

**Suppression of neuropathic pain and comorbidities by recurrent cycles
of repetitive transcranial direct current motor cortex stimulation in mice**

Zheng Gan^{1, #}, Han Li^{1, #}, Paul Vincent Naser¹, Manfred Josef Oswald¹ and
Rohini Kuner^{1,*}

¹Institute of Pharmacology, Medical Faculty Heidelberg, Heidelberg University,
Im Neuenheimer Feld 366, 69120 Heidelberg, Germany

These authors contributed equally to this work.

*Correspondence should be addressed to R.K. (rohini.kuner@pharma.uni-
heidelberg.de)

Supplementary figure legends:

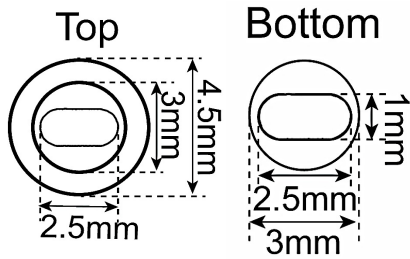
Supplementary Fig. 1: (A) Electrode holder dimensions for the cranial placement of the anode and the cathode on mouse skull. (B) Analysis of plantar sensitivity to mechanical von Frey stimulation applying graded force in M1 tDCS or sham-treated mice prior to nerve injury. (C, D) Analysis of von Frey sensitivity in the same groups of mice at 6 days (C) or 34 days (D) post-nerve injury (SNI) but prior to repetitive M1 tDCS or 0 mA sham stimulation. In panels B and C, n = 7 mice/group; in panel D, n = 7 mice for the sham treatment group and n = 6 mice for repetitive M1 tDCS group; ANOVA for repeated measures was performed, followed by Sidak's test for multiple comparisons; n.s. represents non-significant differences between groups. Data are expressed as Mean \pm S.E.M.

Supplementary Fig. 2: Quantitative analysis of Fos-expressing cells over diverse brain regions and the lumbar spinal cord upon hind paw stimulation with low intensity mechanical force (0.16 g; corresponding to neuropathic mechanical allodynia) at chronic stages of neuropathic pain (43 days post-nerve injury) in SNI mice that received either repetitive M1 tDCS or 0 mA sham stimulation over 35-39 days post-nerve injury. Shown are analyses over the mid-cingulate cortex (MCC, A), primary motor cortex (M1, B), secondary motor cortex (M2, C), primary somatosensory cortex, hindlimb region (S1HL, D),

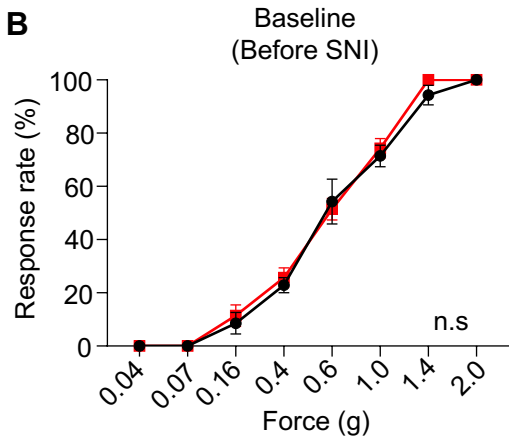
prelimbic cortex (PrL, **E**), ventral posterolateral thalamic nucleus (VPL, **F**), ventral posteromedial thalamic nucleus (VPM, **G**) and the deeper spinal laminae III and IV (**H**). In panel **A** to **G**, n = 9 sections from 3 mice in repetitive M1 tDCS group (without mechanical stimulation) and n = 11-12 sections from 4 mice in each of the other groups. In panel **H**, n = 6-10 sections from 4 mice per group; ANOVA for random measures was performed, followed by Sidak's test for multiple comparisons, n.s. represents non-significant differences between groups. Data are expressed as Mean \pm S.E.M.

Supplementary Fig.1

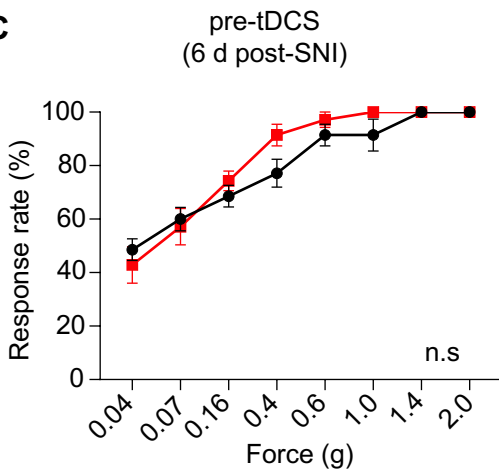
A



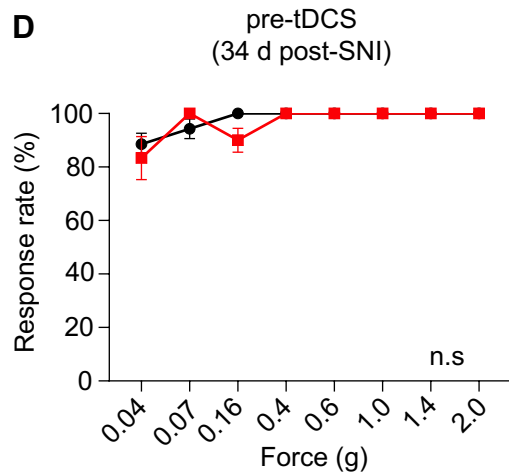
B



C



D



For panels B, C and D: ● Sham treatment ■ Repetitive M1 tDCS

Supplementary Fig.2

