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Neuronal excitability changes depend on the time course of cellular edema induced by water intoxication in young rats

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Abstract

OBJECTIVES: The aim of the study was to determine whether the functional state of neurons is affected by the duration of the induced cellular edema and by the age of animals tested. The cellular edema was induced by water intoxication and neuronal functions were tested by the standard method of electrical stimulation of neurons of the cerebral cortex.
METHODS: water intoxication was induced by standard method of fractionated hyperhydration. Excitability of cortical neurons was tested by cortical stimulation with the intensity required to induce cortical afterdischarge (AD). Animals were divided into three experimental groups (B, C, D) and three control groups (AB, AC, AD). Experimental groups differed in age of water intoxication (12 or 25 days) and age of excitability testing (25 or 35 days). Changes in the duration of AD (seconds) were statistically evaluated.
RESULTS: Duration of cortical afterdischarges (AD) in the control groups was at the level literature data. In all experimental groups (B, C, D), excitability of cortical neurons was markedly inhibited. AD was possible to induce only in some of the animals and its average duration was significantly shorter than in control groups.
CONCLUSION: This inhibitory effect can be explained by persistent impairment of astrocyte-to-neuron communication, which plays a key role in the process of formation of structural and functional changes during cellular edema. Some of the functional manifestations of the developing edema are influenced by the age of experimental animals. At least some events of this process are not influenced by the age of experimental animals.

Abbreviations:

AQP 4	- aquaporin 4
AD	- cortical afterdischarges
BBB	- blood brain barrier
CNS	- central nervous system
EU	- European Union
ADC	- apparent diffusion coefficients
DAI	- diffuse axonal injury

O R I G I N A L A R T I C L E

INTRODUCTION

Water intoxication (hyperhydration, overhydratation) leads to hypoosmolality due to the dilution of solutes in the extracellular fluid. An osmotic gradient which drives water from the extracellular into the intracellular compartment is formed (Go 1997; Kimlberg 1995). Accumulation of water in cells initiates a cascade of events that interferes with cell metabolism and results in cytotoxic edema (Klatzo 1967). Recent view on the pathophysiology of cytotoxic edema brought Liang and colleagues in 2007 (Liang *et al.* 2007). Cytotoxic edema (or cellular edema, oncotic cell swelling, oncosis), represents premorbid cellular process with influx and intracellular accumulation of Na+, and other cations within neurons and astrocytes. In order to maintain electrical neutrality, the influx of cations is followed by influx of anions with subsequent transfer of water into the cells. Water passes membrane via specific water channels – aquaporins (AQP). From the 13 types known in mammals, seven occur in the CNS. An essential role for the movement of water in the CNS and the formation of cellular edema has AQP4 (Agre *et al.* 2004; Hsu *et al.* 2015; Manley *et al.* 2000; Papadopoulos & Verkman 2007; Pasantes-Morales *et al.* 2002; Wells 1998). Water movements result in osmotic cell expansion, the main feature of cytotoxic edema. Intracellular edema does not lead to brain swelling, but depletes the extracellular space of Na^+, Cl^- and water, and induces a new gradient for the transfer of these ions and water across the capillaries of gliovascular complex (BBB). As a result, the selective permeability of the BBB is lost, this new gradient formed by cytotoxic edema leads to transcapilary ionic edema. It is a process known as brain edema which is characterized by an increased content of water and increased volume of the brain (Klatzo 1967, Michinaga and Koyama 2015). Cellular swelling induced by water intoxication is currently used as a standard experimental model (Manley *et al.* 2000; Olson *et al.* 1994; Vajda *et al.* 2000; Yamaguchi *et al.* 1997).

The above given facts allow to suggest a hypothesis on the negative effect of the developing cytotoxic edema on the neuronal activity. The aim of the work was to study cortical excitability in various time intervals from the induction of cellular edema by water intoxication, the role of the duration of edema and the age of experimental animals.

MATERIAL AND METHODS

All experiments were approved by the Ethical Committee of the First Faculty of Medicine (Charles University in Prague) and were in agreement with the Guidelines of the Animal Protection Law of the Czech Republic and Guidelines for the treatment of laboratory animals EU Guidelines 86/609/EEC.

Adult male Wistar strain laboratory rats of our own bred were used in experiments. Animals were divided into three experimental groups (B, C, D) which correspond to the three control groups (AB, AC, AD) – see Table 1. Experimental groups differed in age when which the water intoxication was introduced and when the excitability was tested. Group B was hyperhydrated at the age of 25 days and excitability was tested 19–20 hours after the last dose of water. Animals in group C were hydrated in the age of 12 days and excitability was tested in the age of 25 days. In group D water intoxication started when animals were 25 days old and excitability was tested at the age of 35 days. The number of animals in each group and the reference to the figure with results are shown in Table 1.

Water intoxication

For hyperhydration the standard model of water intoxication was used (Olson *et al.* 1994, Manley *et al.* 2000, Vajda *et al.* 2000, Yamaguchi *et al.* 1997). Animals received distilled water in the amount corresponding to 20% of their body weight. The volume was divided into three parts and administered intraperitoneally in 8 hours interval during 24 hours.

Electrophysiology

Excitability of cortical neurons was tested by the stimulation of the cerebral cortex. Stimulating electrodes were placed and fixed at the right sensorimotor area; registration electrodes were located at the left sensorimotor area and bilaterally at the occipital area. Stimulation parameters were used as follows: rectangular bipolar

Tab. 1. Characteristics of experimental and control groups.

Group	Number of animals	Age at the last dose of DW	Age at the excitability testing	Interval between DW administration and testing	Figure
Control group AB	29		25 days		
Group B	9	25 days	25 days	19-20 hours	1
Control group AC	29		25 days		
Group C	9	12 days	25 days	13 days	2
Control group AD	11		35 days		
Group D	8	25 days	35 days	10 days	3

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pulses, pulse duration 0.5 ms, the intensity required to induce cortical afterdischarge (AD) 3–5 mA, frequency of 8 Hz, duration of stimulation was 15 s. The stimulation was repeated 5 times, always 1 minute after the termination of AD. If AD was not elicited with stimulation intensity up to 5 mA, the trial was completed. AD duration in all groups were statistically evaluated using GraphPad Prism tests (unpaired t test and F test to compare variances; statistical significance was set at 5%).

RESULTS

Differences in excitability in individual experimental groups was assesed according to the duration of AD (in seconds).

Group B (hyperhydration at 25 days, EEG recording within the next 20 hours)

Duration of AD in the control group (AB) was significantly different (p<0.001) during the second, third and fourth stimulation from the first, fifth and sixth stimulation (statistical significance is not given). In the experimental group (B) AD was induced only in three animals, and its average duration was at all stimulations (1–6) significantly shorter than the AD duration in the control group (statistical significance is given by asterisks) (Figure 1).

Group C (hyperhydration at 12 days, EEG recording after 13 days)

Also in the group of youngest rats (12-day-old) the duration of AD in the control group (AC) was significantly different (p<0.001) during the second, third and fourth stimulation from the first, fifth and sixth stimulation (for simplification, statistical significance is not indicated). In the experimental group of the same age (C) afterdischarges were induced only in two animals, and their average duration was at all stimulations (1–6) significantly shorter than the AD duration in the control group (statistical significance is given by asterisks) (Figure 2).

Group D (hyperhydration at 25 days, EEG recording after 10 days)

Duration of AD in the control group (AD) was significantly different (p<0.001) during the second, third and fourth stimulation from the first, fifth and sixth stimulation (statistical significance is not given). In the experimental group (C) ADs were induced only in two animals, and their average duration was at all stimulations (1–6) significantly shorter than the AD duration in the control group (statistical significance is given by asterisks) (Figure 3).

Excitability of cortical neurons was tested by repeated cortical stimulation in order to generate and register cortical afterdischarge (AD). Duration of ADs was monitored to evaluate excitability changes in exper-

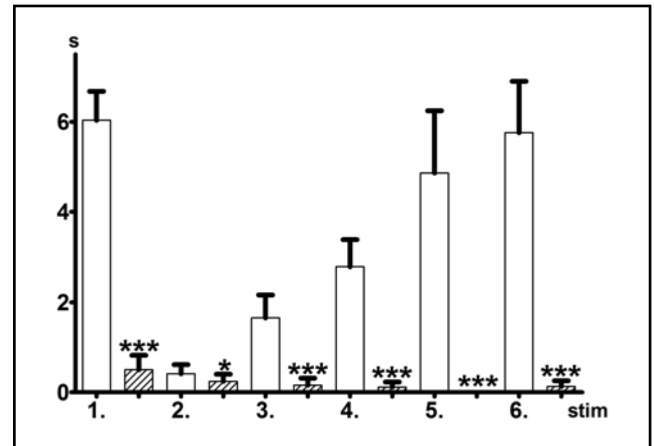

Fig. 1. Duration of AD in 25-day-old animals, tested within 20 hours white columns: control group AB, diagonal lined columns: experimental group B, vertical axis: duration of ADs in seconds (s), horizontal axis: order of stimulation, *=p<0.05, ***=p<0.001, error bars: ±SEM.

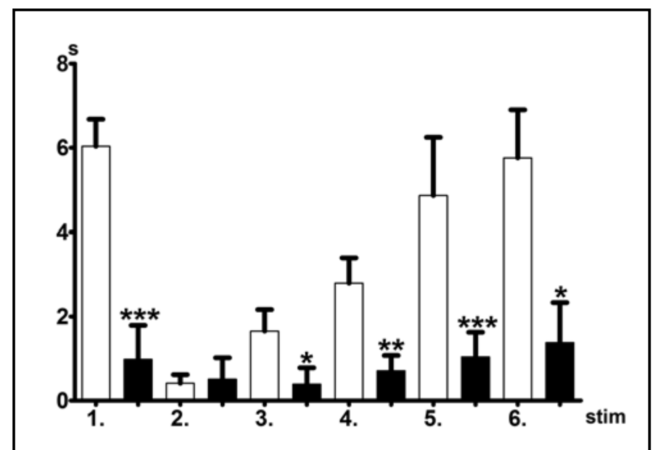

Fig. 2. Duration of AD in 12-day-old animals, tested after 13 days white columns: control group AC, black columns: experimental group C, vertical axis: duration of ADs in seconds (s), horizontal axis: order of stimulation, *=p<0.05, **=p<0.01, ***=p<0.001, error bars: ±SEM.

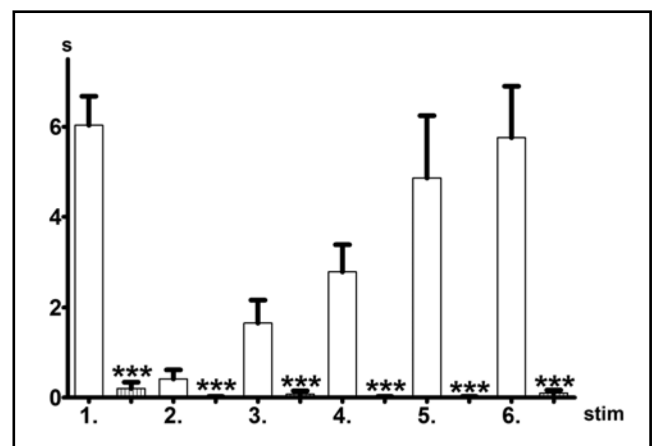

Fig. 3. Duration of AD in 25-day-old animals, tested at 35 days white columns: control group AC, vertical lined columns: experimental group D, vertical axis: duration of ADs in seconds (s), horizontal axis: order of stimulation, ***=p<0.001, error bars: ±SEM.

imental groups B, C and D compared to the control animals. Duration of cortical afterdischarges (AD) in the control groups was at the level literature data (Seifert *et al.* 2010, Fellin and Carmignoto 2004). In all experimental groups (B, C, D), excitability of cortical neurons was markedly inhibited. AD was possible to induce only in some of the animals and its average duration was significantly shorter than in control groups.

DISCUSSION

Our previous work demonstrated that in rat pups aged 12 and 25 days water intoxication, achieved by the same standard method, brought about reduced excitability of cortical neurons tested by the standard method of cortical stimulation (Marešová *et al.* 2014). Attenuation of neuronal excitability was caused by disturbed homeostasis of water and ions in the microenvironment of the brain, which determines the excitability of cortical neurons (Fellin and Carmignoto 2004, Pasantes-Morales *et al.* 2002, Schwartzkroin *et al.* 1998, Seifert *et al.* 2010) A key finding of this phenomenon is that oncotic edema formation and the excitability of cortical neurons are controlled simultaneously and in interaction by both the largest cell populations – astrocytes and neurons. Nase and coworkers studied the penetration of water into astrocytes in the initial stage of cellular edema induced by water intoxication in genetically modified strain of mice whose astrocytes contained fluorescent proteins. With two photon laser scanning microscopy they demonstrated a volume increase of astrocytes and they concluded that astrocytes represent the point of entry of water into the cells in the initial stages of cellular edema formation (Nase *et al.* 2008). Neuronal function cannot be explained without the knowledge of glial regulatory mechanisms. One is the so-called tripartite synapses principle – a link between pre- and post-synaptic neuronal elements by cytoplasmic membrane of astrocytes. Because astrocytes are key cellular elements maintaining extracellular homeostasis by controlling concentration of ions, neurotransmitters and in general all active molecules, tripartite synapse path is responsible for neuronal excitability (Tasker *et al.* 2012, Verkhratsky and Parpura 2010). Astrocytes can recognize the degree of neuronal activity and release signalling molecule which modulate variety of neuronal functions, including neuronal excitability (Fellin 2009).

Irrespective of the method of induction (oncotic, ischemic, traumatic), cytotoxic edema, defined as premorbid cellular process (Liang *et al.* 2007), has its time development and course of which can be revealed by accompanying abnormalities. Creed *et al.* studied behavior and structural changes after a minor injury in adult mice, which led to cerebral edema. They demonstrated histologically degeneration of cortical and hippocampal neurons during the first three days after the injury. It was accompanied by behavioral disorders manifested by the loss of spatial orientation resulting from the loss of memory. These changes disappeared the fourth day after the insult. They also observed inraaxonal accumulation of amyloid precursor protein in the corpus callosum and cingulum during the first three days after the injury, which was associated with dephosphorylation of neurofilament and axonal conductivity deficiency, which was detectable until 14 days after the insult. From these findings they concluded that though the behavior of mice was from the 4th day after insult normal, brain edema induced by an injury resulted in persisting structural and functional axonal disorder (Creed *et al.* 2011). In our previous study we demonstrated by histological examination that cellular edema induced by water intoxication in adult rats led to myelin disintegration already 30 minutes after the completion of hyperhydration, and it remained evident for one following week. Despite the apparent structural lesions in rats, no behavioral changes were observed (Kozler & Pokorny 2012).

Kuroiwa *et al.* by MRI examination found that cellular edema induced by ischemia can be demonstrated by a decrease in apparent diffusion coefficients (ADC). Changes occur already within 15 minutes after ischemia and the rate of decrease corresponds to the amount of intracellulary accumulated water. Decrease the ADC and thus manifestation of cellular edema significantly predates formation of necrotic cells in the ischemia. From these findings they concluded that the mechanism of formation of cellular edema is very fast (Kuroiwa *et al.* 2007). In another MRI study, Engelhorn and co-workers confirmed, that water moves rapidly between the compartments of the brain. They studied the movement of water in brain compartments using ADC in rats after the generalized status epilepticus induced by pilocarpine. They found that very soon after the seizure – between 3 and 10 minutes ADC increases sharply (water is localized extracellularly), then ADC gradually and steadily (measured over 120 minutes) decreases (water becomes localized intracellularly) (Engelhorn *et al.* 2007). Onaya showed that the typical signs of a Diffuse Axonal Injury (DAI) – retraction loop – can be found only in very pale axons, which is a sign of completed cellular swelling. In other words, unless cellular swelling was developed, structural changes in axon – pallor and retraction balls cannot be formed (Onaya 2002).

Overview of literature data shows that the onset of changes induced cellular edema is fast, and some functional and structural changes in the CNS can be prolonged without being accompanied by alterations in the behavior of animals.

Our present study revealed a decrease of excitability of neurons in the group of 25-day-old rats tested 19–20 hours after the completion of hyperhydration (see experimental group B). The same decrease was found in the group of 25-day-old rats tested the 13th day after the end hyperhydration (see experimental group C). In the

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group of 35-day-old rats tested the 10th day after the end hyperhydration (see experimental group D) excitability of neurons was attenuated in a similar manner.

The aim of the present work was to ascertain whether the functional state of neurons depends on the duration of the induced cellular edema and on the age of animals. The results allow following conclusion: In 25-day-old rats changes in the homeostasis of the brain microenvironment induced by water intoxication attenuate excitability of cortical neurons within several hours (19–20). Impaired neuronal function persists for 10 to 13 days, irrespective of the age of the animal during hyperhydration.

The above reviewed findings on prolonged structural and functional changes due to the timing of the cytotoxic (oncotic) edema were obtained in studies with adult animals (mice, rats). Our results correspond to these findings. It means that at least some of the functional changes in the CNS, induced by water intoxication, do not depend on the age of experimental animals. We believe that our findings result from changes in astrocyte-to-neuron communication. It confirms the importance of astrocytes as a pathway of entry of water into the cells in the early stages of the cellular edema formation, and also the role of astrocytes as cells that affect many neuronal functions, inter alia, neuronal excitability (Nase *et al.* 2008, Frollin 2009).

CONCLUSIONS

Water intoxication induced by standard method of fractionated hyperhydration brought about in 25-day-old rats a significant decrease of excitabity of cortical neurons when tested shortly after hyperhydration (for 19–20 hours). Similar inhibition was found in animals hydrated at the 12th day and tested 13 days later, and in animals hydrated at the 25th day and tested 10 days later. It can be caused by a failure of glial regulating mechanisms during the initial phases of cellular edema formation.

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