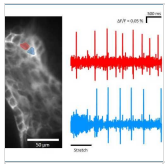

Apr 06, 2021

Mechanosensitive enteric neurons: incidence and abundance in the porcine submucosal plexus with ultrafast neuroimaging and immunhistochemical techniques

Forked from Mechanosensitive enteric neurons: incidence and abundance in the porcine submucosal plexus with ultrafast neuroimaging and immunhistochemical techniques

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1 Works for me dx.doi.org/10.17504/protocols.io.btv3nn8n

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ABSTRACT

This protocol is to investigate the sensitivity of enteric neurons to mechanical stimulation in the inner submucosal plexus of the porcine colon using ultrafast neuroimaging and immunhistochemical techniques. Ultrafast neuroimaging technique combined with a voltage sensitive dye was used to characterize neuronal responses to mechanical stimuli compression and tension. Immunohistochemistry was subsequently used to determine the neurochemical coding of mechanosensitive submucosal neurons.

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FORK NOTE

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KEYWORDS

enteric nervous system, mechanosensitivity, ultrafast neuroimaging technique, voltage sensitive dye, immunohistochemistry

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MATERIALS TEXT

- Samples of porcine colon
- Krebs solution for preparation containing in (mM): 117 NaCl, 11 Glucose, 4.7 KCl, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 2.5 $CaCl_2$; Carl Roth GmbH & Co. KG (Karlsruhe, Germany)
- Krebs solution for experiment containing in (mM): 117 NaCl, 11 Glucose, 4.7 KCl, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 20 $NaHCO_3$, 2.5 $CaCl_2$; Carl Roth GmbH & Co. KG (Karlsruhe, Germany)
- Di-8-ANEPPS; Thermo Fisher Scientific Inc. (Waltham, MA, USA)
- 0.0135 % Pluronic® F-127; Thermo-Fisher Scientific Inc. (Waltham, MA, USA)
- 0.135 % Dimethyl sulfoxide (DMSO); Acros Organics (Geel, Belgium)
- 100 µM nicotine (GL9693); Glentham Life Sciences (Corsham, Great Britain)
- Sylgard® 184; World Precision Instruments (Sarasota, FL, USA)
- Micropipettes: glass capillaries (GB100 F-10); Science Products GmbH (Hofheim, Germany) formed into micropipettes by a micropipette puller (P-1000); Sutter Instrument Company (Novato, CA, USA)
- Inverted microscope (Olympus Corporation, Hamburg Germany)
- DaVinci 1K CMOS camera; Redshirt Imaging LLC (Decatur, GA, USA)
- Turbo SM 64 Software; Redshirt Imaging LLC (Decatur, GA, USA)
- 4% paraformaldehyde; Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany)
- 0.002% picric acid; Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany)
- 0.1 mol/l phosphate buffer; Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany)
- phosphate-buffered saline (PBS): H_2O, Sodium Phosphate Monobasic, Sodium Phosphate Dibasic, NaCl; Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany)
- 0.1% NaN_3; Carl Roth GmbH & Co. KG (Karlsruhe, Germany)
- 4% horse serum; Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany)
- Triton X-100; Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany)
- Object slides; Gerhard Menzel B. V. & Co. KG (Braunschweig, Germany)
- Glycerol; Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany)
- Cover slips; Omnilab-Laborzentrum GmbH & Co. KG (Gehrden, Germany)
- All antibodies from table 1 and table 2
- Epifluorescence microscope; Olympus Corporation (Hamburg, Germany)
- Olympus cell Sens Standard Software, Olympus Corporation (Hamburg, Germany)

A	B	C	D
Primary Antibodies	**Host**	**Dilution**	**Supplier**
ChAT	rabbit	1:1000	Prof. Dr. Schemann
SP	rat	1:1000	Fitzgerald Industries International order code: 10-S15
NOS	mouse	1:1000	Santa Cruz Biotechnology, Inc. order code: sc-3502
anti-Hu C/D Biotin	mouse	1:50	Thermo Fischer Scientific, Inc.

Table 1: List of primary antibodies

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A	B	C	D
Secondary Antibodies	**Host**	**Dilution**	**Supplier**
donkey anti rabbit Cy5	donkey	1:500	Jackson ImmunoResearch Laboratories, Inc.
donkey anti rat Cy2	donkey	1:200	Jackson ImmunoResearch Laboratories, Inc.
donkey anti mouse Cy3	donkey	1:500	Jackson ImmunoResearch Laboratories, Inc.
Streptavidin Cy5		1:500	Jackson ImmunoResearch Laboratories, Inc.

1 Samples of colon were taken from apparently healthy pigs, placed in ice-cold oxygenated Krebs solution for preparation and immediately transferred to the laboratory. Tissues were then dissected in the ice-cold oxygenated Krebs solution for preparation to obtain whole-mount inner submucosal plexus preparations.

2 Tissue samples were pinned on a ring made of Sylgard®184 and transferred into a recording chamber where they were constantly perfused with 37°C oxygenated experimental Krebs solution.

3 The voltage sensitive dye (VSD) Di-8-ANEPPS was dissolved in 1.5 ml experimental Krebs solution to which 0.135% DMSO and 0.0135% Pluronic® F-127 were added, so that it was used at a concentration of 20 µM.

4 In order to stain selected ganglia, a micropipette was filled with Di-8-ANEPPS and then positioned on the surface of the ganglion. The ganglion sheath was scratched with the tip of the micropipette and the VSD was applied into the ganglion at moderate pressure (≤ 0.5 bar).

5 The viability of ganglia was proven by filling a micropipette with nicotine and then applying nicotine on the surface of a ganglion at a pressure of 0.5 bar for 500 ms.

6 Mechanical stimulation included compression and stretch. Compression of submucosal neurons was induced by filling a micropipette with experimental Krebs solution and then applying it into a ganglion at a pressure of 0.5 bar for 500 ms. Stretch of entire ganglia was performed bidirectionally by moving a self-constructed stretching tool in opposite directions perpendicular to the ganglion axis at a speed of 15 µm/s.

7 The preparations were examined with an inverted microscope equipped with an appropriate filter set. Pictures were acquired with a CMOS camera (DaVinci 1 K) connected to a computer and controlled by Turbo SM 64 Software.

8 Tissue specimens were fixed overnight in a solution containing 4% paraformaldehyde and 0.002% picric acid in 0.1 mol/l phosphate buffer and then washed (3 x 10 min) in PBS.

9 The preparations were then incubated in PBS/NaN_3/horse serum for 1h at room temperature followed by 12h and 2h incubation with the primary and secondary antibodies, respectively.

10 Specimens were washed in PBS, mounted on object slides and cover slipped with a solution of PBS (pH 7.0)/NaN3 containing 65% glycerol.

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11 The preparations were examined with an epifluorescence microscope equipped with appropriate filter blocks. Pictures were acquired with a camera connected to a computer and controlled by Olympus cellSens Standard Software.

12 For the ganglia examined the following applied: based on their localisation in the fixed tissue preparation and their specific morphology, they could be clearly identified.

13 Mechanosensitive neurons previously determined by ultrafast neuroimaging technique and subsequently stained with the antibodies could be clearly identified based on their localization in the fixed tissue preparation and their specific morphology. Pictures of the stained ganglia were acquired.

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