Supplemental tables

Table S1: Biological parameters of animals used for histological analysis of GFAP and myelin loss.

	NormoNa	HypoNa	Hypertonic NaCl					
Groups (n)	**1 (n=6)**	**2 (n=6)**	**3 (n=6)**	**4 (n=6)**	**5 (n=6)**	**6 (n=6)**	**7 (n=6)**	**8 (n=6)**
Time of SNa analysis			6hrs	12 hrs	24 hrs	24 hrs	24 hrs	24 hrs
Time of histological analysis (after correction of HypoNa)	NA	0hrs	6hrs	12 hrs	24 hrs	2 days	3 days	6 days
Na before correction (mEq/L)	140 ± 2	102 ± 1	104 ±3	106±4	106±2	103±3	105 ±2	100± 6
Na after the correction*	NA	NA	133± 1	134±3	135±2	132 ±2	130 ±2	126± 2
Delta SNa mEq/L	NA	NA	29 ±4	28±3	30±1	29 ±2	25± 3	26± 4

*p <0.05 for Na after the correction vs Na before the correction in group 3 to 8.

Table S2: Biological parameters of animals used for the quantification of gene expression by quantitative RTPCR

	Controls (n=5)	Hyponatremia (n=5)	NaCl 12hrs (n=5)	NaCl 24hrs (n=7)
SNa before correction	144±1	110±0.5	105 ±1	113± 1
SNa after correction	NA	NA	134 ± 1	142 ± 1*
ΔSNa	NA	NA	29 ± 1	29 ± 1

*p <0.05 for SNa before the correction vs after the correction.

Table S3: Biological parameters of the total of animals used in experiments involving S100B measurements.

	NaCl SNa < 23 (n= 11)	NaCl SNa > 23 (n= 31)
Groups	1	2
SNa before correction	112±1	107±1*
SNa 24 hrs after correction	131±2	137±1*
ΔSNa 24 hrs	19±1	30±1*
S100B before correction	2.61±0.39	2.32±0.24
S100B after the correction	2.06±0.39°	3.78±0,36**°
ΔS100B	-0.50±0.51	1.45, ±0.25**

For SNa before correction: *p<0.05 for 1 vs 2 for SNa after the correction and p= 0.0001 in 1 vs 2 for ΔSNa. ** p=0.008 for S100B after correction in 1 vs 2 and for ΔS100B, p=0.0018 in 1 vs 2. ° S100b before and after the correction: p= NS in group1 and p<0.0001 in group 2.

Table S5: Sequence of the primers used for RT-PCR

Gene accession number	Sense	Antisense
IL1 beta NM_031512.2	AAGACAAGCCTGTGTTGCTGAAGG	TCCCAGAAGAAAATGAGGTCGGTC
TNF alpha NM_012675.3	AAATGGGCTCCCTCTCATCAGTTC	TCTGCTTGGTGGTTTGCTACGAC
IL10 NM_012854	TAAGGGTTACTTGGGTTGCC	TATCCAGAGGGTCTTCAGC
MCP NM_031530.1	TGTCTCAGCCAGATGCAGTTA	TGCTGCTGGTGATTCTCTTGT
Connexin 47 NM_001100784.1	GCATCCAGAGGGAGGGCCTGAT	CGGTTGGCCGCGACACGAA
Connexin 43 NM_012567.2	TTGAGCGCGGTCTACACCTGC	CGCTTCTTCCCTTCACGCGAT
MBP AF439750.1	CCGTTCTAATTCCGAGGAGAGTGTGG	ACTGCAGCTGCGCTGTCTGG
CNPase NM_18630.1	AGACGGCGTGGCGACTAGACT	AATGATCCTGGCCGGCTGTCT
Aldh1L1 NM_001007557.1	AGGTGCCAGGTGCCTGGACA	TGGTGACCACGCCTGGACGA
GFAP NM_017009.1	TCCTGGAACAGCAAAACAAG	CAGCCTCAGGTTGGTTTCAT
Iba 1 NM_017196.3	AGAGGTGTCCAGTGGCTCCGA	GGTCCTCGGTCCCACCGTGTT
GADPH NM_017008	AATGTATCCGTTGTGGATCT	CAAGAAGGTGGTGAAGCAGG

GADPH: glyceraldehyde 3-phosphate dehydrogenase; CNPase: 2'-3' cyclic nucleotide phosphodiesterase. MCP-1: Macrophage chemotactic protein 1; MBP: myelin basic protein. GFAP: glial fibrillary acidic protein. IL: interleukin. GFAP glial fubrillary acidic protein. Aldh1L1: aldehyde dehydrogenase 1 family, member L1. Iba1: Ionized calcium binding adapater molecule 1. Primers were designed using primer blast ® software except for GFAP primer which was from ATRC reagent bank and was designed by Charles R. Vanderburg - Harvard Neurodiscovery Center - Boston, Massachusetts. All primers were synthetized by Eurogentec, Seraing, Belgium.

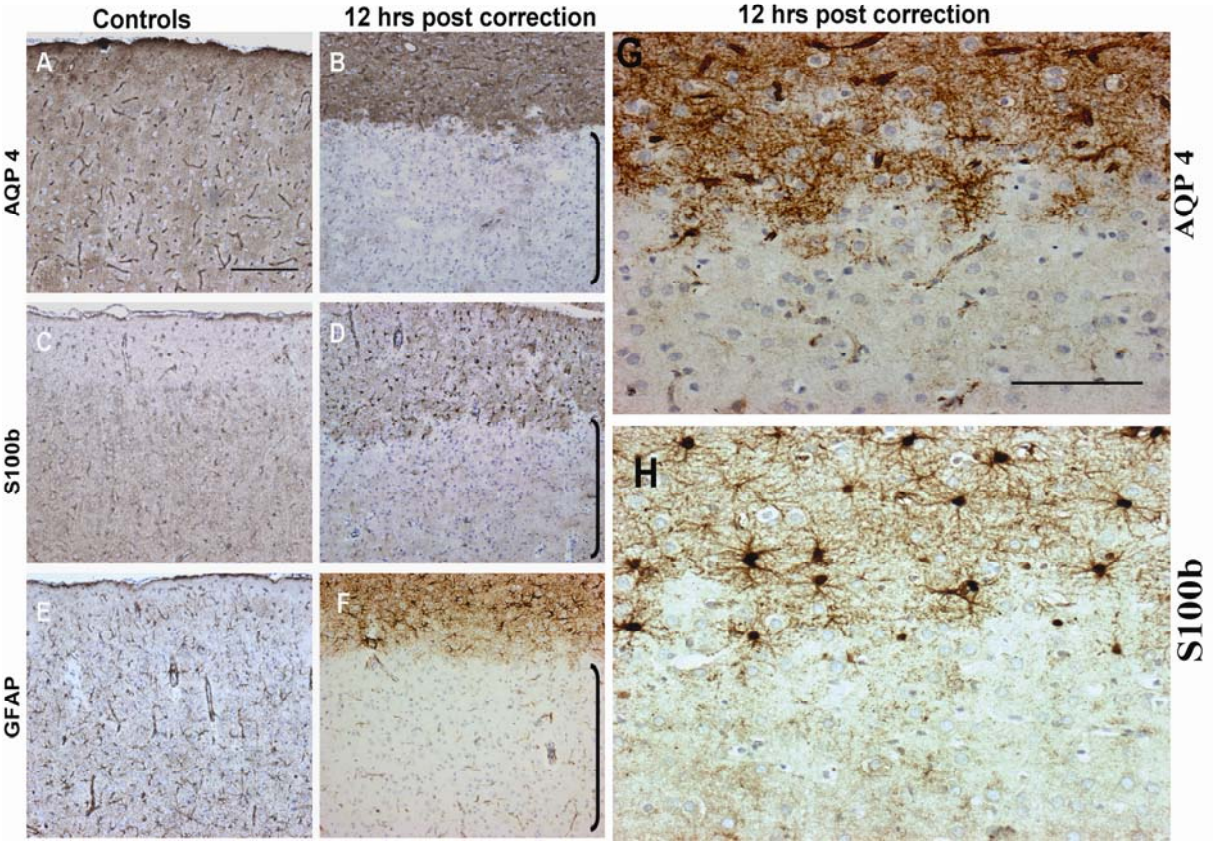
Table S4: Antibodies used for immunohistochemistry and immunofluorescence.

Antibody	Target	Concentration	Supplier
GFAP	Astrocytes	IHC 1/2000; IF: 1/200	Sigma-Aldrich . (Boonen , Belgium)
GFAP	Astrocytes	IHC 1/1000; IF: 1/100	Dako (Heverlee, Belgium)
AQP4	Astrocytes	IHC 1/1000	Chemicon. (Brussels,Belgium)
S100	Astrocytes	IHC 1/1000; IF: 1/100	Dako (Heverlee, Belgium)
MBP	Myelin	IHC 1/2000; IF: 1/100	Abcam. (Cambridge,UK)
CD68 (clone ED1)	Microglia	IHC1/300; IF: 1/100	AbDSerotec (Dusseldorf, Germany)
CD3	Lymphocytes	IF 1/100	BDPharmingen, (Brussels, Belgium)
MPO	Neutrophils	IF: 1/100	Thermofischer (Duiven Belgium)
APC (CC1)	Oligodendrocytes	IF: 1/100	Calbiochem (Merck Cambridge UK)
MAP2	Neurons	IHC 1/2000	JP Brion.
Apoptag Kit	Apoptotic cells	As per manufacturer.	Millipore (Brussels, Belgium)
Anti mouse IgG Alexa 594	Mouse IgG	IF 1/100	Invitrogen (Brussels, Belgium)
Anti Rabbit IgG Alexa 594	Rabbit IgG	IF 1/100	Invitrogen (Brussels, Belgium)
Anti mouse IgG FITC	Mouse IgG	IF 1/100	Invitrogen (Brussels, Belgium)

Abbreviations: GFAP: glial fibrillary acidic protein, AQP4: aquaporin 4, MBP: Myelin basic protein, MPO: myeloperoxidase, APC: adenomatous polyposis cancer, MAP2: microtubule associated protein 2, IHC: Immunohistochemistry, IF: Immunofluorescence

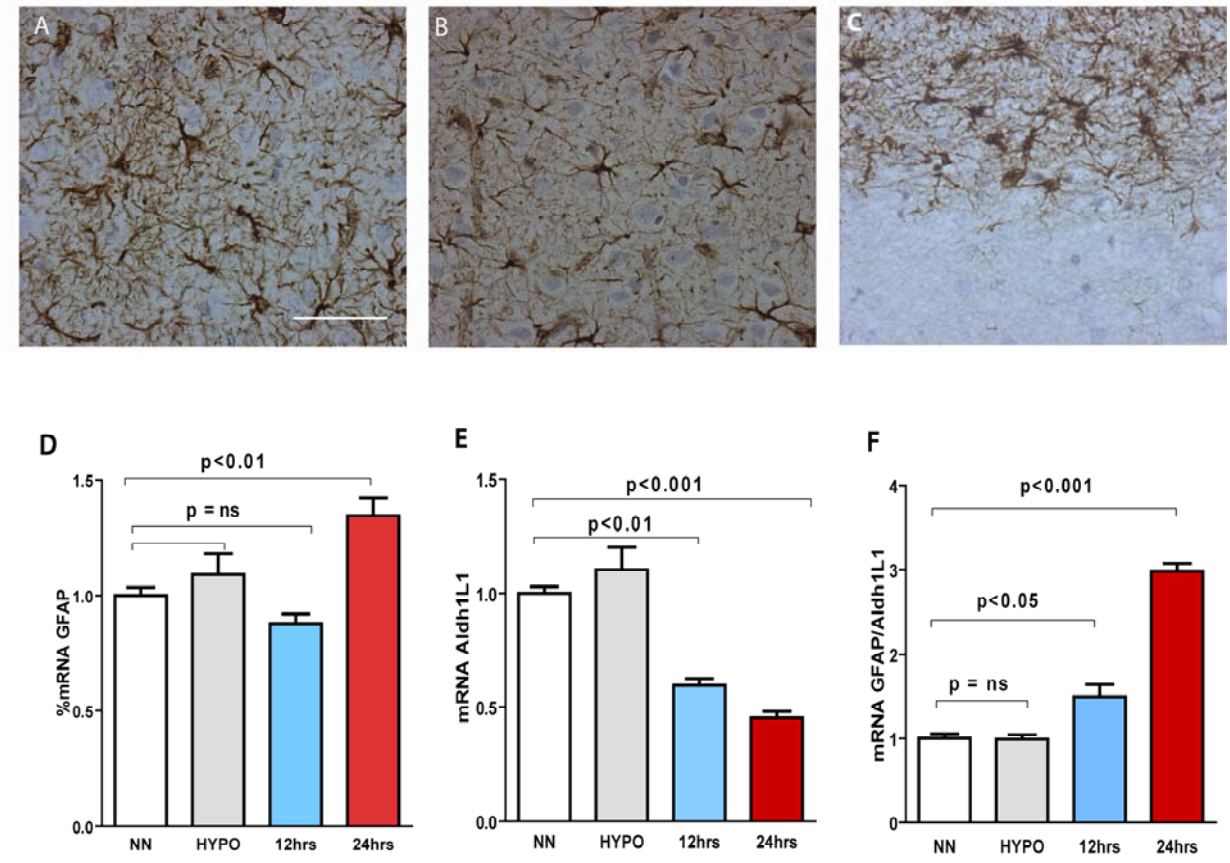
Supplemental figures

Figure S1. Immunoreactivity for the astrocyte-specific markers S100B and aquaporin 4 (AQP4) is lost after rapid correction of chronic hyponatremia.



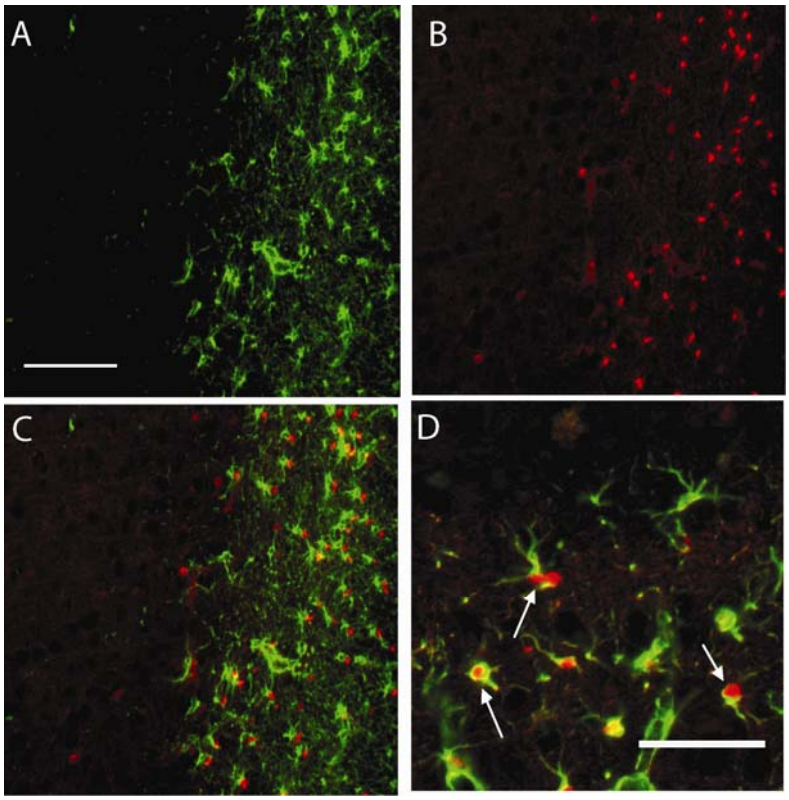
Reactivity for AQP4 reactivity is homogenous and preserved in uncorrected control (A) as opposed to rats 12 hrs post correction (B). The disappearance of S100B reactivity follow the same pattern 12 hrs postcorrection (C) compared to uncorrected controls (D). Those changes are identitical to those observed for GFAP (E and F). A higher magnification of the regions of AQP4 and S100B loss is provided in (G) and (H) respectively. The scale bar is 200 μm.

Figure S2. Astrogliosis is seen after the induction of ODS.



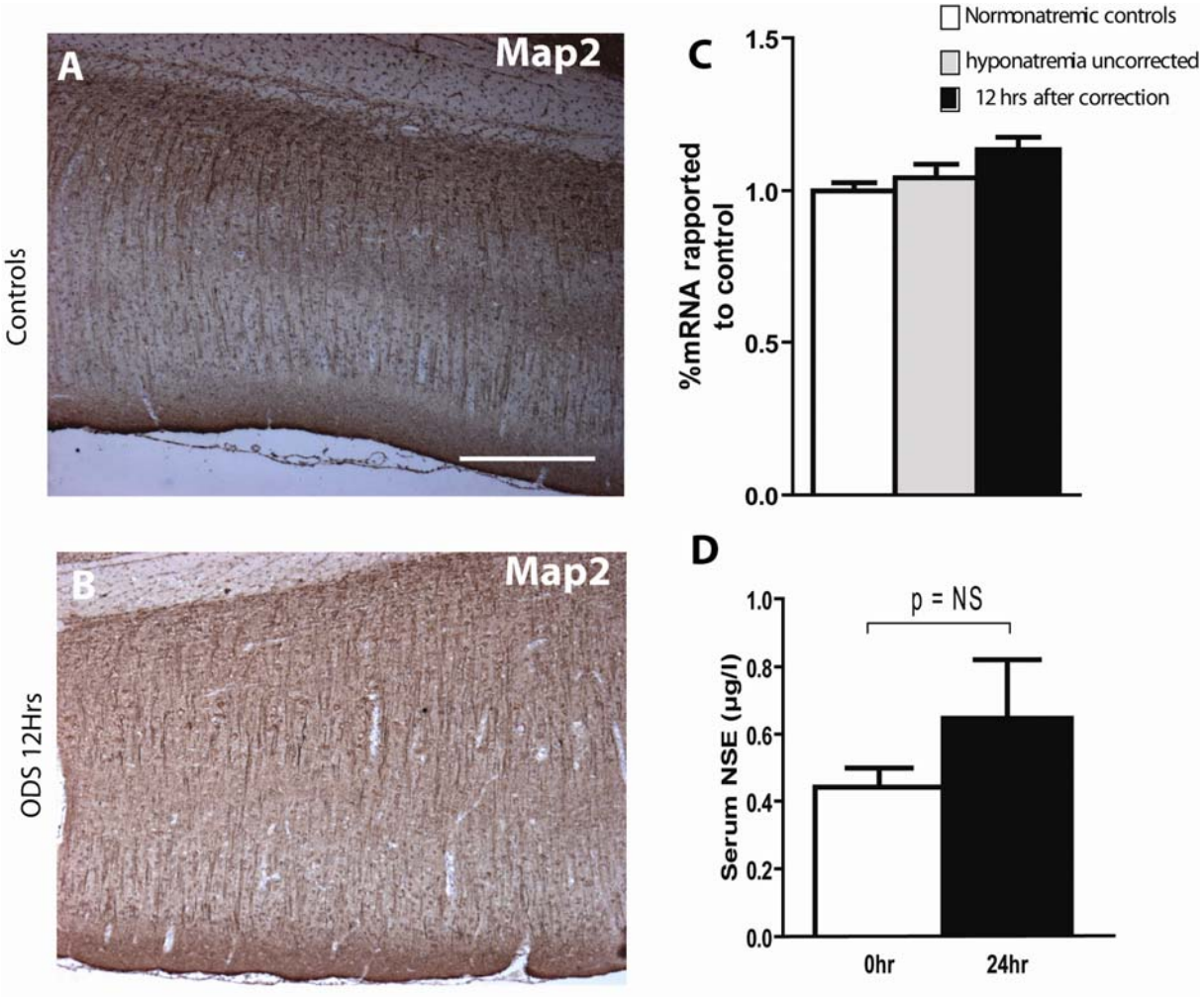
The panels (A) and (B) show the pattern of GFAP staining in normonatremic and hyponatremic rats and (C) shows the GFAP staining pattern 24 h after the correction of hyponatremia. Astrocytes in unaffected areas appear larger and have broader ramifications. In graph (D), quantification of GFAP mRNA expression in normonatremic rats (NN), hyponatremic controls (HYPO) and 12 and 24 h after hyponatremia correction shows increased GFAP transcript levels at 24 h postcorrection, which contrasts with the significant astrocyte death found at that time and suggests an increased production of GFAP in the remaining astrocytes. In graph (E) the expression of astrocyte-specific marker Aldh1L1 mRNA confirmed the significant depletion of astrocytes at 12 and 24 h postcorrection of hyponatremia ($p< 0.01$ and $p< 0.001$, respectively, ANOVA, n=5-7) and in (F) reactive gliosis was assessed by analyzing the ratio of GFAP to Aldh1L1 expression. No changes were detected in chronic hyponatremia, but GFAP:Aldh1L1 ratio significantly increased 12 and 24 h postcorrection ($p<0.05$ and $p<0.001$, respectively, ANOVA, n=5-7)

Figure S3. S100B is produced by reactive astrocytes in ODS.



In panel (A-C) Colocalization of GFAP (green) and S100B (red) in astrocytes is seen 24 h after the correction of hyponatremia. Higher magnification in (D) with arrows pointing to double-positive cells.

Figure S4. Early astrocyte loss in ODS does not result in neuronal damage.



Panel A shows neuron (anti-Map-2) staining in the cortex of uncorrected hyponatremic rats and 12 h postcorrection (B) did not show any apparent neuronal loss. Also, in (C), using RTPCR, no changes in the transcription of NSE were found 12 h postcorrection, and (D) no changes in the serum levels of NSE were detected 24 h postcorrection. The scale bar is 200 µm.