Neuroendocrinology Letters Volume 35 No. 7 2014
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

ORIGINAL ARTICLE

CT density decrease in water intoxication rat model of brain oedema

Petr Kozler, Jaroslav Pokorný

Charles University in Prague, First Faculty of Medicine, Institute of Physiology, Czech Republic

Correspondence to: Prof. Jaroslav Pokorný, MD., DSc.
Institute of Physiology, First Faculty of Medicine
Charles University in Prague
Albertov 5, 128 00, Prague 2, Czech Republic.
tel: +420 224968416; fax: +420 224918816; e-mail: pokorny@lf1.cuni.cz

Submitted: 2014-10-21 Accepted: 2014-11-21 Published online: 2014-12-27

Key words: **CT density; water intoxication; brain oedema; region of interest**

Neuroendocrinol Lett 2014; **35**(7):608–612 **PMID:** 25617884 NEL350714A06

Abstract

OBJECTIVES: The aim of this study was to determine whether water intoxication affects the radiodensity of brain tissue in CT scan examination in the rat model of brain oedema.

METHODS: A standard CT scan of the brain was obtained in a group of rats, first at control conditions (controls – CG) and then after hyperhydration (oedema model – EG) in the region of interest (ROI) corresponding to the area of coronary sections with pixel size 0.125 mm in position A (bregma +2.43 mm), position B (bregma –2.92 mm), position C (bregma –12.73 mm). Densitometrically determined mean values (MV), expressed in Hounsfield units (HU) were processed by standard statistical methods.

RESULTS: The average MV density was 120.49±6.79 HU for the control measurement and 88.01±4.72 HU after the hyperhydration, which represents decrease in the density by 32.48 HU ($p<0.001$). In the control measurement the average value of HU for the position A was 121.98, for position B 112.4 and for position C 127.08. In conditions of hyperhydration, the average MV density in position A was 89.95 HU in position B 84.67 HU and in position C 89.43 HU. The differences between the CG and EG were in all positions A, B, C statistically significant ($p<0.001$). In the control measurement, the differences between position A × B ($p<0.05$) and B × C ($p<0.001$) were statistically significant. After hyperhydration no significant difference between the position A, B, C was found.

CONCLUSION: water intoxication caused by hyperhydration in rats can induce diffuse brain oedema, which is reflected in the CT examination by the decrease of brain tissue density, expressed in HU. The value of the measured density depends on the location and size of the measured brain area.

Abbreviations:

CG	- control group
CT	- computed tomography
EG	- oedema group
HU	- Hounsfield units
MV	- mean value
ROI	- region of interest

INTRODUCTION

Ten years ago, Agre (Agre *et al.* 2004) and Kimelberg (Kimelberg 2004) summarized previously known findings on the brain oedema and thus laid foundations of our current understanding of this clinically serious condition. Oedema represents a life-threatening disorder of water homeostasis in the internal environment of the brain, which brings fluid accumulation in the cellular and extracellular brain compartments. Brain oedema develops as a result of pathological factors (e.g. trauma, ischemia or tumour) by means of brain aquaporins. In the early 70's of the previous century, computerized tomography was introduced into the clinical practice, which allowed identification of brain oedema. According to Cormack and Hounsfield who invented this method, radiodensity of water was set as 0 (zero) Hounsfield units (HU), which means that the density of the brain decreases proportionally with the increase of the water content (Cormack 1973; Hounsfield 1976). The aim of the present study was to ascertain whether the experimental model of brain oedema induced by water intoxication meets this criterion.

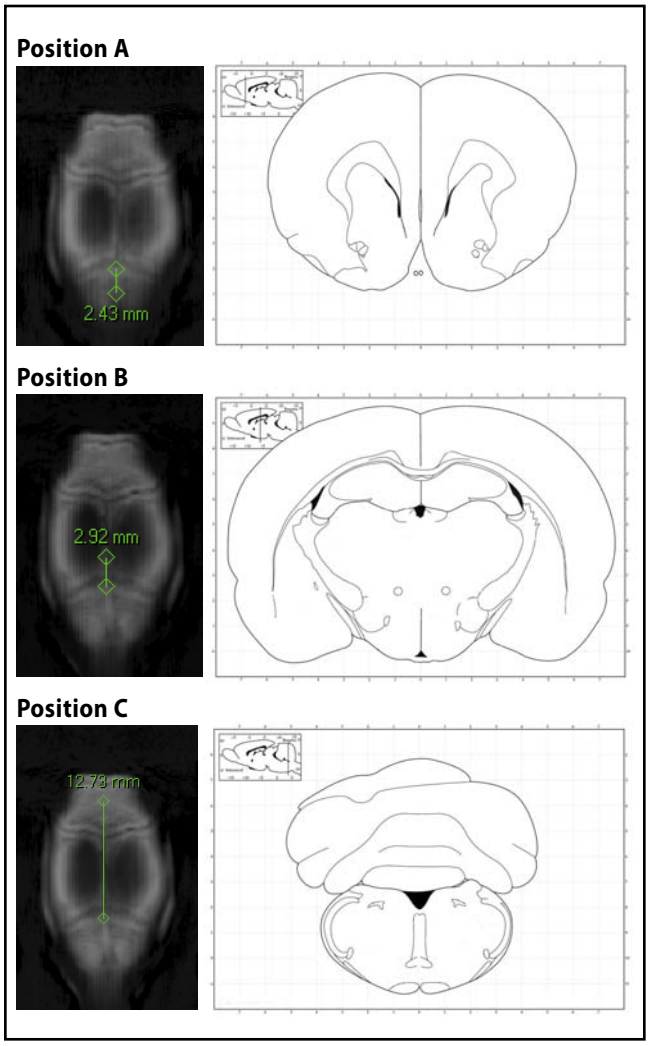

Fig. 1. Positions and corresponding sections from a stereotactic atlas. A: bregma +2.43 mm; B: bregma –2.92 mm; C: bregma –12.73 mm

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 (weight 390–410 g) were used in our experiments. Each animal was monitored at standard conditions (control group – CG) and later after hyperhydration (oedema group – EG).

Water intoxication

For hyperhydration the standard model of water intoxication was used. 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.

The next day (16 hours after the last dose of distilled water), animals underwent CT examination.

CT examination

For identification of brain oedema, Albira PET/CT system (Bruker, Biospin, Spain) was used in collaboration with the Institute of Physiology AV CR.

Three rats were used for standard CT scan of the brain (control group). Subsequently, the same rats were hyperhydrated (oedema group), and a new CT scan was obtained. Altogether six scans were performed and from their set of data, three coronal sections were selected for the evaluation: coronary section at bregma +2.43 mm, 0.125 mm pixel size, area size (ROI – region of interest) 0.52 cm^2 (position A), coronary section at bregma –2.92 mm, 0.125 mm pixel size, area size (ROI) 1.00 cm^2 (position B), coronary section at bregma –12.73 mm, 0.125 mm pixel size, area size (ROI) 0.51 cm^2 (position C). Positions A, B and C were determined with multiplanar reconstruction. For topographical orientation at each position, corresponding sections from a stereotactic atlas (Paxinos & Watson 1998) were assigned (Figure 1).

In all tests, software for determination the density (densitometry) was used to evaluate studied variables: minimal value, maximal value, mean value (MV), all expressed in Hounsfield units (HU). To compare data from individual recordings in both groups, mean value (MV) was selected. Beside the standard densitometry before and after hyperhydration, in one animal (rat No 3) a new evaluation in a smaller area size (ROI) was performed (instead of 1.00 cm^2 only the size 0.05 cm^2

was used). The measurement was carried out in three randomly located ROI within the section B.

The measured values were statistically analyzed using analysis of variance for one factor and repeated measurements.

RESULTS

For each rat, data were obtained from the initial control measurement and from the subsequent hyperhydration stage (oedema) (in total 18 measurements). CT densitometry software was used to evaluate each measurement area size, perimeter, minimum density, maximum density, mean value and standard deviation of the measured densities. For comparison, a constant value of surface fraction (evaluated area size – ROI) at each AP position was used. To demonstrate our procedure, CT images and accompanying densitometric data are given for one rat (No 1) (Figures 2 and 3).

Comparison of CT data before and after hyperhydration revealed that the hyperhydration resulted in a decrease of MV values in all three positions ($p<0.001$) (Table 1). At the same time the average MV values for all three positions measured before and after hyperhydration were also significantly different ($p<0.001$) with the mean density decrease of 32.48 HU.

The next step was to find whether there is a difference in MV between positions A, B and C. It turned out that in the control group the difference in MV between position A and B is a statistically significant ($p<0.05$) as well as between the position B and C ($p<0.001$). After hydration no significant differences between the position A, B, C were found. (Figure 4).

The next task was to determine whether the currently measured MV varies in relation to the size and location of ROI. MV at ROI corresponding to the surface area of the whole section was compared with much smaller ROI placed randomly at three different locations of the same section. The assessment was performed only in one section of a single rat (rat No 3); measured MV was therefore not evaluated statistically. At the position B (ROI size 1.00 cm^2) in the control conditions MV=112.9 HU and after oedema induction MV=84.03 HU (see also Table 1). When the size of ROI was set to a significantly smaller size (0.05 cm^2) and located in three places of the section (Figure 4), MV represented 139.24 HU, 103.70 HU, and 71.97 HU in control conditions and 130.47 HU, 78.43 HU and 55.0 HU in oedema state (Figure 5).

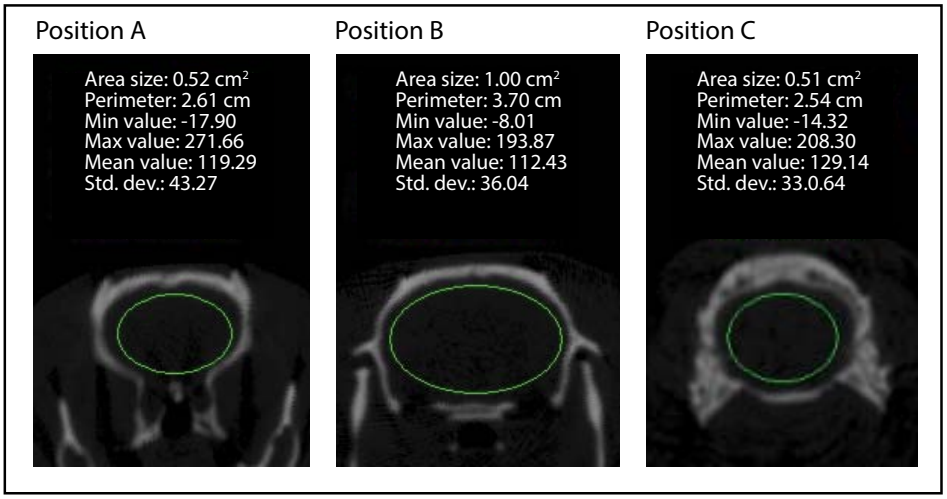

Fig. 2. CT values for three coronar sections in the control group (legend to section position see Fig. 1).

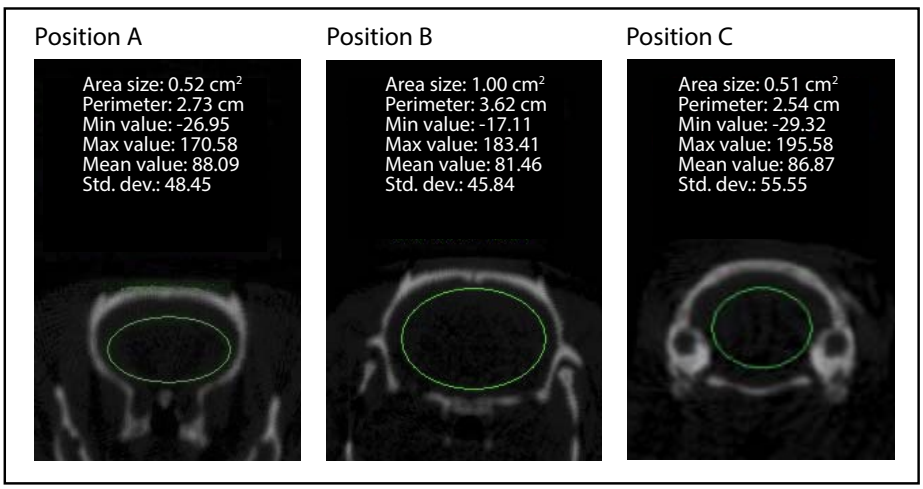

Fig. 3. CT values for three coronar sections in oedema group (legend to section position see Fig. 1).

DISCUSSION

The CT density of the normal human brain ranges from 29 to 38 HU (Mangel *et al.* 2002; Kucinski *et al.* 2002). If the minimum density of the brain tissue is 20 HU or if density is reduced by at least 12 HU, radiological criteria for brain oedema are met. Limit 20 HU considers the observation that density of the brain lower than 20 HU reflects not only the fluid accumulation in the brain tissue, but indicates the presence of necrosis and lipid transformation due to damage of the brain tissue, namely due to long lasting ischemia (Clasen *et al.* 1981; Torack 1982). Literary data indicate that

Tab. 1. Numeric values of MV expressed in HU for each animal in both groups at position A, B and C as well as an average MV±SE for the whole brain (A + B + C) are given.

position	A		B		C		A, B, C average	
	MV (HU)		MV (HU)		MV (HU)		MV (HU)	
# rat	control	oedema	control	oedema	control	oedema	control	oedema
1	119.29	88.09	112.43	81.46	129.14	86.07	120.3	85.2
2	126.23	93.52	111.86	88.52	126.52	96.85	121.54	92.96
3	120.42	88.23	112.9	84.03	125.59	85.36	119.64	85.87
position average	121.98±3.72	89.95±3.10	112.4±0.52	84.67±3.57	127.08±1.84	89.43±6.44	120.49±5.7	88.01±3.36

(legend to section position see Fig. 1).

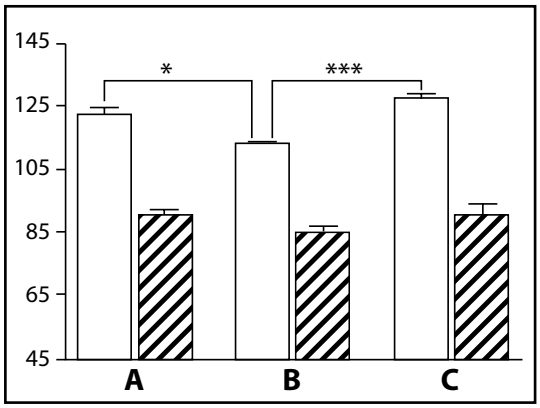

Fig. 4. CT density in Hounsfield units at three coronal sections of the brain. Control animals – empty columns, hyperhydrated animals – striped columns; Density value is given at vertical axis, position of section is on horizontal axis; Significance of differences at $p<0.05$(*) and at $p<0.001$(***) are given (legend to section position see Figure 1).

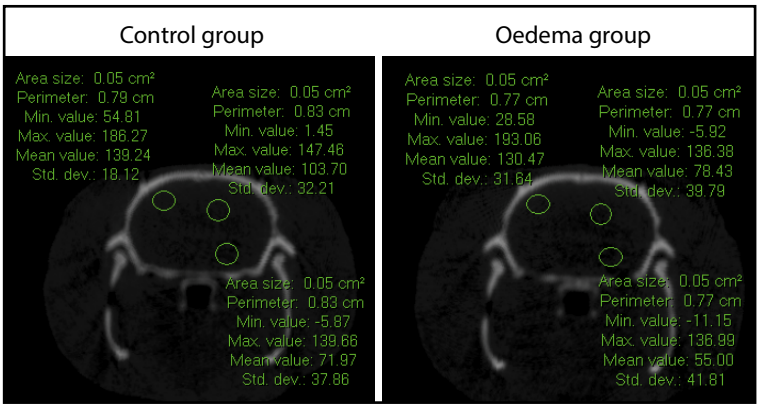

Fig. 5. Densitometric data for rat No 3 in control conditions and after hyperhydration in three randomly located ROI positions. Position B (bregma –2.92 mm), area size (ROI) 0.05 cm^2.

the density of the normal brain in rats is 75.6±2.2 HU, and after induction of ischemic oedema by middle cerebral artery occlusion decreases to 71.7±3.4 HU (Dzialowski *et al.* 2004). Our results showed that the density of the brain of control rats was 120.49±6.79 HU and it dropped after the hydration to 88.01±4.72 HU. The density difference was well above the minimum difference of 12 HU, indicating the presence of brain oedema in terms of densitometry (Clasen *et al.* 1981). This postulate is based on densitometry findings of the human brain. Similarly defined difference in densities that would point to brain oedema in rats, we have not found in the literature. Dzialowski and co-workers, using model of focal ischemia, considered existence of brain oedema in rats when density dropped by 3.9 HU (Dzialowski *et al.* 2004). In monkeys and cats with the same model of oedema, other authors also observed a density decrease (Clasen *et al.* 1981; Kuroiwa *et al.* 1998). In our experiments, the density difference between the normal brain and the hyperhydrated brain was more than 32 HU. The discrepancy between these data can be explained by the fact that, unlike the above-cited authors, we did not induce focal oedema, but we studied animals after water intoxication, leading to diffuse oedema (Olson *et al.* 1990).

In our experiment, densitometry was performed in a precisely defined region of interest (ROI) covering the whole surface area of the brain section (pixel size 0.125 mm) at three different locations along the rostro-caudal axis (positions A, B and C). The averaged values in control animals differed for each position; the difference being significant between positions A and B and between positions B and C (Figure 3). Variations in the brain radiodensity of the whole section area with the pixel size 0.125 mm can result from anatomical inhomogeneity of the brain at each position. In humans, differences were observed between the density of white matter (30 HU), gray matter (34 HU), cerebrospinal fluid (5 HU) and blood (47 HU) (Hounsfield 1976). Although for the brain of rats such values are not available, it can be assumed that differences in the density of brain components can be similar. Position C corresponds topographically with the section in cerebellum (see Figure 1C), which has a strong capillary vascular network with a small spaces with cerebrospinal fluid. Position B topographically corresponds with the sec-

tion in hippocampus with a rich ventricular system (see Figure 1B) and the position A corresponds to the prefrontal cerebral tissue with only very narrow chambers (see Figure 1A). Statistically higher CT density in position A and C versus position B is therefore understandable. Density values recorded after hyperhydration, expressed as average MV in each position, differed substantially less (Table 1, Figure 3).

Measured values clearly suggest the presence of brain oedema, as in all three positions, as the difference between control and hyperhydrated state was greater than 12 HU ($p<0.001$). While in the control state the measured values express the differences in structural composition within each position, in the oedema state such anatomical differences are wiped away by the accumulation of water.

Along with that, the dependence of the density value on the size and location of ROI was identified. It can be demonstrated in the result analysis of the rat No 3 in position B (see Figure 4). When the ROI covered the whole surface of the section, (ROI 1.00 cm^2), density of the normal brain was 112.9 HU. At the same section, with randomly located ROI of a very small size (0.05 cm^2), the density values were different: 139.24 HU, 103.70 HU, and 71.97 HU. In the hyperhydrated state at the same locations the density of the whole area was 84.03 HU and values for the small ROI were 130.47 HU, 78.43 HU and 55.0 HU. It shows that not only examination of the entire section area, but also comparison of individual small ROI of hyperhydrated animals indicate the presence of brain oedema. The measured values were not statistically processed because only one rat was evaluated. Nevertheless, we believe that also these data indicate the heterogeneity of the brain areas.

Earlier experiments revealed that alteration of brain microenvironment induced by hyperhydration results in functional changes manifested with increased neuronal excitability (Maresova *et al.* 2014) with elevation of the water content in the brain (Kozler *et al.* 2013).

From the above results it is possible to draw several conclusions. First of all, water intoxication achieved by hyperhydration can induce diffuse brain oedema in rats, which is expressed as a significant decrease of CT densities by more than 12 HU in all three measured positions. Normal values of the CT density vary depending on densitometric heterogeneity of structural components within each studied position of the brain (position A, B, C). They can also depend on the size and location of the ROI (rat No 3, position B). Our results are consistent with findings of other authors who studied brain oedema in the model of focal ischemia in rats (Dzialowski *et al.* 2004). Our results also support the importance of standardization of the size and location of the ROI for the densitometry in the rat brain (Unger *et al.* 1988, Kucinski *et al.* 2002).

CONCLUSION

Water intoxication achieved by hyperhydration can induce diffuse brain oedema in rats, which in the CT examination is expressed by a significant decrease in the CT density.

From densitometric point of view it proves increased water content in brain tissue. Values of CT densities vary depending on the densitometric heterogeneity of structural components of the brain tissue and depend on the size and location of the ROI.

ACKNOWLEDGEMENTS

Supported with grant P – 34/LF 1/7.

REFERENCES

1 Agre P, Nielsen S, Ottersen OP (2004). Towards a molecular understanding of water homeostasis in the brain. Neuroscience. **129**: 849–850.
2 Clasen RA, Huckman MS, Von Roenn KA, Pandolfi S, Laing I, Lobick JJ (1981). A correlative study of computed tomography and histology in human and experimental vasogenic cerebral edema. J Comput Assist Tomogr. **5**: 313–327.
3 Cormack AM (1973). Reconstruction of densities from their projections, with applications in radiological physics. Phys Med Biol. **18**: 195–207.
4 Dzialowski I, Weber J, Doerfler A, Forsting M, von Kummer R (2004). Brain tissue water uptake after middle cerebral artery occlusion assessed with CT. J Neuroimaging. **14**: 42–48.
5 Hounsfield GN (1976). Picture quality of computed tomography. AJR Am J Roentgenol. **127**: 3–9.
6 Kimelberg HK (2004). Water homeostasis in the brain: basic concepts. Neuroscience. **129**: 851–60.
7 Kozler P, Pokorný J (2012). Effect of methylprednisolone on the axonal impairment accompanying cellular brain oedema induced by water intoxication in rats. Neuroendocrinol Lett. **33**(8): 782–786.
8 Kucinski T, Väterlein O, Glauche V, Fiehler J, Klotz E, Eckert B, Koch C, Röther J, Zeumer H (2002). Correlation of apparent diffusion coefficient and computed tomography density in acute ischemic stroke. Stroke. **33**: 1786–1791.
9 Kuroiwa T, Nagaoka T, Ueki M, Yamada I, Miyasaka N, Akimoto H (1998). Different apparent diffusion coefficient: water content correlations of gray and white matter during early ischemia. Stroke. **29**: 859–865.
10 Mangel L, Vönöczky K, Hanzély Z, Kiss T, Agoston P, Somogyi A, Németh G (2002). CT densitometry of the brain: a novel method for early detection and assessment of irradiation induced brain edema. Neoplasma. **49**: 237–242.
11 Marešová D, Kozler P, Pokorný J (2014). Neuronal excitability after water intoxication in young rats. Neuroendocrinol Lett. **35**(4): 101–106.
12 Olson JE, Mishler L, Dimlich RV (1990). Brain water content, brain blood volume, blood chemistry, and pathology in a model of cerebral edema. Ann Emerg Med. **19**: 1113–1121.
13 Paxinos G, Watson C (2006). The Rat Brain in Stereotaxic Coordinates: Hard Cover Edition, Academic Press.
14 Torack RM (1982). Computed tomography and stroke edema: case report with an analysis of water in acute infarction. Comput Radiol. **6**: 35–41.
15 Unger E, Littlefield J, Gado M (1988). Water content and water structure in CT and MR signal changes: possible influence in detection of early stroke. Am J Neuroradiol. **9**: 687–91.