Electrical and Mechanosensitive Activity of the Mouse Fetal Enteric Nervous System

Richard Amedzrovi Agbesi, Nick Spencer, Tim Hebberd, Nicolas R. Chevalier

▶ **To cite this version:**

Richard Amedzrovi Agbesi, Nick Spencer, Tim Hebberd, Nicolas R. Chevalier. Electrical and Mechanosensitive Activity of the Mouse Fetal Enteric Nervous System. The Journal of Physiology, In press. hal-04287080

HAL Id: hal-04287080
https://hal.science/hal-04287080

Submitted on 15 Nov 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License

Electrical and Mechanosensitive Activity of the Mouse Fetal Enteric Nervous System

Richard J. Amedzrovi Agbesi,* Nick Spencer,† Tim Hibberd,‡ and Nicolas R. Chevalier§
Laboratoire Matière et Systèmes Complexes UMR 7057,
Université Paris Cité / CNRS and College of Medicine and Public Health,
Flinders University(Adelaide, South Australia)

(Dated: August 14, 2023)

Gut motility undergoes a switch from myogenic to neurogenic control in late embryonic development. Here, we report on the first electrical events underlying neurogenic motility in the developing mouse, using a transgenic line expressing the GCamp6f reporter in neural crest cell derivatives. We find that both nitrergic inhibitory and cholinergic excitatory neurons exhibit spontaneous calcium activity and that this activity requires L-type calcium channels and intracellular store-operated calcium channels (IP3R). This spontaneous electrical activity translates in terms of motility in periodic, high-frequency contraction complexes, which are inhibited by tetrodotoxin. We demonstrate that the ENS is mechanosensitive from the earliest stages on (E14.5) and that this behavior is tetrodotoxin and 2-APB resistant, but requires synaptic transmission. We extend our result on spontaneous L-type channel-dependent spontaneous activity and TTX-resistant mechanosensitivity to the adult colon. Our results shed light on the critical transition from myogenic to neurogenic motility in the developing gut.

I. INTRODUCTION

The gastrointestinal tract is a tube with the complex task of propelling content along its length to allow sequential flow and facilitate the mixing and transport of nutrients to the gut wall for absorption[1]. These functions are made possible by the coordinated action of smooth muscle, interstitial cells of Cajal, and the enteric nervous system(ENS)[2–5]. The ENS is an extensive network of neurons and glial cells connected to extrinsic autonomic and primary afferent innervation[6]. The ENS arises from the neural crest cells, mostly from the vagal region of the neural tube[7]. During embryonic development in mice, neural crest-derived cells migrate into the developing foregut around E9.5 and populate it in a wave-like pattern[8, 9]. The ENS performs its function by responding to mechanical and chemical cues[10–12]. Myenteric neurons, whether isolated or in situ, can transduce the mechanical forces that act on the gut wall into action potential firing, including changes in length (strain), tension (stress), and compression[13, 14]. In the colon of mature mice, $KCa_{1.1}$ and transient receptor potential(TRP) have been demonstrated to be mechanosensitive channels[15]. The embryonic mouse has been found to respond to electrical field stimulation and this response was mediated by L, N, P/Q, and R calcium channels[16]. Pharmacological studies have revealed that the main intestinal motility patterns in mature animals - segmentation, peristalsis, and migrating motor complex all depend on neuronal activity[17, 18]. In the mouse embryo, the first motor activity at E13.5 is purely myogenic and takes the form of spontaneous circular muscle contractile waves that propagate along the intestine. The ENS is present in the whole gut from E14.5 but remains in a state of functional quiescence until E18.5 when the first neurogenic motility patterns can be detected[19, 20]. The developmental transition from myogenic to neurogenic patterns yields great insight into the basic mechanisms by which the ENS affects motility. In the chicken embryo, our group has found that the ENS gives rise to a descending, inhibitory, mechanosensitive coupling of the circular and longitudinal muscle layers. This antagonism gives rise to a new motor pattern, the migrating motor complex(MMC). The MMC has the same frequency as longitudinal smooth muscle contractions, which act as a trigger for this motor pattern[21]. The polar, descending character of neural inhibition gives rise to the stereotyped "ascending contraction-descending inhibition" response to mechanical stimulation, known as "the law of the intestine"[22]. We wondered to what extent the results we obtained in chicken applied to a mammalian model, the mouse. We take advantage here of a mouse model expressing the GCamp fluorescent intracellular calcium reporter in neural crest-derived cells. We study the development of ENS calcium activity from E18.5-P3, which to the best of our knowledge has not been examined so far. We were in particular keen on preserving an intact muscular activity (imaging without muscle inhibitors like nicardipine) to observe the coupling of contractions with neuronal activity. The embryonic gut is small, enabling the study of relatively expansive ENS areas and network-wide effects that may not be evident from visualizing only a few ganglia, as is typical of adult gut studies. Because ENS electrical activity in the embryo was sensitive to mechanical stimulation, we further investigated which ion channels could relay this mechanosensitive response.

* richard-junior.amedzrovi-agbesi@etu.u-paris.fr
† nicholas.spencer@flinders.edu.au
‡ tim.hibberd@flinders.edu.au
§ nicolas.chevalier@paris7.jussieu.fr

II. MATERIALS AND METHOD

A. Ethics

All experiments were performed in accordance with the ethics and guidelines of INSERM and CNRS.

B. Animal Model

Mice were hosted at the Institut Jacques Monod animal husbandry. The Cre reporter mice Gt(ROSA)26 Sortm1(EYFP)Cos_{43} is referred to as GCampfl/fl. A transgenic mouse line in which the transgene is under the control of the 3-kb fragment of the human tissue plasminogen activator (Ht-PA) promoter Tg(PLATcre) 116Sdu16, was referred to as Ht-PA::Cre. GCampfl/fl males were crossed with Ht-PA::Cre females to generate embryos carrying the calcium fluorescent reporter in neural crest cells and their derivatives. The day a plug was observed following mating overnight was defined as E0.5. The day of birth was considered as post-natal day P0=E19.5. The mice were dissected at E18.5(embryos were taken at 18.5 days of gestation), P1, and P3. The gastrointestinal tracts were dissected from the stomach to the hindgut in phosphate-buffered saline (PBS) with Ca^{2+} and Mg^{2+}, and the mesentery was removed.

C. Calcium Imaging and Analysis

After dissection, a 0.2 mm diameter nylon thread was inserted in the lumen of a $\sim$ 2 cm long gut segment. The end of the thread was inserted non-invasively in a block of soft PDMS (Sylgard XXX) in a way that the nylon gently pressed the gut segment against the bottom of a 30mm diameter petri dish(Greiner). This method has been detailed in [23]. It allows muscle contractions to take place but prohibits bulk movements of the sample in and out of the imaging field. The gut was covered with 5 mL of Dulbecco's Modified Eagle Medium(DMEM) supplemented with 1% penicillin and constantly bubbled with 95% O_2 – 5% CO_2 at 36.5 $\pm$ 1°C. Imaging of the gut was captured using an inverted spinning disk confocal microscope (Olympus) with x10 objective, the illumination wavelength was set to 488 nm. Fluorescence was collected within the range of 492-630 nm, and the acquisition rate was set to 2 Hz. The video duration was 2 to 3 minutes. Drugs were administered from a stock solution by directly adding them to the petri dish under the microscope. To ensure proper mixing, the solutions were gently agitated by an up-and-down movement using a 1 mL pipette. To analyze calcium transients, the region of interest(ROI) was selected over the ENS area. The pixel intensity changes (calcium transients) over time were obtained using the 'findpeaks' function in MATLAB. We set the minimal peak prominence, and minimal and maximal peak width to take into consideration. The number of calcium flashes is the total number of peaks measured over the region of interest. We next assessed the area of ENS (mm^2) in the imaging field using the internodes thresholding method in Fiji. Normalized calcium activity was finally reported as the total number of flashes / per unit area ENS / per unit time.

D. Immunohistochemistry

Samples were fixed for 1h in 4% PFA in PBS, and washed 3 times. They were then dehydrated for 24 h in 30% sucrose solution in water. 1-2 cm pieces of the samples were embedded in OCT and frozen with a -80°C freezer pack. Frozen longitudinal sections were performed on a Leica cryostat machine set to -20°C at 14 μm thickness. Thin slice sections were blocked and permeated in 1% BSA and 0.1% triton in PBS overnight. The antibodies used were AntiCav1.2(ACC003AN7125 - alomone labs), AF 647(α rabbit), Cy3(α rabbit), Choactase(E-7, AF 546 conjugated), NOS1(A-11, AF 647 conjugated).

E. Statistics and Reproducibility

All sample numbers indicated in this article correspond to different embryos (guts, biological replicates). For mice, data were collected from a minimum of 3 different litters per age group to ensure robustness. Each group presented in this study comprises a minimum of n=5 samples, ensuring an adequate sample size for statistical analysis. Statistical analysis was performed with 2-way ANOVA and t-tests and was considered significant at $p<0.05$.

III. RESULTS

A. Embryonic Enteric Nervous System Calcium Activity

Veratridine(a depolarizer that causes persistent opening of the voltage-gated Na^+ channels) and tetrodotoxin(a Na^+ channel blocker) were used to excite and inhibit the ENS, respectively. Veratridine(10μM) increased the spontaneous calcium activity of E18.5(Fig. 1a, S1)neurons by more than 10-fold(Fig. 1b). Tetrodotoxin(1μM) abolished the stimulating effect of veratridine and almost no neuronal calcium activity was recorded after application(Fig. 1b, S1). These results indicate that the observed calcium signals stem from enteric neural electric activity.

Calcium activity in enteric neural crest cells has been reported at earlier stages (E11.5-E14.5)[16, 24] and is absent in the period E14.5-E18.5. We first examined how calcium activity evolved in the late fetal to early postnatal period from E18.5 - P3 small intestine. The average

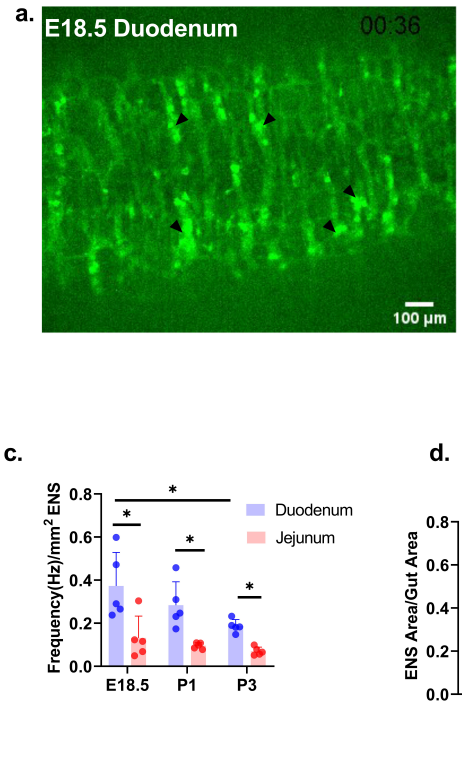

FIG. 1. **ENS developmental changes, E18.5-P3.** (a) Spontaneous calcium activity of the ENS of E18.5 mice. (b) Effect of veratridine(10 μM) and TTX(1 μM) on spontaneous calcium activity of E18.5 mice (c) Frequency of calcium activity per unit area of the ENS of E18.5 to P3 mice. (d) Fractional ENS area covered to the area of mice gut from E18.5 to P3.

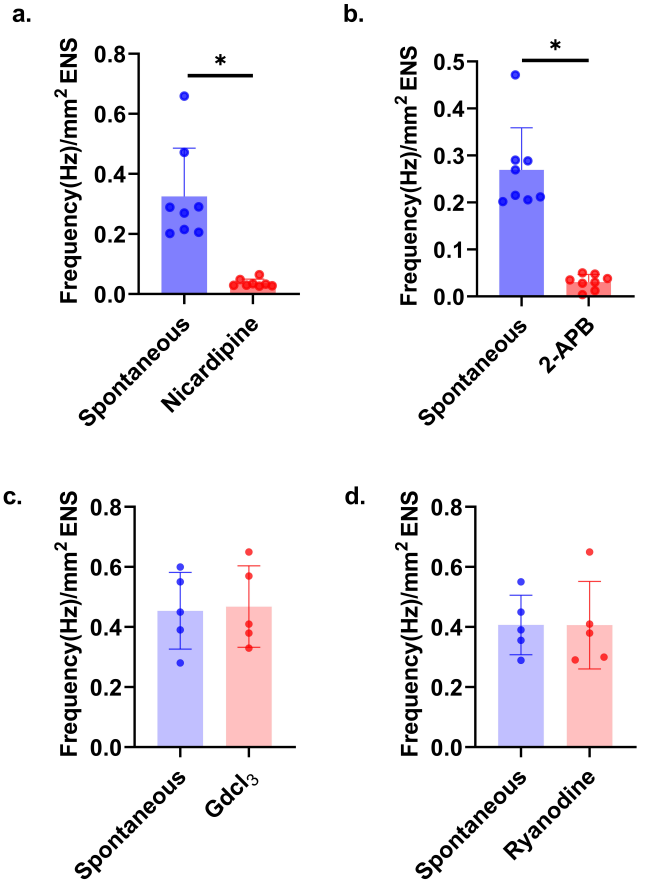

FIG. 2. **Voltage-Gated Calcium Channels Responsible for Spontaneous Calcium Activity of the ENS.** (a-d) Effects of nicardipine(10 μM), 2-APB(20 μM), $GdCl_3$(100 μM), and ryanodine(10 μM) on spontaneous calcium activity respectively.

frequency of calcium activity per unit area of ENS decreased from E18.5 to P3 in both the duodenum and jejunum(Fig. 1c); this decrease was statistically significant for the duodenum between E18.5 and P3. The frequency of calcium activity per unit area of the duodenum was always a factor ∼2 higher than in the jejunum at each stage considered (Fig. 1c). We note that the fraction area occupied by the ENS was lower in the duodenum than in the jejunum (Fig. 1d), consistent with previous reports[25]. Nicardipine(10μM, n=8) - an L-type calcium channel blocker almost completely abolished spontaneous calcium activity(Fig. 2a, S2). 2-APB(20μM, n=8), a known inhibitor of IP3R and store-operated Ca^{2+}(SOC) channels significantly reduced the frequency of spontaneous calcium activity of the ENS(Fig. 2b, S3). In contrast, $GdCl_3$(100 μM, n=5) - a transient receptor potential(TRP) channel blocker, and ryanodine (10μM, n=5) - RyRs receptors blocker found on the endoplasmic reticulum had no effect on calcium activity(Fig. 2c,d). Nicardipine is a known smooth muscle blocker. It is not expected that this drug acts directly on enteric neurons, yet was effective in blocking spontaneous patterns of neuronal activity. To investigate the possibility of a direct neuronal action of nicardipine, we investigated the presence of L-type calcium channels on neurons in the myenteric plexus. Immunohistochemistry studies revealed the presence of the L-type voltage-gated calcium channel, $CaV_{1.2}$ on populations of myenteric neurons that also contained ChAT or NOS immunoreactivity. (Fig. 3).

B. Mechanosensitivty of the Embryonic Mouse

Because the ENS plays a key role in mediating the mechanosensitive response of the gut[5, 15], we were interested in finding out if mechanosensitive properties are established at the fetal and early postnatal ages(E18.5-P3). We applied mechanical force using a 0.3 mm diameter blunt glass probe to gently pinch the gut wall. Intracellular calcium concentration in the ENS promptly rose to high levels upon pinching(Fig. 4b). We observed contraction of the circular smooth muscle(n=7/10) that was synchronous with the intracellular calcium rise in the ENS(S4). We investigated what ion channels and

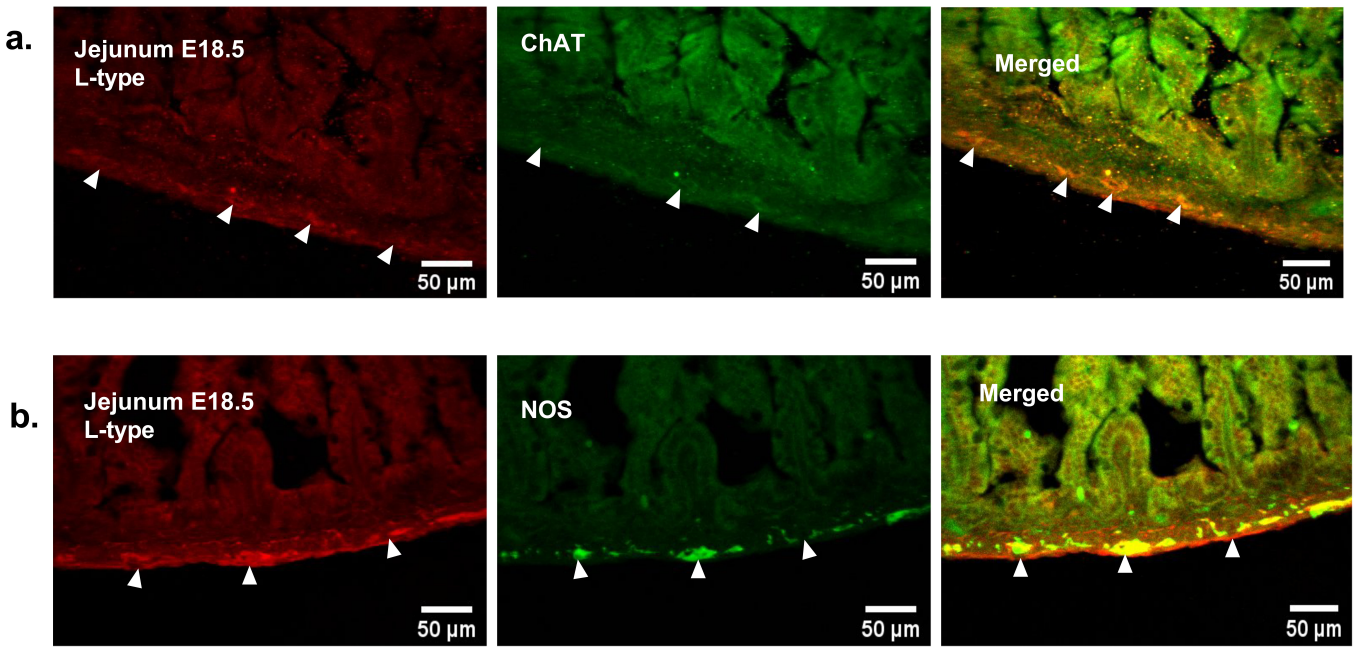

FIG. 3. **Presence of L-type, NOS, and ChAT labeled enteric neurons in E18.5.** the white arrows point to labeled myenteric nerve cell bodies (a,b) L-type colocalization with nitrergic(NOS) and cholinergic(ChAT) neurons in the jejunum of E18.5 mouse.

molecular pathways could mediate the mechanosensitive behavior of the fetal ENS. The three compounds that abrogated spontaneous calcium activity, 2-APB(100 μM, n=10), nicardipine(10 μM, n=10), and TTX(1 μM, n=10) had no effect on the mechanosensitive calcium flash when the gut was pinched(Fig. 4a, S5). We further tested NOLA(100 μM, n=5) - an inhibitor of nitric oxide synthase and $GdCl_3$(100 μM, n=5) - a TRP channel blocker; neither of these compounds inhibited the calcium uprise in the ENS upon pinching(Fig. 4a, S5). We finally found that the same mechanosensitive response was also present at E14.5, a stage at which the ENS does not present any spontaneous calcium activity(Fig. 4c). Blocking synaptic transmission(Mg^{2+} (10 μM), Ca^{2+} (0.25 μM)(n=3) of the ENS abolished the pinch-induced calcium flash. Spontaneous ENS calcium activity was also significantly reduced(n=3).

C. Relation between ENS spontaneous calcium activity and motility

Motility is purely myogenic at stages E13.5 to E17.5. We were interested in understanding how the ENS affects motility when the first spontaneous neurogenic activity settles in at E18.5. We further wondered if the contractile response to pinching would differ for an active or inactive (TTX-inhibited) ENS. E18.5 motility is characterized by a propagating circular contraction and longitudinal contraction. The duodenum(n=4/10), and jejunum(n=5/10) showed an alternating high(with a dominant frequency $>$ 18 cpm) and low(with a dominant frequency $<$ 10 cpm) frequency of contraction(Fig. 5a, S6). The periods of high-frequency contractility occurred at a rate of 0.55 ± 0.1 cpm and lasted for about 60 ± 5 s. After TTX(1 μM) was added, the alternating high and low frequency wave pattern was abolished(Fig. 5b). This pattern, therefore, represents one of the first neurally mediated motility patterns of E18.5 mice. The gut in TTX showed a rhythmic contraction with a frequency of 11 ± 2 cpm, i.e. comprised between the high and low dominant frequencies when TTX was introduced (Fig. 5b). There was a circular contraction in response to the pinch after TTX(1 μM) was added.

D. Mechanosensitivty and Spontaneous Calcium Activity of the Adult Mouse

The embryonic gut responds to pinching by an increased calcium activity even in the presence of TTX. We were curious to find out if this mechanism is conserved in the adult mouse. We applied mechanical force using a 0.3 mm diameter blunt glass probe to gently pinch the gut wall of the mouse colon. We observed an instantaneous increase in calcium activity(Fig.6a, S7) in the ENS(n=3). Instantaneous calcium increase of the colon ENS(n=3) persisted in the presence of TTX(1 μM)(Fig.6b, S8). The region of the gut pinched is a few millimeters away from the point imaged. We blocked synaptic transmission(Mg^{2+} (10 μM), Ca^{2+} (0.25 μM) [26]) as a proof of concept that pinch-induced calcium activity requires chemi-

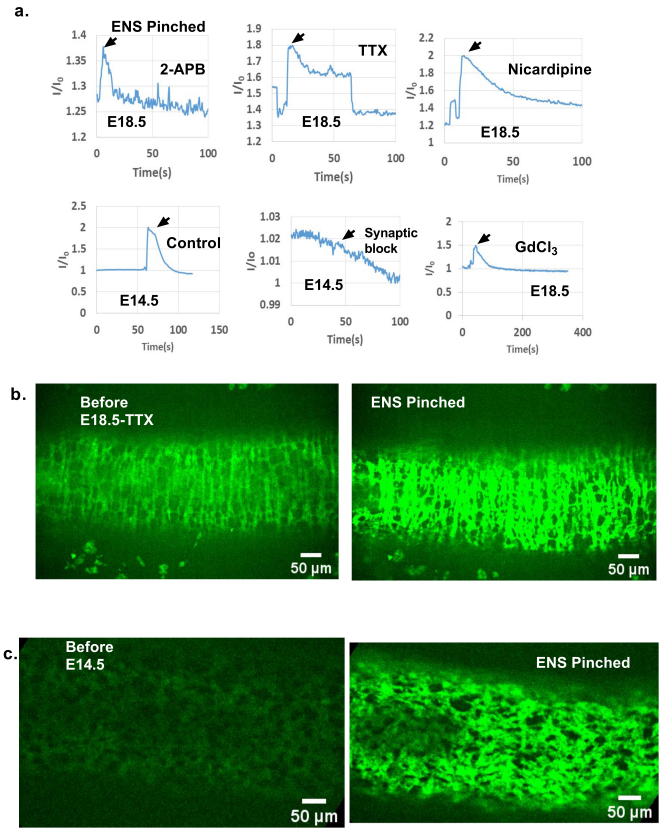

FIG. 4. **Pinching effect of ENS.** ENS intensity change when pinched (a) after 2-APB(100 μM), TTX(1 μM), nicardipine(10 μM), Krebs solution([26]), $GdCl_3$(10 μM), were added. (b) Calcium flash of the ENS when pinched after TTX(1 μM) was added (c) E14.5 jejunum response to pinching.

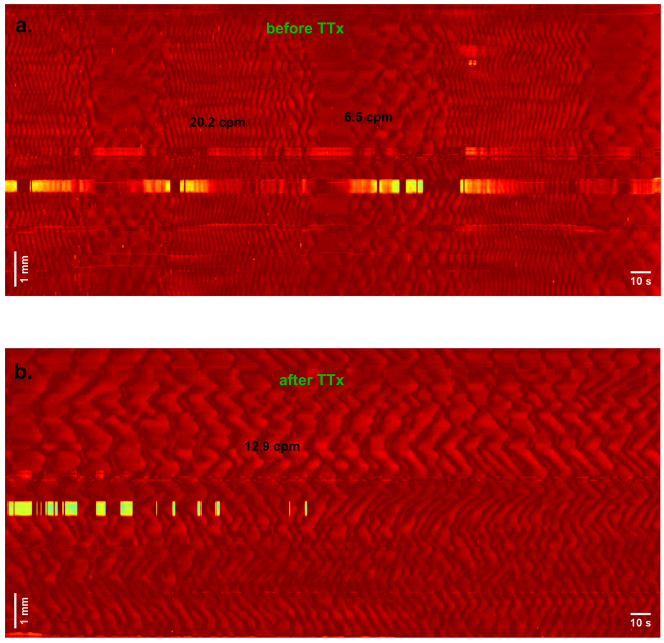

FIG. 5. **Motility patterns of E18.5.** (a) Alternating high and low frequency wave pattern. (b) Rhythmic contraction after TTX(1μM) was added.

cal synapses. No flash or increased calcium activity of the ENS was evident(S9) in these conditions. Spontaneous ENS calcium activity was significantly reduced(n=3). Contraction of the smooth muscles persisted when synaptic transmission was blocked and a pinched-induced contraction of the smooth muscles was recorded(n=1/3). We also found that nicardipine(10 μM), as in the embryonic mouse, almost completely abolished spontaneous calcium activity in the adult mouse(n=7) colon(Fig.6c).

IV. DISCUSSION

In this study, we showed that spontaneous neuronal calcium activity is present in the E18.5 mouse small intestine and that the frequency of this activity decreased as the mouse aged from E18.5-P3. This spontaneous calcium activity requires L-type calcium channels (Fig. 2, S2), intracellular store-operated calcium channels, and IP3R (2, S3). Consistent with this, L-type calcium channels immunoreactivity was identified in both excitatory and inhibitory neurons (Fig. 3). L-type channel dependence of spontaneous activity was further observed in the adult colon (Fig.6). We identified that the fetal ENS can transduce mechanical force into calcium activity. Mechanical probing promptly increased intracellular calcium in ENS cells (Fig. 4, S7). This behavior was from E14.5 (Fig. 4), was TTX resistant both in the fetal and adult gut (Fig. 6), but was inhibited when synaptic transmission was blocked (Fig. 4). We detected a motility pattern of alternating high- and low-frequency circular smooth muscle contractions that disappeared when tetrodotoxin was added at E18.5 (Fig. 5).
Spontaneous calcium activity has been recorded in many parts of the developing central nervous system and is known to control essential aspects of development [27–32]. We found that E18.5 mouse enteric neurons in the duodenum and jejunum present spontaneous calcium activity. This activity is TTX-dependent and mediates a motility pattern characterized by alternating high and low contractile frequencies in the duodenum and jejunum. These results corroborate and complement previous electrophysiological and motility observations at E18.5 [19]. As this neurally mediated motility pattern develops just prior to birth, it presumably facilitates absorption of milk-derived nutrients during weaning; it might prefigure neurogenic motility patterns like segmentation or migrating motor complexes that are crucial in mature animals [1, 2]. We note that the spontaneous neuronal activity we observe at E18.5 is distinct from the early calcium events observed in neuronal and migrating enteric neural crest cells [16]. This latter activity has been shown to peak at E12.5

and disappear by E16.5, and to be driven by purinergic signaling [16].

We found that calcium activity was higher in the duodenum than in the jejunum at each of the stages considered (E18.5-P3). Because enteric neurons colonize the duodenum first and mature earlier in more rostral regions of the gut [8, 33], this higher activity in the duodenum probably results from increased ion channel expression or developmental changes in ion channel properties. Neurogenic motility in the hindgut for example only develops from stage P10 [19]; the amplitude of calcium response to electrical stimulation from E15.5 and E18.5 duodenum has been reported to be higher than those from the colon at the same ages [16]. Unexpectedly, we found that spontaneous calcium activity decreased from E18.5-P3 in the duodenum and jejunum. This observation parallels studies in the central nervous system showing that spontaneous depolarizing potentials are observed during the first eight postnatal days in the hypothalamus, but decrease from P9 to P12 and disappear by P12.2 [27].

L- type channels are prominently expressed in smooth muscle [34–37] and are often used in electrophysiological and calcium imaging studies of the ENS to limit movements of intestinal preparations. Our protocol constrained movements by gently pressing the gut against the Petri dish bottom and we were thus able to monitor the effects of L-type channels on spontaneous neural activity. Strikingly, we found that nicardipine application almost completely abolished spontaneous activity, both in the fetal E18.5 mouse foregut and the adult mouse colon. Because L-type calcium channels are present on both NOS and ChAT neurons, these results support a direct neuronal effect of nicardipine and further indicate that the spontaneous ENS calcium activity we observe stems from both NOS and ChAT neurons. Given the widespread use of L-type channel blockers to treat hypertension[38, 39], it is noteworthy that they have extensive repercussions on the ENS as well.

We further found that 2-APB abolished spontaneous neural activity at E18.5, indicating the prominent role played by intracellular calcium stores. We note that 2-APB affects spontaneous ENS activity and smooth muscle(both spontaneous and pinch-induced contraction), and not pinch-induced ENS activity. Synaptic blocking affects both spontaneous and pinch-induced ENS activity and not pinch-induced smooth muscle contraction. This helped us to dissociate ENS and smooth muscle mechanosensitivity response.

Distension results in mechanical deformation of the cell body of neurons and changes in calcium activity. Mechanosensitive neurons detect mechanical distension and relay the bioelectric signal to excitatory or inhibitory effector neurons [40]. Recent studies suggest that a broad population of enteric neurons respond to mechanical stress by a change in their firing behavior and or

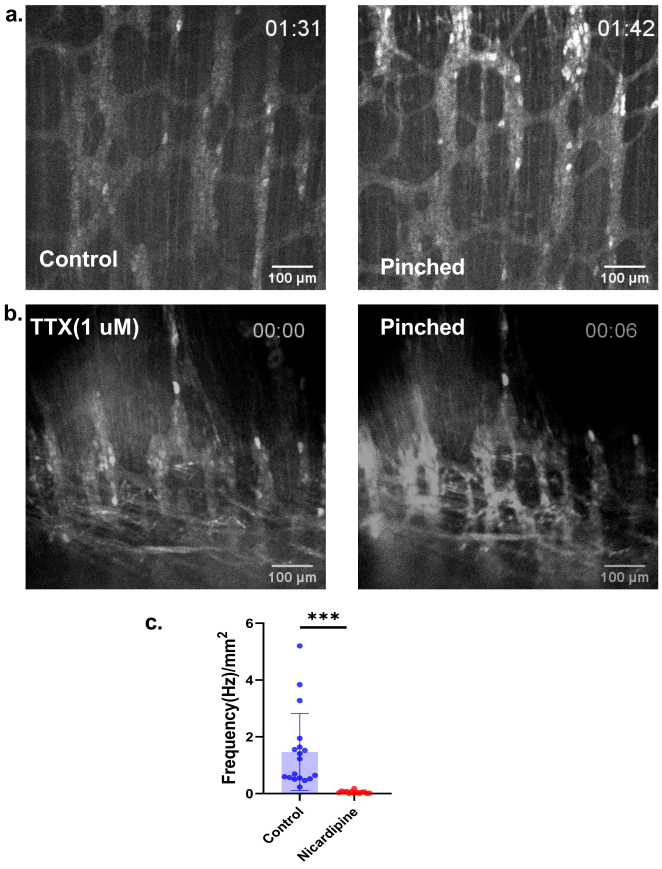

FIG. 6. **Spontaneous and Pinch-Induced Calcium Activity of the Adult Mouse Colon.** (a) Calcium activity of adult mouse colon before and when pinched(S7). (b) Calcium activity in the presence of TTX(1 μM) before and when pinched(S8). (c) Effects of nicardipine(10 μM) on spontaneous calcium activity(n=7, we looked at different regions of the colon of seven different mice).

frequency [13, 41]. Intestinal distension in the small and large intestines of adult mouse colon affects most of the neurons in the ENS by locally modulating their levels of intracellular calcium ions[15]. Similar observations were made in primary cultures of neurons from the esophagus [42]. What triggers the increase of intracellular calcium ions in response to mechanical stress in the late embryonic and early postnatal ages remain to be fully understood. Candidates of ion channels considered were Piezo1[43], which is widely expressed in enteric neurons and considered mechanosensitive, TRP[15], which has been shown to be involved in mechanosensitivity, L-type channel, and intracellular SOC, which we have shown to be involved in spontaneous calcium activity. In the colon of adult mice, distension-induced regulation of intracellular calcium was shown to be dependent on KCa1.1 channels activity [15]. We found that when the embryonic gut was pinched, the ENS responded with a conspicuous increase in intracellular calcium. Because

whole-organ mechanical stimulation elicits both an instantaneous myogenic contractile response and a compression of the ENS, we could not dissociate whether the ENS calcium increase was due to muscle contraction, direct ENS mechanical stimulation, or both. Strikingly, this response was resistant to $GdCl_3$, 2-APB, nicardipine, and tetrodotoxin, and was present even at very early embryonic stages (E14.5). We further demonstrated that this TTX-resistant response is present in the adult colon. Our results allude to the fact that the increase of intracellular calcium in the ENS might be a generic reaction resulting from compression-induced disruption of the endoplasmic reticulum within cells, that triggers a calcium release even in the presence of 2-APB. Similarly, electric field stimulation-induced calcium transients still persisted after the addition of a cocktail of antagonists to block all five types of voltage-gated calcium channels in E12-E18 mice [16]. Our results suggest that mechanical (and perhaps electrical) stimulation can trigger calcium release by cytoplasmic phenomena that are independent of voltage-gated channels at the membrane.

An interesting result that needed exploring was the calcium flash in the presence of TTX at the early embryonic and late postnatal stages. It has been well established that nerve conduction along axons in the ENS cannot occur without Na^+ channels[44–46]. We were curious to find out if this result is observed in the adult mouse as it poses a potential alternative mechanism for cell-cell propagation. Increased calcium activity persisted in the ENS of adult mouse colon when pinched in the presence of TTX. This indicates synaptic axonal propagation even when Na^+ channels were blocked. One possibility is a different mechanism that could mediate cell-cell propagation without Na^+ channels. It has been found in our work and other works of ENS[15] and CNS[44, 45], the presence of TTX-resistant neurons. Although three to five times slower than the kinetics of fast TTX sensitive neurons[44], could be a possible means of synaptic propagation in the presence of TTX.

ACKNOWLEDGMENTS

The authors thank Sylvie Dufour for the HTPA:Cre, Isabelle Annoux and Nathalie Rouach for the Gcamp, and acknowledge the generous support from the IDEX Université de Paris ANR-18-IDEX-0001 grant and from the Agence Nationale de la Recherche ANR GASTROMOVE-ANR-19-CE30-0016-01 grant.

[1] J. B. Furness, *The Enteric Nervous System* (John Wiley & Sons, 2008).
[2] J. Bornstein, M. Costa, and J. Grider, Enteric motor and interneuronal circuits controlling motility, Neurogastroenterology & Motility **16**, 34 (2004).
[3] G. Farrugia, Interstitial cells of cajal in health and disease, Neurogastroenterology & Motility **20**, 54 (2008).
[4] K. Sanders, Regulation of smooth muscle excitation and contraction, Neurogastroenterology & Motility **20**, 39 (2008).
[5] J. D. Huizinga, K. Ambrous, and T. Der-Silaphet, Cooperation between neural and myogenic mechanisms in the control of distension-induced peristalsis in the mouse small intestine, The Journal of Physiology **506**, 843 (1998).
[6] K. A. Sharkey and G. M. Mawe, The enteric nervous system, Physiological reviews **103**, 1487 (2023).
[7] N. M. L. Douarin and M.-A. Teillet, The migration of neural crest cells to the wall of the digestive tract in avian embryo, Development **30**, 31 (1973).
[8] R. P. Kapur, C. Yost, and R. D. Palmiter, A transgenic model for studying development of the enteric nervous system in normal and aganglionic mice, Development **116**, 167 (1992).
[9] R. Anderson, A. Stewart, and H. M. Young, Phenotypes of neural-crest-derived cells in vagal and sacral pathways, Cell and tissue research **323**, 11 (2006).
[10] N. W. Bellono, J. R. Bayrer, D. B. Leitch, J. Castro, C. Zhang, T. A. O'Donnell, S. M. Brierley, H. A. Ingraham, and D. Julius, Enterochromaffin cells are gut chemosensors that couple to sensory neural pathways, Cell **170**, 185 (2017).
[11] P. Bertrand, W. Kunze, J. Bornstein, J. Furness, and M. Smith, Analysis of the responses of myenteric neurons in the small intestine to chemical stimulation of the mucosa, American Journal of Physiology-Gastrointestinal and Liver Physiology **273**, G422 (1997).
[12] W. A. Kunze, Y.-K. Mao, B. Wang, J. D. Huizinga, X. Ma, P. Forsythe, and J. Bienenstock, Lactobacillus reuteri enhances excitability of colonic ah neurons by inhibiting calcium-dependent potassium channel opening, Journal of cellular and molecular medicine **13**, 2261 (2009).
[13] G. Mazzuoli and M. Schemann, Mechanosensitive enteric neurons in the myenteric plexus of the mouse intestine, PLoS One **7**, e39887 (2012).
[14] N. J. Spencer, G. W. Hennig, and T. K. Smith, A rhythmic motor pattern activated by circumferential stretch in guinea-pig distal colon, The Journal of Physiology **545**, 629 (2002).
[15] J.-B. Cavin, P. Wongkrasant, J. B. Glover, O. B. Balemba, W. K. MacNaughton, and K. A. Sharkey, Intestinal distension orchestrates neuronal activity in the enteric nervous system of adult mice, The Journal of Physiology **601**, 1183 (2023).
[16] M. M. Hao, W. Boesmans, V. Van den Abbeel, E. A. Jennings, J. C. Bornstein, H. M. Young, and P. V. Berghe, Early emergence of neural activity in the developing mouse enteric nervous system, Journal of Neuroscience **31**, 15352 (2011).
[17] W. L. Hasler, Motility of the small intestine and colon, Textbook of gastroenterology **1**, 231 (2009).
[18] R. M. Gwynne, E. Thomas, S. Goh, H. Sjövall, and J. Bornstein, Segmentation induced by intraluminal fatty

acid in isolated guinea-pig duodenum and jejunum, The Journal of Physiology **556**, 557 (2004).
[19] R. R. Roberts, J. F. Murphy, H. M. Young, and J. C. Bornstein, Development of colonic motility in the neonatal mouse-studies using spatiotemporal maps, American Journal of Physiology-Gastrointestinal and Liver Physiology **292**, G930 (2007).
[20] R. M. Lindley, D. B. Hawcutt, M. G. Connell, S. N. Almond, M.-G. Vannucchi, M. S. Faussone-Pellegrini, D. H. Edgar, and S. E. Kenny, Human and mouse enteric nervous system neurosphere transplants regulate the function of aganglionic embryonic distal colon, Gastroenterology **135**, 205 (2008).
[21] N. R. Chevalier, N. Dacher, C. Jacques, L. Langlois, C. Guedj, and O. Faklaris, Embryogenesis of the peristaltic reflex, The Journal of Physiology **597**, 2785 (2019).
[22] W. C. Alvarez, Bayliss and starling's law of the intestine or the myenteric reflex, American Journal of Physiology-Legacy Content **69**, 229 (1924).
[23] N. R. Chevalier, Y. Ammouche, A. Gomis, C. Teyssaire, P. de Santa Barbara, and S. Faure, Shifting into high gear: how interstitial cells of cajal change the motility pattern of the developing intestine, American Journal of Physiology-Gastrointestinal and Liver Physiology **319**, G519 (2020).
[24] M. B. Carey and S. G. Matsumoto, Spontaneous calcium transients are required for neuronal differentiation of murine neural crest, Developmental biology **215**, 298 (1999).
[25] N. R. Chevalier, R. J. A. Agbesi, Y. Ammouche, and S. Dufour, How smooth muscle contractions shape the developing enteric nervous system, Frontiers in Cell and Developmental Biology **9**, 678975 (2021).
[26] J. Dean and J. A. Boulant, Effects of synaptic blockade on thermosensitive neurons in rat diencephalon in vitro, American Journal of Physiology-Regulatory, Integrative and Comparative Physiology **257**, R65 (1989).
[27] Y. Ben-Ari, E. Cherubini, R. Corradetti, and J. Gaiarsa, Giant synaptic potentials in immature rat ca3 hippocampal neurones., The Journal of physiology **416**, 303 (1989).
[28] J. Holliday and N. C. Spitzer, Spontaneous calcium influx and its roles in differentiation of spinal neurons in culture, Developmental biology **141**, 13 (1990).
[29] R. Wong, A. Chernjavsky, S. Smith, and C. Shatz, Early functional neural networks in the developing retina, Nature **374**, 716 (1995).
[30] L. D. Milner and L. T. Landmesser, Cholinergic and gabaergic inputs drive patterned spontaneous motoneuron activity before target contact, Journal of Neuroscience **19**, 3007 (1999).
[31] J. Gust, J. Wright, E. Pratt, and M. Bosma, Development of synchronized activity of cranial motor neurons in the segmented embryonic mouse hindbrain, The Journal of physiology **550**, 123 (2003).
[32] X. Gu and N. C. Spitzer, Distinct aspects of neuronal differentiation encoded by frequency of spontaneous ca2+ transients, Nature **375**, 784 (1995).
[33] H. Young, C. Hearn, D. Ciampoli, B. Southwell, J.-F. Brunet, and D. Newgreen, A single rostrocaudal colonization of the rodent intestine by enteric neuron precursors is revealed by the expression of phox2b, ret, and p75 and by explants grown under the kidney capsule or in organ culture, Developmental biology **202**, 67 (1998).
[34] A. Lacampagne, F. Gannier, J. Argibay, D. Garnier, and J.-Y. Le Guennec, The stretch-activated ion channel blocker gadolinium also blocks l-type calcium channels in isolated ventricular myocytes of the guinea-pig, Biochimica et Biophysica Acta (BBA)-Biomembranes **1191**, 205 (1994).
[35] I. Bodi, G. Mikala, S. E. Koch, S. A. Akhter, A. Schwartz, *et al.*, The l-type calcium channel in the heart: the beat goes on, The Journal of clinical investigation **115**, 3306 (2005).
[36] C. Dessy and T. Godfraind, The effect of l-type calcium channel modulators on the mobilization of intracellular calcium stores in guinea-pig intestinal smooth muscle, British journal of pharmacology **119**, 142 (1996).
[37] M. Ragy and E. Elbassuoni, The role of nitric oxide and l-type calcium channel blocker in the contractility of rabbit ileum in vitro, Journal of physiology and biochemistry **68**, 521 (2012).
[38] S. Nozato, K. Yamamoto, Y. Nozato, M. Takeda, K. Hongyo, M. Takeya, H. Akasaka, Y. Takami, Y. Takeya, K. Sugimoto, *et al.*, Comparison between l-type and n/l-type calcium channel blockers in the regulation of home blood-pressure variability in elderly hypertensive patients, Hypertension Research **41**, 290 (2018).
[39] M. T. Johnson, A. Gudlur, X. Zhang, P. Xin, S. M. Emrich, R. E. Yoast, R. Courjaret, R. M. Nwokonko, W. Li, N. Hempel, *et al.*, L-type ca2+ channel blockers promote vascular remodeling through activation of stim proteins, Proceedings of the National Academy of Sciences **117**, 17369 (2020).
[40] C. Fung and P. Vanden Berghe, Functional circuits and signal processing in the enteric nervous system, Cellular and Molecular Life Sciences **77**, 4505 (2020).
[41] G. Mazzuoli-Weber and M. Schemann, Mechanosensitive enteric neurons in the guinea pig gastric corpus, Frontiers in Cellular Neuroscience **9**, 430 (2015).
[42] H. Dong, Y. Jiang, J. Dong, and R. K. Mittal, Inhibitory motor neurons of the esophageal myenteric plexus are mechanosensitive, American Journal of Physiology-Cell Physiology **308**, C405 (2015).
[43] E. Drokhlyansky, C. S. Smillie, N. Van Wittenberghe, M. Ericsson, G. K. Griffin, G. Eraslan, D. Dionne, M. S. Cuoco, M. N. Goder-Reiser, T. Sharova, *et al.*, The human and mouse enteric nervous system at single-cell resolution, Cell **182**, 1606 (2020).
[44] G. Baranauskas, Ionic channel function in action potential generation: current perspective, Molecular neurobiology **35**, 129 (2007).
[45] B. P. Bean, The action potential in mammalian central neurons, Nature Reviews Neuroscience **8**, 451 (2007).
[46] B. D. Clark, E. M. Goldberg, and B. Rudy, Electrogenic tuning of the axon initial segment, The Neuroscientist **15**, 651 (2009).
[47] M. M. Hao, R. E. Moore, R. R. Roberts, T. Nguyen, J. B. Furness, R. B. Anderson, and H. M. Young, The role of neural activity in the migration and differentiation of enteric neuron precursors, Neurogastroenterology & Motility **22**, e127 (2010).
[48] L. N. Borodinsky, C. M. Root, J. A. Cronin, S. B. Sann, X. Gu, and N. C. Spitzer, Activity-dependent homeostatic specification of transmitter expression in embryonic neurons, Nature **429**, 523 (2004).