Supplementary Information

Ammonia short-circuits astroglial potassium buffering resulting in cortical disinhibition and seizures

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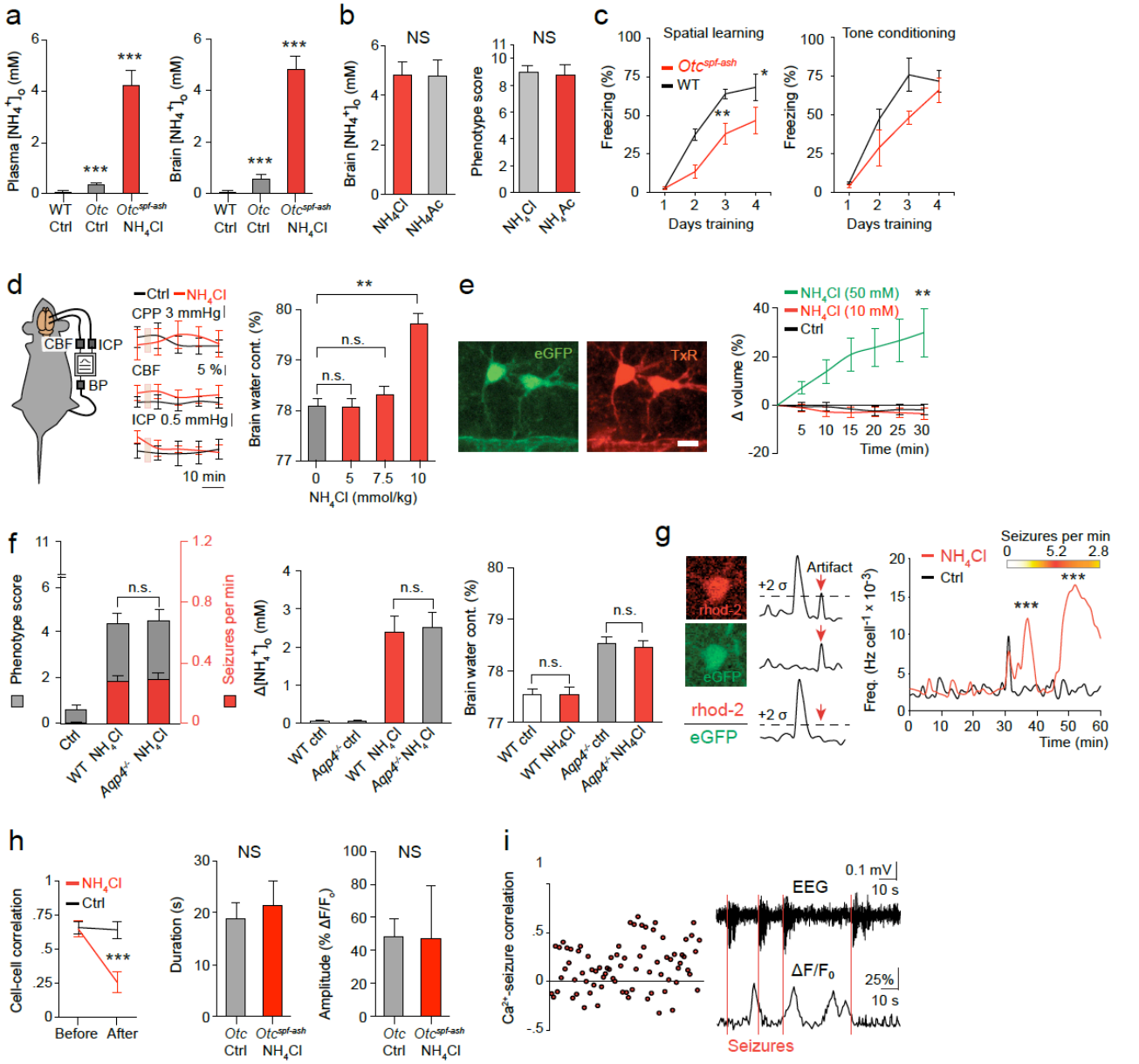
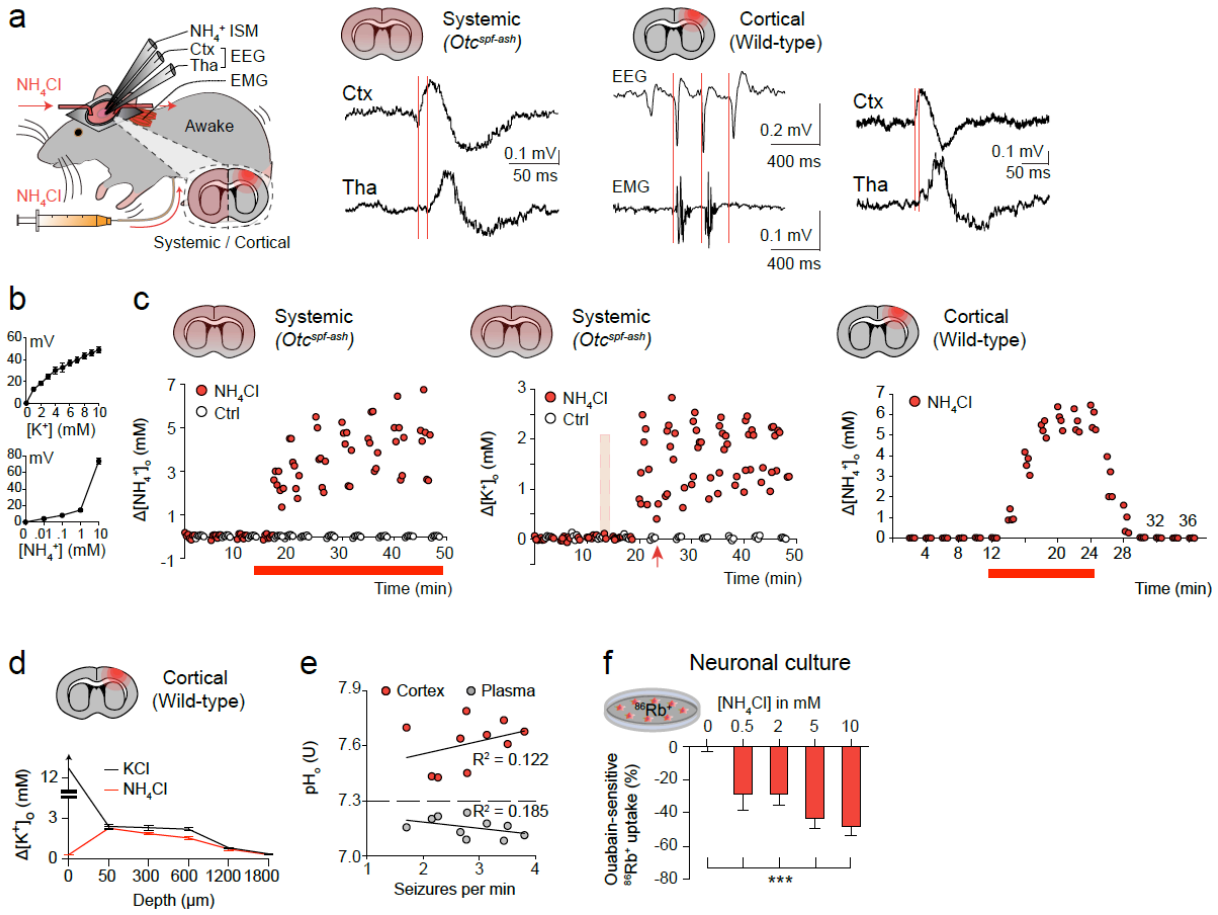
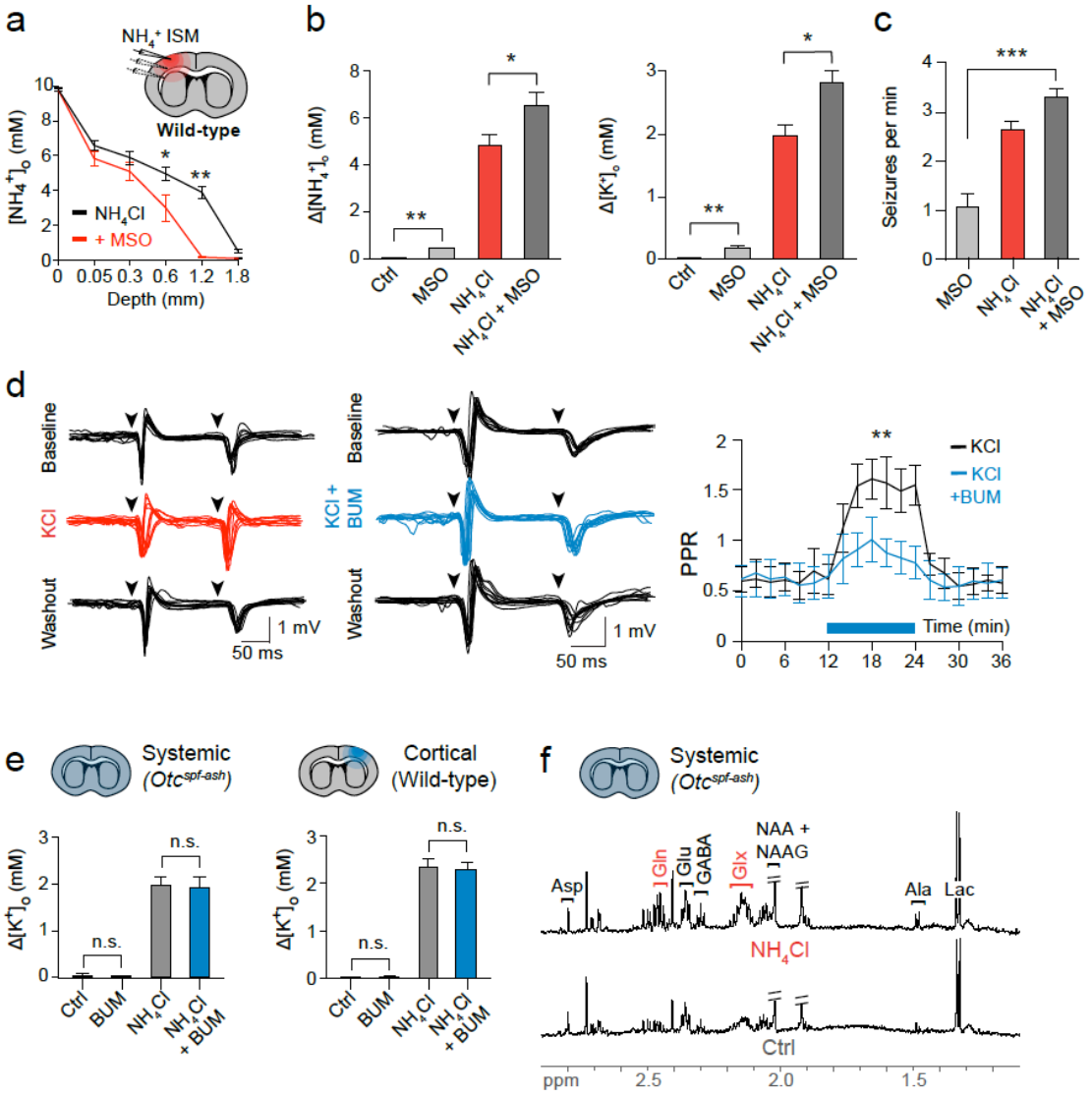


Supplementary Figure 1 Characterization of acute ammonia neurotoxicity. (**a**) Ammonia levels in blood plasma (left) and brain (right) before and 15 min after 7.5 mmol kg^{-1} NH_4Cl. ***$P < 0.001$, n = 7 for each group, Mann-Whitney U test. (**b**) Cortical $[NH_4^+]_o$ increase (left) and phenotype score (right) in NH_4Cl and NH_4Ac (ammonium acetate) injected mice. $P = 0.88$, $P = 0.73$, $n = 7$ (NH_4Cl) and 5 (NH_4Ac), Mann-Whitney U test. (**c**) *Otc*$^{spf\text{-}ash}$ and WT mouse freezing behavior upon testing for learning impairment after training for 4 days. *$P = 0.014$, *$P < 0.01$, $n = 9$ for both groups, Mann-Whitney U test. (**d**) Left, diagram of hemodynamic recordings and line graph of mean change following ammonia administration. Cerebral perfusion pressure (CPP), cerebral bloodflow (CBF) and intracranial pressure (ICP). Right, brain water content after mild, moderate and severe (lethal) ammonia intoxication. n.s. = not significant, **$P < 0.01$, , $n = 7$ (0 mmol kg^{-1}), 5 (5 mmol kg^{-1}), 6 (7.5 mmol kg^{-1}), 5 (10 mmol kg^{-1}), Mann-Whitney U test. (**e**) Volume analysis of texas red hydrazide (TxR) loaded astrocytes in cortical slices. Scale bar represents 10 µm. **$P < 0.01$, $n = 13$ (Control),

12 (10 mM), 10 (50 mM), unpaired *t* test. (**f**) Left, phenotype score (grey) and myoclonic seizure frequency (red) in wild-type (WT) and *Aqp4*$^{-/-}$ mice injected with 7.5 mmol kg^{-1} NH_4Cl. n.s. = not significant, $P = 0.829$ (seizures), $P = 0.357$ (phenotype), $n = 10$ (ctrl) and 9 (*Aqp4*$^{-/-}$), Mann-Whitney *U* test. Middle, cortical $[NH_4^+]_o$ in WT and *Aqp4*$^{-/-}$ mice before and after ammonia injection. n.s. = not significant, $P = 0.94$, $n = 6$ for each group, Mann-Whitney *U* test. Right, brain water content in control and ammonia injected WT and *Aqp4*$^{-/-}$ mice. n.s. = not significant, $P = 0.59$ (WT), $P = 0.69$ (*Aqp4*$^{-/-}$), $n = 10$ (WT ctrl), 5 (WT NH_4Cl), 5 (*Aqp4*$^{-/-}$ ctrl) and 5 (*Aqp4*$^{-/-}$ NH_4Cl), Mann-Whitney *U* test. (**g**) Left, change in calcium indicator rhod-2 flourescence ($\Delta F/F_0$) was normalized to eGFP to reduce movement artifacts. Right, running average of calcium transient frequency following ammonia administration (at time = 30 min) and in control (saline). Heat bar indicates mean myoclonic event (ME) frequency per min. ***$P < 0.001$, $n = 52$ (ammonia) and 62 (control) cells sampled from 12 animals, unpaired *t* test with Bonferroni correction. (**h**) Left, Pearson correlation coefficient of $\Delta F/F_0$ between different astrocytes before and after ammonia administration. ***$P < 0.001$, $n = 52$ (ammonia) and 62 (control) cells, paired *t* test. Middle and right, duration and amplitude of calcium transients before vs. after ammonia administration. (**i**) Left, scatterplot of correlation coefficients for individual astrocyte calcium signals and myoclonic seizures. Co-efficient < 0.3, $n = 52$ cells. (**d**) Representative calcium ($\Delta F/F_0$) and corresponding EEG trace showing temporal correlation between individual myoclonic seizures and astrocyte calcium transients. Data are shown as mean ± SEM.



Supplementary Figure 2 Electrophysiological and ion-sensitive microelectrode recordings. (**a**) Left, diagram showing systemic and cortical models of ammonia neurotoxicity. Right, representative EEG, EMG and local field recordings demonstrating the cortical focus of poly-spike and wave discharge in myoclonic seizures induced by either systemic or cortical ammonia application. Ion-sensitive microelectrodes (ISM), electroencephalogram (EEG), electromyogram (EMG), cortex (Ctx), thalamus (Tha). n = 478 events, 6 mice. (**b**) ISM calibration curves. (**c**) Scatterplots of $[NH_4^+]_o$ and $[K^+]_o$ recordings during either systemic or cortical ammonia administration (red bar, n = 4–10). (**d**) Line graph of $[K^+]_o$ at different cortical depths during either 12.5 mM KCl (black) or 10 mM NH_4Cl (red) application. Depth 0 μm represents ISM in the solution. n = 5 for each depth. (**e**) Linear regression of seizure frequency on pH_o during systemic ammonia intoxication in *Otc*$^{spf\text{-}ash}$ mice. (**f**) Na^+-K^+-ATPase-dependent (ouabain-sensitive) uptake of potassium analogue $^{86}Rb^+$ in cultured neurons, normalized to vehicle (0 mM NH_4Cl). ***P < 0.001, n = 20 (0 mM), n = 4 (0.5 mM), n = 4 (2 mM), n = 16 (5 mM), n = 4 (10 mM), Kruskall-Wallis test. Data are shown as mean ± SEM.



Supplementary Figure 3 Treatment strategies for ammonia neurotoxicity. (**a**) $[NH_4^+]_o$ at different depths in the brain during direct cortical application of ammonia (10 mM) with the glutamine synthetase inhibitor L-methionine sulfoximine (MSO, 1.5 mM). Depth 0 µm represents ISM in the solution. **P* = 0.019, ***P* < 0.01, *n* = 5 for each depth, Mann-Whitney *U* test. (**b**) Cortical $[NH_4^+]_o$ (left) and $[K^+]_o$ following administration of ammonia systemically, with vs. without MSO pre-treatment (0.83 mmol kg^{-1} i.p., administered 3 h before the ammonia) in *Otc*$^{spf\text{-}ash}$ mice. ***P* < 0.01, **P* = 0.048 ($[NH_4^+]_o$) and 0.013 ($[K^+]_o$), *n* = 5–10, Mann-Whitney *U* test. (**c**) Myoclonic seizure frequency following ammonia with vs. without MSO (0.83 mmol kg^{-1} i.p.) in awake *Otc*$^{spf\text{-}ash}$ mice. ****P* < 0.001, *n* = 7–22, Kruskall-Wallis test. (**d**) Left and middle, 10 consecutive sweeps of field excitatory post-synaptic potentials elicited by paired-pulse whisker stimulation (arrowheads) before, during, and after cortical application of KCl (12.5 mM, red box) or KCl with bumetanide (BUM, blue box). Right, paired-pulse ratio for whisker stimulation with KCl and KCl/BUM. **P* = 0.028 (KCl vs. BUM), ***P* < 0.01 (KCl vs. control), *n* = 5–6, Wilcoxon signed ranks test. (**e**) Left, increase in cortical $[K^+]_o$ following a systemic NH_4Cl load in *Otc*$^{spf\text{-}ash}$ mice with vs. without BUM. n.s. = not significant, *P* = 0.53, *P* = 0.92, *n* = 7 (Ctrl), 6 (BUM), 8 (NH_4Cl) and 7 (NH_4Cl + BUM), Mann-

Whitney *U* test. Right, increase in cortical $[K^+]_o$ following a cortical NH_4Cl with vs. without BUM. n.s. = not significant, $P = 0.94$, $P = 0.66$, $n = 6$ (Ctrl), 6 (BUM), 7 (NH_4Cl) and 8 (NH_4Cl + BUM), Mann-Whitney *U* test. (**f**) Representative ^{1}H-NMR spectra 15 min after injection with saline or ammonia in *Otc*$^{spf\text{-}ash}$ mice. ppm (parts per million), aspartate (Asp), glutamine (Gln), glutamate (Glu), γ-aminobutyric acid (GABA), glutamine + glutamate (Glx), N-acetylaspartic acid (NAA), N-acetylaspartylglutamate (NAAG), alanine (Ala), lactate (Lac). Data are shown as mean ± SEM.

Supplementary Table 1. Ion-sensitive microelectrode recordings from cortex.

CORTICAL SUPERFUSION						
	$[NH_4^+]_o$ (mM)	**n (animals)**	***P* value Mann-Whitney *U* test**	**$\Delta[K^+]_o$ (mM)**	**n (animals)**	***P* value Mann-Whitney *U* test**
Control	0.45 ± 0.17	6		0.0058±0.019	6	
BUM	0.52 ± 0.11	6	0.59	0.0025±0.018	6	0.94
NH_4Cl	5.82 ± 0.18	6		2.24 ± 0.17	10	
NH_4Cl + BUM	5.78 ±0.13	6	0.87	2.17 ± 0.12	9	0.66
INTRAPERITONEAL INJECTION						
	$[NH_4^+]_o$ (mM)	**n (animals)**	***P* value Mann-Whitney *U* test**	**$\Delta[K^+]_o$ (mM)**	**n (animals)**	***P* value Mann-Whitney *U* test**
Control	0.48 ± 0.12	6		0.015 ± 0.025	9	
BUM	0.42 ± 0.16	6	0.39	0.0073± 0.015	6	0.53
NH_4Cl	4.83 ± 0.52	7		1.93 ± 0.19	10	
NH_4Cl + BUM	4.70 ± 0.32	6	0.83	1.97 ± 0.17	6	0.92

Ammonia was applied either via cortical superfusion (wild-type mice) or intraperitoneal injection ($Otc^{spf\text{-}ash}$ mice). Data are shown as mean ± SEM.

Supplementary Table 2. Biochemical changes during acute ammonia neurotoxicity.

Genotype:	**WT**	**WT**	**WT**	***Otc*$^{spf-ash}$**	***Otc*$^{spf-ash}$**	***Otc*$^{spf-ash}$**	***Otc*$^{spf-ash}$**
Exposure:	**Ctrl**	**BUM**	**NH_4Cl**	**Ctrl**	**BUM**	**NH_4Cl**	**BUM + NH_4Cl**
Glutamate	14.29 ± 0.19	14.38 ± 0.22	12.49 ± 0.15	14.67 ± 0.68	14.27 ± 0.23	13.24 ± 0.33	12.51 ± 0.37
Glutamine	4.85 ± 0.47	4.52 ± 0.18	8.69 ± 0.33	7.58 ± 0.52	8.70 ± 1.54	9.25 ± 0.34	11.44 ± 0.46
GABA	2.00 ± 0.13	2.55 ± 0.05	2.19 ± 0.12	2.53 ± 0.12	2.39 ± 0.13	2.46 ± 0.13	2.23 ± 0.08

^{1}H-NMR was used on whole brain homogenates to determine amino acid concentrations (μmol g^{-1} wet weight) 15 min after saline (Ctrl) or ammonia administration. Bumetanide is abbreviated BUM. Data are shown as mean ± SEM.