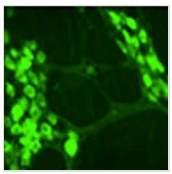

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Imaging of Calcium Dynamics in Vasoactive Intestinal Peptide-expressing Neurons of Enteric Nervous System

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SPARC
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The protocol was published on behalf of the investigators by the SPARC project team.

The University of Toledo Health Science Campus animal care and use committee approved animal care, breeding procedures, and experimental protocols. Animals were housed in an Association of Laboratory animal Care-approved facility with a 12-hour light cycle with food (standard chow) and water ad libitum.

One male VIP-GCAmP reporter mouse, age 4 months, was used in this study. The mouse was purchased from the Jackson Laboratories, and all genotyping was performed using primers recommended by the Jackson Laboratories according to their protocols.

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protocol

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Mouse strains:

A	B	C	D	E	F
Formal strain name	**Commonly used name**	**Mouse genome informatics ID**	**Jax Lab stock number**	**RRID**	**Strain background**
Vip-tm1(cre)Zjh-J	Vip-IRES-cre	4431361	031628	IMSR_JAX:031628	C57BL/6J
B6J.Cg-Gt(ROSA)26Sortm95.1(CAGGCaMP6f)Hze/MwarJ	Ai95(RCL-GCaMP6f)-D (C57BL/6J) or Ai95D (C57BL/6J)	5558090	028865	IMSR_JAX:028865	C57BL/6J

Mice that contained a floxed GCaMP6f construct within the Gt(ROSA)26 locus were bred with mice that expressed Cre recombinase under the control of the Vip promoter.

Artificial cerebrospinal fluid:

NaCl - catalogue #: S9888, Sigma, St. Louis, MO
KCL - catalogue #: P3911, Sigma, St. Louis, MO
$NaHCO_3$ - catalogue #: S6014, Sigma, St. Louis, MO
NaH_2PO_4 - catalogue #: S8282, Sigma, St. Louis, MO
$MgSO_4 \cdot 7H_2O$ - catalogue #: 230391, Sigma, St. Louis, MO
$CaCl_2$ - catalogue #: C1016, Sigma, St. Louis, MO
D-glucose - catalogue #: G5767, Sigma, St. Louis, MO
sodium butyrate - catalogue #: B5887, Sigma, St. Louis, MO
sodium acetate - catalogue #: S2889, Sigma, St. Louis, MO

Equipment:

Picospritzer II, Parker Instrumentation Corp, Barnstaple, UK
DM6000 FS Leica fluorescence microscope - Leica, Buffalo Grove, IN
Prime 95B CMOS camera - Teledyne Photometrics, Tuscon, AZ
Metamorph software - Molecular Devices, Downington, PA

1 On the day of the experiment prepare solutions of:

1. *Artificial cerebrospinal fluid (CSF)* containing:

- 117.9 mmol/L NaCl
- 4.7 mmol/L KCL
- 25 mmol/L $NaHCO_3$
- 1.3 mmol/L NaH_2PO_4
- 1.2 mmol/L $MgSO_4 \cdot 7H_2O$
- 2.5 mmol/L $CaCl_2$
- 11.1 mmol/L D-glucose
- 2 mmol/L sodium butyrate
- 20 mmol/L sodium acetate

2. *Artificial CSF with test reagents*, e.g., agonists.

2 Euthanize the mouse.

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3 Remove the colon.

4 Open the excised proximal colon segment along the mesenteric border.

5 Pinn the colon segment mucosal side down onto a Sylgard surface lining a glass coverslip attached to the bottom of a plastic imaging chamber containing artificial CSF perfused and bubbled with Carbogen (95% O_2/5% CO_2) to oxygenate and achieve pH **7.4** .

In experiments that involve recording neural activity leading up to spontaneous Colonic Motor Complexes (CMCs) heat the artificial CSF to **35 °C** .

6 Detect GCaMP-positive neurons by their basal Ca^{2+} fluorescence.

To do this we used an upright DM6000 FS Leica fluorescence microscope (Leica, Buffalo Grove, IL) and Prime 95B Scientific CMOS camera (Photometrics, Tucson, AZ).

Images were collected with Metamorph software (Molecular Devices, Downington, PA) at a 40-Hz sampling rate and 25-ms exposure time.

7 Record spontaneous and evoked changes in GCaMP6f-mediated Ca^{2+} fluorescence intensity.

We acquired 12-bit images using a 1.44-megapixel CMOS camera capable of capturing at 40 frames/sec (Prime 95B; Teledyne Photometrics, Tuscon, AZ) controlled by Meta-Morph software (version 7.10.1.161; Molecular Devices, Silicon Valley, CA).

8 At second 20 focally deliver an agonist by pressure microperfusion (10psi,10 sec; via Picospritzer II; Parker Instrumentation Corp, Barnstaple, UK) from blunt glass micropipettes (diameter, 5–10 mm) delivered within 50 µm of an adjacent myenteric ganglion.

Here we used dimethylphenyl-piperazinum (DMPP) [M] **10 micromolar (µM)** .

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