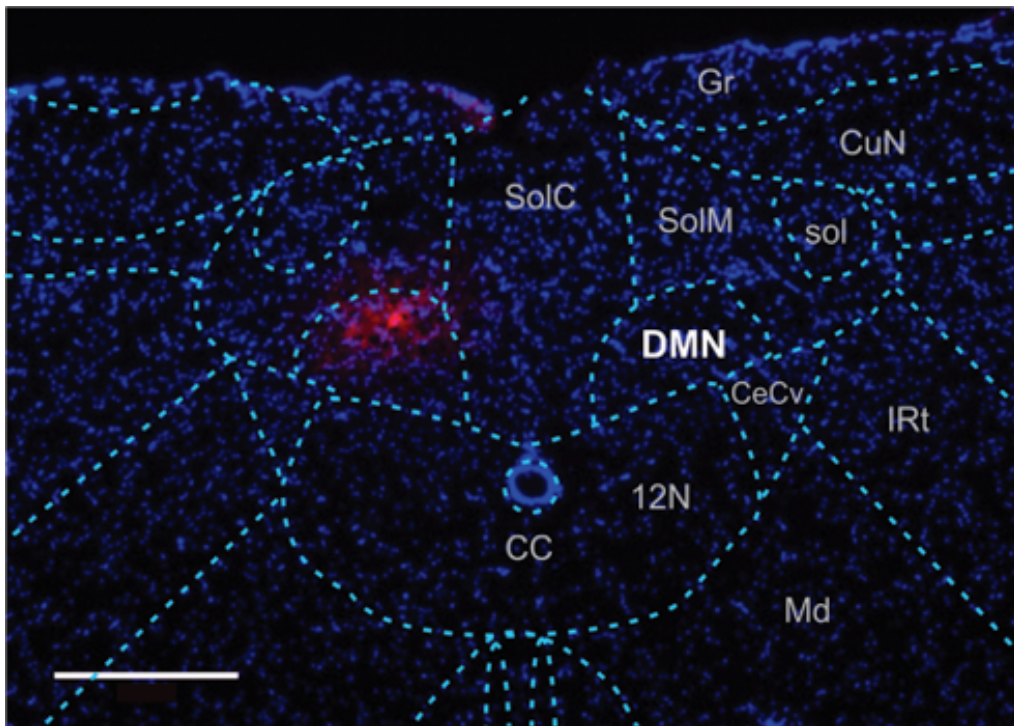


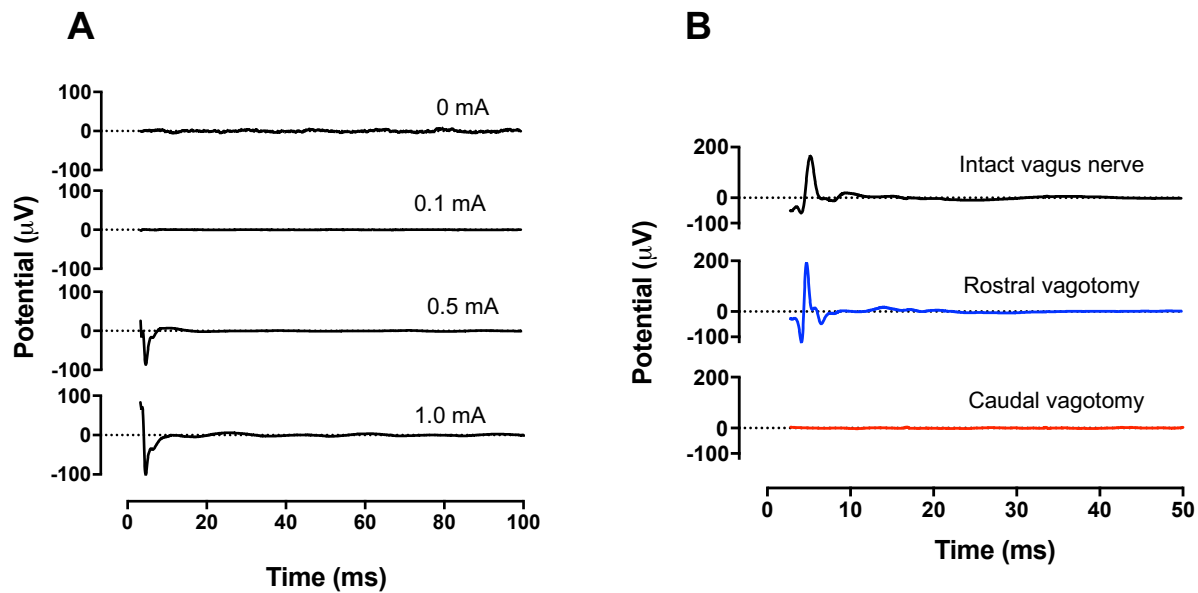
Identification of a Brainstem Locus That Inhibits Tumor Necrosis Factor

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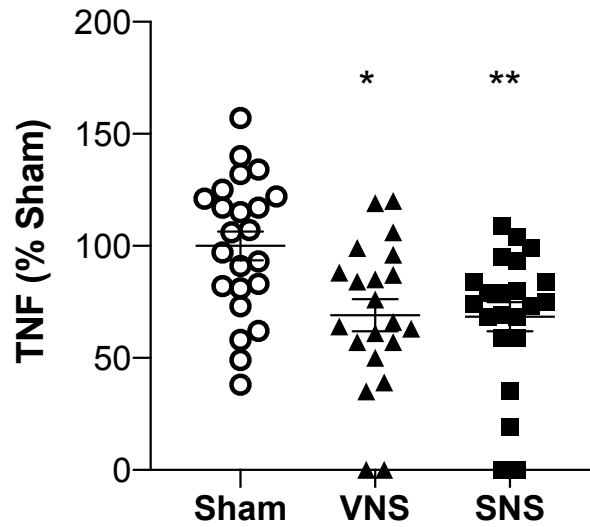
Supplementary Figures



Supplementary Fig. 1. Targeting brainstem DMN for optogenetic stimulation. A representative image of a brain section in the region of DMN. Mice were placed in a stereotactic frame and 1% Dil (1,18-dioctadecyl-3,3,38,38-tetramethylindocarbocyanine perchlorate) solution was injected in the left DMN under stereotaxic guidance with the head tilted 45° to allow an approach via the 4th ventricle (see Methods). Immediately following injection, the mouse was euthanized and 20 µm frozen sections of the brainstem were imaged. The location of visualized dye corresponds with the location of the DMN according to the mouse brain atlas (1), indicating that the DMN in mice can be repeatedly identified and accessed. 12N, hypoglossal nerve; CC, central canal; CeCv, central cervical nucleus of the spinal cord; CuN, cuneate nucleus; DMN, dorsal motor nucleus of the vagus; Gr, gracile nucleus; IRt, intermediate reticular nucleus; Md, medullary reticular nucleus; sol, solitary tract; SolC, solitary nucleus commissural part; SolM, solitary nucleus medial part. Scale bar: 250 µm.



Supplementary Fig. 2. Vagus nerve induced EMG activity in laryngeal muscles. Recordings of EMG signals in the laryngeal muscles, an established target function of VNS, was performed after **(A)** vagus nerve stimulation carried out at 0.25 ms biphasic pulses of 0, 0.1, 0.5, 1.0 mA and after **(B)** caudal or rostral vagotomy. Vagus nerve stimulation induced EMG activity in the laryngeal muscles which is abolished by caudal but not rostral vagotomy.



Supplementary Fig. 3. Splenic nerve stimulation attenuates LPS-induced serum TNF response in mice. Mice were subjected to either sham surgery or electrical vagus nerve stimulation (VNS) or splenic nerve stimulation (SNS) (750 μ A, 200 μ s pulse width at 10 Hz for 60 s). After overnight recovery, animals were challenged intraperitoneally with LPS. Serum TNF was analyzed 90 min post-LPS administration. Data is represented as individual mouse data point with mean \pm SEM. n=22-23 per group. One-way ANOVA followed by Dunnett's multiple comparisons test between groups: sham versus VNS ($p < 0.05$), sham versus SNS ($p < 0.01$).

REFERENCES

1. K. B. J. Franklin, G. Paxinos, *The Mouse Brain in Stereotaxic Coordinates (map)* (2007).