HR3.0 inhibition was used to modulate the response to graded, isobaric colorectal distension (20-60 mmHg) measured via a visceromotor behavioral response (VMR) quantified as the number of abdominal muscle contractions in freely moving rats. **Results:** were analyzed with a repeated measure two-way ANOVA with Bonferroni's post-hoc analysis (mean ± standard deviation). **Results:** Optogenetic activation of CeA fibers at the BNST in sham-stressed rats induced colonic hypersensitivity to luminal distension compared to sham-stressed rats with optogenetic inhibition of the same pathway (60 mmHg: SHAM-ChR2: 44.7±2.4 vs. SHAM-HR3.0: 23.0±0.0, P< 0.0001). In WAS exposed rats, activation of CeA fibers at the BNST did not further potentiate the VMR to distension (60 mmHg: WAS-ChR2: 45.7±2.9, P= 0.99 vs SHAM-ChR2). In contrast, inhibition of CeA fibers at the BNST completely inhibited the WAS-induced colonic hypersensitivity (60 mmHg: WAS-HR3.0: 20.7±6.4, P< 0.0001 vs. WAS-ChR2; P= 0.99 vs. SHAM-HR3.0). **Summary:** In sham-stressed rats, activation of the CeA-BNST pathway induced colonic hypersensitivity that was comparable to WAS-induced colonic hypersensitivity. In rats exposed to repeated WAS, inhibition of the CeA-BNST pathway completely inhibited the stress-induced colonic hypersensitivity. **Conclusions & Inferences:** This study demonstrated that signaling from the CeA-BNST pathway is required for behavioral expression of stress-induced colonic hypersensitivity to luminal distension in a freely moving rat model. Thus, therapies directed at normalizing the brain-gut axis via central limbic pathways should be sufficient to relieve chronic visceral pain in IBS patients.

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GUT SENSING OF NUTRIENT CALORIC CONTENT

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One of the most satisfying, yet dangerous, things we do everyday is eat. Overconsumption is linked to diseases including obesity. However, how the gut transduces the caloric content of nutrients to the brain remains unknown. Recent studies have shown that post-ingestive signaling from sucrose can elicit a robust preference. But sucralose, a non-nutritive sweetener, does not have the same effect. We believe this discrepancy occurs at the level of sensory transduction at the gut epithelium. The sensory epithelial cell of the gut is the enteroendocrine cell. Though classically studied from an endocrine perspective, we recently discovered that a subset of enteroendocrine cells synapse with vagal neurons. We call them neuropod cells. These cells transduce glucose stimuli using glutamate as a neurotransmitter. Here, we sought to establish the molecular mechanisms by which neuropod cells differentially sense and synaptically communicate the calories of sugar to the brain. This sensory transduction mechanism forms the basis of a gut sensor for calories. First, we determined how murine small intestinal neuropod cells sense and transduce nutritive versus non-nutritive sugars. Using calcium imaging in acutely dissociated neuropod cells, we established that these cells can be either unimodal or multimodal in sugar sensing. In whole nerve recordings of the cervical vagus, optogenetic silencing of small intestinal neuropod cells abolished the vagal response to both intraluminal sucrose and sucralose infusions. Because these cells are necessary to sense and synaptically communicate both caloric and non-caloric sugars, we next defined how neuropod cells sort the signals. Using pharmacological inhibition of nutrient receptors, we found that the sodium glucose co-transporter SGLT1 is necessary for sucrose sensation while the sweet taste receptor T1R2/3 is responsible for sucralose sensation. In addition, by inhibiting glutamate receptors, we found that glutamate release depends on SGLT1 activation. Taken together, these results show that sugar calories activate SGLT1 to trigger glutamate release from neuropod cells. Finally, we sought to determine the role of neuropod cells in preference of caloric over non-caloric sugars. We adapted optogenetic tools widely used to probe behavior in the brain, to the gut. This allowed us to specifically target neuropod cells in awake, behaving mice. When neuropod cells are optogenetically silenced, the mice's preference for sucrose over sucralose was significantly reduced. These data show that neuropod cells sense and communicate sugar calories and that inhibition of these cells greatly attenuates calorie preference. This neuroepithelial circuit represents a therapeutic target to alter the sensory transduction of calories from gut to brain and to modulate sugar preference.

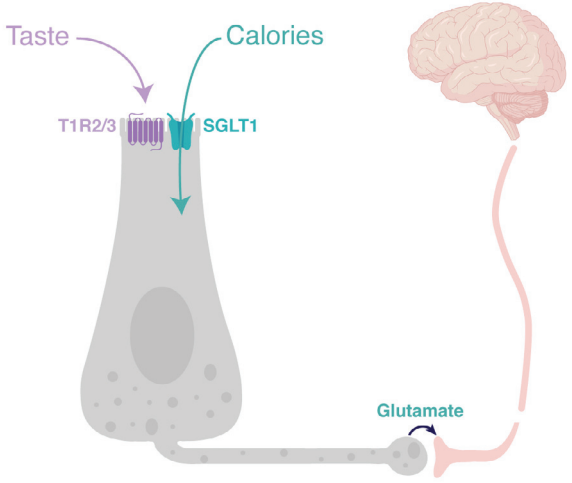

Figure 1 - Neuropod cells release glutamate upon SGLT1 activation and synapse with the vagus nerve to synaptically communicate caloric content to the brain.

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ENTERIC ALPHA-SYNUCLEIN INCLUSIONS, COLONIC INFLAMMATION, INCREASED MUCOSAL PERMEABILITY AND ALTERATIONS OF BOWEL NEUROMUSCULAR FUNCTIONS PRECEDE CENTRAL NEURODEGENERATION IN A TRANSGENIC MOUSE MODEL OF PARKINSON'S DISEASE

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Introduction. Gastrointestinal dysfunctions represent one of the most common non-motor symptoms of Parkinson's disease (PD). Several lines of evidence suggest that enteric accumulation of α-synuclein (αS) inclusions (a hallmark of PD) and colonic inflammation could contribute to bowel motor dysfunctions since the earliest stages of PD. However, current knowledge does not allow to establish a clear relationship between altered mucosal permeability, enteric inflammation, bowel dysmotility and PD pathology. This study examined concomitantly enteric αS accumulation, mucosal permeability, bowel inflammation and in vitro colonic motor activity in a transgenic model of PD, before onset of neurodegeneration in the CNS. **Methods.** PrP human A53T αS transgenic (Tg) mice, Line G2-3 develop a progressive PD-like neurological and motor deficiency after 9 months of age, accompanied by neuronal degeneration and pathological accumulation of aggregated αS in the central nervous system (CNS). Animals were sacrificed at the age of 3, 6 and 9 months, in order to evaluate the presence and timing of enteric αS inclusions, alterations of intestinal mucosal barrier and colonic inflammation since the very early phases of the disease, before the overt development of CNS dysfunction. Blood samples were collected to evaluate circulating lipopolysaccharide (LPS) levels (an indirect index of intestinal permeability) by ELISA assay; then distal colon was excised and processed for: 1) expression and aggregation of αS (western blot); 2) tissue tumor necrosis factor (TNF) and interleukin-1beta (IL-1β) (ELISA). Colonic longitudinal and circular muscle preparations were set up in organ baths with Krebs solution, and connected to isometric transducers to record contractions elicited by electrical stimulation (ES, 10 Hz, 0.5 ms, 30 mA). **Results:** An accumulation of insoluble and aggregated αS was present in the enteric neurons of the distal colon in Tg mice since 3 months of age, as compared with controls. In addition, at 3, 6 and 9 months of age Tg mice displayed a significant elevation of circulating LPS along with increased colonic TNF and IL-1β levels. Electrically evoked contractions in longitudinal and circular muscle preparations were impaired in Tg mice at 3, 6 and 9 months of age, as compared with controls. **Conclusion.** The present findings suggest that a concomitance of enteric αS accumulation, altered mucosal permeability, bowel inflammation and impaired colonic motor activity represent early events in PD, occurring before the onset of CNS neurodegeneration. These changes could contribute to the pathogenesis of intestinal motor dysfunctions known to be associated with PD in humans.

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NEURONAL SEROTONIN AS A LINK BETWEEN DEPRESSION AND CONSTIPATION: A ROLE FOR SIMULTANEOUS TREATMENT WITH A NOVEL SLOW-RELEASE FORMULATION OF THE SEROTONIN PRECURSOR, 5-HYDROXYTRYPTOPHAN

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Depression and chronic constipation are often comorbid, yet their shared etiologies have rarely been explored. Neuronal serotonin (5-HT) affects central and enteric nervous system (CNS and ENS, respectively) development and long-term functions, including gastrointestinal (GI) motility and mood, and could thus be a nexus connecting the brain and intestine. A genetic variant of the rate-limiting enzyme critical for neuronal 5-HT synthesis, tryptophan hydroxylase 2 (TPH2; TPH2-R441H) was identified in a cohort of individuals with severe depression. The analogous murine mutation, R439H, was genetically engineered into mice and resulted in a 60-80% decrease in CNS 5-HT levels and depressive-like behaviors. We found that the TPH2-R439H mice also manifest low enteric neuronal 5-HT. By immunocytochemistry, we found significantly less 5-HT neurons and 5-HT neurite density as well as decreased levels of 5-HT release in response to tryptamine superfusion, as measured by Ca^{2+} imaging (all $P< 0.01$). Low neuronal 5-HT was accompanied by ENS hypoplasia in the myenteric and submucosal plexuses with an exaggerated hypoplasia in 5-HT-dependent subsets ($P< 0.005$). Further, TPH2-R439H mice exhibited slowed total and colonic transit ($P< 0.05$) and an enteric microbial composition coincident with human depression and/or constipation. To evaluate whether the slowed motility resulted from abnormal ENS development, in vitro peristaltic contractions (a direct measure of ENS function) were quantified. The frequency and velocity of peristaltic contractions were also significantly lower in TPH2-R439H mice ($P< 0.001$). We next sought to utilize a treatment to reverse the defects associated with low neuronal 5-HT. 5-HT itself is ineffective because of its rapid inactivation. We considered 5-hydroxytryptophan (5-HTP), the immediate precursor of 5-HT, because it enhanced the effects of SSRIs on neuroendocrine biomarkers of elevated extracellular 5-HT in humans. The rapid elimination of acute 5-HTP, however, reduces its ability to maintain the 5-HT levels necessary for effective treatment. A novel slow-release formulation of 5-HTP (5-HTP SR) was thus created and administered to TPH2-R439H and WT mice for four weeks and was found to treat depression. Strikingly, adult TPH2-R439H mice treated with 5-HTP SR also exhibited ENS neurogenesis with a normalization of ENS neuroanatomy, reversal of slowed in vivo and in vitro GI motility and a normalization of enteric microbial composition. Our findings thus reveal an important role for neuronal 5-HT in linking constipation and depression and, further, demonstrate the utility of 5-HTP SR as a novel therapy for both conditions.