Table 1: SW and USW characteristics in healthy subjects vs constipated patients; no significant difference was demonstrated between the 2 groups ($P > 0.5$).

	Slow Waves		Ultra-Slow Waves	
	Health; n = 44	Constipation; n = 90	Health; n = 44	Constipation; n = 90
Prevalence of SW and USW	57% (25/44)	59% (53/90)	2% (1/44)	30% (27/90)
Frequency (number of waves in 60 seconds)	12.8 (±3.2)	13.1 (±3.9)	1.0	1.4 (±0.4)
Mean Amplitude (mmHg)	6.5 (±2.7)	6.9 (±3.4)	10.0	30.6 (±13.3)

367 | Chromosome topology analysis reveals remote super-enhancers for neuronal nitric oxide synthase 1 (Nos1) expression and the mechanisms of their association with Nos1-proximal regulatory elements

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Background/Objectives: The mechanisms of transcriptional regulation of *Nos1* are poorly understood. Previously we found that physiologically low O_2 (physioxia) stimulates *Nos1* transcription by stabilizing hypoxia-inducible factor 1α (HIF1A). By chromatin immunoprecipitation-sequencing (ChIP-seq) and targeted chromosome conformation capture (3C) analysis we discovered 3 *Nos1*-proximal enhancer-promoter pairs (E1-P1, E2-P2, E3-P3) within ~100 kb upstream of *Nos1*. Physioxia/HIF1A stimulated transcriptional initiation at all 3 promoters by relaxing interactions between E1-E3 and P1-P3 and facilitating interactions between cognate enhancers and promoters. Here, we aimed to discover enhancers outside the *Nos1* locus and analyze the structural basis of their interactions with the *Nos1*-proximal regulatory elements.
Methods: $NOS1^+$ N1E115 cells were cultured at high (20%) and physioxic (4%) O_2 for 3 days. Binding of HIF1A, the cohesin component RAD21, the chromatin loop anchor CTCF, and 6 histone marks was determined by ChIP-seq. Chromosome topology analysis was performed by 3C-sequencing (HiC).
Results: HiC identified an ~800-kb region that contained all interactions involving the *Nos1*-proximal regulatory elements. At 20% O_2, ChIP-seq revealed 3 enhancer clusters (super-enhancers; SEs) within this region: 2 upstream (5': SE1, SE2) and 1 downstream (3': SE4) of *Nos1*. At 4% O_2, binding of HIF1A and H3K27ac (a histone mark for active enhancers) increased within these SEs and one remote 5' enhancer gained SE status (SE3). E3, the most *Nos1*-proximal enhancer, interacted with all 3 SE sites at 20% O_2, but only the SE1(5')-E3 and SE4(3')-E3 interactions were RAD21- and CTCF-anchored architectural loops (conventional and coiled, respectively), while others were dynamic/temporary. At physioxia, the SE4(3')-E3 loop dissolved, whereas the SE2(5')-E3 and SE3(5')-E3 interactions became conventional architectural loops; and E1 and E2 formed new dynamic interactions with a HIF1A-bound typical enhancer 5' of *Nos1* and with SE1, respectively.
Conclusions: Physioxia stimulates *Nos1* transcription by reconfiguring structural and functional interactions between *Nos1*-proximal and remote regulatory elements.

368 | Toll-like receptor 4 interaction with enteric dopaminergic pathways in a mouse model of dextran sulfate sodium-induced colitis

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Objective: Changes in dopamine levels, deregulated dopaminergic machinery and altered Toll-like receptor 4 (TLR4) expression have been consistently associated with IBD in patients and in animal models. Thus, we aimed to assess the crosstalk of enteric dopaminergic system and TLR4 signaling in a mouse model of dextran sulfate sodium (DSS)-induced colitis.
Methods: Male $TLR4^{-/-}$ mice (8 ± 1 weeks old; N = 32 mice) received 1.5% DSS in their drinking water for 5 days, then switched to regular drinking water for 3 days. Small intestine inflammation was measured by disease activity index and histological analysis. Gastrointestinal transit was measured by nonabsorbable-FITC-labeled dextran distribution. Changes in ileal muscle tension were isometrically recorded following: i) cumulative addition of dopamine (0.1-300 μM); ii) electric field stimulation (EFS, 4 Hz) in presence of 30 μM dopamine with or without 10 μM SCH-23390 (D1R antagonist) or 10 μM sulpiride (D2R antagonist). Immunofluorescence distribution of the neuronal HuC/D and glial (GFAP and S100β) markers and dopamine β-hydroxylase (DBH) and dopamine transporter (DAT) were determined in longitudinal-muscle-myenteric plexus whole-mounts (LMMPs) by confocal microscopy.
Results: In WT mice, DSS treatment determined a delayed gastrointestinal transit, a reduction of dopamine-induced relaxation (-26%, N = 5, $P < 0.05$), reactive gliosis and 1.2-fold increase in DBH immunoreactivity. After DSS treatment $TLR4^{-/-}$ mice showed a significant increase in dopamine-induced relaxation (+30%, N = 5, $P < 0.01$),

together with a 2.3-fold increase in 4-Hz EFS-elicited contraction (N = 5, $P < 0.001$), sensitive to D1R and D2R activation. Changes in ENS neurochemical coding were evidenced by a reduced number of HuC/D^{+} neurons (-12%, N = 5, $P < 0.05$), together with a 1.4-fold increase of DBH immunoreactivity and no changes in GFAP and S100β immunofluorescence in DSS-treated TLR4$^{-/-}$ LMMPs.

Conclusions: In mice, TLR4 signaling influences the severity of small intestine inflammation as well as ENS activity and neurochemical coding, markedly increasing small intestine dopaminergic-mediated neuromuscular function.

369 | Involvement of toll-like receptor 4 signaling and dopaminergic neurotransmission in high-fat diet-induced small intestine dysmotility

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Objective: Recent clinical and preclinical studies have shown that high-fat diet (HFD) intake is associated with gastrointestinal dysmotility, gut microbiota dysbiosis, endotoxaemia and colonic neurodegeneration via Toll-like receptor 4 (TLR4). Excessive intake of dietary fats leads to diminished brain dopaminergic function. In this study, we assessed the role of TLR4 in tuning small bowel contractility and dopaminergic signaling pathways affected by HFD-induced obesity.

Methods: Male TLR4$^{-/-}$ mice and wild-type (WT) C57BL/6J male mice (7 ± 1 weeks old) were fed with standard-diet (SD; 18% kcal fat) or HFD (60% kcal fat) for 8 weeks. Changes in ileal muscle tension were isometrically recorded following: i) cumulative addition of dopamine (0.1-300 μM); ii) electric field stimulation (EFS, 4 Hz) in presence of 30 μM dopamine, with or without 10 μM SCH-23390 (D1R antagonist) or 10 μM sulpiride (D2R antagonist). Immunofluorescence distribution of the neuronal HuC/D and glial GFAP markers and dopamine transporter (DAT) were determined in longitudinal-muscle-myenteric plexus whole-mounts (LMMPs) by confocal microscopy.

Results: After 8-week SD, TLR4 deficiency determined a significant reduction in dopamine-induced relaxation response (Emax = -23%, N = 5, $P < 0.001$), 4-Hz EFS-elicited contraction (N = 5, $P < 0.01$) sensitive to D1R activation (N = 5, $P < 0.05$), a 1.4-fold increase in GFAP and DAT immunoreactivity. However, TLR4 deficiency protected against body-weight gain after HFD (+21% in TLR4 mice vs +33% in WT mice, N = 16, $P < 0.05$). In WT mice, HFD caused a marked reduction in dopamine-induced relaxation (Emax = -50%, N = 5, $P < 0.001$), in 4-Hz EFS-elicited contraction (N = 5, $P < 0.05$) sensitive to dopamine and involving D1R together with a 1.5 fold increase of DAT immunoreactivity and onset of reactive gliosis. In TLR4$^{-/-}$ mice, HFD impaired markedly 4-Hz EFS-elicited neurotransmission and D1R-mediated response (N = 5, $P < 0.01$).

Conclusions: In mice, TLR4 signaling appears to partially protect against the detrimental metabolic effects of HFD and to be highly involved in finely tuning enteric dopaminergic neurotransmission controlling neuroglial crosstalk.

371 | Effect of the adaptive immune system on the homeostasis and function of the enteric nervous system

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A complex set of tissues of distinctive embryological origin (such as the intrinsic immune and nervous systems) continuously interact to maintain a normal physiology of the gastrointestinal (GI) tract, which is constantly threatened by several and sometimes severe challenges originated within the wall or the lumen of the gut. The enteric nervous system (ENS) is composed of an intricate network of enteric glial cells (EGCs) and neurons localised into different compartments within the GI wall. These cells are involved in regulating virtually all aspects of GI physiology, including intestinal peristalsis. The ENS shares the same intestinal environment with the highly active immune system, due to its positioning at the interface between the internal and external milieu of the body, and therefore is expected to be influenced by immune responses. However, the methodology to explore these questions and consequently our understanding of neuro-immune communications in the mammalian gut is limited. In this work, we examined the effects of the absence of the adaptive immune system on the homeostasis and function of the ENS in adult animals. We have effectively generated a robust technology to isolate and evaluate the molecular landscape of enteric neurons from the adult gut. Moreover, by using immunodeficient mouse models lacking T and B lymphocytes (*Rag1*$^{-/-}$ and *Rag2*$^{-/-}$) and only T cells (*Tcrα*$^{-/-}$), our experiments revealed that in the absence of the adaptive immune cells, there is an impaired network of EGCs and neurons within the myenteric ganglia. These changes are followed by alterations on the expression of important genes by ENS cells. Besides, the frequency of the neuronal colonic motility (CMMCs) is decreased in mice lacking the adaptive immune system. Together, our data suggest that the adaptive immune system is crucial for maintaining the integrity and functional equilibrium of the ENS.