Central cholinergic function and metabolic changes in streptozotocin-induced rat brain injury

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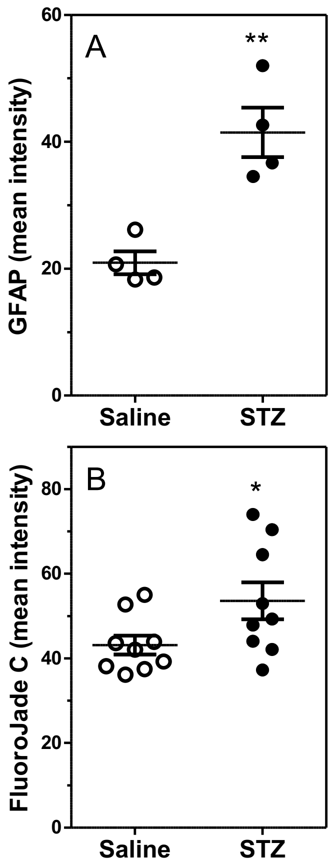
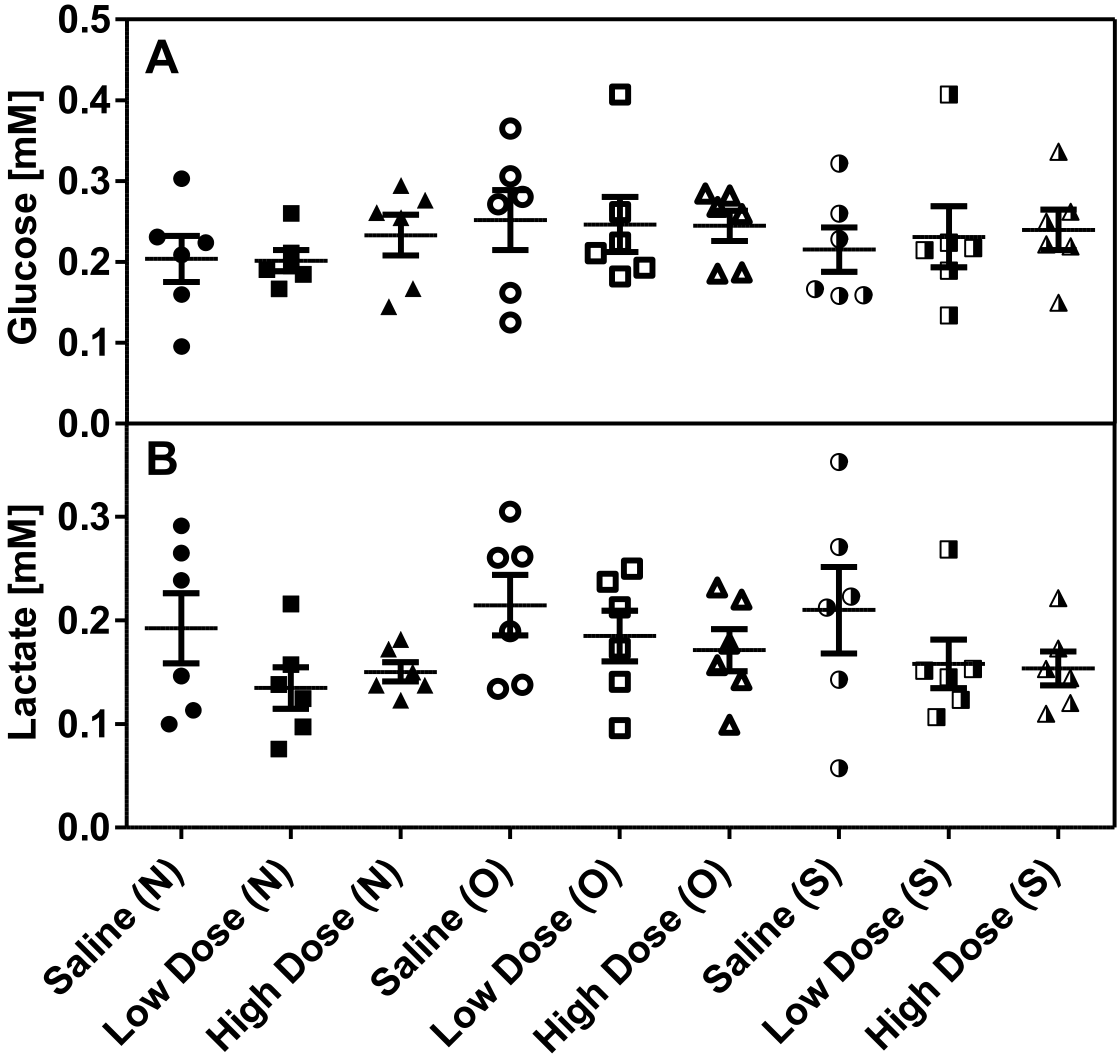
Supplemental Table 1. Animal groups and numbers of animals. HPC = hippocampus; STR= striatum. For cholinergic parameter experiments, hippocampus and striatum samples were obtained from the same rats.

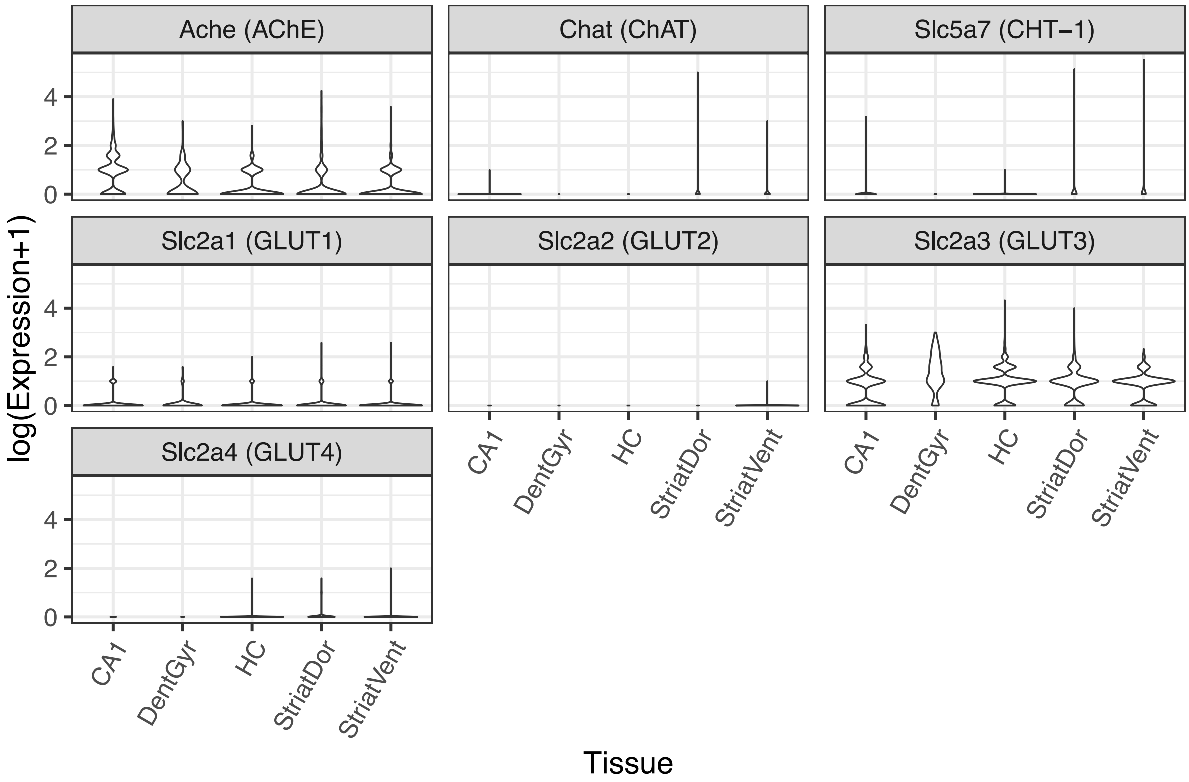
	Saline		Low Dose		High Dose	
Total number of animals receiving i.c.v. injection	44		15		44	
Brain region	HPC	STR	HPC	STR	HPC	STR
Total number of animals for histology study	9	0	0		9	0
Total number of animals for mitochondria study	12	0	0		12	0
Total number of animals for cholinergic marker study	8		0		8	
Total number of animals for microdialysis study	7	8	7	8	7	8
Dialysates was used in the statistics (n)	6	8	6	8	6	8
Exclusion criterion	1	0	1	0	1	0



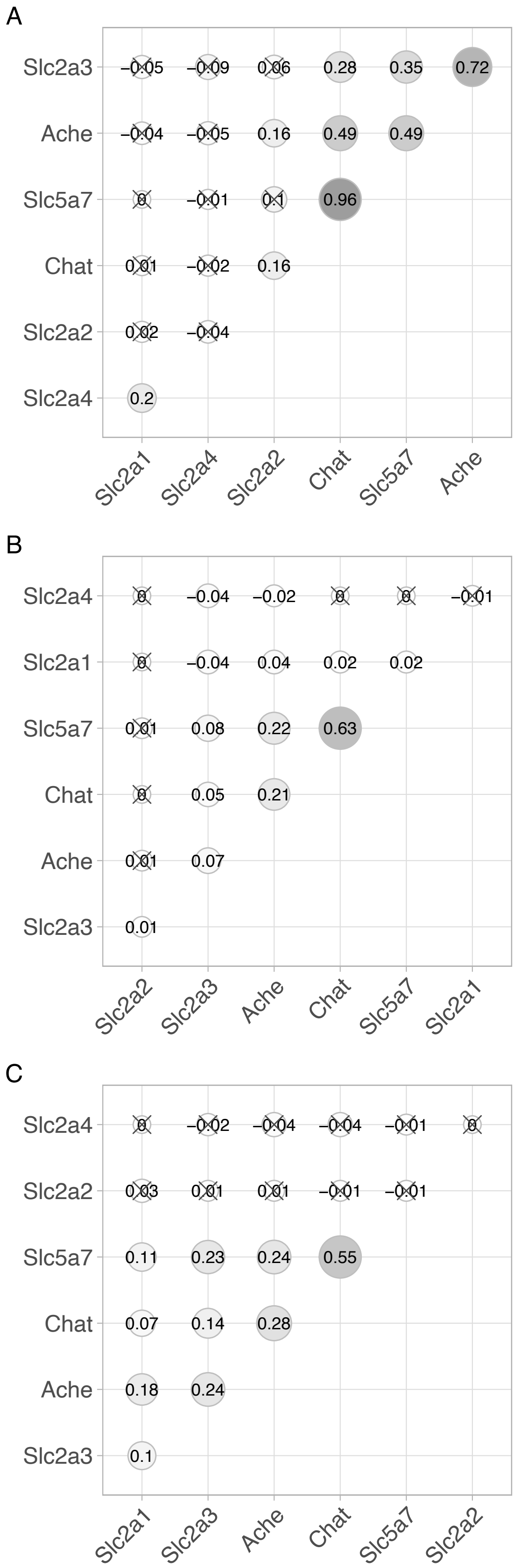
Supplementary Figure 1. Quantification of fluoroscence intensity from coronal cryosections of hippocampus (14 µm) at AP -5.6 mm from bregma (for exemplary pictures, see Fig. 2). GFAP staining was performed according to Kallenborn-Gerhardt *et al.* (2014) and Fluoro-Jade C staining was performed based on Schmued *et al*. (2005). Fluorescence signal was detected using a filter system (a TRITC filter for GFAP; a FITC filter for Fluoro-Jade C) and was quantified using ImageJ software. Data were analyzed by unpaired t-test. (A) Glial fibrillary acidic protein (GFAP) immunoreactivity in the rat hippocampus 21 days after i.c.v. injection of saline vs. streptozotocin (STZ). $t=4.6$, $p = 0.003$, $N=4$ animals; (B) Fluoro-Jade C signals of the CA1 region of the hippocampus 21 days after i.c.v. injection of saline vs. streptozotocin (STZ). $t=3.8$, $p = 0.048$, $N=9$ animals.



Supplemental Figure 2. Metabolite levels in hippocampal microdialysates under basal conditions (N), during open field exploration (O) and during infusion of scopolamine (S). Statistics: one-way ANOVA followed by Tukey post-test. N = 6 animals. Data are averages of six samples (90 minutes) each. (A) Glucose $F_{2,15} = 0.11$, $p = 0.897$; (B) Lactate $F_{2,15} = 1.38$, $p = 0.282$.



Supplementary Figure 3. Single-cell expression of cholinergic-associated transcripts and glucose transporters in neurons of hippocampus and striatum of the adult mouse. Hippocampal regions: CA1, dentate gyrus (DentGyr), complete hippocampus (HC); striatal regions: dorsal striatum (StriatDor), ventral striatum (StriatVent). Y-axis shows $\log_2$(expression + 1).



Supplementary Figure 4. Correlation of expression of cholinergic-associated transcripts and glucose transporters in single cells of the murine nervous system. Size and depth of colour of circles denote strength of correlation (Pearson's product-moment correlation coefficient, PPMCC), numbers denote PPMCC (range: -1 to 1). Non-significant correlation coefficients ($p > 0.05$) are crossed out. Rows and columns are ordered by hierarchical clustering, representing similarity of PPMCC values (Euclidian distance). (A) Correlation of transcripts in all tissues (cell-type-level) of the murine nervous system. (B) Correlation in all neurons (single-cell-level) of the murine nervous system. (C) Correlation in cholinergic neurons (single-cell-level) as determined by expression of the vesicular ACh-transporter, SLC18A3.