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1 The thorax of adult mice of either sex were removed under deep Isoforane anesthesia according to approved protocols by the Institutional Animal Care and Use Committee at Oregon Health and Science University, and guidelines of the National Institutes of Health. The heart and SG were perfused with modified Ringers that also served as the external recording solution containing: 146mM NaCl, 4.7mM KCl, 0.13mM NaH_2PO_4, 0.6mM $MgSO_4$, 1.6mM $NaHCO_3$, 2.0 mM $CaCl_2$, 7.8mM D-glucose, 20mM Hepes, adjusted to pH 7.31 with NaOH,

adjusted to 300mOsm.

2 Right and left stellate ganglia, Rostral Ansa, and iCN were identified, traced to the heart and separated from surrounding tissue using a high magnification (250x-400x) stereo microscope. Ganglia with at least 3mm of outflow nerves were carefully transferred to a custom recording chamber and treated with 0.7 mg /ml Collagenase Type 2, (Sigma Aldrich) and 0.3mg/ml Collagenase Type 1 (Worthington Biochemical) dissolved into oxygenated Leibowitz, supplemented culture media, L-15 (Thermo-Fisher Scientific).

3 The ganglia, RA/iCN were positioned in the recording chamber for optimal placement of recording and stimulating electrodes and gently weighted down with a custom Teflon coated platinum harp with non-fluorescent angled strands to prevent crushing the minutia nerves.A gentle stream of 100% O2 was directed over the chamber to gently circulate the enzyme solution and loosen connective tissue. After approximately one hour in the enzyme solution, any remaining connective tissue that adheres to the patch pipettes was gently teased away from the ganglia with extra fine ceramic Dumont #55 forceps or gently suctioned away using a custom glass Furst Baron Suction Tube with 0.3mm inner diameter. Care was taken to prevent touching or damaging the nerves

4 After the ganglia was clear of loose tissue, the recording chamber was transferred to a Zeiss Axioskop 2, FS Mot microscope with 40X water immersion objective, epifluorescence and Differential Interference Contrast (DIC) optics.Images were captured using a SPOT Pursuit Camera with custom imaging software (Spot Imaging Solutions).

5 Two ultra-fine platinum Iridium, concentric bipolar stimulating electrodes (Fredriech Heare) with a 25 µM inner pole and a 50 µm outer pole diameter were positioned on isolated nerve trunks as far as possible from the ganglia

6 Minimal stimulation was used to recruit and isolate single synaptic inputs. Constant current Square wave stimuli ranged from 10- 1500 µA with pulse durations ranging from 40 to 100uSec. Stimulus train inter-pulse intervals ranged from 100 mSec, to 6 Sec using a Master 9 programmable stimulator and Iso-Flex optical isolation unit (AMPI industries, Jerusalem, Israel)

7 Patch Pipettes for Intracellular voltage and current clamp recordings were pulled to a final tip resistance of 2.5-4.0 M.Ohms using 5251 borosilicate glass (WPI) and filled with internal solution containing: 94mM K-gluconate, 30mM KCl, 10mM Phosphocreatine, 10mM Hepes, 0.2mM EGTA, 4mM MgATP, 0.3mM Na_2GTP, osmolarity adjusted to 290mM with D-glucose.

pH was adjusted to 7.31 using a Tris buffer, and brought to a final concentration of 290mOsm using D-Glucose. Voltage and current clamp recordings were sampled at 20 kHz with a Multiclamp 700B amplifier, and pClamp 9.2 software (Axon Instruments, Union City, CA). Junction potentials were calculated using pClamp algorithms and applied where appropriate. Pipette Capacitance was calculated and corrected before, and after each recording. Series resistance compensation was applied during voltage clamp protocols where membrane steps were required

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8 Labeled neurons were identified for recording using epifluorescent optics and patch pipettes were maneuvered to selected neurons under direct DIC visualization.Positive pressure was kept on the pipette to gently push through, and inflate the glial cell covering each neuron to provide a clear surface on the neuronal membrane inside the glia to establish a 1.2- 3.5giga-ohm seal

9 In some neurons, Neurobiotin 5 mg/ ml (Vector Laboratories) was added to the internal solution for measurements of axonal and dendrite anatomy using confocal microscopy. In these instances, the internal solution D-Glucose concentration was corrected such that the final osmolarity was 290 mOSM with a small volume vapor pressure osmometer (Wescor).

10 When ganglia were to be recovered for confocal analysis, after recording was complete, the bath temperature was cooled from 32 degrees to 26 degrees for 10 minutes and very slight positive pressure was applied to the pipette to separate it from the neurolemma without tearing it.

11 Reference photographs of the cell were taken 5 minutes after the recording electrode was removed to verify that the soma remained intact, and to establish the position of the cell in the ganglia. The recording chamber was then transferred back under the surgical microscope to carefully remove the retaining harp and to transfer the ganglia with attached nerves into to 4% Paraformaldehyde (PFA) as quickly as possible. The ganglia was then flash fixed, using a 2450 MHz, 600 KiloWatt microwave irradiation for 5 seconds, rinsed in 9% Phosphate Buffered Saline, and stored in high concentration sucrose storage solution at -20 degrees.

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