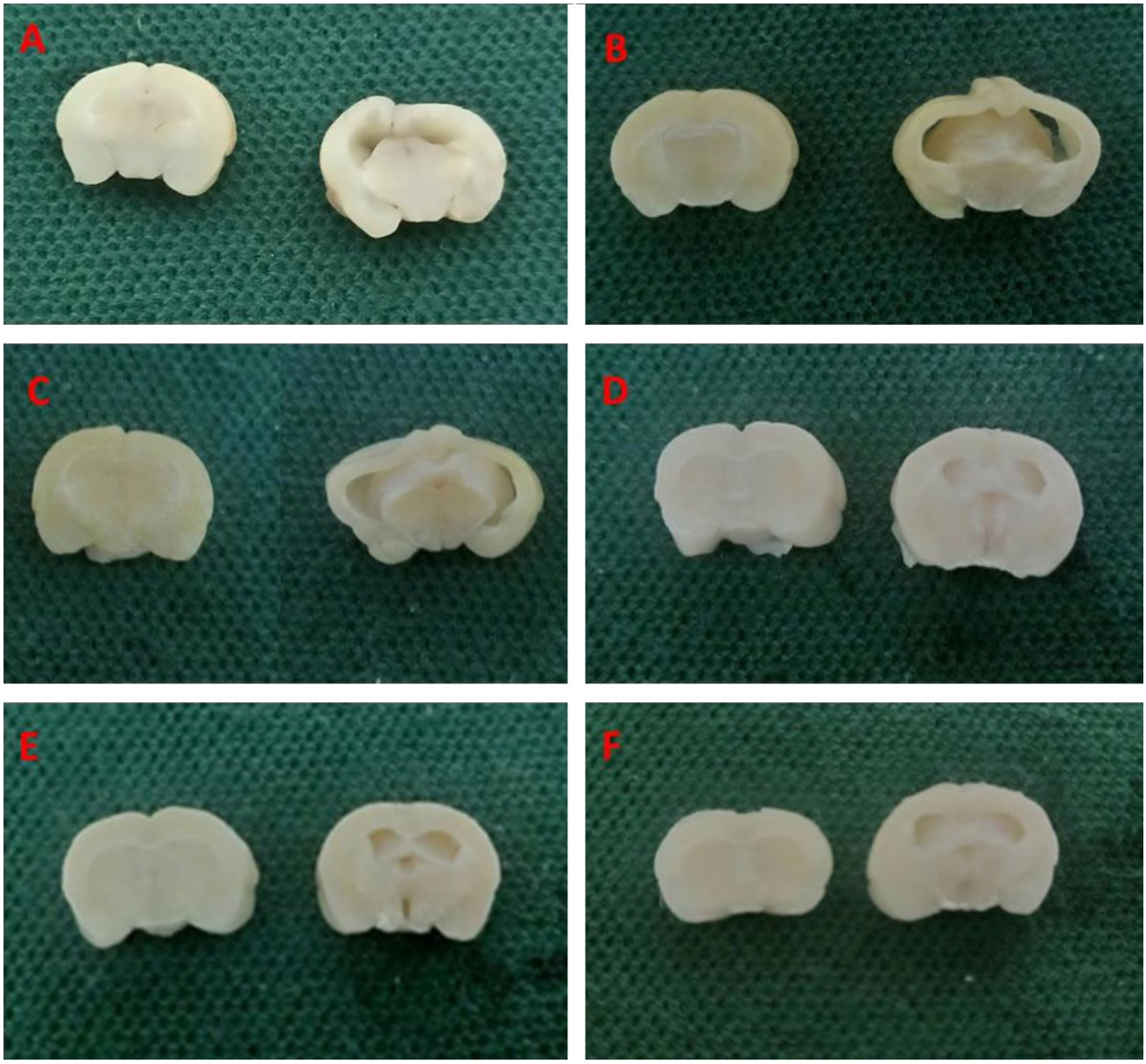

Fig. 3.- Representative gross photographs of dissected rat brains (control and hydrocephalic). The hydrocephalic brains show distended ventricles compared to their age matched controls. **A**: N2W; **B**: N4W; **C**: J4W; **D**: J8W; **E**: A4W; **F**: A8W. (A: adult; N: neonate; J: juvenile; W: weeks).

weeks) hydrocephalus, the differences were not statistically significant.

However, in the CA3 sub-region the pyknotic indices were significantly increased only in neonates with acute hydrocephalus and in juveniles with the chronic form (Table 1).

Neuronal cell density

The total number of neurons (spared and dead cells) in the CA1 and CA3 hippocampal sub-regions was counted per square area using Motic image plus 2.0 to obtain neuronal cell density. Statistical analysis (Table 2) between the hydrocephalic and controls of the CA1 and CA3 sub-region of the hippocampus showed no significant difference in the neuronal cell density among the groups.

Modified Golgi stain

Dendritic arborization of the pyramidal neurons of the hippocampal layers were assessed by the modified Golgi stain. The pyramidal neurons selected for analysis must meet these criteria: (a) completely impregnated, (b) isolated from the other neurons. Well-defined pyramidal neurons with pear-shaped soma were observed in the control animals. Each soma gave rise to a single apical dendrite, which courses through the surface of the hippocampus and divides into

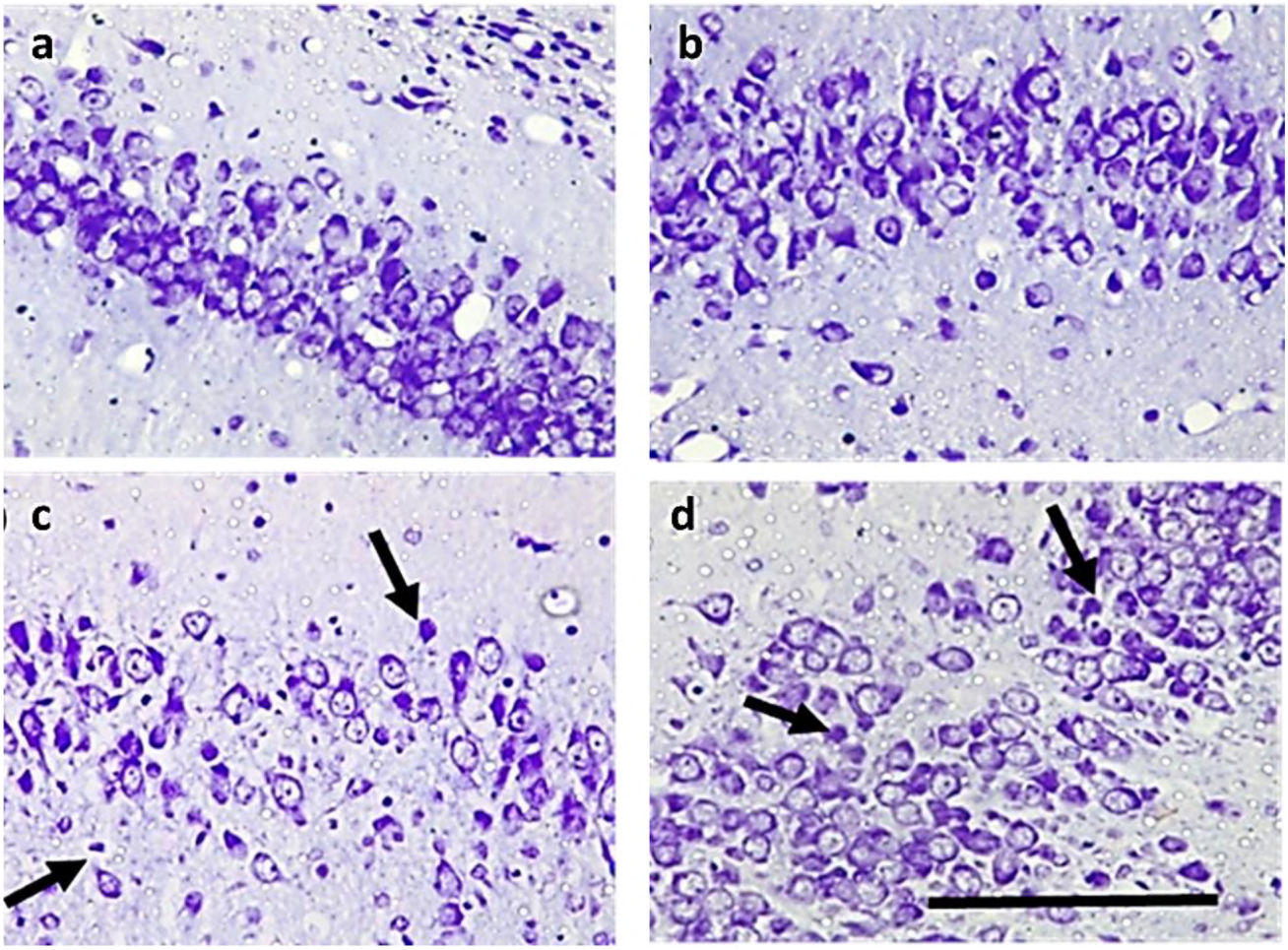

Fig. 4.- Cresyl violet stained CA1 & CA2 subareas of the hippocampus of neonates 2 weeks old (**a-d**). Note the derangement of the layered CA1 and CA2 subareas in the hydrocephalic sections (c,d) and pyknotic neurons (black arrows). (**a, b**: control rats; **c, d**: hydrocephalic rats; a: CA1 control; b: CA2 control. Scale bar: 50 µm).

Table 1. Pyknotic indices of hippocampal CA1 and CA3 subregion.

	CONTROL CA1	**HYDROCEPHALIC CA1**	**CONTROL CA3**	**HYDROCEPHALIC CA3**
GROUPS	(n=4)	(n=4)	(n=4)	(n=4)
N, acute	6.10 ± 1.16	24.50 ± 7.25*	12.55 ± 1.30	23.63 ± 1.26*
N, inter	12.10 ± 1.07	20.85 ± 1.02*	16.60 ± 1.19	22.38 ± 6.75
J, inter	9.68 ± 1.06	22.48 ± 5.28*	14.50 ± 1.22	22.20 ± 2.22*
J, chronic	8.83 ± 0.59	29.50 ± 12.61	13.68 ± 1.10	15.95 ± 3.66
A, inter	6.13 ± 1.11	10.98 ± 1.18*	13.40 ± 0.08	16.35 ± 4.22
A, chronic	8.03 ± 0.53	9.85 ± 1.16	13.13 ± 1.76	15.35 ± 0.84

(N: neonate; J; Juvenile; A: adult; inter: intermediate); *p<0.05

several terminal branches. The hydrocephalic animals demonstrated somatic features similar to that of control. However, major differences were observed in the dendritic branching of the experimental animals (Fig. 7). Although the branching patterns of the apical dendrites were similar to that of control animals, they possess fewer terminal branches.

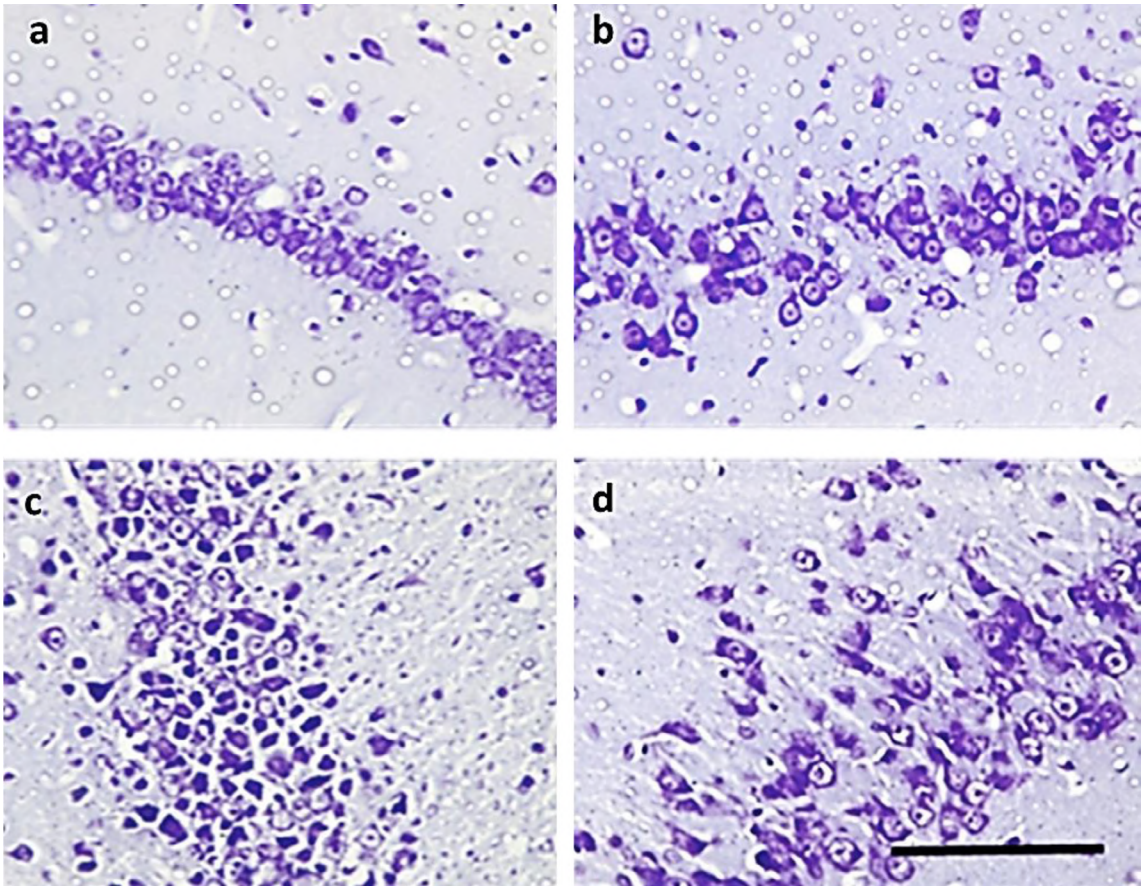

Fig. 5.- Cresyl violet stained hippocampus of juvenile 8 weeks old (**a-d**). Note the derangement of the layered CA1 and CA2 subareas in the hydrocephalic sections (c, d). (**a, b**: control rats; **c, d**: hydrocephalic rats; a: CA1 control; b: CA2 control. Scale bar: 50 µm).

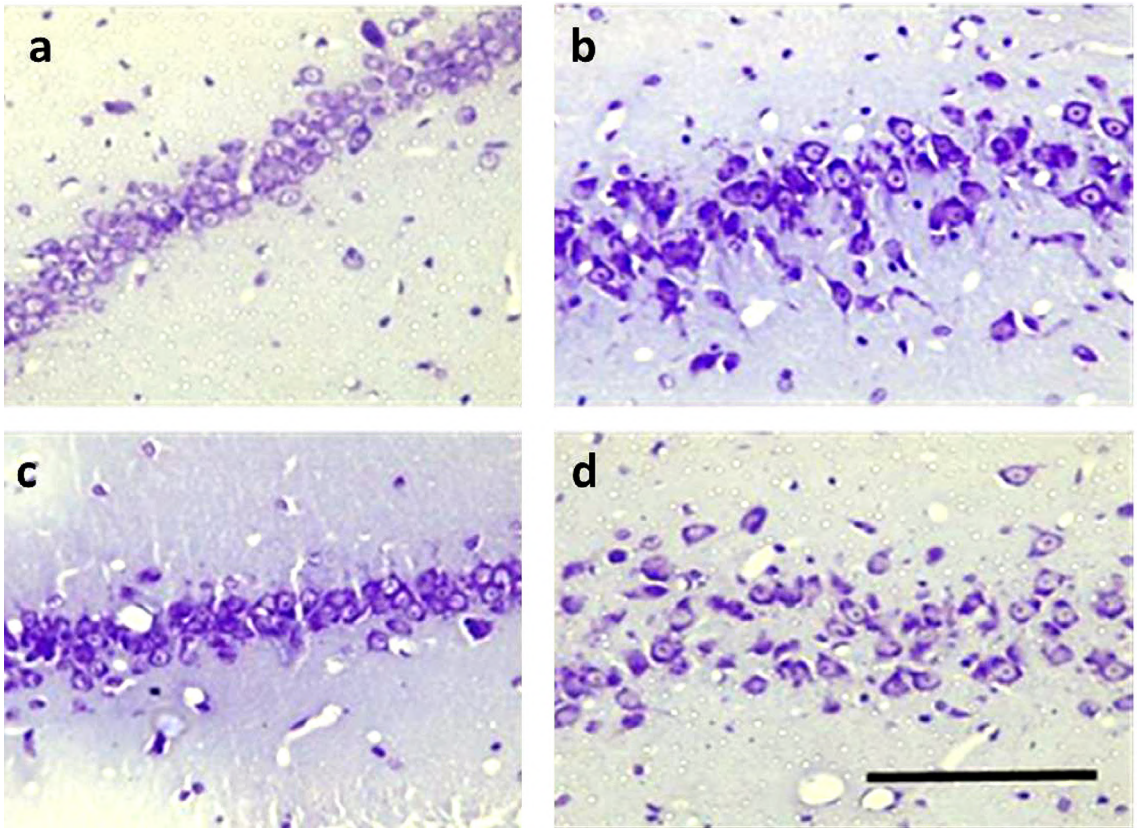

Fig. 6.- Cresyl violet stained hippocampus of adult 4 weeks old rats. Note the dispersed and fewer cells in the CA2 subarea of the hydrocephalic section. (**a**: CA1 control; **c**: CA1 hydrocephalic; **b**: CA2 control; **d**: CA2 hydrocephalic. Scale bar: 50 µm).

Table 2. Neuronal cell density of hippocampal CA1 and CA3 subregions.

	CONTROL CA1	HYDROCEPHALIC CA1	CONTROL CA3	HYDROCEPHALIC CA3
GROUPS	(n=4)	(n=4)	(n=4)	(n=4)
N, acute	41.43 ± 4.56	36.35 ± 2.45	27.65 ± 1.81	25.48 ± 2.93
N, inter	36.05 ± 1.23	39.35 ± 3.71	27.15 ± 0.64	31.75 ± 1.75
J, inter	32.78 ± 2.54	41.13 ± 5.05	31.33 ± 2.18	32.50 ± 1.48
J, chronic	34.25 ± 3.10	33.60 ± 4.60	28.18 ± 1.53	26.20 ± 2.24
A, inter	38.75 ± 2.04	31.83 ± 3.19	26.33 ± 0.78	28.03 ± 2.00
A, chronic	32.00 ± 0.86	37.98 ± 2.30	25.43 ± 0.70	27.48 ± 0.84

(N: neonate; J; Juvenile; A: adult; inter: intermediate); $p > 0.05$ in all subgroups, in comparison to control.

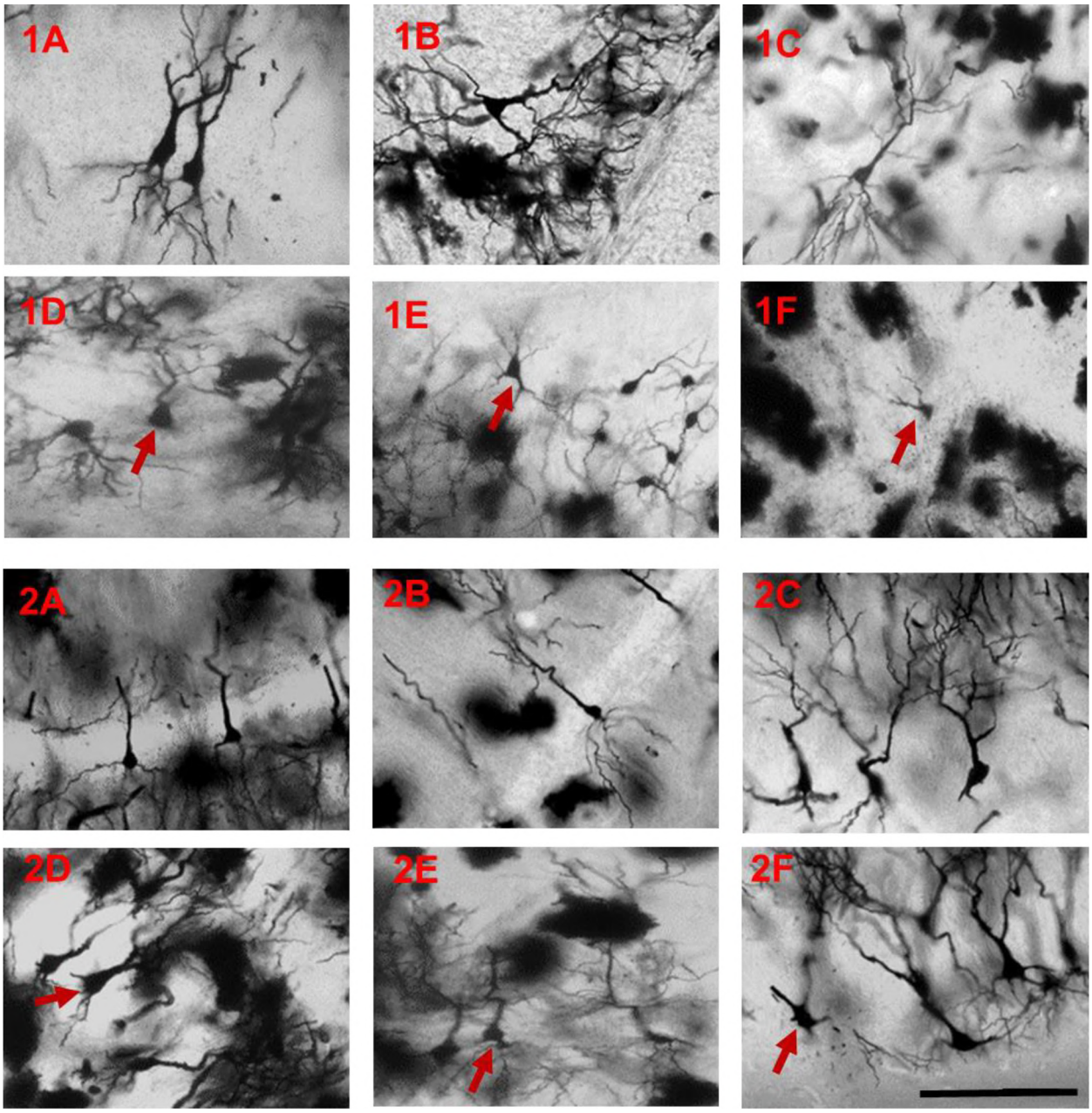

Fig. 7.- Modified Golgi-stained pyramidal cells of the hippocampus of neonates, acute (**1A,1D**); neonate, intermediate (**1B,1E**); juvenile, intermediate (**1C,1F**); juvenile, chronic (**2A,2D**); adult, intermediate; (**2B,2E**) and adult, chronic (**2C,2F**). Note the fewer basal dendrites (red arrows) in the hydrocephalic groups. (1A,1B,1C,2A,2B,2C: control rats; 1D,1E,1F,2D,2E,2F: hydrocephalic rats; Scale bar: 50 µm).

DISCUSSION

In this study, we examined the early, intermediate and long-term changes in the structure of the pyramidal neurons of the hippocampus in relation to learning and memory in hydrocephalic rats at different age groups. We found an age-related vulnerability and a tendency to recovery over time.

We have previously described changes in the density of neurons in the sensorimotor cortex and impaired neurodevelopmental progression in neonatal hydrocephalic mice (Femi-Akinlosotu and Shokunbi, 2020). We showed that in these animals, the pyramidal cells of the sensorimotor cortex suffered diminution of basal dendritic arbor, synaptic spine density and synaptophysin immunostaining (Femi-Akinlosotu et al., 2019). We have also demonstrated that in adult-onset hydrocephalus in this rodent species, the pyramidal layer of the CA1 (but not CA3) region of hippocampus showed increased pyknosis and cells with reduction of dendritic arbor (Shokunbi et al., 2020). In these studies, we provided supportive evidence for significant cellular injury in the sensorimotor and hippocampal cortices for the abnormal neurodevelopmental outcomes in neonatal and adult murine experimental hydrocephalus.

In the present investigation, we explored hydrocephalic neuronal injury further by examining the dendritic arborization of the pyramidal neurons of the CA1 and CA3 regions of the hippocampus at different stages of development in the rat hydrocephalus model, and related our findings to the neurobehavioural status of the animals. Our study revealed the following key findings: a) loss of basal dendrites in pyramidal cells of the hippocampus in all age groups; b) excessive death of pyramidal cells of the CA1 of the hippocampus throughout the neonatal period, but evident only early in the hydrocephalic process in juvenile and adult rats; c) loss of basal dendrites in the early phase of the disease in the CA3 region only in neonates and juveniles; d) relative resistance of the pyramidal cells in CA3 to pyknosis; e) learning and spatial memory were impaired by hydrocephalus. These results indicate a regional gradient in the vulnerability of the pyramidal cells of the hippocampus to hydrocephalus, with the CA1 region showing higher predilection for hydrocephalic injury. They also suggest a greater vulnerability of neonates and juveniles and better recovery in adult rats.

Intra-cisternal injection of kaolin has been an effective method for inducing hydrocephalus in experimental animals producing variable degree of ventricular dilatation (Khan et al., 2006; Del Bigio et al., 2003; Karinna et al., 2012; Olopade and Shokunbi, 2012, Femi-Akinlosotu et al., 2019). In this study, intra-cisternal injection of 150mg of kaolin in 1ml of sterile water successfully induced hydrocephalus in the neonate, juvenile and adult rats, with reduction in mortality rate, probably due to a slower rate of ventricular enlargement (Kondziella et al., 2008). Despite the use of the same volume of kaolin suspension for rats of the same age, the degree of hydrocephalus was variable with substantial ventricular distension often observed in adult rats with chronic hydrocephalus. This is similar to previously reported observations (Brinker et al., 1998; Klinge et al., 2003). Experimental animals showed enlarged dome-shaped head, unsteady gait and poorly groomed fur. These are consistent with findings in previous reports (Ding et al., 2001; Olopade et al., 2012; Olopade and Shokunbi, 2016.). The body weights of the hydrocephalic rats in the three age groups were significantly reduced in the first week post-injection of kaolin. This was followed by a slower rate of gain which was observed among the controls. This has been also previously observed and may likely be due to reduced activity and loss of appetite (Del Bigio, 2001; Olopade and Shokunbi, 2016; Femi-Akinlosotu et al., 2019).

Learning and memory were impaired as revealed in the Morris water maze tasks. The hydrocephalic rats, especially the chronic ones (8 weeks P.I) had significantly higher escape latency, i.e.. they took longer time to locate the hidden platform during the training periods, suggesting impairment in their learning ability. The result of the probe trial showed that the hydrocephalic rats' memories were impaired as the number of crossing made by them were significantly fewer than those of their age-matched controls. These

findings are consistent with earlier studies that also tested rats' performance in multiple days using the Morris Water Maze task (Olopade et al., 2012, 2016; Williams et al., 2014; Chen et al., 2016).

Several previous studies have described increased pyknosis and neuronal dispersal in the hippocampus in hydrocephalic animal brains (Kriebel and McAllister, 2000; Taveira et al., 2012; Chen et al., 2016.) and have related these changes to impaired memory and behavior. However, they did not differentiate the hippocampal subareas, but only examined subarea CA3. Histological staining of the hippocampal pyramidal neurons showed cytoarchitectural distortion of the pyramidal layer of CA1 and CA3 subareas. Dispersed pyramidal neurons and loss of the normal 4-8 rows of pyramidal neurons of the CA1 hippocampal sub-region were observed in hydrocephalic rat brains. These observations had been earlier reported by Chen et al. (2016), where the neurons of the CA1 and CA3 hippocampal sub-regions were altered and dispersed in hydrocephalic rats. Our study revealed that the cytoarchitectural alteration was more pronounced in the neonates and juveniles than in the adults. We observed this across all the groups despite the alteration in the cytoarchitecture of these neurons. However, this is consistent with the report by Chen et al. (2016) that there was no increase in neuronal density of hydrocephalic rats compared to the control rats in both acute and chronic stages of hydrocephalus.

The hippocampus CA1 region is known to be very susceptible to hypoxia and ischemia, and is considered to be an important target for the secondary neuronal injury originating in hydrocephalus (Karinna et al., 2012; Schultz and Engelhardt, 2014). However, other studies have shown neuronal tolerance during hydrocephalus in the CA1 sub-region of hydrocephalic rats, as the CA1 neurons do not show the neuronal degeneration and death such as observed in ischemic brain injury. CA1 sub-region might not be critical in the pathology of hydrocephalus as there was no apparent increase in neuronal death of the hippocampal pyramidal neurons but rather reduction in their connectivity (Ding, 2001; Chen et al., 2016). Nevertheless, dark shrunken neurons, especially at the early hydrocephalic stage have been observed in other studies (Kriebel and McAllister, 2000; Del Bigio, 2003; Cabuk et al., 2011; Taveira et al., 2012; Karinna et al., 2013; Turgut et al., 2018).

Our study revealed that the pyknotic index was significantly increased in the CA1 in the two groups of hydrocephalic neonates, but only in the early (4 weeks) groups of juveniles and adult hydrocephalic rats. The diminished amount of neuronal death observed in the chronic state (8 weeks) can be attributed to a tolerance to the ischemic injury developed during that hydrocephalic insult. Cortical and hippocampal blood flow might have decreased in early hydrocephalus and return to normal in chronic hydrocephalus due to collateral supply (Klinge et al., 2003). Their observations suggest that neuronal tolerance to ischemia might develop in hydrocephalic rats.

The geometry of these pyramidal neurons may influence their propagation and /or integration of their synapses (Piskorowski and Chevaleyre, 2012). Dendritic arborization of hippocampal pyramidal neurons have characteristics morphology, with the apical and basal dendrites of the CA1 pyramidal neurons having a single apical dendrite which may or may not bifurcate from the soma. Several secondary oblique dendrites may also emerge from the apical dendrites. However, the apical dendrite of CA2 neurons bifurcates close to the soma into two or three apical dendrites. The pyramidal neurons of the hippocampus are similar to their counterparts in the forebrain, with extensive apical and short basal dendrites. A typical pyramidal neuron receives tens of thousands of excitatory and inhibitory synaptic inputs (Piskorowski and Chevaleyre, 2012). Alterations in the branching of the neuronal dendritic morphology have essential significance functionally. Our study revealed alterations in the basal dendrites of the pyramidal neurons of the CA1 and CA3 regions of the hydrocephalic rats. Several studies have documented various morphological alterations in the networking of the hippocampal pyramidal neurons (Kirino, 1982; Kawamata et al., 1988; Kriebel and McAllister, 2000; Klinge et al., 2003; Cabuk et al., 2011; Chakraborti et al.,

2012; Piskorowski and Chevaleyre, 2012; Chen et al., 2016). Chakraborti et al. (2012) reported a decrease in the total dendritic length and dendritic spine densities of the CA1 pyramidal neurons of the hydrocephalic hippocampus in young adult mice. They suggested that these changes might reduce excitatory connectivity, which could underlie the learning and memory deficits observed in hydrocephalic states.

CONCLUSION

We have described the acute, intermediate and chronic changes in hippocampal pyramidal neurons in experimental hydrocephalus in neonatal, juvenile and adult rats and the varying effects on learning and memory in these animals. Our results suggest that the CA1 region in young animals is particularly vulnerable, with functional recovery occurring over time and to a greater extent in older animals. These results may have implications for clinical decision making in the management of hydrocephalus.

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Ethics approval: Approval was obtained from the ethics committee of University of Ibadan, Nigeria. The procedures used in this study adhere to the tenets of the EU Directive 2010/63/EU for animal experiments.

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