Role of Estrogen and Stress on the Brain-Gut Axis

Yanyan Jiang[1], Beverley Greenwood-Van Meerveld[2,3], Anthony C. Johnson[3,4], R. Alberto Travagli[1,*]

[1]Department of Neural and Behavioral Sciences,
Penn State-College of Medicine, Hershey, PA
and
[2]Oklahoma Center for Neuroscience,
University of Oklahoma Health Science Center, Oklahoma City, OK

[3]VA Health Care System, Oklahoma City, OK

[4]Department of Neurology,
University of Oklahoma Health Science Center, Oklahoma City, OK

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* Corresponding author:
Dr. R. Alberto Travagli
Department of Neural and Behavioral Sciences
Penn State College of Medicine
500 University Drive
MC H109
Hershey, PA 17033
rtravagli@pennstatehealth.psu.edu

Introduction

The gastrointestinal (**GI**) tract performs the processes of digestion under semi-autonomous control of the enteric nervous system (**ENS)**. Parasympathetic (vagal and pelvic) and sympathetic (thoracolumbar) pathways convey sensory information to the central nervous system (**CNS**) and modulate motility via descending brain-gut pathways impinging onto ENS neurons. Disorders of the brain-gut axis contribute to the development of functional gastrointestinal disorders (**FGID**), such as functional dyspepsia (**FD**) and irritable bowel syndrome (**IBS**), that involve altered motility and/or altered sensitivity (21).

Multiple epidemiological studies have established that the prevalence of FGID is higher among women (25). Although the exact pathophysiology is largely unknown, clinical evidence suggests that GI dysmotility, including impaired gastric accommodation, delayed gastric emptying, and gastric hypersensitivity contributes to FD symptoms (25). Women are more likely to report symptoms of FD, such as nausea, early satiety, bloating and both upper and lower abdominal pain, and meet diagnostic criteria for FGID, suggesting the involvement of circulating gonadal hormones, estrogen and progesterone (25). GI motility is decreased in women, including a shorter migrating motor complex, prolonged proximal gastric relaxation, altered distal gastric motor function, and attenuated postprandial antral contractions, during the follicular phase when estrogen levels are high (4). These observations suggest that circulating female hormones play a major role in the delayed gastric emptying observed in women, although the effects of the menstrual cycle on gastric emptying rate seem inconclusive, likely due to a disparity in measurement methodology as well as the size, age, and intrinsic variation of the selected sample (44, 46, 92). Notably, pre- as well as post-menopausal women receiving hormone therapy replacement have gastric emptying rates slower than that of post-menopausal women without hormone therapy, which is similar to that of age-matched men (46). Studies in animals have also shown that gastric emptying rates are slower in intact compared to ovariectomized females, and that estradiol administration delays gastric emptying and inhibits gastrointestinal motility (9, 19, 36). Conversely, testosterone, or androgens in general, do not appear to have any effect on GI motility, or gastric hypersensitivity (3, 19, 36).

Abdominal pain is one of the major symptoms in IBS patients (24). Clinical evidence suggests that patients with IBS exhibit abnormal bowel habits in part due to altered smooth muscle function, abnormal mucosal transport, and/or increased epithelial permeability (24). There is a strong sex-related bias in the prevalence of IBS, with a female to male ratio ranging from 2:1 to 4:1 in developed countries (16). The observed sex difference in the prevalence and severity of GI symptoms in IBS could be, at least in part, explained by circulating ovarian

hormones (65), since many symptoms, such as bloating, change in bowel habits, and abdominal pain vary during the menstrual cycle and pain severity scores are reduced in IBS patients following menopause (22, 39, 71). Furthermore, in rodent models, colonic sensitivity is increased during proestrus and estrus, and diminished during diestrus or metestrus (37, 48), while estrogen replacement in ovariectomized rats increases visceromotor response to nociceptive stimuli (18, 47).

Brain-Gut Axis Regulation of Motility

The coordinated autonomic processes of the GI tract, from the lower esophagus to the transverse colon, are under a prominent extrinsic, parasympathetic modulatory control. Upper GI functions are regulated by the efferent vagus nerve, the output of which is controlled by neurons of the dorsal vagal complex (**DVC**), consisting of the dorsal motor nucleus of the vagus (**DMV**), the nucleus tractus solitarius (**NTS**), and the area postrema. Sensory signals from the upper GI tract are relayed by afferent vagal fibers to the NTS, where they are integrated with information from other CNS centers involved in the regulation of autonomic and homeostatic functions. The integrated signal is then transmitted from the NTS to the efferent preganglionic neurons of the DMV, which project to either cholinergic (excitatory) or non-adrenergic non-cholinergic (**NANC**, inhibitory, mainly vasoactive intestinal peptide and nitric oxide, but also ATP) post-ganglionic myenteric neurons (12, 32, 85).

NTS neurons project to the DMV primarily via GABA-ergic, glutamatergic, and catecholaminergic synapses, with GABAergic inputs exerting the strongest influence on the activity of gastric-projecting DMV neurons. Microinjection of the $GABA_A$ receptor antagonist, bicuculline, into the DVC increases gastric motility, for example, whereas microinjections of glutamatergic or catecholaminergic antagonists have limited effects on gastric motility and tone under basal conditions. Using a brainstem slice preparation, we and others have shown that bicuculline increases the firing rate in the majority of DMV neurons, suggesting a robust GABAergic synaptic input onto these neurons, which tonically regulates their excitability. Notably, these GABAergic NTS-DMV synapses are not static, but undergo a great deal of plastic changes that enable an appropriate response of vagally regulated gastric motility to variable physiological and pathophysiological conditions. The vagal output that modulates gastric motility, or smooth muscle contractility, is thus largely dependent on the activity of DMV neurons. Both the intrinsic spontaneous pacemaking properties as well as the synaptic inputs to the DMV neurons shape their excitability and by consequence, determine the vagal motor output to the stomach (85). Lower GI motility is modulated by the parasympathetic fibers originating in the pelvic ganglia that innervate the distal colon.

Both the upper and lower GI tract is also innervated by sympathetic fibers from the prevertebral ganglia which project to the esophagus, stomach and proximal small intestine (celiac ganglia), duodenum (superior mesenteric ganglia), and distal small intestine and colon (inferior mesenteric ganglia). These ganglia play an essential role in the inhibition of motility, via activation of presynaptic α_2 receptors (29).

Brain-Gut Axis Regulation of Sensitivity

Sensory information, including noxious somatic stimuli, visceral pain, and responses to neuromodulators released from the enteric neurons, is detected by nociceptors located throughout the layers of the GI tract (35). While vagal afferent fibers play a significant role in upper GI pain signaling, the majority of nociceptive signaling occurs via thoracolumbar sympathetic afferents (30). The nociceptive neurons have cell bodies located in the dorsal root ganglia, and transmit the noxious signal to the dorsal horn of the spinal cord (2, 8). Ascending fibers transmit pain signals to higher centers via various tracts, and is relayed by the thalamus to cortical areas for localization of pain, and to limbic areas, such as the amygdala, insula and nucleus accumbens, for the processing of the emotional component of pain (14). Descending inhibitory brainstem pathways are activated by outputs from both the cortical and the limbic systems in response to the pain signals, decreasing noxious signaling by inhibiting dorsal horn neurons (38).

Chronic visceral pain is associated with sensitization that occurs in both peripheral sensory receptors and in the neuronal network mediating pain responses in the brain. Peripheral sensitization in response to injury or infection is associated with receptor activation by inflammatory mediators, such as cytokines, chemokines, or prostaglandins, and/or algesic chemicals such as bradykinin or histamine (75). The downstream signaling further sensitizes visceral afferents via modification of existing cell-membrane receptors that increases excitability of the afferent fibers as well as via changes in gene expression that leads to insertion of more or different classes of receptors into the cell membrane. These changes in sensory neurons modify the amount and pattern of neurotransmitters released within the dorsal horn of the spinal cord, and amplifies pain signals via both increased centripetal synaptic transmission and decreased descending inhibitory modulation (26).

In the brain, a similar mechanism to promote and maintain chronic pain can be evoked in the thalamus and brainstem. Increased afferent nociceptive neurotransmission due to peripheral or spinal sensitization leads to hypersensitivity and central remodeling in the thalamus, and enhances signaling to the other cortical and limbic regions (74). The integration nuclei, including

prefrontal cortex, cingulate cortex, amygdala, and insula, are subsequently sensitized in response to increased afferent stimulation, which can produce an enhanced negative emotional response, and/or disrupt the descending inhibitory pathways (89).

Estrogen Receptor Signaling and Expression

The biological effects of estrogen are mediated through two subtypes of genomic/nuclear receptors, estrogen receptor (**ER**)α and ERβ, as well as membrane bound/non-genomic receptors, G protein-coupled receptor 30 (**GPR30**)/G protein-coupled estrogen receptor 1 (**GPER**). The mechanisms of estrogen action involve a long-term, slow genomic effect via actions at nuclear receptors, and a rapid, non-genome action via activation of membrane-bound GPER receptors (40).

Estrogen receptors are expressed throughout the brain, including the hypothalamus, amygdala and midbrain, all of which have been shown to send extensive projections to preganglionic vagal neurons of the DMV, and, hence, modulate GI functions (12, 57, 63, 80). Estrogen receptors are also expressed on the myenteric plexus of both rodents and humans (1, 58, 59, 90, 93).

Estrogen or its non-selective agonist, 17β-estradiol, inhibit voltage-gated potassium channels in CNS regions (23) as well as in the GI tract resulting in inhibition of smooth muscle contractility in both stomach and colon (1, 59, 90, 93), and modulates synaptic transmission and neuronal firing rate via actions on both glutamate and GABAergic transmission (45, 64, 70, 88).

Estrogen Effect on GI Motility

Recent evidence indicates that estrogen receptors are abundant in the brainstem neuronal population, including NTS and DMV neurons, thus providing the neuroanatomical support for the direct effect of estrogen on either the DMV membrane and/or the critical GABAergic synapses between NTS and DMV, hence vagal efferent output to the stomach (76, 87). Furthermore, estrogen promotes increased density of vagal afferent projections to the NTS (20), suggesting that estrogen may also facilitate GABAergic neurotransmission to the gastric-projecting neurons of the DMV, thereby decreasing their excitability and vagal efferent output to the stomach. Additionally, direct administration of estrogen onto isolated gastric smooth muscle decreases gastric contractions, likely via a cGMP-dependent nitric oxide (**NO**) production (1, 77). Importantly, such effects of estrogen are also sex-dependent, since the relaxation in response to estrogen is greater in females compared to males (1).

In general, estrogen has been shown to delay colonic motility in in vivo and in vitro rodent models via the release of NO (7, 58, 93). However, short-term sex hormone supplementation and withdrawal in healthy post-menopausal women was not found to affect colonic transit, suggesting the effects of estrogen on GI motility may be influenced by the dosage and timing of hormonal exposure (31).

Estrogen Effect on GI Sensitivity

Estrogen receptors (ERs) are distributed at all levels of the visceral pain sensation pathways, including the ENS, spinal cord, and the brain centers mediating pain responses (81). In peripheral visceral afferent terminals, estrogen can modulate nociception by altering ion channel opening and regulation of receptor expression. Furthermore, estrogen also activates colonic tachykinin NK1 receptor and probably induces substance P release, in addition to modulating inflammatory pathways, secretion, and barrier function (10, 61, 78). Intrathecal administration of an ERα agonist increases the visceromotor behavioral response to colonic distension in ovariectomized rats, suggesting an important role of spinal ER in mediating visceral sensation (17, 47).

An emerging body of evidence suggests that estrogen modulates not only pain perception, but also the processing of visceral information in the CNS. Brain imaging studies have shown that, compared to men with IBS, women with IBS have increased activation in emotional circuits, including the amygdala and locus coeruleus, in response to aversive visceral stimuli (54, 55). Elevation of estrogen levels by implantation of estradiol in the amygdala has been shown to increase visceromotor pain response to colorectal distension in ovariectomized rats (67). Although the underlying mechanisms of the central estrogen actions have not been fully investigated, several studies have suggested that estrogen may alter expression of specific receptors related to pain signaling, such as the glucocorticoid receptor (**GR**) (73). The estrogen-mediated mechanism may also involve opioid systems, as evidence suggests that estrogen can promote μ-opioid receptor activation in several brain areas, such as the amygdala and bed nucleus of the stria terminalis, related to pain processing (15).

Stress Modulation of GI Motility

Stress can be defined as a stimulus or event that challenges the physiological and psychological homeostasis of an individual (27, 86). A rapid, appropriate response to stress is a reflexive mechanism that allows for necessary adaptive processes of relatively brief duration to maintain physiological homeostasis. Conversely, prolonged stress represents a more serious

challenge and requires more sustained modifications. Stressful situations promote a complex and integrated re-arrangement of neuroendocrine and autonomic stress systems, including the vagal neurocircuits that control GI motility (41, 80, 85). Stress activates the hypothalamic-pituitary-adrenocortical (**HPA**) axis resulting in release of corticotrophin-releasing hormone (**CRH**) from the paraventricular nucleus of the hypothalamus (**PVN**) and elevations in circulating glucocorticoids. CRH release delays gastric emptying and inhibits gastric motility profoundly through actions that involve vagal motoneurons in the DVC (57). Indeed, functional GI disorders, including FD and IBS, are correlated highly with stress, and stressful situations trigger and exacerbate GI symptoms in susceptible individuals (21, 28). A lack of resilience, habituation, or adaptation to stress results in dysfunction of both stomach (delayed gastric emptying) and colon (accelerated colonic motility) (6). The response of individuals to stress, however, differs such that some individuals exhibit high level of resistance, whereas other individuals show vulnerability, to stress. It is crucial and urgent to recognize and elucidate the underlying mechanisms that determine the degree of stress resilience or susceptibility to enable a better understanding of stress-associated GI-related dysfunctions.

Cumulative evidence strongly supports the anxiolytic and stress-attenuating effects of oxytocin, including the restoration of impaired gastric and colonic motility by oxytocin. A series of studies pioneered by Takahashi's group have highlighted the essential role of central oxytocin in adaptive GI response following chronic repetitive stress (5, 6, 91). Furthermore, oxytocin is involved in restoring the delayed gastric emptying and impaired gastric motility following acute stress or chronic stress maladaptation (91). Although several beneficial effects of oxytocin on GI motility is attributed to its action to reduce the expression and release of CRH in the PVN and, by consequence, the prominent systemic effect on the HPA axis (13, 53, 68), one cannot downplay the direct influence of hypothalamic oxytocin on vagal neurocircuits innervating the GI tract. In fact, oxytocin projections from the PVN are present in the DVC at birth, and increase markedly with age. In adult rats, oxytocin axons occur throughout the rostrocaudal extent of the DVC, and appose closely to GI-projecting DMV neurons (60). This anatomical evidence suggests that oxytocin may regulate the activity of vagal neurocircuits directly, thus influencing the vagal output to the peripheral organs, including the GI tract (60). Indeed, upon its release onto the brainstem vagal neurons, oxytocin excites DMV neurons, and inhibits glutamate, but not GABAergic, neurotransmission resulting in gastric relaxation through the activation of a postganglionic nitric oxide-mediated pathway (11, 42, 72).

It is important to note that the oxytocinergic connection from the PVN to the DVC undergoes a high level of neuroplasticity in both morphology and physiology, especially in conditions related to stressful stimuli. In naïve, non-stressed rats, oxytocin mediated modulation of

previously unresponsive NTS-DMV GABAergic neurotransmission is uncovered by pretreatment with CRH. Furthermore, the gastric relaxation induced by microinjection of oxytocin into the DVC is attenuated, abolished, or even reversed in CRH-exposed rats, possible via a cAMP-dependent translocation of oxytocin receptors to the terminals of GABAergic NTS-DMV synapses. Interestingly, following stress load, the mechanism of action of oxytocin engages another distinct pathway; in fact, in naïve conditions, the oxytocin-mediated effects occur via activation of a NANC-NO pathway, while after stress, they involve the activation of postganglionic VIP- and cholinergic- vagal pathways (11, 42).

Furthermore, we demonstrated recently that rats that undergo chronic repetitive stress display a higher number of oxytocin-IR neurons that project from the PVN to the DVC, as well as an increased density of oxytocin-IR fibers in the DVC (50). Such an upregulation of oxytocin in the hypothalamic-vagal neurocircuits may contribute to stress adaptation and restoration of GI motility, although its precise physiological effect and the modulation by sex hormones need further investigation.

Although the mechanisms of neuroplasticity in vagal neurocircuits induced by chronic stress are still largely unknown, the receptor translocation seems to be one important candidate that can explain the rearrangement of brainstem wiring that determines the level of adaptive response following chronic stress exposure. Indeed, we have shown recently that following chronic stress exposure, the response of vagal neurocircuits to α2-adreneceptor activation varies according to the type of chronic stress. Rats which underwent chronic variable stress showed a larger inhibition of antrum tone in response to α2-adreneceptor activation, compared to control or rats which underwent chronic repetitive stress. The translocation of α2-adreneceptor on GABAergic terminal of NTS-DMV synapses, combined with changes in intrinsic DMV neuronal excitability, may be responsible for the maladaptive response to α2-adreneceptor activation on gastric tone and motility (49). More detailed investigations on the mechanisms of neuroplasticity of vagal neurocircuits occurred following chronic stress, as well as how these changes contribute to the adaptive or maladaptive response to stress, are certainly needed.

Stress Modulation of GI Sensitivity

Stress maladaptation and negative emotions also play a significant role in the modulation of colorectal hypersensitivity, which contributes to IBS. Clinically, evidence implicates that periods of stress exhibit a high comorbidity with anxiety, depression, and other psychiatric disorders in the exacerbation of IBS symptoms.

An emerging body of evidence has shown that stress enhance visceral hypersensitivity through multifactorial mechanisms, e.g. psychological stress increases colonic permeability, epithelial secretion, and the structure and composition of the ENS, likely via CRH1 mediated actions (56, 69, 80).

In addition to peripheral mechanisms that mediated stress-induced visceral hypersensitivity, activation of central neuroendocrine and pain facilitatory mechanisms by stress appears to play a prominent role in colonic hypersensitivity (34). Neuroimaging studies in IBS patients have shown a greater response to nociceptive stimuli in limbic regions (62) that regulate sensory processing and emotion. In particular, several studies have suggested that neuronal remodeling in the CeA following chronic stress exposure exacerbates nociception and promotes visceral hypersensitivity (34). This neuronal remodeling involves regulation of CRH expression as well as the corticosterone (**CORT**) receptors, mineralocorticoid (**MR**) and GR. Chronic stress or stereotaxic delivery of CORT in the CeA induces visceral hypersensitivity, which can be attenuated by central application of GR or MR antagonist to the CeA (66), or systemic administration of a GR antagonist (43). Furthermore, a persistent decrease in GR expression in the CeA and an upregulation of CRH has also been observed following visceral hypersensitivity induced by either stress or CeA administration of CORT (33, 82, 83). Selectively knockdown of GR or MR in the absence of CORT exposure in the CeA is sufficient to promote visceral hypersensitivity in stress-naïve rats, indicating a significant role of GR and MR signaling in the CeA for modulation of colonic sensitivity (51). In addition, CRH expression in the CeA is a further regulator in mediating stress-induced visceral hypersensitivity. Indeed, intra-CeA CRH administration increases colonic sensitivity via CRH_1 receptor activation, and similar findings were demonstrated in female rats that had undergone an early life stressor (73) (79). Knockdown of CRH in the CeA attenuates visceral hypersensitivity induced by adult or early life stress, as does exposure of CeA to elevated CORT (52, 73). Furthermore, recent evidence also suggests stress-induced visceral hypersensitivity involves central epigenetic mechanisms within the CeA (82, 84).

Summary and Conclusion

The incidence of FGIDs is disproportionately higher in women, possibly due to a complex interaction between sex hormone signaling and stress reactivity on the function of the brain-gut axis. Specifically, both preclinical and clinical evidence has demonstrated that estrogen can affect GI motility and sensitivity via direct activation of its receptors, which are located throughout the brain-gut axis, and indirectly via modulation of other receptor systems. Many women with FGIDs have also experienced multiple stressors across their lifespan, the additive

effects of which can lead to peripheral and central sensitization along the brain-gut axis to affect motility and sensitivity throughout the GI tract. By further investigating sex- or stress-specific mechanisms underlying FGID pathophysiology, targeted therapies can be developed to provide relief for these patient populations.

Figure 1: The effect of estrogen or stress in the brain-gut axis. The brain-gut axis, illustrated on the left of the figure, is comprised of bidirectional communication from the visceral organs to the brain, via spinal and parasympathetic connections. Within the stress and pain responsive areas in the brain, such as the amygdala (AMY), cingulate cortex (CING), hippocampus (HIP), and hypothalamus (HYPO) integrate signals from the gastrointestinal (GI) tract are transmitted through brainstem areas such as the dorsal vagal complex (DVC). The bidirectional communication is relayed and modified within parasympathetic ganglia, such as the nodose ganglia (NG), and/or sympathetic dorsal root ganglia (DRG), with further regulation of noxious signals within the dorsal horn of the spinal cord. Within the GI tract, the stomach and small intestine (Sm. Intest.) are primarily innervated by vagal afferents, while the majority of the large intestine (Lg. Intest.) is innervated by spinal afferents. For each region of the brain-gut axis, the summarized effect of estrogen signaling or stress on sensation (sen) or motility (mot) is indicated with up arrows (↑) for increased responses, down arrows (↓) for decreased responses, or both arrows (↓↑) when the response can both increase and decrease depending on the receptor subtype. Changes are measured compared to ovariectomized females for estrogen or non-stressed baselines for stress. A (--) indicates that there is no literature consensus on the effect at the listed region.

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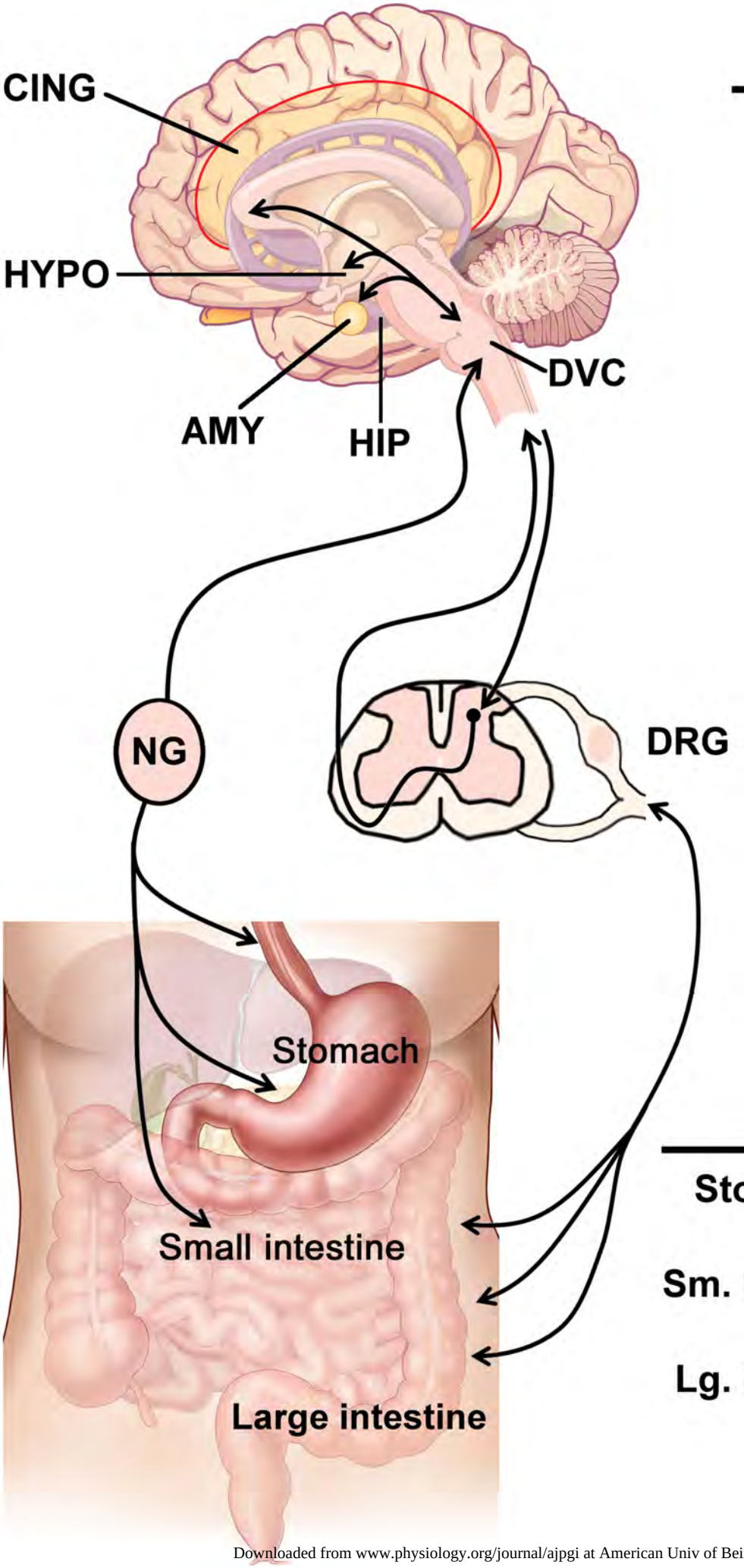
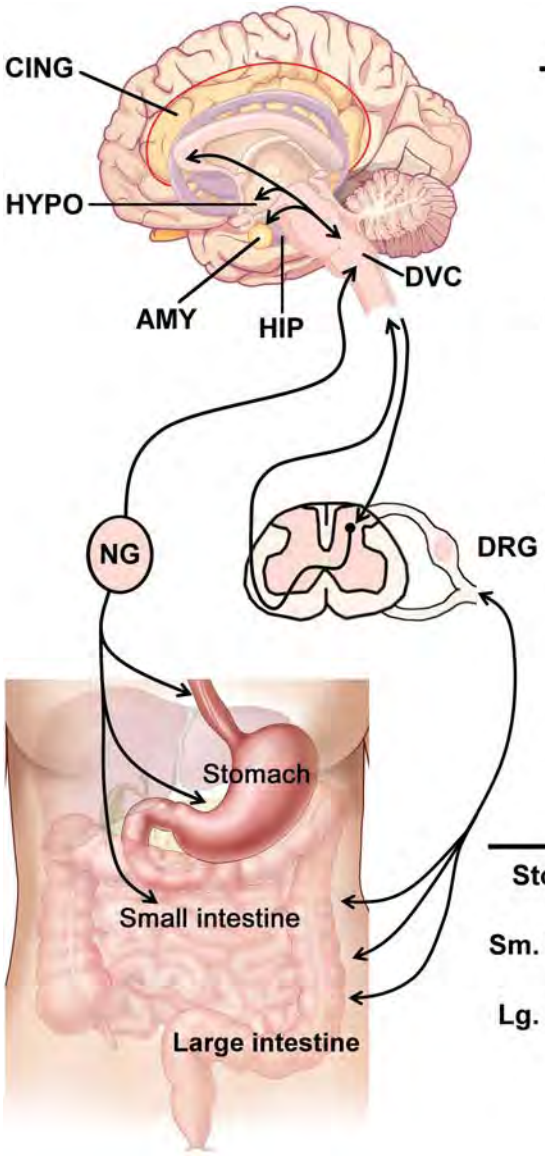


	Estrogen	Stress
AMY	↑ sen	↑ sen
CING	↑ sen	↑ sen
DVC	↓↑ sen ↓ mot	--
HIP	↑ sen	↑ sen
HYPO	--	↑ sen

	Estrogen	Stress
NG	↓↑ mot	--
DRG	↑ sen	↑ sen

	Estrogen	Stress
Stomach	↓ mot	↑ sen ↓ mot
Sm. Intest.	↓ mot	↑ sen ↑ mot
Lg. Intest.	↓↑ sen ↓ mot	↑ sen ↑ mot



	Estrogen	Stress
AMY	↑ sen	↑ sen
CING	↑ sen	↑ sen
DVC	↓↑ sen ↓ mot	--
HIP	↑ sen	↑ sen
HYPO	--	↑ sen

	Estrogen	Stress
NG	↓↑ mot	--
DRG	↑ sen	↑ sen

	Estrogen	Stress
Stomach	↓ mot	↑ sen ↓ mot
Sm. Intest.	↓ mot	↑ sen ↑ mot
Lg. Intest.	↓↑ sen ↓ mot	↑ sen ↑ mot