SUPLEMENTARY MATERIAL

In vivo studies: test for gastric emptying

Gastric emptying for solids was measured as previously reported. (Yamamoto *et al.*, 2008) After a 24-h fasting period (9:00 AM - 9:00 AM) with free access to water, mice had free access to pre-weighed Western diet for 1 h (9:00 AM - 10:00 AM). Food intake was calculated by weighing food left uneaten. Mice were returned to the cages without food and water, and sacrificed after 1.5 h. After clamping the esophagus at the cardia and pylorus, the stomach was exposed, and the content of the stomach was dried and weighed. Gastric emptying was calculated according to the following formula: gastric emptying (%) = [1 - (weight of food remaining in the stomach/weight of food intake)]/100.

Small intestinal transit

Small intestinal transit was determined by assessing the distribution of the 70 kDa FITC conjugated dextran maker (Sigma) throughout the gastrointestinal tract as previously described. (Aube, 2006) Briefly, mice were fasted overnight with free access to water. In the morning between 8:00 and 9:00 AM, each mouse was gavaged with 0.2 ml of 5 mg/ml FITC-dextran prepared in PBS. After 20 min postgavage, mice were sacrificed by cervical dislocation. The entire gastrointestinal tract was removed and divided into 10 equal segments of small intestine. The intestinal segments were opened, and the luminal contents were rinsed out into 2 ml of PBS and centrifuged at 1200 rpm for 5 min, and fluorescence activity of the supernatant was measured using a fluorimeter (Perkin-Elmer, Courtaboeuf, FR). For measurement of small intestinal transit, the geometric center of the fluorescence distribution present in the small intestine only was calculated as previously described. (Aube, 2006)

Ex vivo studies: gastric motility

Mice were killed by cervical dislocation. The whole stomach of each mouse was then quickly excised and placed in an ice-cold Krebs solution (NaCl, 117 mM; KCl, 4.7 mM; MgCl2, 1.2

mM; NaH2PO4, 1.2 mM; NaHCO3, 25 mM; CaCl2, 2.5 mM and glucose, 11 mM). Circular muscle strips (10–15 mm in length and 0.5 mm in width) were cut from distal antrum region. Muscle preparations were suspended vertically in an organ bath filled with Krebs solution warmed at 37 °C and gassed with 95% O2 + 5% CO2. After an equilibration period of 45-60 min at initial tension of 0.5 g, muscle strips were stimulated with two platinum rod electrodes. Three electrical field stimulation (EFS, train duration: 10 s; stimulation frequency: 20 Hz; pulse duration: 200 μs; pulse amplitude: 15 V) were applied at 10-min intervals.

EFS-induced responses in preparations from ND and WD mice were compared in basal condition, in the presence of the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME 500 μM; Sigma) and L-NAME + atropine (1 μM; Sigma). Atropine and L-NAME were applied 15 min before nerve stimulation. At the end of the experiment, after multiple washings, dose-response curves were generated by exposing the tissue to increasing concentrations of the muscarinic agonist carbachol (1 nM to 10 mM; Sigma). Lastly, the response of the antrum preparation to the nitric oxide (NO) donor sodium nitroprusside (SNP 10^{-4} M; Sigma) was measured. The effects of EFS and carbachol on tension were evaluated by measuring the area under the curve (AUC) using baseline as the 0 value. For EFS, AUC was measured during stimulation (10 s). For carbachol, AUC was measured for 2 minutes following the addition of a dose of carbachol respectively. All values were normalized to the wet weight of the tissue. Data are expressed in mN/ g of tissue x duration of measurement of the AUC. The contractile response of the circular muscle was continuously recorded, using isometric force transducers (Basile no 7005; Comerio, VA, IT), and a MacLAb/4s System (ADI, Spechbach, DE). All EFS-induced responses were of neuronal origin as they were abolished in the presence of tetrodotoxin (1 μM; Sigma) (data not shown). All tested substances were administered in a volume which did not exceed 1% of the total bath volume.

Calcium imaging of antral myenteric neurons

[Ca2+]i transients in antral myenteric neurons were investigated in 4 ND and 4 WD C57BL/6J mice (Charles Rivers, Sulzfeld, Germany). Stomachs were sampled and dissected to obtain preparations of the myenteric plexus (final size, 10 x 20 mm) that were continuously perfused with ice cold Krebs solution of the following composition; in mM: MgCl2: 1.2, CaCl2: 2.5, NaH2PO4: 1.2, NaCl: 120.9, NaHCO3: 14.4, Glucose: 11.5, KCl: 5.9, at pH 7.4, gassed with 95% O2, 5% CO2, ph 7.4) (all chemicals from Sigma-Aldrich, Taufkirchen, Germany).

Ultra fast neuroimaging technique was used to record intracellular calcium concentrations as described previously. (Vanden Berghe *et al.*, 2000) Tissue preparations were incubated for 120 min at room temperature with 30µM Fluo-4 AM (Invitrogen, Darmstadt, Germany) and 1.25 mM Probenecid (Sigma-Aldrich) in Krebs solution followed by a 60 min equilibration period with superfusion of Krebs solution at 37°C. Tissue chamber was mounted onto an inverted microscope IX50 (Olympus, Hamburg, Germany) equipped with appropriate filter blocks and a high-speed charge coupled device camera (CCD) with a spatial resolution of 80x80 pixels (NeuroCCD, Redshirt Imaging Decatur, GA, USA). The NeuroCCD SM system consists of a fast CCD camera and the Neuroplex 8.3 software for experiment control and data analysis. The fluorescence filterset for the Fluo-4 AM used was a FITC filterset (excitation: HC482/35, dichroic: BS506, emission: HC536/40, AHF Analysentechnik). The light source was a Luxeon LXHL-LB3C blue LED (operated at constant current 700 mA, Philips Lumileds Lighting Company, San Jose, CA, USA) and light illumination time was controlled by the Neuroplex software. Acquisitions of 10 s duration were done at 40 Hz frame rate (x40 objective, UAPO 340, NA 1.35, oil immersion, Olympus).

Electrical stimulation of interganglionic nerve strands was performed with a bipolar electrode (uncoated diameter: 51 µm, Science Products, Hofheim, Germany). Train pulses stimulations (500 µA, 10 Hz, 0.6 ms pulse duration) were done with a Universal Stimulus Isolator Model

501 (University of Cologne, Department of Zoology, Köln, Germany). Data were displayed as % changes of resting fluorescence (ΔF/F).

Immunohistochemistry was used to characterize whether responding cells were neurons or glia cells. After the neuroimaging experiments, tissues were fixed overnight in the fridge in phosphate buffered 4% paraformaldehyde and 0.2% picric acid, then washed (3 x 10 minutes) in phosphate buffered and incubated for 1 hour at room temperature in phosphate-buffered saline containing 0.5% Triton X-100 and 4% horse serum to block nonspecific binding. Tissues were then incubated for 16 hours overnight at room temperature with the primary antibodies sheep anti-PGP 9.5 (1:10000, PH164, The Binding Site, Birmingham, United Kingdom) and rabbit anti-S100 (1:20000, Z0311, Dako, Glostrup, Denmark). This was followed by incubation for 1.5 hours at room temperature with species specific secondary antibodies (Cy5-conjugated donkey anti-sheep, 1:500, 713-175-147 and Cy3-conjugated donkey anti-rabbit, 1:500, 711-165-152, Dianova, Hamburg, Germany). The fluorescence was detected by using an Olympus microscope (BX61 WI; Olympus, Hamburg, Germany) equipped with appropriate filter blocks, a Fview II CCD camera and software (Cell^P, Olympus) as previously described. (Schemann *et al.*, 2010)

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Schemann M, Hafsi N, Michel K, Kober OI, Wollmann J, Li Q, Zeller F, Langer R, Lee K & Cellek S (2010). The beta3-adrenoceptor agonist GW427353 (Solabegron) decreases excitability of human enteric neurons via release of somatostatin. *Gastroenterology* **138,** 266–274.

Vanden Berghe P, Tack J, Coulie B, Andrioli A, Bellon E & Janssens J (2000). Synaptic transmission induces transient Ca2+ concentration changes in cultured myenteric neurones. *Neurogastroenterol Motil* **12,** 117–124.

Yamamoto T, Watabe K, Nakahara M, Ogiyama H, Kiyohara T, Tsutsui S, Tamura S, Shinomura Y & Hayashi N (2008). Disturbed gastrointestinal motility and decreased interstitial cells of Cajal in diabetic db/db mice. *J Gastroenterol Hepatol* **23,** 660–667.

Supplementary Table 1. Characteristics of primary and secondary antibodies.

Primary Antibodies

Antigen	Host species	Dilution	Sources
Active caspase-3	Rabbit	1:2000	Sigma; C8487
ChAT	Goat	1:200	Millipore; AB144P
GDNF	Rabbit	1:100	Santa Cruz Biotechnology; (D-20) sc-328
GFAP	Rat	1:200	Calbiochem; 345860-100UG
GFR-alpha 1	Goat	1:50	Santa Cruz Biotechnology; (C-20) sc-6157
Hu C/D (tissues)	Mouse-biotin	1:50	Invitrogen; A21272
Hu C/D (cultures of ENS)	Mouse	1:200	Invitrogen; A21275
nNOS	Rabbit	1:1000	Enzo Life Sciences; ALX-210-501
Ob-R	Goat	1:50	Santa Cruz Biotechnology; (M-18) sc-1834
PGP 9.5	Rabbit	1:10000	UltraClone Limited; RA95101
Ret	Goat	1:50	R&D Systems; AF482
Sox-10	Goat	1:500	Santa Cruz Biotechnology; (N-20) sc-1732

Secondary Antibodies

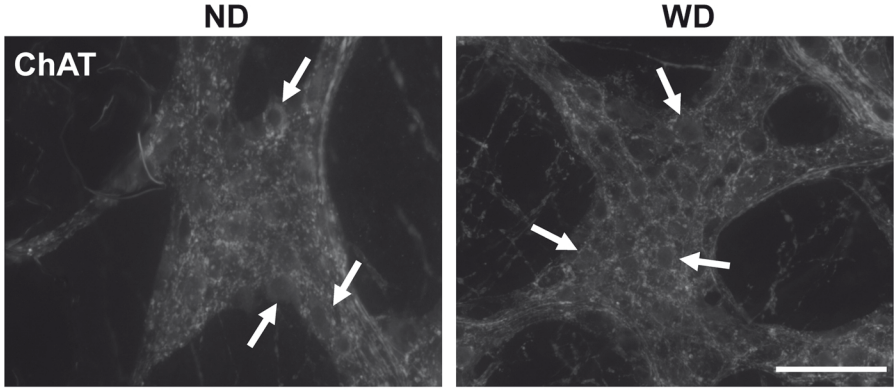
Antibody	Dilution	Sources
Cy3-conjugated donkey Anti-goat	1:500	Jackson ImmunoResearch; 705-165-003
Cy3-conjugated donkey Anti-rabbit	1:500	Jackson ImmunoResearch; 711-165-152
Cy5-conjugated donkey Anti-rat	1:500	Jackson ImmunoResearch; 712-175-150
FluoProbes 488 donkey Anti-mouse	1:200	Interchim innovations; FP-SA4110
FluoProbes 488 donkey Anti-rabbit	1:200	Interchim innovations; FP-SA5110
Streptavidin Alexa Fluor 488	1:200	Invitrogen; s11223

SUPPLEMENTARY FIGURES LEGENDS

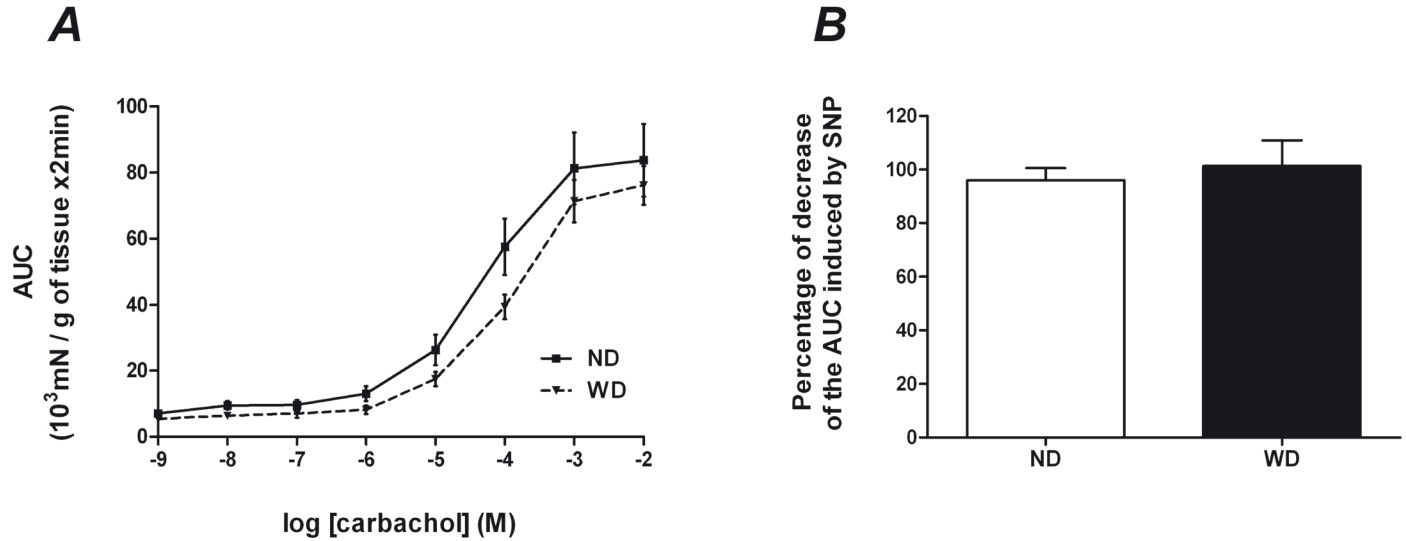
Supplementary Figure 1. ChAT-immunoreactive labeling of antral myenteric plexus in mice after 12 weeks of ND and WD. The faint staining of ChAT-IR neurons and the network of ChAT-IR fibres overlaying the ganglia prevented the precise evaluation oh the number of ChAT-IR neurons per ganglia. Scale bar=50µm.

Supplementary Figure 2. Impact of diet-induced obesity upon circular muscle response to NO donor and carbachol. (A) Quantitative analysis of the area under the curve (AUC) of the contractile response induced by increasing concentrations of carbachol in ND and WD mice (n = 25). (B) Amplitude of the AUC induced by sodium nitroprusside (SNP) and normalized to the AUC induced by carbachol (10^{-2} M) (n = 7-10).

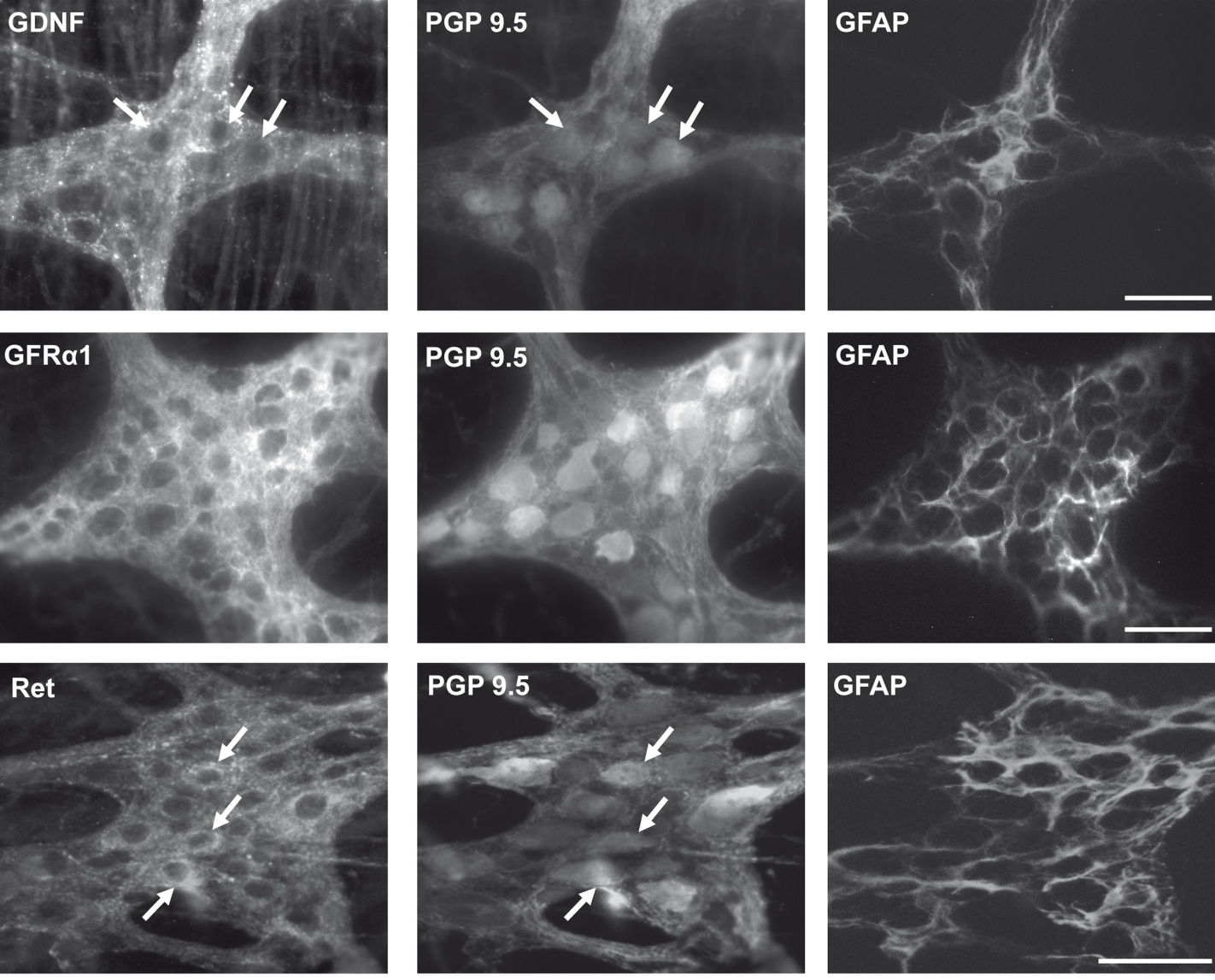
Supplementary Figure 3. Immunohistochemical labeling of antral myenteric plexus with anti-GDNF, anti-GFRα1, anti-Ret, anti-PGP 9.5 and anti-GFAP antibodies. GDNF-IR and Ret-IR cells coexpressed PGP 9.5 (arrows) while GFRα1-IR cells coexpressed PGP 9.5 and GFAP. Scale bar=50µm.



Supplementary Figure 1.



Supplementary Figure 2.



Supplementary Figure 3.