Hydration-sensitive Gene Expression in Brain

Cai Tang^{1*}, Christine Zelenak^{1*}, Jakob Völkl¹, Melanie Eichenmüller¹, Ivonne Regel¹, Henning Fröhlich¹, Daniela Kempe¹, Liliana Jimenez², Laurent Le Bellego², Sebastien Vergne² and Florian Lang¹

¹Department of Physiology, University of Tübingen; ²DANONE Research, Palaiseau; *contributed equally and thus share first authorship

Key Words

Dehydration • Cell volume • SGK1 • Forkhead box P1 • Serpinh1 • Gap junction protein beta 1

Abstract

Dehydration has a profound influence on neuroexcitability. The mechanisms remained, however, incompletely understood. The present study addressed the effect of water deprivation on gene expression in the brain. To this end, animals were exposed to a 24 hours deprivation of drinking water and neuronal gene expression was determined by microarray technology with subsequent confirmation by RT-PCR. As a result, water deprivation was followed by significant upregulation of clathrin (light polypeptide Lcb), serum/glucocorticoid-regulated kinase (SGK) 1, and protein kinase A (PRKA) anchor protein 8-like. Water deprivation led to downregulation of janus kinase and microtubule interacting protein 1, neuronal PAS domain protein 4, thrombomodulin, purinergic receptor P2Y - G-protein coupled 13 gene, gap junction protein beta 1, neurotrophin 3, hyaluronan and proteoglycan link protein 1, G protein-coupled receptor 19, CD93 antigen, forkhead box P1, suppressor of cytokine signaling 3, apelin, immu-

nity-related GTPase family M, serine (or cysteine) peptidase inhibitor clade B member 1a, serine (or cysteine) peptidase inhibitor clade H member 1, glutathion peroxidase 8 (putative), discs large (Drosophila) homolog-associated protein 1, zinc finger and BTB domain containing 3, and H2A histone family member V. Western blotting revealed the downregulation of forkhead box P1, serine (or cysteine) peptidase inhibitor clade H member 1, and gap junction protein beta 1 protein abundance paralleling the respective alterations of transcript levels. In conclusion, water deprivation influences the transcription of a wide variety of genes in the brain, which may participate in the orchestration of brain responses to water deprivation.

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Introduction

Compelling evidence suggests that excitability and survival of neurons is modified by hydration [1, 2]. For instance neuroexcitability has been shown to be sensitive to subtle changes of hydration [3, 4]. The ingestion of 750 ml of either water or saline led to significant differ-

ences in cortical synchronization and attention [3, 4]. Plasma hyperosmolarity decreases and plasma hypoosmolarity increases the susceptibility to epileptic seizures [5-7].

The impact of osmolarity on brain function may be either due to movements of water across the blood-brain barrier, leading to the respective changes of extracellular space [7-12] or due to alterations of neuronal or glial cell volume [13].

Neuronal cell volume changes stimulate release [14-17] or uptake [18] of organic osmolytes including inositol, glutamate, aspartate, GABA, glycine, and taurine [13]. The organic osmolytes are partially powerful neurotransmitters [13], which could, upon release from swollen cells, modify the function of neighboring cells [14, 16]. Cell shrinkage activates the Na^+ , K^+ , 2Cl^- cotransporter [19, 20], which counteracts the cell shrinkage and by the same token modifies cytosolic Cl^- concentration and thus excitability [20, 21].

Alterations of cell volume may influence neurotransmitter receptors, such as NMDA (N-methyl D-aspartate) receptors [22], or modify neuronal excitability by altering the trafficking of secretory vesicles [23]. Changes of neuronal cell volume may further affect neuronal function by modifying the activity of Cl^- channels [13, 24-28], Na^+/H^+ exchangers [13, 29], G-proteins [30], Ca^{2+} signaling [31] and the organisation of cytoskeletal proteins [32, 33].

Several genes have been defined which are differentially regulated by exposure of neurons to altered osmolarity [34, 35]. The dehydration-sensitive genes may be related to cell volume regulation [36], salt appetite [37], ADH release [38] and thirst [39]. Dehydration or hyperosmolar stimulation has previously been shown to inhibit Fos-dependent translation [40] and to upregulate the expression of the gonadotropin-inducible transcription factor in the hypothalamo-neurohypophyseal system [41]. Hypoosmolarity is known to activate the Sp1 transcription factor [42] and water deprivation upregulates the expression of L-type Ca^{2+} channels [43]. Cell shrinkage enhances the neuronal expression of the serum- and glucocorticoid inducible kinase SGK1 [44], a kinase regulating a wide variety of channels and transporters in the brain including glutamate receptors and transporters [45].

Excitability of neurons is modified by glial cells, which are again sensitive to cell volume [46]. Functions of glial cells include the regulation of the extracellular K^+ concentration by cellular K^+ accumulation, which is accomplished by uptake of K^+ through Na^+ , K^+ , 2Cl^- cotransport in parallel to the Na^+/K^+ -ATPase [47]. The cellular K^+

accumulation is expected to be compromised during glial cell swelling, which has indeed been implicated in several disorders affecting brain function [48-55].

Cell dehydration, on the other hand, is a precipitating cause of suicidal cell death or apoptosis of a wide variety of cells [13, 56-60] including neurons [59, 61, 62]. Cell volume-sensitive survival may be affected by SGK1, which has previously been shown to modify several cellular events important for the regulation of cell survival or apoptosis [45]. Target proteins for SGK1-dependent phosphorylation include tau [63, 64]. Hyperphosphorylation of tau, which is observed in cell shrinkage [65] fosters apoptosis of neuronal cells [65, 66]. SGK1 is activated by the PI3 kinase pathway [45] and is thus expected to be triggered by nerve growth factors, which play a role in apoptosis following hyperosmotic stress [67].

To analyze the influence of water deprivation on the gene expression of neuronal cells the present study employed a gene array analysis in hydrated mice and in mice subjected to a 24 hours period of water deprivation.

Materials and Methods

Experiments were carried out on wild-type BALB/c mice of either sex. The animals were housed under controlled environmental conditions (22-24°C, 50-70% humidity and a 12 h light/dark cycle). Throughout the study the mice had free access to standard pelleted food (C1310, Altromin, Heidenau, Germany). The mice had either free access to tap water or were water-deprived for 24 hours. All animal experiments were conducted according to the German law for the care and welfare of animals and were approved by local authorities.

The mice were anesthetized and the blood was withdrawn into heparinized capillaries by puncturing the retro-orbital plexus. Thereafter plasma was separated by centrifugation at 4000 rpm for 10 min. Plasma osmolarity was measured by the vapour pressure method.

The whole brains were removed from the animals that had been anesthetized and subsequently killed by cervical dislocation.

A gene array was performed (3 + 3 animals) to identify genes sensitive to water deprivation. To this end, total RNA was isolated from the brain using the Qiagen RNeasy Fibrous Tissue Midi Kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). Using total RNA, second-strand synthesis was performed by means of a commercially available kit (Invitrogen Life Technologies, Rockville, MD) and an oligo d(T)₂₄ T7 primer. cRNA was generated using biotin-labeled CTP and UTP by *in vitro* transcription with a T7 promoter-coupled double stranded cDNA as template and the T7 RNA transcript labelling kit (ENZO Diagnostics, Farmingdale, NY). The cRNA was fragmented and hybridized to the mouse genome MOE430A oligonucleotide array chip (Affymetrix, Santa Clara,

Gene Symbol	forward	reverse
Jakmip1	CACAGCATCCAGAGGATGGA	TTAGCGAGGCCTGAGCAGA
Npas4	ACTCAGAAGGTCCAGAAGGCC	TCAGAGTTTAGCTGCTGGCG
Thbd	TTTTGGCGCTTCTCTGTCAC	ACTACCTCCTTGGCGGAAGAT
P2ry13	CAACTAACATTTGTATGGACCCCT	CTTGCCCTTCCCCATCTCA
Gjb1	TCGGCCACCGCCTCT	CGGCGCAGTATGTCTTTTCAG
Ntf3	CAAACCTATGTCCGAGCACTGA	ACAAGGCACACACACAGGAAGT
Hapln1	CTGCTGTGGTGGCATTGGA	CAGACAAGCCTGGCGTGC
Gpr19	AGAGCTCCCTTGTCTTCACGG	CCCTCTCCGAAAATTGGCAT
Cd93	AGCAAGCCGACACATGAAGA	CCACCACCGTCCCCAG
Foxp1	CAGTCCAGGCAGATCCCTTA	GGACAGAGGGCCTTCAGCT
Socs3	CTCCAGCATCTTTGTGCGAA	CCAGGAACTCCCGAATGG
Apln	CTGCTGCTCTGGCTCTCCTT	AGCGCATGCTTCCTTCTTCTA
Irgm	CCCCCGTGTTCCTGGTAT	TCACAGCACCTGATGTTGGAGA
Serpnb1a	CTGCCTAAAGACATTGAGGACG	CACGTTTGGTCCATTCAAGC
Serpinh1	ATCTCCCTGCCCAAGGGT	TGTTCTTGTCATGGCCTCC
2310016C16Rik	ATTTTAGGGCCGGAAGCAGA	CCCTCAGGGTTGACCAGATACTT
Dlgap1	CTCATGGCGCAGAAATTCTACC	CAAAACCCCGCCAAGTCCT
Zbtb3	CCAGGTCCAGGGCAGTATCA	GCTGCTTCATACTCAGGAGGAGG
H2afv	CGCCACTGCTGCTGTGTACA	TGCGCTTCACCTTGAGATCTTTG
Cltb	AACCAGCGCCAAAGTGAACA	CATGTGCTTCGCGGCC
Sgk1	CTCCAGAGGAGTCCTGTTCTCTG	GGGTCCGGCTTGGTCAG
Akap81	CCTGTGACCTTTTCATCCCC	TCCATCATAAGCCTGCGGT

Table 1. Primer Sequences (5'→3'orientation)

CA). The array chips were then stained by phycoerythrin-conjugated streptavidin (Molecular Probes, Invitrogen Life Technologies, Rockville, MD) and the fluorescence intensities determined by a laser confocal scanner (Agilent, Affymetrix). The intensity of the scanned images was analysed by Microarray Suite Version 5 (Affymetrix). Global scaling was applied to all arrays such that the mean intensity of each array was equivalent. In global scaling, the raw signal value of each probe was multiplied by a scaling factor. Genes whose expression varied significantly with a signal log ratio of 0.5 were identified using Data Mining Tool (Affymetrix).

Additional RT-PCR experiments were performed utilizing brain tissue from additional 3 euvoletic and 3 dehydrated mice. Total RNA was extracted from mouse tissue in Trizol (Peqlab, Erlangen, Germany) according to the manufacturer's instructions. After DNase digestion reverse transcription of total RNA was performed using random hexamers (Roche Diagnostics, Mannheim, Germany) and SuperScriptII reverse transcriptase (Invitrogen, Carlsbad, CA, USA). Polymerase chain reaction (PCR) amplification of the respective genes were set up in a total volume of 20µl using 40ng of cDNA, 500nM forward and reverse primer and 2x iTaq Fast SYBR Green (Bio-Rad, Hercules, CA, USA) according to the manufacturer's protocol. Cycling conditions were performed as follows: initial denaturation at 95°C for 2min, followed by 40 cycles of 95°C for 15sec, 55°C for 15 sec and 68°C for 20 sec. Specificity of PCR products was confirmed by analysis of a melting curve. Real-time PCR amplifications was performed on a CFX96 Real-time System (Bio-Rad) and all experiments were done in doublets. The re-

sults were normalized against the house-keeping gene *Tbp* to standardize the amount of sample RNA. Relative quantification of gene expression was done using the $\Delta\Delta C_t$ method previously described [68]. The pairs of primers utilized are listed in Table 1.

Western blotting was performed to explore whether alterations in mRNA expression levels were comparable to differences in protein abundance. To this end, the brains from hydrated and dehydrated mice were removed and immediately shock-frozen in liquid nitrogen. The brain tissues were then homogenized in lysis buffer containing 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, beta-mercaptoethanol, and a protease inhibitor cocktail (Complete mini, Roche Diagnostics). The homogenates were centrifuged at ~20.000 g at 4°C for 30 min, and the supernatant was removed for Western blotting. Total protein (60 µg) was separated by SDS-PAGE (10% Tris-Glycine), transferred to PVDF membranes, blocked for 1 h in blocking buffer (5% fat-free milk in TBS containing 0.1% Tween), and incubated over night at 4°C with a polyclonal anti forkhead box P1 antibody (1:1000; Cell Signaling, Frankfurt am Main, Germany), Hsp47 antibody (1:1000; Abcam, Cambridge, MA, USA), or Connexin 32 antibody (1:100; Invitrogen). After incubation with a horseradish peroxidase-conjugated anti-rabbit secondary antibody (1:2000; Amersham, Freiburg, Germany or 1:2000; Cell Signaling, Frankfurt am Main, Germany) in case of forkhead box P1 and Connexin 32, or with horseradish peroxidase-conjugated anti-mouse secondary antibody (1:2000; Cell Signaling, Frankfurt am Main, Germany) in case of Hsp 47, the bands were visualized with

Fig. 1. Body weight and plasma osmolarity of hydrated and water deprived mice. Left panel: Arithmetic means $\pm$ SEM (n = 8) of plasma osmolarities in hydrated (open bars) or 24 hours water deprived (closed bars) mice. Right panel: Arithmetic means $\pm$ SEM (n = 8) of the body weight of mice prior to and following the indicated time periods of water deprivation. * and *** indicate significant ($p < 0.05$, $p < 0.001$) difference to hydrated animals.

Gene Symbol	Gene Title	FC-Absolute	p-value	Probe Set ID	(putative) function
Cltb	clathrin, light polypeptide (Lcb)	2.757	0.022	1456797_at	membrane traffic
Sgk1	serum/glucocorticoid regulated kinase 1	1.747	0.036	1416041_at	PI3K-regulated Kinase
Akap8l	A kinase (PRKA) anchor protein 8-like	1.669	0.009	1417734_at	PKA signaling

Table 2. Genes upregulated by water deprivation. FCAbsolut = fold change as compared to hydrated animals, Probe Set ID = Identity number of the transcript on the chip, p-value = statistical significance level.

enhanced chemiluminescence according to the manufacturer’s instructions. Homogenates were also probed with a primary β -actin (1:1000; Cell Signaling) antibody as a loading control. Densitometric analysis of protein abundance was performed using Quantity One software (Bio-Rad Laboratories). Data are provided as arithmetic means $\pm$ SEM. All data were tested for significance using Student’s unpaired two-tailed *t*-test or ANOVA (Dunnets test), as appropriate. $P < 0.05$ is considered to indicate statistical significance.

Results

As illustrated in Fig. 1, the body weight of the mice was significantly decreased in a time dependent manner during water deprivation. The water deprivation further resulted in a significant increase of plasma osmolarity (Fig. 1). The gene array disclosed the differential genomic regulation of a variety of genes in brain. The genes upregulated by water deprivation are listed in Table 2, the genes downregulated by water deprivation in Table 3. Only genes are listed, where the regulation has been confirmed by RT-PCR and/or Western blotting. As compiled in Table 2, water deprivation was followed by significant upregulation of the signaling mol-

ecules clathrin, serum/glucocorticoid- regulated kinase 1, and A kinase (PRKA) anchor protein 8-like. As apparent from Table 3, water deprivation led to downregulation of several signaling molecules (janus kinase and microtubule interacting protein 1, neuronal PAS domain protein 4, hyaluronan and proteoglycan link protein 1, forkhead box P1 (FoxP1), suppressor of cytokine signaling 3, immunity-related GTPase family M, discs large (Drosophila) homolog-associated protein 1, zinc finger and BTB domain containing 3, and H2A histone family member V), receptors (purinergic receptor P2Y, G protein-coupled receptor 19, CD93, mediators (thrombomodulin, neurotrophin 3, apelin), connexins (gap junctional protein beta 1(Gjb1)), and enzymes or enzyme inhibitors (serine (or cysteine) peptidase inhibitor clade B member 1a, serine (or cysteine) peptidase inhibitor clade H member 1 (Serpinh1), putative glutathion peroxidase 8). Additional Western blotting experiments were performed to elucidate whether the downregulation of FoxP1, Serpinh1 and Gjb1 transcription levels were paralleled by respective alterations of protein expression. As shown in Fig. 2, water deprivation was followed by significant decrease of FoxP1, Hsp47 (Serpinh1) and Connexin 32 (Gjb1) protein abundance.

Gene Symbol	Gene Title	FC-Absolute	p-value	Probe Set ID	(putative) function
Jakmip1	janus kinase and microtubule interacting protein 1	2.378	0.044	1433815_at	Interaction with GABA receptors
Npas4	neuronal PAS domain protein 4	2.030	0.031	1459372_at	Development of inhibitory synapses
Thbd	Thrombomodulin	1.898	0.034	1448529_at	anticoagulant protein
P2ry13	purinergic receptor P2Y, G -protein coupled 13	1.755	0.017	1428700_at	endocytosis of high density lipoproteins
Gjb1	gap junction protein, beta 1	1.724	0.017	1448766_at	Connexin
Ntf3	neurotrophin 3	1.704	0.028	1434802_s_at	neurotrophic factor
Hapln1	hyaluronan and proteoglycan link protein 1	1.690	0.011	1421633_a_at	stabilizing aggregates of hyaluronic acid and aggrecan
Gpr19	G protein-coupled receptor 19	1.682	0.005	1445480_at	orphan receptor
Cd93	CD93 antigen	1.653	0.034	1456046_at	regulator of immunity
Foxp1	forkhead box P1	1.637	0.033	1421142_s_at	Transcription factor, cell survival
Socs3	suppressor of cytokine signaling 3	1.627	0.021	1455899_x_at	Inhibition of JAK2 kinase, suppression of activation/differentiation of immune cells
Apln	Apelin	1.613	0.021	1451038_at	Body weight
Irgm	immunity-related GTPase family M	1.602	0.009	1418825_at	GTPase participating in inflammation
Serpinb1a	serine (or cysteine) peptidase inhibitor, clade B, member 1a	1.584	0.041	1416318_at	Peptidase inhibitor
Serpinh1	serine (or cysteine) peptidase inhibitor, clade H, member 1	1.508	0.002	1450843_a_at	Collagen accumulation
2310016 C16Rik	Glutathion peroxidase 8 (putative)	1.574	0.005	1424099_at	Antioxidative defence
Dlgap1	discs, large (Drosophila) homolog-associated protein 1	1.574	0.018	1438098_at	scaffolding proteins participating in synaptogenesis
Zbtb3	zinc finger and BTB domain containing 3	1.562	0.003	1440180_x_at	Regulation of gene expression
H2afv	H2A histone family, member V	1.512	0.014	1436596_at	Regulation of gene expression

Table 3. Genes downregulated by water deprivation. FCAbsolute = fold change as compared to hydrated animals, Probe Set ID = Identity number of the transcript on the chip, p-value = statistical significance level.

Discussion

The present study disclosed a variety of genes regulated by water deprivation. The vast majority of genes analysed did not change to the extent that safe conclusions could be derived. Since the absence of statistical difference cannot be equated with absence of difference, the potential sensitivity of those genes to water deprivation remained elusive and those genes are thus not shown in the tables.

In the following, the function of the regulated genes will be briefly discussed. It must be pointed out that alterations of transcript levels are not necessarily paralleled by the respective alterations of protein expression.

Three of the genes were in addition studied at the protein level. It should further be kept in mind that gene array has been performed in the whole brain, which is composed by heterogeneous neurons and glial cells. This approach does not disclose alterations of gene expression in a small subset of cells.

Water deprivation upregulated the transcript levels of CLTB (clathrin, light polypeptide LCB). CLTB participates in the regulation of the clathrin heavy chains, which ubiquitously form clathrin-coated vesicles involved in membrane traffic [69]. The functional role of CLTB in neurons is not known.

Expectedly, the genes upregulated by water deprivation include the serum- and glucocorticoid-inducible

Fig. 2. Protein abundance of FoxP1, Hsp 47 and Connexin 32 in hydrated and dehydrated brain tissue. Left panels: Original Western blot illustrating FoxP1 (upper panel), Hsp 47 (middle panel) and Connexin 32 (lower panel) protein abundance in hydrated and dehydrated brain tissue. Right panels: Arithmetic means $\pm$ SEM ($n = 3-6$) of the FoxP1 (upper panel, $n = 3$), Hsp 47 (middle panel, $n = 6$) and Connexin 32 (lower panel, $n = 6$) protein band intensity in hydrated (open bars) and dehydrated (closed bars) brain tissue. * indicates significant ($p < 0.05$) difference to hydrated animals.

kinase SGK1. The human SGK1 has been cloned as a gene particularly sensitive to cell volume changes and upregulated by cell shrinkage [70] and later shown to be upregulated in the rat brain by water deprivation [44]. SGK1 has been shown to activate a wide variety of channels and carriers [71-74] including the kainate receptor [75] and all glutamate transporter isoforms [76-81]. Thus, SGK1 participates in the regulation of neuroexcitation by enhancing the sensitivity of neurons to glutamate and by fostering the clearance of glutamate from the extracellular space. Further effects of SGK1 relevant for brain functions include phosphorylation of tau [63, 64], and huntingtin [82]. Upregulation of SGK1 coincides with the onset of dopaminergic cell death in a model of Parkinson's disease [83, 84]. Excessive expression of SGK1 has been observed in Rett syndrome (RTT), a disorder with severe mental retardation [85]. Thus, SGK1 may contribute to a variety of neuronal functions and pathological conditions.

Water deprivation further upregulates A kinase (PRKA) anchor protein 8-like. Kinase-anchoring proteins (AKAP) participate in the scaffolding of cAMP-

dependent protein kinase (PKA), protein kinase C (PKC), and protein phosphatase 2B/calcineurin 8 [86-88]. Nothing is known about the specific role of AKAP81 in neuronal function.

Water deprivation down regulates a variety of genes, as listed in Table 3. The genes do serve a multitude of functions.

JAKMIP1 (janus kinase- and microtubule-interacting protein 1) inhibits cytotoxic T lymphocytes [89]. In the brain it interacts with GABA receptors [90, 91] and has been implicated in the pathophysiology of autism [92].

NPAS4 (neuronal PAS domain protein 4) regulates the development of inhibitory synapses by influencing the expression of activity-dependent genes, which in turn control the number of GABA-releasing synapses that form on excitatory neurons [93]. Reduced expression of Npas4 may contribute to the impairment of neurogenesis, memory and aggression during social isolation [94]. Moreover, NPAS4 expression is altered by ischemia of the brain [95].

Thrombomodulin (Thbd) is an endothelial-associated anticoagulant protein thought to control hemostasis in specific vascular beds [96].

The purinergic receptor P2RY13 regulates the endocytosis of high density lipoproteins [97, 98], inhibits the ATP release from erythrocytes [99] and may contribute to the regulation of mast cell degranulation [100].

GJB1 (gap junctional protein beta 1) is a connexin (32) defective in the peripheral neuropathy Charcot-Marie-Tooth syndrome [101, 102]. Its role in the central nervous system remained elusive.

NTF3 (Neurotrophin 3) is a neurotrophic factor [103, 104] conferring neuronal cell survival [105] and implicated in various diseases, such as Hirschsprung's disease [106], adult attention deficit hyperactivity disorder (ADHD) [107, 108], eating disorders [109], deafness [105, 110, 111], bipolar disorders [112], and schizophrenia [113].

Hapln 1 (hyaluronan and proteoglycan link protein 1) belongs to the link proteins stabilizing aggregates of hyaluronic acid and aggrecan [114]. The link proteins are deposited in the extracellular matrix of the central nervous system and limit the plasticity of brain [115].

GPR19 (G protein-coupled receptor 19) is an orphan receptor expressed during embryonic development and in the adult brain [116]. In the adult brain, transcripts of GPR19 are particularly high in olfactory bulb, the hippocampus, hypothalamic nuclei, and the cerebellum [116]. Nothing is known about its function.

CD93 (CD93 antigen), a transmembrane glycoprotein, is an important regulator of innate and adaptive immunity [117-119]. For instance, it is expressed during early B-cell development and reinduced during plasma-cell differentiation, is critically important for the maintenance of antibody secretion and bone-marrow plasma-cell numbers [117]. Nothing is known about its function in the brain.

FOXP1 (Forkhead Box P1) is a member of the FOX family of transcription factors, which are widely expressed and play a role in cardiac, lung, adipocyte and lymphocyte development [120, 121]. FOXP1, which is localised on chromosome 3p 14.1, a tumour suppressor locus, is differentially expressed in a variety of malignant cell types and is thus considered a tumour suppressor gene [121]. It is expressed in several structures of the brain [122-124].

Suppressor of cytokine signaling 3 (SOCS3) is a member of the STAT-induced STAT inhibitor (SSI) [125]. SOCS3 inhibits JAK2 kinase [125] and thus the signaling of a variety of hormones and mediators including IL-6, erythropoietin and leptin [126]. Functions suppressed by SOCS3 include activation and/or differentiation pathways in macrophages, dendritic cells, and T lymphocytes [127] as well as regulation of body mass [128].

Apelin is a widely expressed hormone, stimulating the APJ receptor [129]. Apelin is coexpressed with vasopressin in magnocellular neurons and sensitivity of the gene to hydration is thus expected [130]. Apelin receptors, like V1a-type and V1b-type vasopressin receptors, are synthesized by magnocellular, vasopressin expressing neurons and apelin may inhibit the phasic electrical activity of those neurons [130]. Accordingly, apelin acts as a vasopressin antagonising diuretic neuropeptide [130]. Apelin is further involved in the regulation of cardiovascular functions, angiogenesis and cell proliferation [131, 132]. Its expression is up-regulated in obesity and may participate in the regulation of fat depot development, energy storage, metabolism or eating behaviour [131]. It contributes to the pathophysiology of type 2 diabetes [131] and heart failure [129, 133]. As one would expect, lack of apelin has been shown to compromise fluid homeostasis [134].

The immunity-related GTPase IRGM induces autophagy [135]. Notably, it is associated with Crohn's disease, a disorder with excessive expression of SGK1 [136]. Nothing is known about the role of IRGM in neuronal function.

The serine (and cysteine) peptidase inhibitors clade B (Serpina1a) and clade H (Serpinh1) are collagen chaperones [137]. Expression of Serpinb1 is sensitive to tumor growth factor TGF β [137], which is a strong regulator of SGK1 [136]. Both, SGK1 [138] and Serpinb1 [137] are involved in lung fibrosis. Serpinb1 further participates in the defense against lung infections [139]. Serpinh1 may similarly be involved in fibrosis [140]. Conversely, defective Serpinh1 is associated with preterm rupture of membranes leading to premature birth [141].

The putative glutathione peroxidase 8, similar to other glutathione peroxidases (GSH-PX) protects against oxidative stress [142]. It is upregulated by neurotrophin and thus contributes to the protective effect of this mediator [142]. Defective GSH-PX may accelerate ageing [143].

The discs large (*Drosophila*) homolog-associated protein (DLGAP1, guanylate kinase-associated protein GKAP) is a scaffolding protein participating in synaptogenesis [144]. It participates in scaffold protein complex-forming postsynaptic hotspots for the establishment of new functional excitatory synapses [144, 145].

ZBTB3 (zinc finger and BTB containing 3) belongs to the family of zinc finger proteins (ZFPs), DNA-binding molecules regulating gene expression [146]. Nothing is known about the specific role of ZBTB3 in the regulation of gene expression.

H2A histone family, member V is presumably involved in the regulation of gene expression [147].

In conclusion, the present study reveals an influence of water deprivation on the transcript levels of a wide variety of genes participating in the accomplishment and regulation of a myriad of functions. Additional experimental effort is required to verify that the differences in transcript levels are paralleled by the respective differences in protein abundance and function. Moreover, fu-

ture studies may elucidate the contribution of those genes to the function or dysfunction of the dehydrated brain.

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