Supplementary information

Title: Reduced tonic inhibition after stroke promotes motor performance and epileptic seizures

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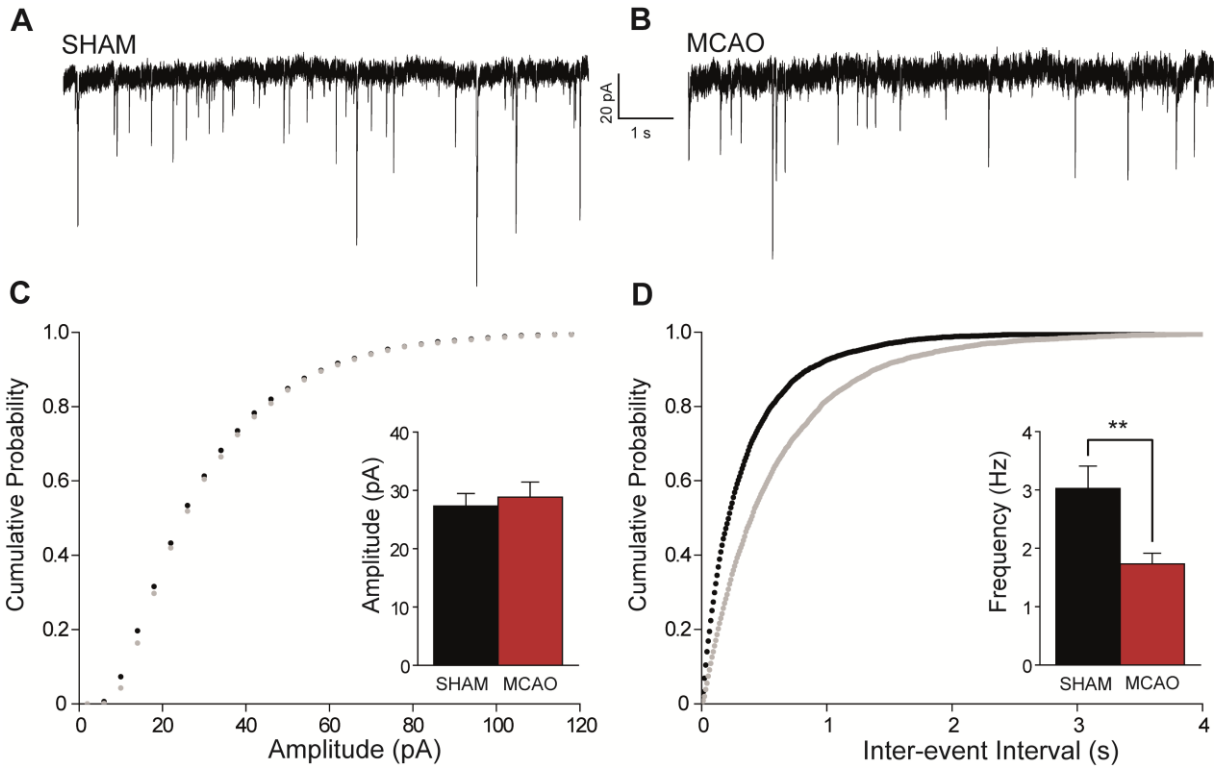
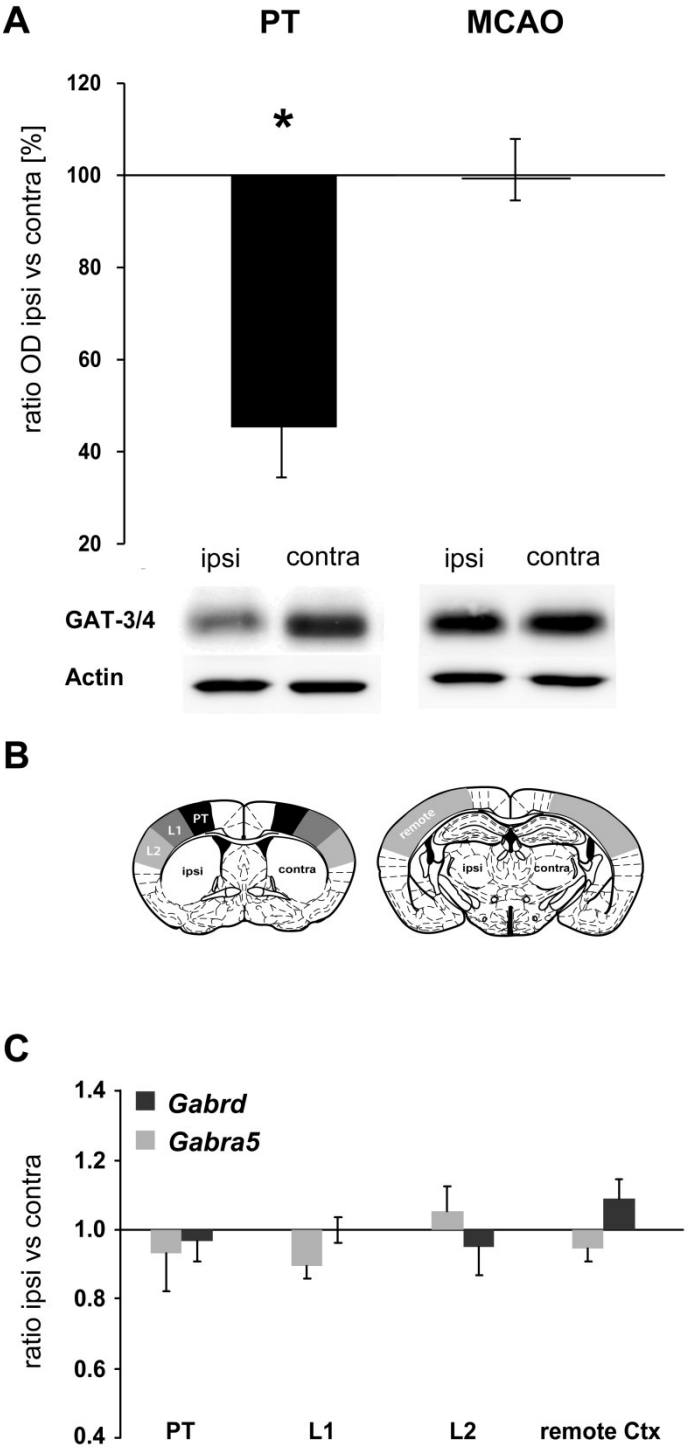
Supplementary Table 1: Primers and antibodies

Primers for qPCR of rat brain samples			
mRNA	**NM number**	**Sequence 5'→ 3'**	**Product bp**
Gapdh	NM_017008	Fwd: GCATTGCTCTCAATGACAACTT Rev: GGCCTCTCTCTTGCTCTCAGT	162
Tubb3	NM_139254.2	Fwd: GGCAACTATGTGGGGGACT Rev: GCACCACTCTGACCGAAGAT	191
Gabra1	NM_183326.2	Fwd: GATGGCAAAAGCGTGGTTC Rev: TCGGTTCTATGGTCGCACTT	160
Gabra2	NM_001135779.1	Fwd: CAGCGAGAACTGTGTTTGGA Rev: CCACTTTGGGAAGGGAATTT	84
Gabra3	NM_017069.1	Fwd: TGGTCATGTTGTTGGGACAG Rev: TGGCAAGTAGGTCTGGATGA	118
Gabra4	NM_080587.3	Fwd: AGGAGTCTGTTCCAGCCAGA Rev: AAAGAATGCCGAGCACTGAT	85
Gabra5	NM_017295.1	Fwd: CAGACGTACCTTCCCTGCAT Rev: GGTTGTCATGGTCAGCACTG	120
Gabrb1	NM_012956.1	Fwd: CAAGACCAGAGTGCCAATGA Rev: CCAGGGTGCTGAGGAGAATA	88
Gabrb2	NM_012957.2	Fwd: GTCAACAAGATGGACCCACA Rev: GAGGCATCATAGGCAAGCAT	128
Gabrb3	NM_017065.1	Fwd: GACAGCCAAGGCCAAGAAT Rev: TGAACATCCATCGGTGCTAG	90
Gabrg1	NM_080586.1	Fwd: AACCACCAGAGACAGGAAGC Rev: TTCCCCTTGAGGCATAGAAA	106
Gabrg2	NM_183327.1	Fwd: TGTCCTGGGTATCCTTCTGG Rev: AGAGACTTCCGGGCTATGGT	112
Gabrg3	NM_024370.3	Fwd: TCCCCTGCATACTGACTGTG Rev: CGTGGTGATGCCTAATGTTG	92
Gabrd	NM_017289.1	Fwd: AGAAACGGAAAGCCAAGGTC Rev: CCTCCTTCTTTGCCTCCACT	189
Primers for qPCR of mouse brain samples			
mRNA	**NM number**	**Sequence 5'→ 3'**	**Product bp**
Gapdh	NM_008084.2	Fwd: CAACAGCAACTCCCACTCTTC Rev: GGTCCAGGGTTTCTTACTCCTT	164
Tubb3	NM_023279.2	Fwd: GCCTTTGGACACCTATTCAGG Rev: ACTCTTTCCGCACGACATCT	133
Gabra1	NM_010250.4	Fwd: GATGGCAAAAGCGTGGTTC Rev: TCGGTTCTATGGTCGCACTT	160
Gabra2	NM_008066.3	Fwd: TTGGGACGGGAAGAGTGTAG Rev: TGGCTTGTTCTCTGGCTTCT	184

Gabra3	NM_008067.4	Fwd: GCCGTCTGTTATGCCTTTGT Rev: CCTTGGCCAGATTGATAGGA	199
Gabra4	NM_010251.2	Fwd: CCCATGAGACTGGTGGATTT Rev: ACAGTCTGCCCAATGAGGTC	176
Gabra5	NM_176942.4	Fwd: AAGAAAGCCCTGGAAGCAG Rev: GTTTGGAGGATGGGTCAGC	105
Gabrb1	NM_008069.4	Fwd: CAAGACCAGAGTGCCAATGA Rev: TGGTCTCGTTCCTGATTTCC	106
Gabrb2	NM_008070.3	Fwd: TGCCAACAATGAGAAGATGC Rev: CCCATTACTGCTTCGGATGT	114
Gabrb3	NM_008071.3	Fwd: ACAATCCTCTCGTGGGTGTC Rev: GAGTCTCCCGAAGGTGAGTG	118
Gabrg1	NM_010252.4	Fwd: TGGAATACGGAACCTTGCAT Rev: TGCTGTTCATGGGAATGAGA	132
Gabrg2	NM_008073.2	Fwd: GGGCTACTTCACCATCCAGA Rev: GACCTTGGGCAGAGATTTTC	172
Gabrg3	NM_008074.2	Fwd: GAAGACTCCCCATCAAACCA Rev: ATTCCAATGTCCGGTCTCAG	122
Gabrd	NM_008072.2	Fwd: CCACTTCAATGCCGACTACA Rev: TGAGAGGGAGAAAAGGACGA	106
Antibodies for Western blotting			
Ab	**Dilution**	**Company**	**kDa**
ß-Actin	1:90.000	beta-actin; ab8227, Abcam, UK	42
TUJ1	1:90.000	Neuronal Class III ß-Tubulin; TUJ1, Covance, USA	51
GABRD	1:250	$GABA_A$ Rδ; sc25705, Santa Cruz Biotechnology, USA	51
GABRD	1:500	$GABA_A$ subunit δ, kindly provided by Werner Sieghart, Center for Brain Research, Medical University Vienna, Austria	51
GABRA5	1:2000	$GABA_A$ receptor α5 antibody, [1]	52
GAT-3	1:5000	Rabbit anti-GABA transporter-3 (GAT-3) polyclonal antibody, AB1574, Millipore, Germany	70
goat @ rbt IgG	1:5000	HRP-conjugated goat anti-rabbit IgG antibody; 1:5000, sc-2004; Santa Cruz Biotechnology, USA	
goat @ mouse IgG	1:5000	HRP-conjugated goat anti-mouse IgG antibody; 1:5000, sc-2002; Santa Cruz Biotechnology, USA	
goat @ guinea pig IgG	1:5000	HRP-conjugated goat anti-guinea-pig IgG antibody; 1:5000, sc-2438; Santa Cruz Biotechnology, USA	



Supplementary Fig. 1. Synaptic GABAergic inhibition 7days after stroke. The frequency of sIPSCs, but not the amplitude, decreased in cortical neurons of MCAO-treated mice. **(A, B)** Representative traces of a sIPSC recorded in cortical neurons (layer 2/3) of control and MCAO mice. **(C)** Cumulative plots and the means of sIPSC amplitudes did not differ between the groups (cells n=10/12, mice n=3). **(D)** Cumulative plots of inter-event intervals and means of frequencies revealed a shift to longer intervals in neurons of the ischemic cortex (cells n=10/12, mice n=3, **p≤0.01).



Supplemetary Fig. 2. GABA transporter GAT3/4 and $GABA_A$ receptor subunits *Gabrd* and *Gabra5* at 7days following stroke in mice. (A) GAT3/4 was reduced following photothrombosis but remained stable following MCAO. Optical densities of GAT3/4 in a ratio to ß-actin are diagrammed as the percent relative to the contralateral hemisphere±s.e.m (PT: n=3, MCAO: n=4, *p≤0.05). **(B)** Scheme of analyzed brain areas (PT: injured tissue inclusive glial scar; L1, L2: perilesional lateral regions and remote cortical area), modified from The Mouse Brain in Stereotaxic Coordinates[2]. **(C)** Normalization of *Gabrd* and *Gabra5*

to *Tubb3* showed stable post-photothrombotic RNA expression. Data are displayed as the geomean of ratios (ipsi vs. contra)±s.e.m. (PT: n=5).

1. Fritschy, J.M. & Mohler, H. GABAA-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. *J Comp Neurol* **359**, 154-194 (1995).
2. Paxinos, G. & Franklin, K.B.J. *The Mouse Brain in Stereotaxic Coordinates* (Academic Press, San Diego, 2001).