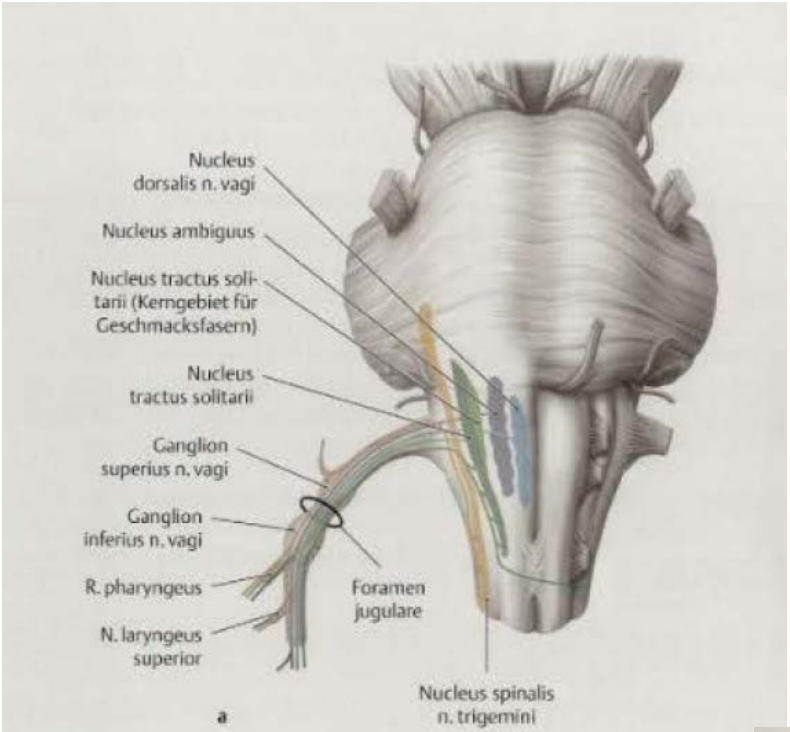
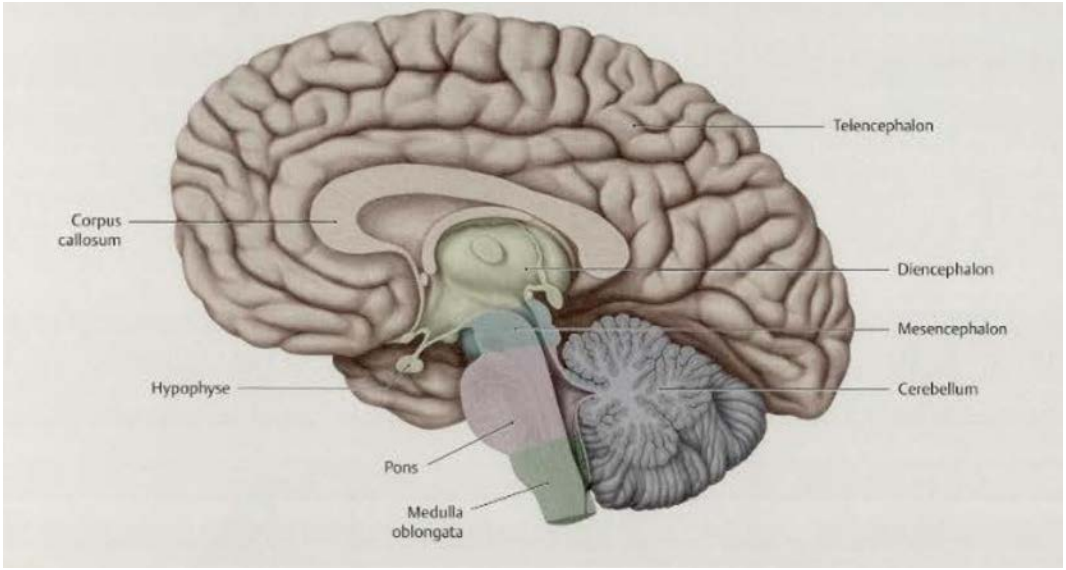
Identification of a brainstem locus that inhibits tumor necrosis factor

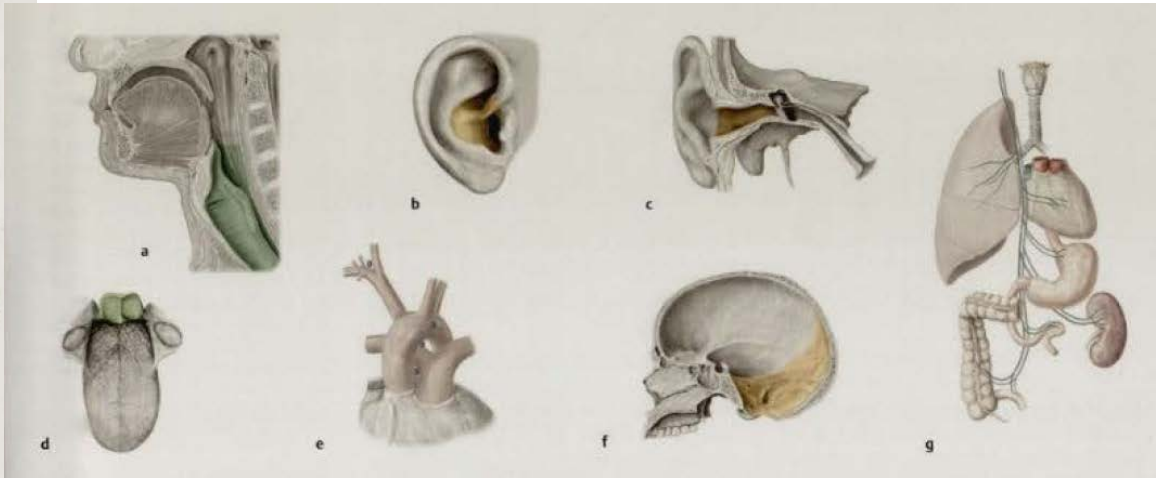
Kressel AM, Tsaava T, Levine YA, Chang EH, Addorisio ME, Chang Q, et al. In Proceedings of the National Academy of Sciences (2020) 117(47):29803-10. doi: 10.1073/pnas.2008213117.

Präsentation im JC Applied Immunology
Daniel Bormann n01118880
22.03.2021

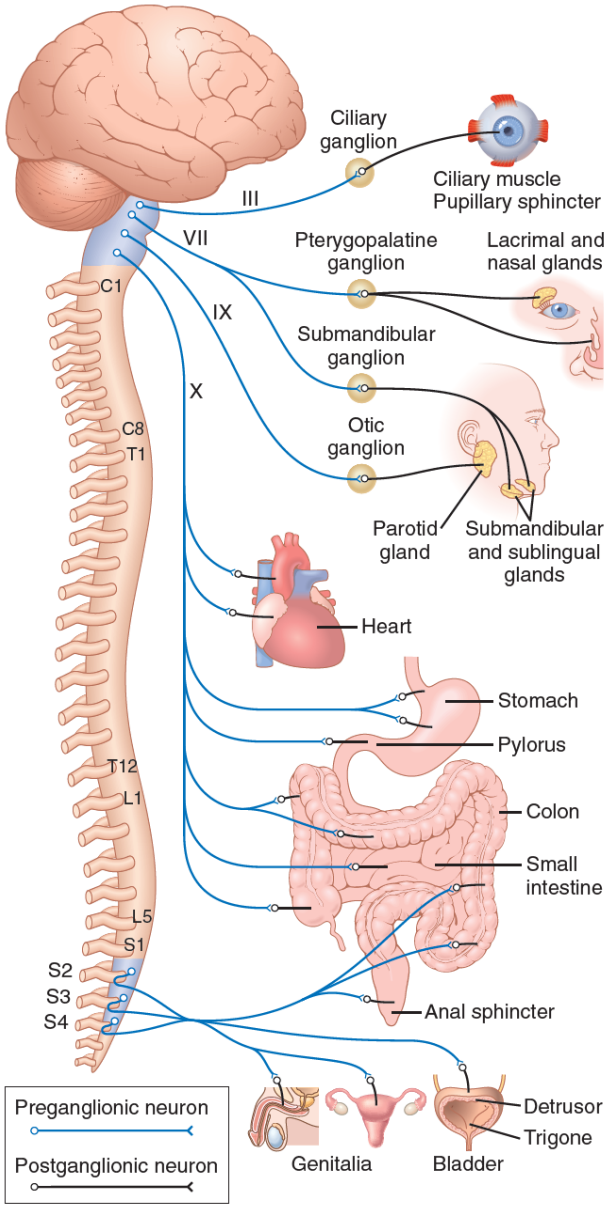
Review: Neuroanatomy





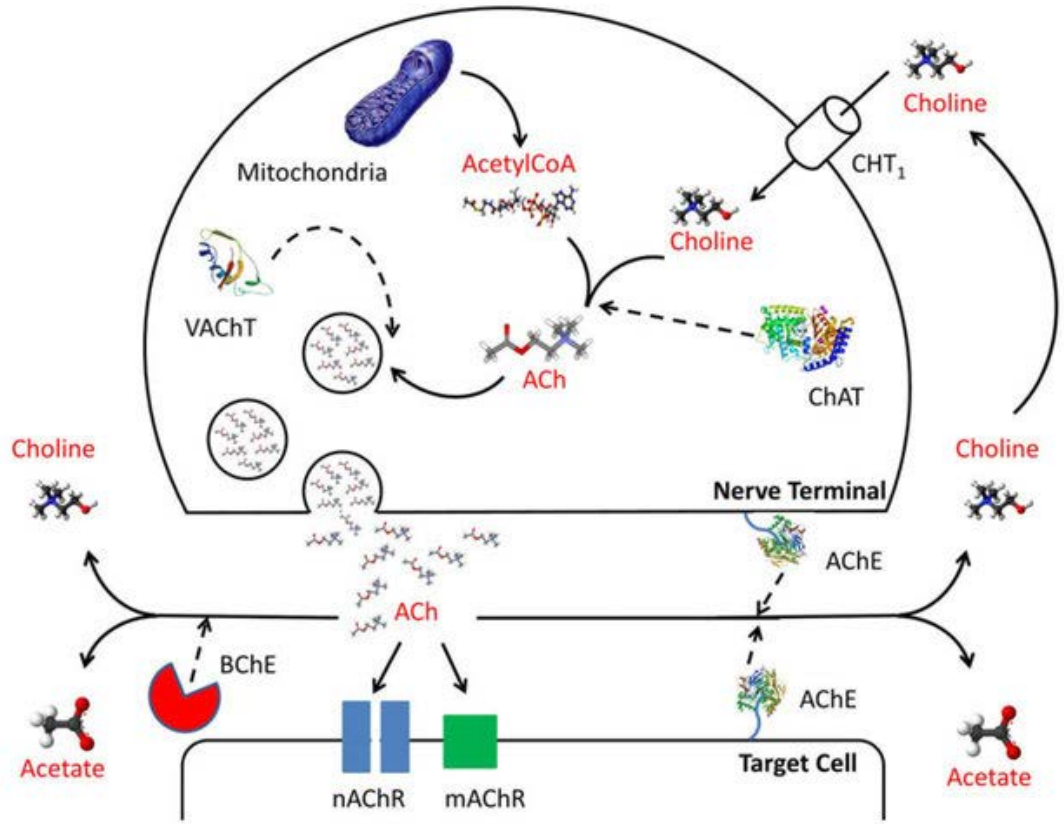


In a nutshell



- The brainstem harbours a network of interconnected nuclei
- This network, in crosstalk with the spinal cord and the peripheral nervous system, governs the majority of the homeostatic innervation of the innervation
- This includes gastrointestinal motility, glandular functions, heart rate, blood pressure, bladder function etc…

Review: Neurophysiology and -chemistry

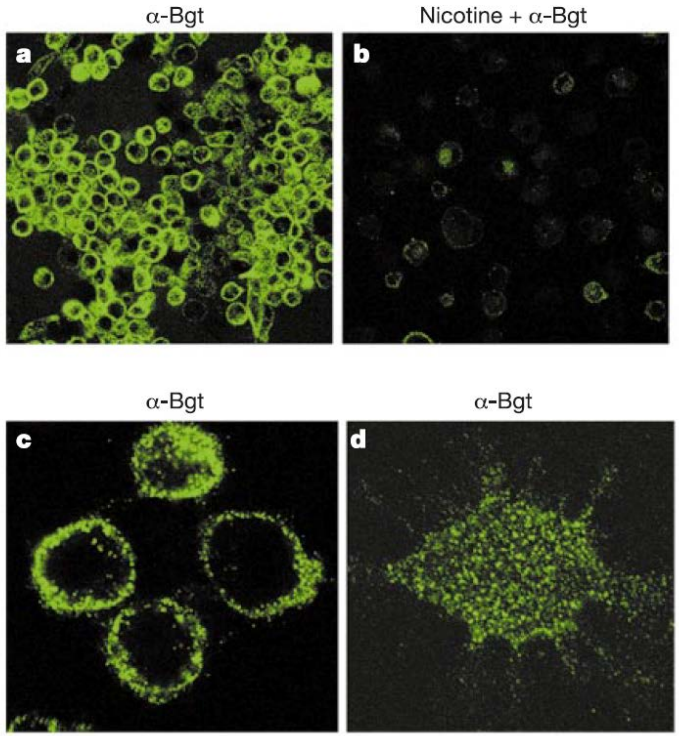


- What are the autonomous nervous systems favorite „languages“

→Catecholaminergic and cholinergic

https://www.researchgate.net/publication/229012492_Acetylcholine_and_the_alpha_7_nicotinic_receptor_A_potential_therapeutic_target_for_the_treatment_of_periodontal_disease

Wang et al. 2003 – Pioneering research on the cholinergic neuroimmunological crosstalk



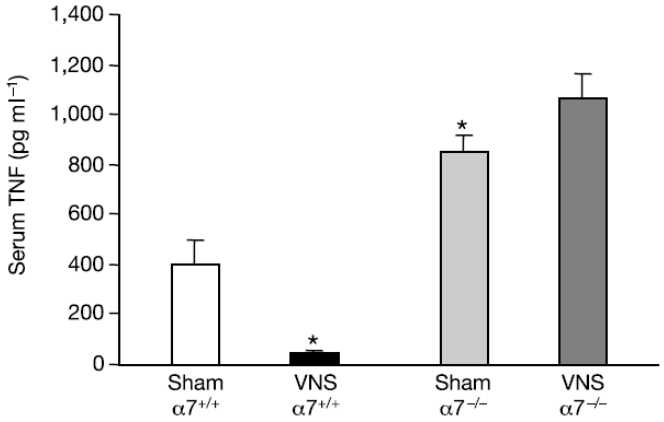
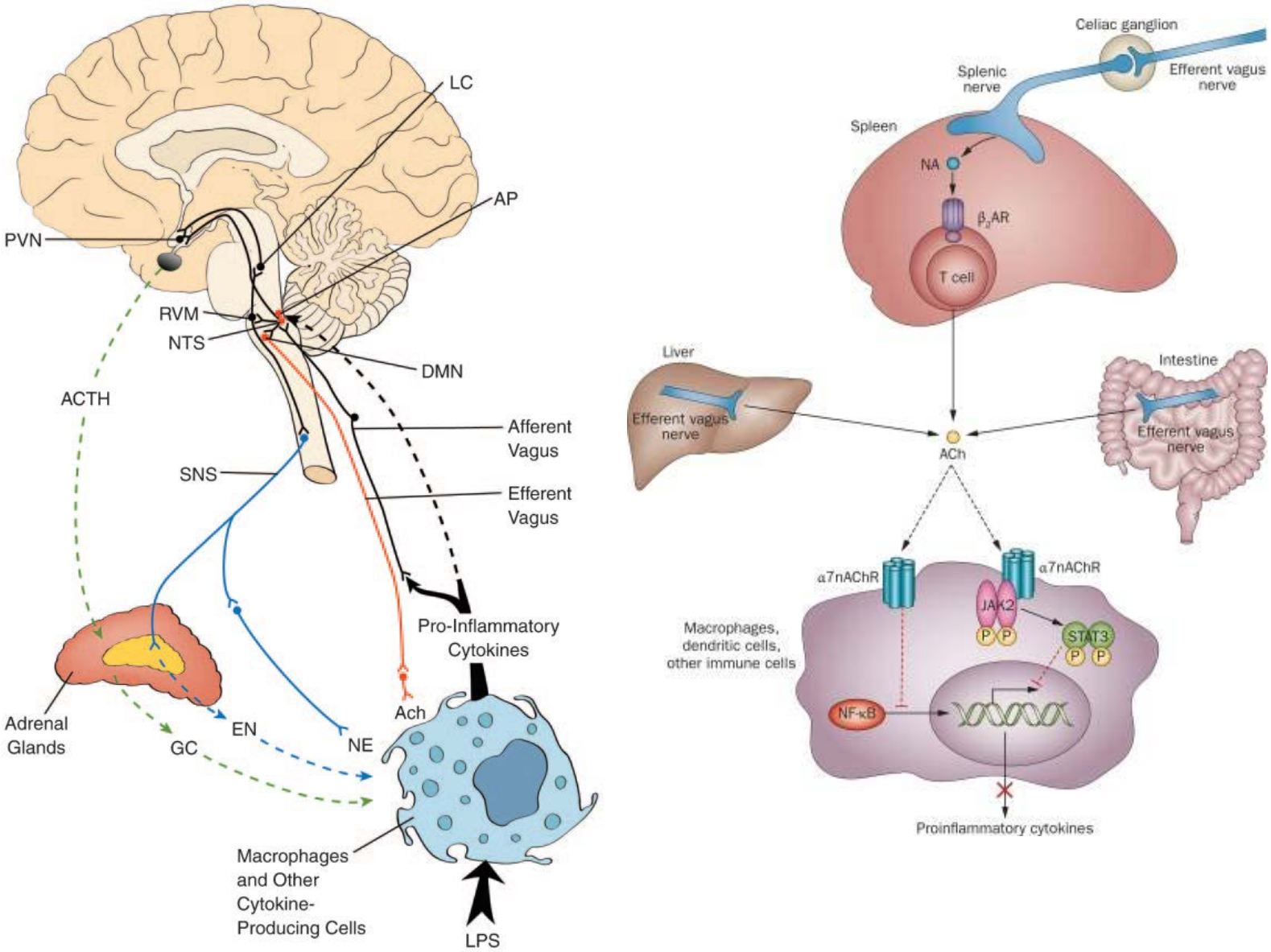


Figure 5 Vagus nerve stimulation does not inhibit TNF production in α7-subunit-deficient mice. α7-subunit-deficient mice (−/−) or age- and sex-matched wild-type mice (+/+) were subjected to either sham operation or vagus nerve stimulation (VNS, left vagus; 1 V, 2 ms, 1 Hz); blood was collected 2 h after LPS administration. Serum TNF levels were determined by ELISA. $n = 10$ (sham $\alpha7^{+/+}$); $n = 11$ (VNS $\alpha7^{+/+}$, sham $\alpha7^{-/-}$, VNS $\alpha7^{-/-}$). Asterisk, $P < 0.05$ versus sham $\alpha7^{+/+}$.

- Left inset: fluorescein isothiocyanate (FITC)-labelled a-bungarotoxin was used to label nAChR on Macrophages, pretreatmen with Nicotine (affine nAChR Partialagonist) prevents the binding

Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, Li JH, Wang H, Yang H, Ulloa L, Al-Abed Y, Czura CJ, Tracey KJ. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature. 2003 Jan 23;421(6921):384-8. doi: 10.1038/nature01339. Epub 2002 Dec 22. PMID: 12508119.

The Cholinergic Anti-inflammatory Pathway



Pavlov VA, Wang H, Czura CJ, Friedman SG, Tracey KJ. The cholinergic anti-inflammatory pathway: a missing link in neuroimmunomodulation. Mol Med. 2003 May-Aug;9(5-8):125-34. PMID: 14571320; PMCID: PMC1430829.

Scope of the study

- N. vagus mediated autonomous innervation has been shown to modulate the production of proinflammatory cytokines
- Certain PBMCs have been shown to express Acetylcholin receptors (predominantly α7nAChR on macrophages), and are able to synthesize and release Acetylcholine (e.g. T-cells).
- Here Kressel et al. combined classical electrophysiology, neuropharamacology, optogenetics and functional mapping in an effort to mechanistically elucidate the „cholinergic anti-inflammatory reflex“

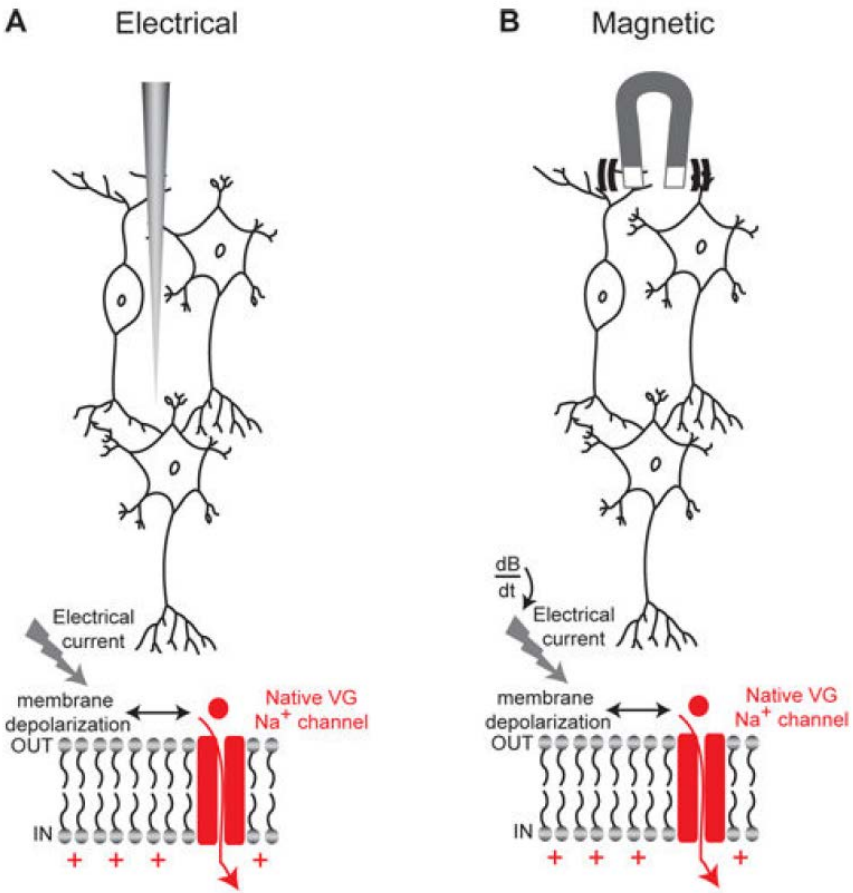
Materials and Methods

1. Selective activation of DNM cholinergic nuclei using optogenetics
2. Functional mapping using a viral vector approach
3. „Classic“ Electrophysiological Recordings

1. Selective activation of DNM cholinergic nuclei using optogenetics

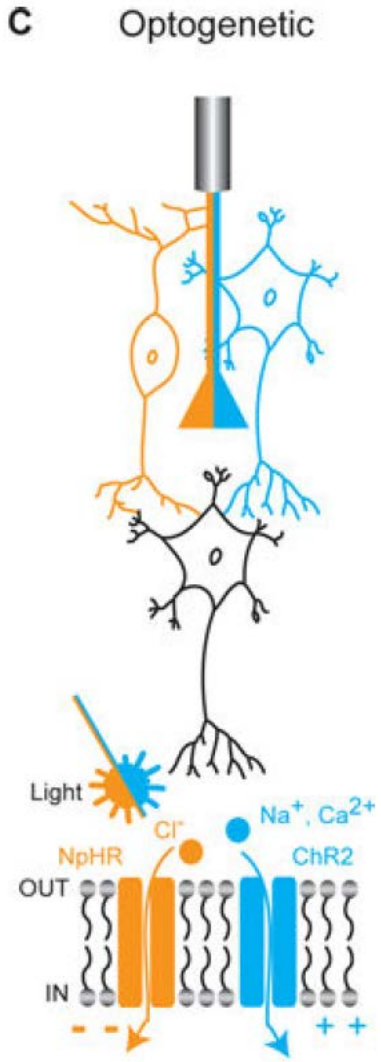
- Background

„Conventional stimulation of neural Tissue“



A,B. Extracellular capacitative current (A) or alternating magnetic fields (dB/dt) (B) induce a Depolarization of the membrane -> Voltage gated cation channels open-> Generation of Action potentials

→ Spatiotemporally restricted stimulation of all surrounding cells irregardless of cell population



Optogenetics provides a method to specifically target specific cells

E.g. only neuronal cells which express a certain photosensitive Channel

-> E.g. ChR2 = channelrhodopsin 2 expressing cells (Cation-channel)-> can be activated by blue light of a certain wave length

-> Halorhodopsin NpHR expressing cells can be selectivles inhibited (Chloride permeable channel)

The authors used **ChAT**-**ChR2**-**eYFP** BAC transgenic male mature mice.

To parpaphrase: Mice that express **ChR2** („a light sensitve ON switch“) coupled to **eYFP** (a flourecent protein to check if the switch is were intended) **SPECIFICALLY** in cells that contain a **ChAT** promotor (= Acetylcholine synthezising cells).

Basic Optogenetic stimulation protocol

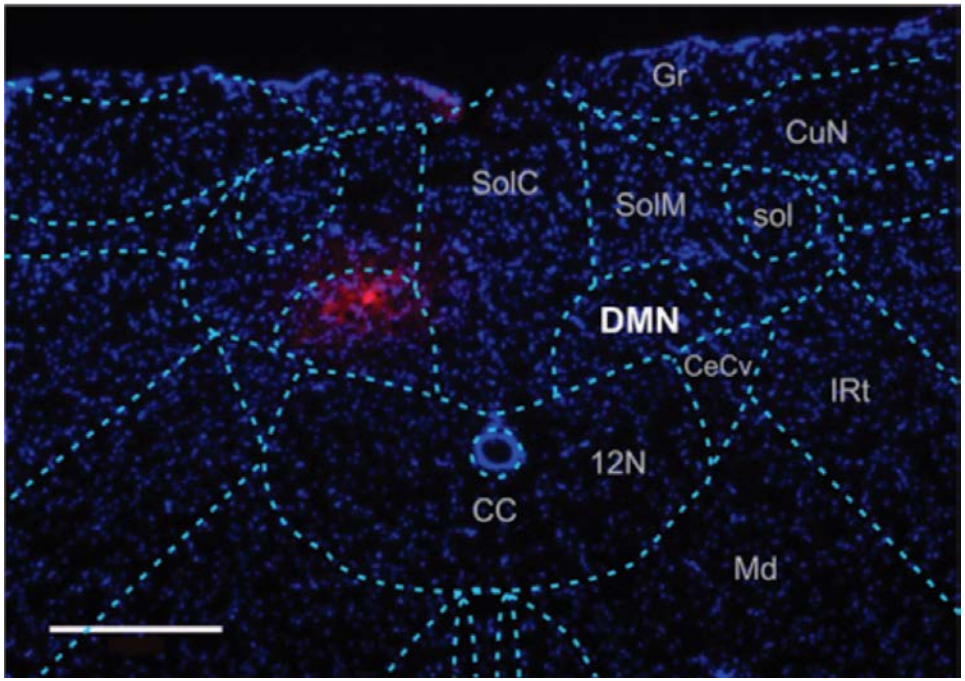
Experiment I: Optogenetic „priming“ before endotoxemia with an i.p. single shot of LPS: 0.25 mg/kg.

- Stereotactic insertion of a fibreoptic cannula in the left DMN delivering light pulses, blue light (473 nm), frequency: 20 Hz, 25% duty cycle, 5-min duration.
- Control conditions:

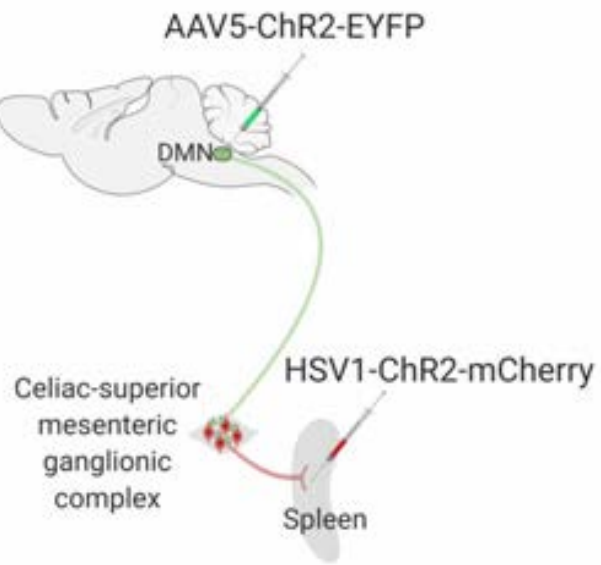
-> Yellow light (593.5nm) which does not activate ChR2

-> Stimulation of DMN neurons in littermate controls (noncarriers)

-> Readout: Serum TNF-Alpha Levels (ELISA)



Experiment II : Functional mapping using a viral vector approach



- Syn-Cre-mice DNMs were microinjected with an **anterograde** AAV5-ChR2-eYFP Adeno viral vector.

→ Selective eYFP staining of axons and presynaptic terminals decending from the DNM

- The spleens of the same animals were injected with a **retrograde** HSV1-ChR2- mCherry viral vector.

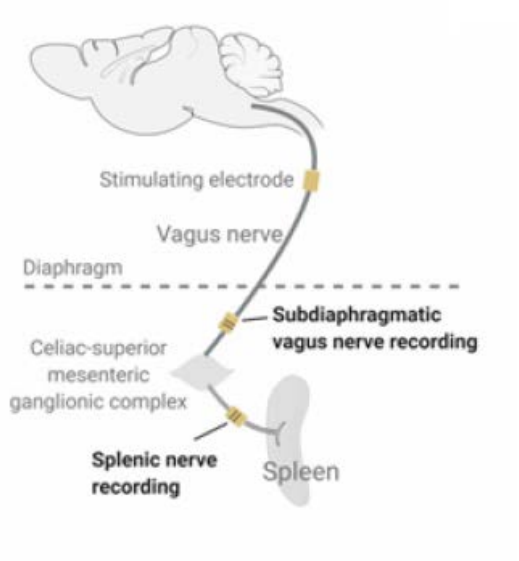
→ Selective labeling of splenic neurons with mCherry

- ChAT-eGFP transgenic mice were used to visualize Celiac-superior mesenteric ganglion complex

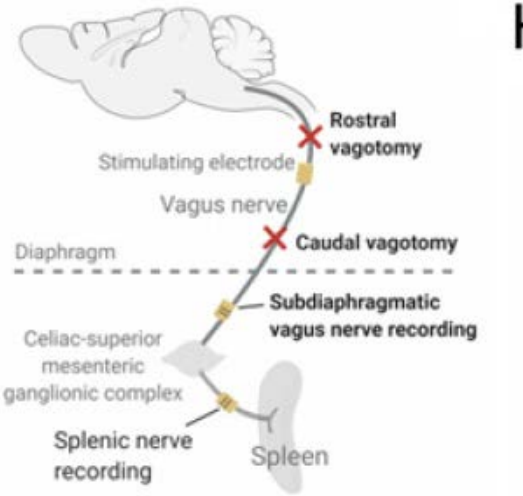
Experiment III

- A micro cuff recording electrode was implanted on the splenic nerve bundle of ChAT-ChR2-eYFP and non-ChR2 littermate control mice prior to optogenetic stimulation of the brainstem DMN cholinergic neurons.
- Firing frequency was recorded in the splenic nerve over a 2-min stimulation period, during optogentic DNM stimulation
- Bupivacaine (a sodium channel blocker) was used as a control (Blockage of voltage dependent sodium channels, should block Action potential generation).

Experiment IV



- Mapping out the peripheral pathway of the vagal Innervation of the spleen.
- Sprague-Dawley rats were anaesthetized and stimulating and recording electrodes positioned at different levels of the vagal and splenic nerve.
- In some experiments vagatomy and neuroectomy of the splenic nerve bundle were performed at different levels



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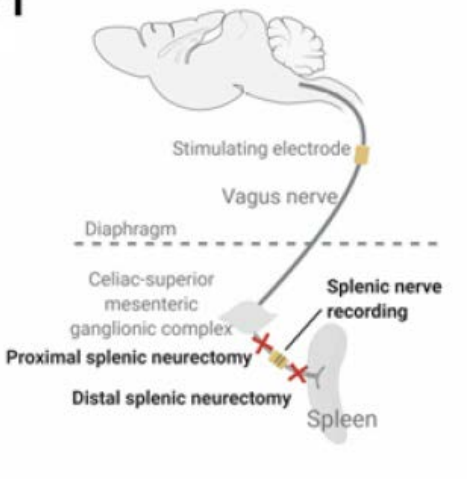
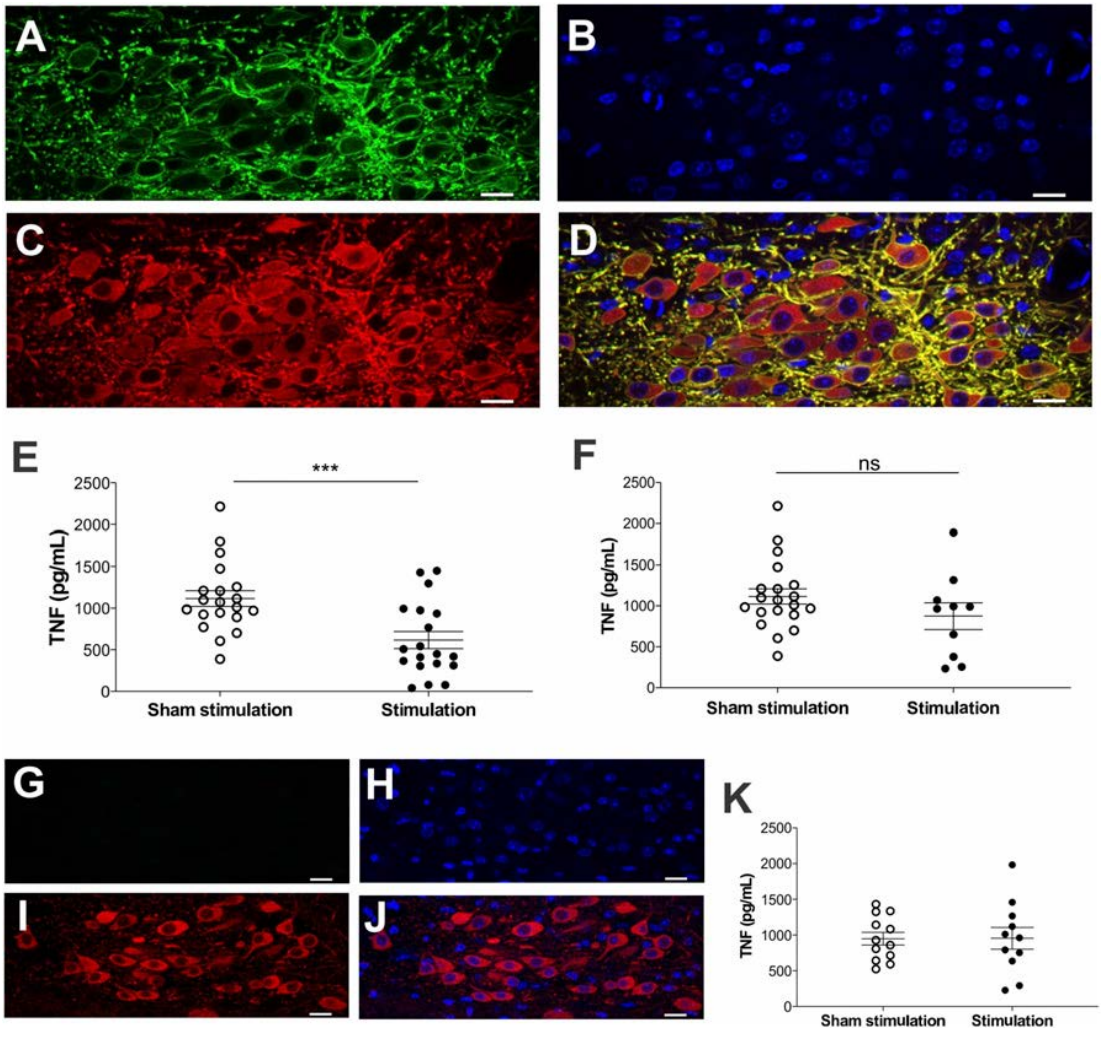


-> Additionally, needle EMG Electrodes were placed adjacent to the laryngeal muscles

-> In some experiments hexamethonium bromide was used to block ganglionar transmission

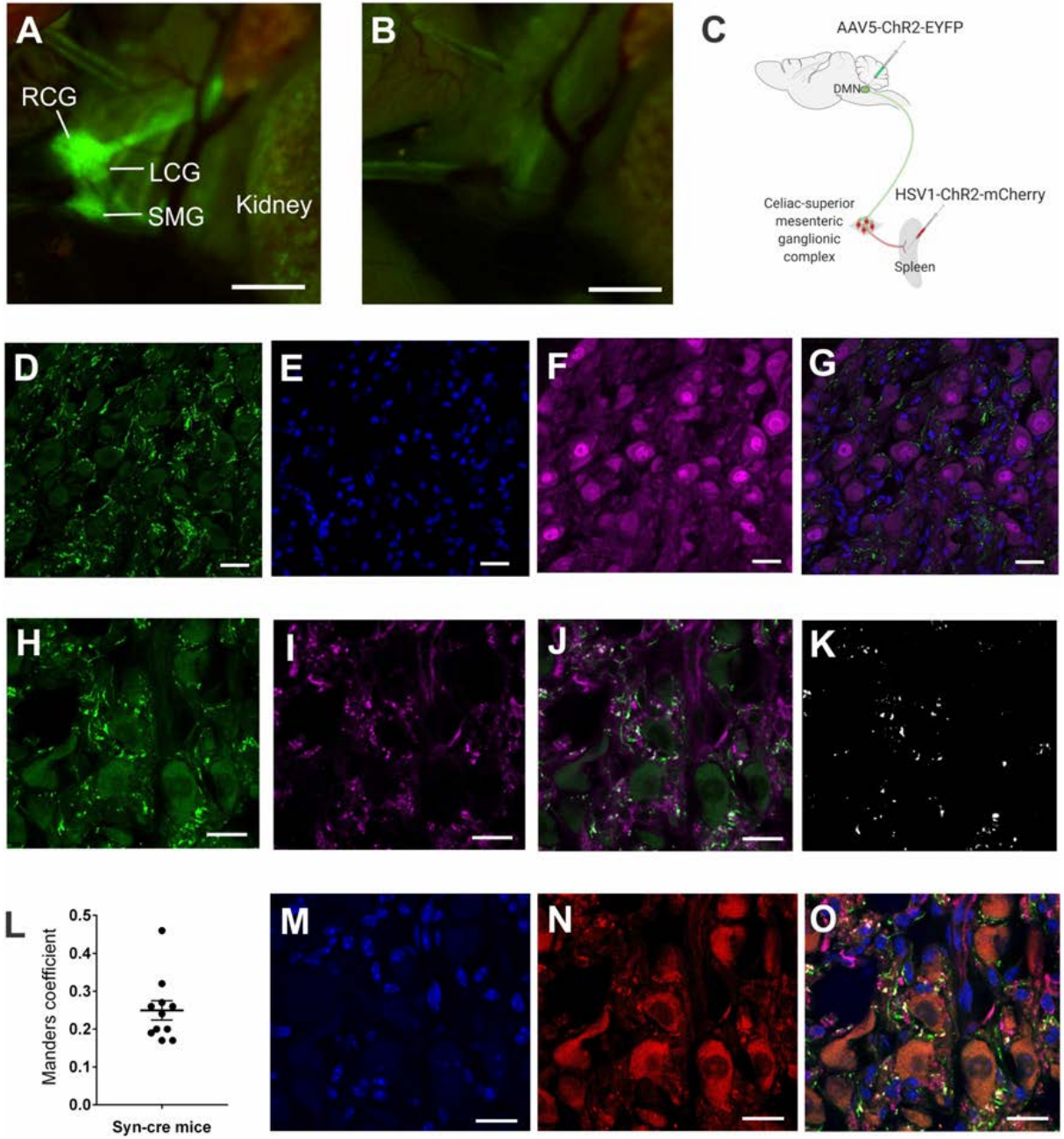
-> LPS was used to induce systemic inflammation

Selective activation of DMN cholinergic neurons inhibits TNF production during endotoxemia.



- (A) Anti-eYFP staining, (B) DAPI, (C) anti-ChAT staining, and (D) merged image of anti-eYFP, DAPI, and anti-ChAT staining
- Optogenetic stimulation with blue light in the DMN attenuated serum TNF in endotoxemic ChAT-ChR2-eYFP mice (E)
- Yellow light (593.5 nm) does not attenuate TNF-Alpha production (F)
- Optogentic stimulation of WT littemates (K) has not effect on TNF-Alpha Serum levels

DMN Cholinergic Fibers Terminate in the Celiac-Superior Mesenteric Ganglion Complex



A, B: celiac-superior mesenteric ganglion complex in ChAT-eGFP mice (A) and littermate controls (B)

D) anti-eYFP staining, (E) DAPI, (F) anti-NeuN staining, (G) merged image of anti-eYFP, anti-NeuN, and DAPI staining

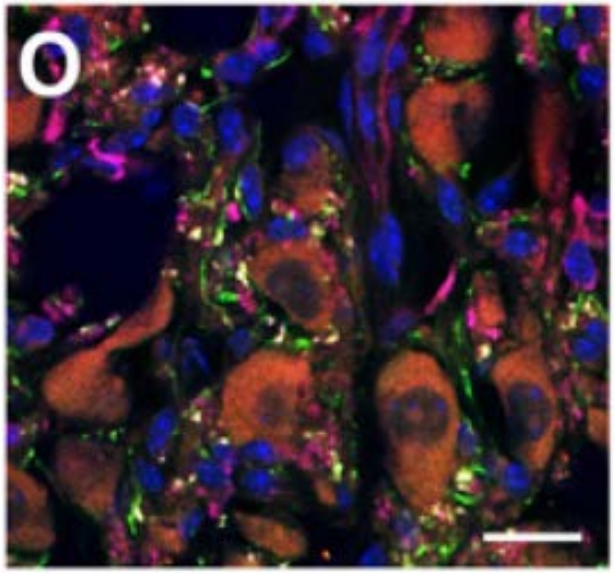
(H) Anti-eYFP staining, (I) anti-synaptophysin staining, (J) merged image of anti-eYFP, and anti-synaptophysin staining.

(K) Colocalization mask showing overlap regions of eYFP and synaptophysin labeling. (L) Mander's coefficient values for overlap proportion

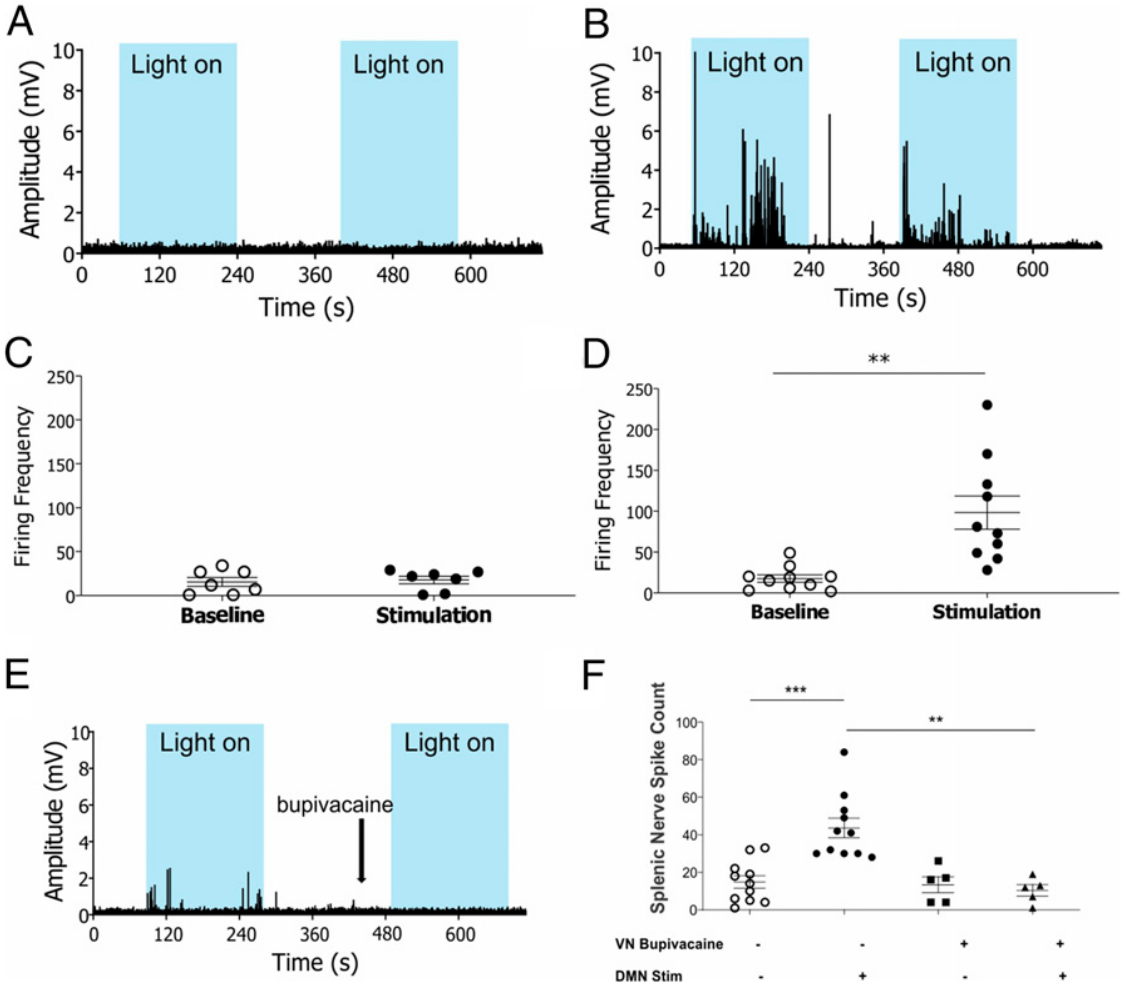
(M) DAPI, (N) mCherry, (O) merged image of anti-eYFP, anti-synaptophysin, mCherry and DAPI staining.

DMN Cholinergic Fibers Terminate in the Celiac-Superior Mesenteric Ganglion Complex

- More than 40% of synaptophysin+ eYFP-expressing efferent vagus nerve terminals were in close proximity (≤300 nm) to mCherry-expressing splenic nerve cell bodies in the celiac-superior mesenteric ganglia

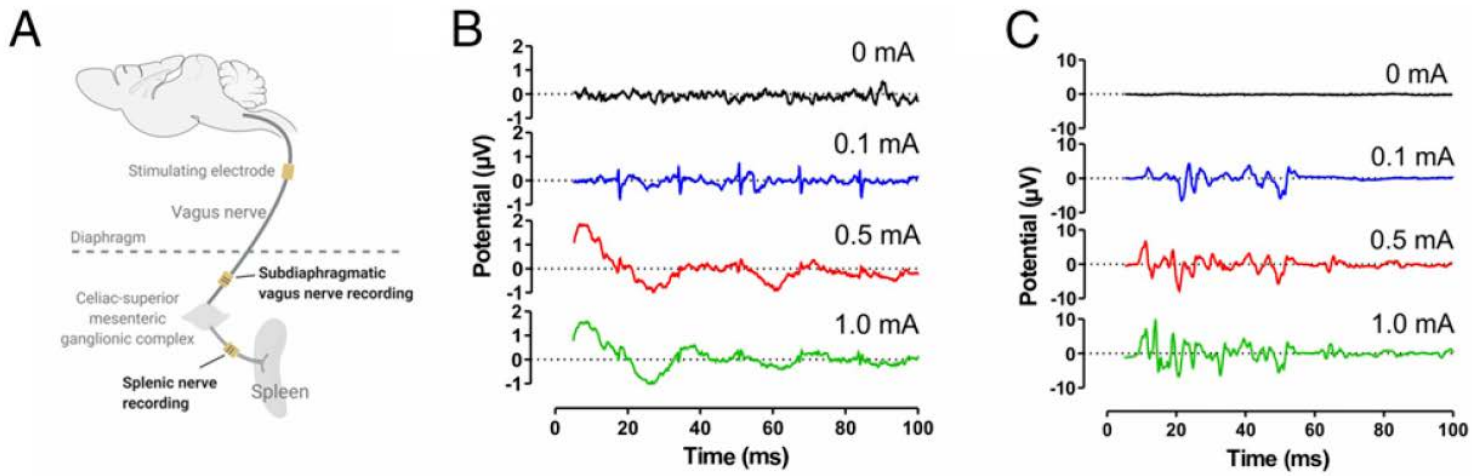


Optogenetic Stimulation of DMN Cholinergic Cell Bodies Induces Evoked Action Potentials in the Splenic Nerve.

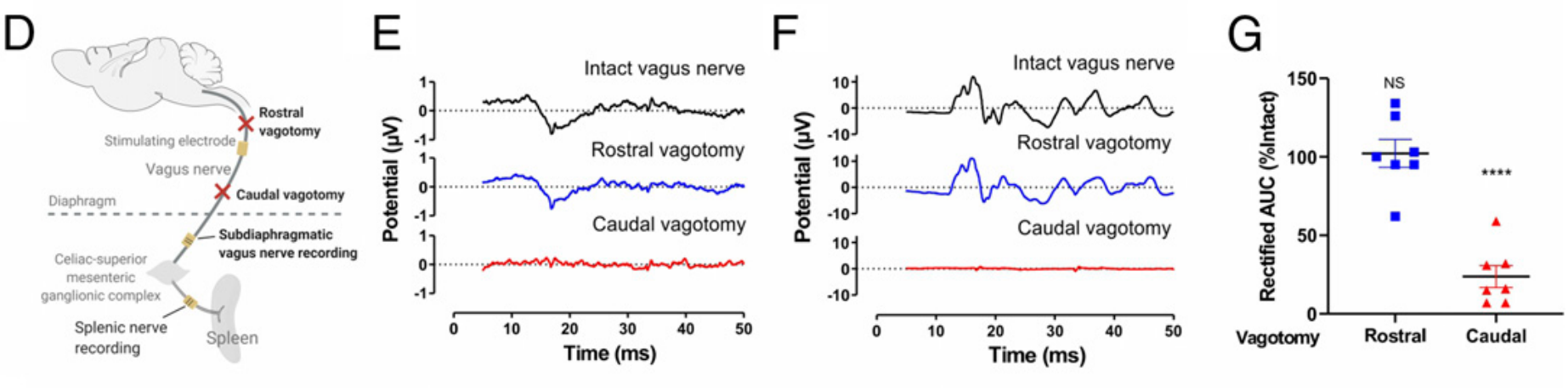


- (A, C) non-ChR2 littermate control (B, D) ChAT-ChR2-eYFP mice
- E and F Optogentically induced Action potentials in the splenic nerve are blocked by bupivacaine

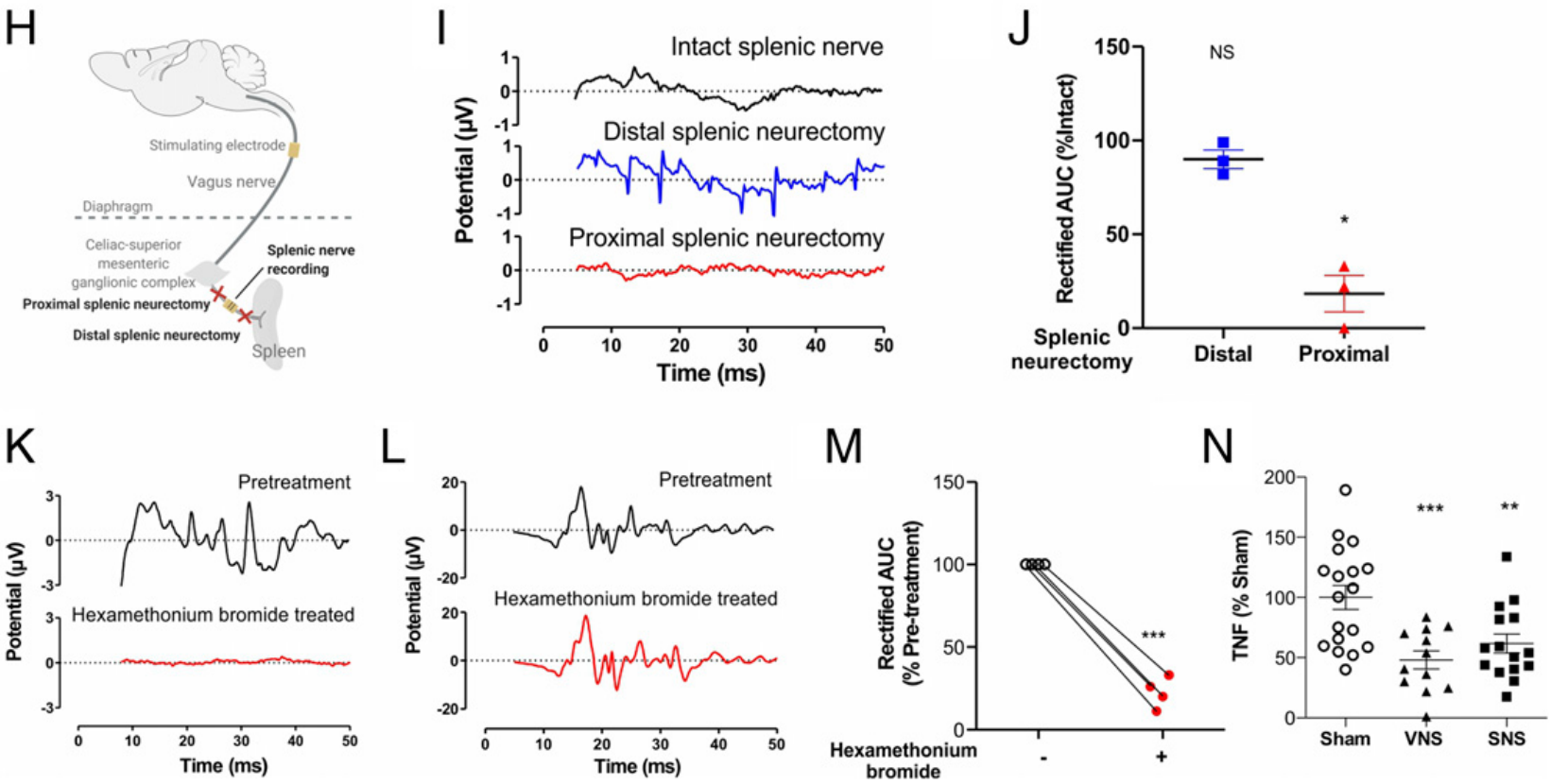
Electrical Stimulation of the Vagus Nerve Induces Efferent Signals to Evoke Action Potentials in the Splenic Nerve.



- Stimulation of ascending intensities (0.25 ms biphasic pulses of 0, 0.1, 0.5, 1 mA) was delivered to the cervical vagus nerve, and evoked compound action potentials were recorded on the splenic nerve and subdiaphragmatic vagus nerve. Representative traces of (B) splenic nerve, (C) subdiaphragmatic vagus nerve.



- Efferent signals transmitted in the cervical vagus nerve induce evoked action potentials in the splenic nerve.
- Caudal but not rostral vagotomy abrogates evoked action potentials in the splenic nerve.



- Splenic nerve activity in response to vagus nerve stimulation recorded after splenic neurectomy (proximal or distal to the splenic nerve recording electrode)
- Splenic neurectomy that was proximal but not distal to the splenic nerve recording electrode abrogates evoked action potentials in the splenic nerve.
- Blocking of cholinergic signaling with hexamethonium bromide (10 mg/kg) abrogates cervical vagus nerve-originating evoked potentials in the splenic nerve. Vagus nerve stimulation-induced evoked potentials were recorded in the (K) splenic nerve and (L) sub-diaphragmatic vagus nerve before injection and at 20 min post-injection.
- Splenic nerve stimulation attenuates LPS-induced serum TNF response

Discussion

- Selective activation of efferent cholinergic vagus nerve fibers originating in the DMN is sufficient to activate the inflammatory reflex and inhibit the production of TNF.
- Preganglionic efferent vagus nerve fibers originating in the brainstem DMN -> terminate in the celiac-superior mesenteric ganglia in close proximity to splenic nerve cell bodies, forming highly varicose synaptic-like structures around the principle ganglion cells in the celiac-superior mesenteric ganglia
- Nerve branches canonically disassociated as part of the sympathetic and parasympathetic division of the autonomous nervous system appear to synergistically mediate a neuroimmunological crosstalk.
- Translational perspective: Selective electrical stimulation of the vagal nerve or Brainstem TMS in the treatment of autoinflammatory diseases?

Literature

- If not otherwise indicated the figures were derived from:
- Kressel AM, Tsaava T, Levine YA, Chang EH, Addorisio ME, Chang Q, et al. Identification of a brainstem locus that inhibits tumor necrosis factor. Proceedings of the National Academy of Sciences (2020) 117(47):29803-10. doi: 10.1073/pnas.2008213117.

Additional References:

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