Central Network Dynamics Regulating Visceral and Humoral Functions

Rita J. Valentino,¹ Patrice Guyenet,² Xun Helen Hou,³ and Melissa Herman⁴

¹Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, Pennsylvania 19104, ²University of Virginia, Charlottesville, Virginia 22908,

³Harvard University, Boston, Massachusetts 02115, and ⁴University of North Carolina, Chapel Hill, North Carolina 27599

The brain processes information from the periphery and regulates visceral and immune activity to maintain internal homeostasis, optimally respond to a dynamic external environment, and integrate these functions with ongoing behavior. In addition to its relevance for survival, this integration underlies pathology as evidenced by diseases exhibiting comorbid visceral and psychiatric symptoms. Advances in neuroanatomical mapping, genetically specific neuronal manipulation, and neural network recording are overcoming the challenges of dissecting complex circuits that underlie this integration and deciphering their function. Here we focus on reciprocal communication between the brain and urological, gastrointestinal, and immune systems. These studies are revealing how autonomic activity becomes integrated into behavior as part of a social strategy, how the brain regulates innate immunity in response to stress, and how drugs impact emotion and gastrointestinal function. These examples highlight the power of the functional organization of circuits at the interface of the brain and periphery.

Key words: autonomic; gastrointestinal; immune; medulla; micturition; pons

Introduction

The maintenance of homeostasis requires complex reciprocal communication between the brain and viscera as well as the immune system. This allows for information from these peripheral systems to be integrated with other sensory inputs to modulate the state of arousal, focus of attention, and executive function. This communication also allows the brain to regulate the autonomic nervous system that controls visceral and immune function in response to internal and environmental challenges. In addition to maintaining homeostasis, the same circuitry that underlies reciprocal communication between the brain and periphery also provides routes through which pathology at either end can adversely influence the other.

Elucidating the complex interplay between the brain and peripheral systems that is involved in the coordination of behavior with visceral activity requires the dissection of complex circuits and, in particular, the ability to specifically manipulate them and to record neural activity and visceral endpoints simul-

taneously under natural conditions. Tools developed over the last few years have enhanced our ability to achieve these goals and advanced our knowledge in this area. Here we provide examples of knowledge gained through these approaches that has led to a better understanding of how the brain and periphery interact and the importance of this interaction in health and disease.

The brain-bladder intersection and its role in social behavior and disease

The pontine micturition center (PMC), also referred to as Barrington's nucleus, is an integral component of a circuit that regulates the descending limb of the micturition reflex. PMC axonal projections extend to the lumbosacral spinal cord and terminate within the preganglionic parasympathetic nucleus that provides the parasympathetic input to the detrusor and distal colon (Loewy et al., 1979; Hida and Shimizu, 1982) (Fig. 1). PMC lesions disrupt the micturition reflex, whereas electrical or chemical stimulation of this region elicits bladder contractions and micturition, the act of passing urine (Barrington, 1925; Willette et al., 1988; Noto et al., 1989; Pavcovich and Valentino, 1995). Although the PMC is neurochemically heterogeneous, many neurons express the stress-related neuropeptide, corticotropin-releasing factor (CRF) (Vincent and Satoh, 1984; Valentino et al., 1994, 1996; Hou et al., 2016). This makes the PMC amenable for manipulation by genetically driven tools and provides an opportunity to probe this system with a much higher specificity than was previously allowed using lesions or chemical or electrical stimulation. The use of these tools is both validating and refining our views of the neurophysiology and function of micturition.

In male mice, the electrophysiological and molecular characterization of CRF-positive (CRF⁺) PMC neurons reveals that they are distinct from their CRF-negative neighbors and

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Correspondence should be addressed to Dr. Rita J. Valentino, Division of Neuroscience and Behavior, National Institute on Drug Abuse, 6001 Executive Blvd., Room 4269, MSC 9555, Bethesda, MD 20892-9555. E-mail: valentinorj@nida.nih.gov.

R. J. Valentino's current address: National Institute on Drug Abuse, 6001 Executive Blvd., Bethesda, MD 20892-9555.

X. H. Hou's current address: Columbia University, 701 W. 168 St., Hammer Bldg. Rm 510, New York, NY 10032.

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Figure 1. Schematic showing circuits linking specific brain nuclei with viscera that are discussed in this review. Based on dual retrograde labeling, PMC axons putatively branch to the nearby LC (white arrowhead) and to the parasympathetic column of preganglionic neurons in the lumbosacral spinal cord. The latter project through the pelvic nerve and major pelvic ganglion to regulate pelvic visceral activity, including micturition. The LC projects to the cortex and limbic nuclei, where it can function in central aspects of voiding behavior. The dorsal motor nucleus of the vagus (DMV, box at the bottom left with part of a transverse section through the dorsal medulla) innervates the upper gastrointestinal tract and spleen. C1 neurons in the ventrolateral medulla can regulate immune function through projections to spinal sympathetic ganglia that project to celiac/suprarenal ganglia that innervate the spleen. Modified with permission from (Pavcovich and Valentino, 1995).

that they are sacral cord-projecting glutamatergic neurons (Hou et al., 2016). Combining transgenic mouse lines with molecular tools enabled genetic control over urine output, and it was demonstrated with fiber photometry that the population activity of CRF⁺ PMC neurons correlates with micturition (Hou et al., 2016). Selective activation of CRF⁺ PMC neurons with light-activated cation channel channelrhodopsin-2 is sufficient to trigger bladder contractions. The fast time course of bladder contraction following light onset supports the hypothesis that glutamate release from CRF⁺ PMC neurons in the spinal cord drives contraction. Conversely, reversible chemogenetic silencing of CRF⁺ PMC neurons with hM4Di, an engineered Gi protein-coupled receptor, impairs micturition.

In addition to serving an essential physiological function of waste elimination, micturition plays an important role in social communication due to the distinguishing information about an animal's identity carried by the chemical components in urine (Yamaguchi et al., 1981; Singer et al., 1997; Hurst and Beynon, 2004). As a result, voiding behaviors that determine where and when adult animals micturate are tightly regulated by internal and external sensory inputs and can be altered by experience and context (Desjardins et al., 1973).

The interaction of olfactory cues and social rank regulates the pattern of micturition in male mice. The presence of estrous female urine in an open-field arena increased the frequency and changed the spatial distribution of micturition selectively in dominant male mice (Hou et al., 2016). That the average location

of the urine spots was not predicted by the average location of the mouse supports the existence of a central circuit that actively regulates micturition in a social setting. Notably, social stress regulates micturition in an opposing manner in subordinate males, inhibiting micturition frequency to the extent of producing pathology (Desjardins et al., 1973; Henry et al., 1982; Wood et al., 2009).

CRF⁺ PMC neurons receive input from multiple forebrain areas that can relay diverse promicturition and antimicturition signals (Hou et al., 2016). Whole-brain analysis of male mice reveals a widespread micturition regulatory network with a large degree of convergence onto ~500 CRF⁺ PMC neurons. Candidate presynaptic neurons were found in cerebral cortices, olfactory relay nuclei, and hypothalamic and brainstem nuclei; among them are areas that process olfactory and social hierarchical information.

Rabies virus tracing in male mice identified a robust putative input to CRF⁺ PMC neurons from the medial preoptic area (MPOA) (Hou et al., 2016). The MPOA is a heterogeneous structure previously shown to be connected to multiple regions involved in social behaviors (Simerly and Swanson, 1986, 1988; Simerly et al., 1986). MPOA neurons retrogradely labeled from the PMC are homogeneously GABAergic, and chemogenetic inhibition of GABAergic MPOA neurons altered micturition patterns and reduced differences in micturition

between subordinate and dominant males, indicating that GABAergic MPOA neurons normally modulate micturition in the marking assay (Hou et al., 2016). These results are consistent with a model in which silencing inhibitory MPOA inputs disinhibits CRF⁺/glutamatergic PMC neurons.

Together, these results in male mice indicate that CRF⁺/glutamatergic neurons in the PMC comprise a brainstem command output that controls urine release. These neurons have the capacity, anatomically and functionally, to integrate promicturition and antimicturition inputs from relevant brain areas and transmit these signals into urine output, a process that exemplifies the integrative capacity of brainstem nuclei to regulate visceral activity and behavior. The whole-brain analysis of candidate input areas to CRF⁺ PMC neurons in male mouse may reveal additional factors that regulate micturition. Future work involving a detailed intranucleus cell type and connectivity characterization of the PMC, combined with population recording and circuit perturbation, will reveal the neural mechanisms by which specific contextual cues, such as sensory stimuli, bladder pressure, social hierarchy and past experience, are integrated to regulate micturition.

The descending limb of the micturition reflex described above that regulates urine release must be coordinated with a central limb that regulates voiding behaviors that determine when and where micturition occurs. For micturition to occur in safe and socially appropriate environments, arousal must be elevated and attention focused on the visceral stimulus. Ongoing behavior unrelated to voiding must be interrupted and replaced by voiding

Figure 2. Relationship between bladder pressure and cortical (CTX) activity in rats exposed to partial bladder outlet obstruction (PBOO) or sham surgery. Middle, Mean bladder pressure over 5–6 micturition cycles and centered at the micturition threshold (time = 0). Heat map above each trace represents bladder pressure for each micturition cycle. For sham, bladder pressure increases gradually and uniformly up to micturition threshold. PBOO1 shows nonmicturition contractions as sporadic episodes (lighter blue blocks interspersed within darker blue) that occur up to micturition threshold. PBOO2 does not exhibit a gradual increase in pressure or nonvoiding contractions. Bottom, Corresponding heat maps represent the mean relative power in different EEG frequency bands (0–20 Hz, ordinate) over the same time period as the bladder pressure recording. In sham rats, a decrease in power in all frequencies (i.e., desynchronization) precedes the micturition threshold and is maintained. PBOO disrupts the relationship between bladder pressure and cortical EEG activity. PBOO1 CTX activity exhibits greater power in higher frequencies (7–10 Hz and 14–15 Hz) that fluctuate like the contractions. CTX activity in PBOO2 is desynchronized throughout the session, and increases in bladder pressure up to the micturition threshold are without further effect. Modified with permission from Rickenbacher et al., (2008).

behaviors that determine temporal and spatial aspects of micturition. All of this must occur in tune with changes in bladder pressure and before micturition. The norepinephrine-containing pontine nucleus, locus ceruleus (LC), is central in a circuit through which bladder sensations can be transmitted to the cortex (Valentino et al., 2011) (Fig. 1). The broad axonal projections arising from LC neurons densely innervate the cortex (Swanson and Hartman, 1976). In anesthetized rats, LC neurons are activated by relatively low magnitudes of bladder or distal colon distention, and this is associated with cortical desynchronization (Elam et al., 1986; Svensson, 1987; Page et al., 1992; Lechner et al., 1997). LC activation by salient sensory stimuli of diverse modalities has been demonstrated to precede the motor response of orientation to the stimulus (Aston-Jones and Bloom, 1981). Pelvic visceral information could be transmitted to the LC from the PMC, as a discrete population of PMC neurons are retrogradely labeled from the LC (Valentino et al., 1996). Alternatively, the periaqueductal gray area, which is hypothesized to relay information about urodynamic status to Barrington's nucleus, may also communicate bladder sensory information to the LC (Bajic et al., 2000; de Groat and Wickens, 2013).

Simultaneous recordings of LC single-unit activity, LC local field potentials, and prefrontal cortical local field potential activity during cystometry in unanesthetized rats are revealing the temporal relationship between urodynamic status and neural activity within different nodes of the pontine-cortical micturition circuit. Preliminary studies in female rats suggest that LC neurons consistently increase their tonic discharge rate 10–30 s before the point of peak bladder pressure and micturition. During this premicturition period, LC local field potential recordings reveal a shift from relatively high-amplitude, low-frequency activity to a prominent theta oscillation and increased LC-PFC coherence. This shift in LC network activity triggers activation of the PFC as indicated by a decrease in amplitude of power at all frequencies, typical of a desynchronized cortical EEG. The desynchronization of cortical activity before micturition has also been reported in male rats (Kiddoo et al., 2006; Rickenbacher et al., 2008). Because LC and cortical responses precede the onset of micturition, it is speculated that these changes in LC-cortical network activity serve to increase arousal and redirect behavior to facilitate appropriate voiding behaviors before urination.

The same network that underlies the adaptive viscerobehavioral response to bladder stimuli described above is also implicated in central symptoms of bladder disorders. Male rats that have surgery for partial bladder outlet obstruction, which has been used to model the partial obstruction that occurs in benign prostatic hypertrophy, develop abnormal urodynamic patterns (Rickenbacher et al., 2008). This model of chronic uropathy altered neuronal firing patterns throughout the pontine-cortical micturition pathway. By 2 weeks after surgery, both PMC and LC neurons became unresponsive to bladder distention, although baseline LC neuronal discharge was elevated above that in sham-operated controls, suggesting a state of hyperarousal in these animals. Consistent with this, from 1 to 4 weeks after surgery, the power spectrum of the cortical EEG shifted to the right toward higher frequencies, indicative of increased arousal, and this effect progressed with time after surgery. A cortical theta oscillation developed in many subjects, particularly those exhibiting nonmicturition contractions, and this appeared to be temporally correlated to the contractions, suggesting that it may be a cortical signature of urgency (Fig. 2). The theta oscillation is likely driven by LC hyperactivity because chemical lesioning of LC-cortical projections prevented its development while leaving the nonmicturition contractions intact. Notably, in this model of bladder pathology, the temporal relationship between bladder pressure and cortical activity becomes disrupted such that the cortical desynchronization that typically precedes micturition is diminished or is not apparent because the cortex is either desynchronized by default or is exhibiting prominent theta oscillations (Fig. 2). These neural alterations that develop in concert with urodynamic alterations in this model of chronic pathology may be the basis of central symptoms of this visceral disease. For example, increased LC tone that contributes to cortical theta oscillations and an enduring desynchronization of cortical EEG could underlie the sensation of urgency, sleep disruption, and anxiety that characterize lower urinary tract disorders (Kirby, 2000; Huang et al., 2017). Importantly, the loss of the cortical response that should precede the micturition event to initiate voiding behaviors may underlie the decreased sensation of bladder fullness that occurs in men with lower urinary tract symptoms and could contribute to enuresis (Griffiths, 1998). This example highlights how circuits that are the foundation of communication between

the brain and viscera support both ongoing physiological functions as well as the expression of comorbid cognitive and visceral symptoms. Dysfunctions of this circuit may be common to other pelvic visceral disorders that are characterized by both central and visceral symptoms, such as irritable bowel syndrome.

Acute stress and inflammation: role of the autonomic nervous system (ANS) and C1 neurons

The C1 neurons are catecholaminergic/glutamatergic/peptidergic cells located in the rostral ventrolateral medulla that regulate both divisions of the ANS via direct projections to preganglionic neurons (Abbott et al., 2013; Guyenet et al., 2013). During hypoxia, general anesthesia, or hypoglycemia, activation of the presympathetic C1 neurons has a homeostatic function that includes minimizing hypotension or restoring blood glucose (Madden et al., 2006; Wenker et al., 2017). C1 cell activation has an allostatic role during pain, fear, restraint, or exercise, increasing glycemia, blood pressure, and respiration (Barna et al., 2012; Chen et al., 2012; Guyenet et al., 2013; Burke et al., 2014; Zhao et al., 2017). The C1 cells may operate as a switchboard for the elaboration of autonomic response patterns. Subsets of presympathetic C1 neurons differentially activate specific groups of preganglionic neurons, thereby producing patterns of sympathetic nerve activation best suited to assist a given behavior or to mitigate the adverse effects of a given stressor (Guyenet et al., 2013). A subset of C1 neurons contribute to the activation of the hypothalamic-pituitary-adrenal axis via direct, and probably indirect, projections to the paraventricular hypothalamic nucleus (Tucker et al., 1987; Schiltz and Sawchenko, 2007). The C1 cells that innervate parasympathetic preganglionic neurons (Loewy et al., 1994; DePuy et al., 2013) must also shape the autonomic responses to stressors, but their exact contribution is unexplored. The activity of C1 neurons is reflexly regulated and under the control of nuclei distributed throughout the brain (Janig, 2006).

The ANS regulates innate immunity. For example, vagal parasympathetic efferent neurons are the efferent arm of an anti-inflammatory reflex mediated by the spleen (Pavlov and Tracey, 2017). Anti-inflammatory reflexes can also be elicited via the sympathetic system. In anesthetized rats, splanchnic nerve section enhances the production of TNF α elicited by lipopolysaccharide injection, suggesting that the production of this inflammatory cytokine is normally restrained by ongoing sympathetic tone (Martelli et al., 2014). Also, sympathetic hyperreflexia following high spinal cord lesions depresses the immune system of mice (Ueno et al., 2016). The central pathways mediating these reflexes are unexplored as are the brain structures that modulate them. In the remainder of this section, we summarize recent evidence that acute stress reduces inflammation and tissue damage via activation of C1 neurons and sympathetic nerves but independently of corticosterone release. This work was done in male mice using renal ischemia-reperfusion (IR) as a model of organ damage.

Renal IR carries a major risk of permanent injury to these organs; the damage is caused or at least exacerbated by inflammation (Inoue and Okusa, 2015). Restraint stress, 24 h before IR, substantially reduced renal damage in mice (Abe et al., 2017). This protective effect was transferable to naive mice by injecting splenocytes harvested from stressed mice and could also be induced by injection of CD4 splenic T cells harvested from control mice and incubated with noradrenaline *in vitro* (Abe et al., 2017). Protection against IR damage was also elicited by moderate opiate activation of C1 cells in conscious mice. The protective effect of restraint stress was greatly attenuated in mice with selective C1 cell lesions or if restraint was applied while C1 neurons

were selectively inhibited (Abe et al., 2017). The protective effect of C1 stimulation disappeared after splenectomy or by silencing the ANS with a ganglionic blocker during C1 stimulation but could not be explained by corticosterone elevation. Finally, the protection persisted after subdiaphragmatic vagotomy. Thus, restraint stress activates a splenic anti-inflammatory mechanism that protects the kidneys from IR injury (Pavlov and Tracey, 2017). In this particular instance, the splenic noradrenergic innervation was activated predominantly via preganglionic sympathetic rather than parasympathetic neurons.

In short, acute stress activates C1 cells causing anti-inflammation and tissue protection. This effect, along with other potentially beneficial consequences of C1 cell stimulation, such as increased vigilance, cardiorespiratory and metabolic stimulation, presumably enhance the chances of surviving an injury.

Anti-inflammatory effects can be elicited by a host of seemingly unrelated interventions, such as stimulation of the vagal nerve, the auricular nerve or somatic nerve afferents via acupuncture (Inoue et al., 2016; Abe et al., 2017; Pavlov and Tracey, 2017). Vagal nerve stimulation attenuates selected signs (circulating cytokines) and symptoms (pain) of rheumatoid arthritis in humans, although the underlying mechanism is not completely elucidated (Koopman et al., 2016). It is possible that the discharge pattern produced by nerve stimulation is distinctly unphysiological and may be interpreted by the CNS as untoward regardless of its origin in the body. A shared effect of peripheral nerve stimulation could be the recruitment of C1 neurons with one of the consequences being activation of the splenic anti-inflammatory pathway via a sympathetic or vagal efferent route or both. In support of this speculation, C1 neurons receive most of their input from the pontomedullary reticular core and respond to innumerable noxious or innocuous stimuli, including restraint stress, infection (lipopolysaccharide, interleukin-1), hypoglycemia, hypotension, and electrical activation of subsets of vagal or somatic sensory afferents (Guyenet et al., 2013; Stornetta et al., 2016; Dempsey et al., 2017).

Nucleus tractus solitarius (NTS)-central amygdala circuitry: target of ethanol

Central regulation of gastric function occurs through sensory vagal afferent projections into the NTS, which synapse onto the dorsal motor nucleus of the vagus to regulate motor vagal efferent signals back to the gut (Travagli et al., 2006). Local GABA_A receptor activity in this circuit is a critical regulator of gastric function, as GABA_A receptor blockade in the NTS of male rats reduced gastric tone and motility (Herman et al., 2009). Gastric-projecting as well as unlabeled NTS neurons receive local GABAergic input that modulates vagal afferent transmission and NTS neuronal excitability in rats and mice (Davis et al., 2004; Glatzer et al., 2007; Herman et al., 2009, 2012). The NTS also makes reciprocal connections with the central amygdala (CeA) (Geerling and Loewy, 2006) and thus is poised to integrate peripheral viscerosensory input with cognitive emotional state. This may be relevant to diseases with comorbid affective and gastrointestinal symptoms (Folks, 2004). However, little is known regarding how alterations in NTS circuitry contribute to neurobehavioral pathology. Here we discuss how ethanol can target this circuitry to influence gastric function and affect.

Given its role in peripheral gastric function, NTS circuits are vulnerable to the effects of ethanol through direct actions in the brain as well as direct effects on the gastrointestinal system. Acute ethanol has been shown to increase local inhibitory transmission and decrease the firing of the majority of NTS neurons (Aimino et

al., 2017). Acute ethanol has also been shown to increase c-fos expression in the NTS (Thiele et al., 1996). Preliminary data suggest that chronic intragastric administration of ethanol and acute withdrawal in male rats increase inhibitory transmission but also result in increased baseline excitability in NTS neurons, suggesting cell-type specific neuroadaptations and/or the recruitment of other signaling systems. Genetically selective manipulation of NTS neurons is necessary to dissect the effects of acute and chronic ethanol on specific components NTS circuitry.

Reciprocal projections link the NTS and the central nucleus of the amygdala (CeA) (Saper, 2002; Geerling and Loewy, 2006), and these are activated by visceral afferent stimulation (McDougall et al., 2017). The CeA functions as an integrative hub that converts emotionally relevant sensory information about the external environment and internal milieu into appropriate behavioral and physiological responses (Gilpin et al., 2015). Although the amygdala is involved in appetitive conditioning processes, it plays a major role in aversive conditioning and negative emotional states. It has been implicated in fear, the behavioral consequences of stress, and alcohol dependence. The CeA is primarily a GABAergic nucleus (Pitkänen and Amaral, 1994; Veinante and Freund-Mercier, 1998), and evidence suggests an involvement of neuroadaptations in CeA GABAergic transmission in the effects of acute ethanol and in the development of alcohol dependence (Roberto et al., 2004, 2010). Acute and chronic ethanol disinhibits CeA output neurons through effects on a local inhibitory microcircuit (Herman et al., 2013; Herman and Roberto., 2016; Herman et al., 2016). Chronic ethanol exposure increases ambient GABA in the CeA (Roberto et al., 2004), although the specific source of this GABA is not yet identified. Whereas attention has focused on forebrain afferents to the CeA (PFC and adjacent amygdala structures), the role of brainstem afferents, such as the NTS, has been relatively neglected.

Preliminary studies, examining the effects of acute and chronic ethanol on CeA-projecting NTS neurons from male rats, suggest that these neurons are under a significant amount of inhibitory control that is enhanced by ethanol exposure. Acute ethanol decreased the firing of most CeA-projecting NTS neurons, consistent with previous work (Aimino et al., 2017). Chronic ethanol increased the baseline inhibitory tone of CeA-projecting NTS neurons; however, this appeared to occur in parallel with a paradoxical increase in basal firing, suggesting the possibility of negative feedback and/or compensatory mechanisms. Collectively, these preliminary data suggest that acute and chronic ethanol exposure alters the function of the NTS and NTS-CeA circuits. Increased inhibitory tone in the NTS could dampen the impact of afferent signals important for the maintenance of physiological gastric control. Increased excitability of NTS neurons following chronic ethanol exposure would simultaneously result in reduced central control over the gastrointestinal system, via increased inhibition of dorsal motor nucleus of the vagus motor neurons, and increased inhibitory drive to the CeA. This has potentially negative consequences for the central control of gastric function and the relay of visceral input to the amygdala, which could collectively enhance the aversive outcomes of chronic ethanol exposure and contribute to the development of alcohol dependence.

Conclusions

In conclusion, here we highlighted examples of circuits that support the interdependence between the brain and specific peripheral organs, with the functional endpoint of one organ (spleen) being regulation of an immune response. These circuits allow for the integration of visceral information with ongoing multimodal

sensory signals and the computation of a response that coordinates visceral activity with motor activity, behavior, and cognition. The examples presented here demonstrate how the ongoing activity of these circuits assures optimal functioning of the whole organism but can also be conduits for the synchronized expression of pathology at central and peripheral sites. Continuing advances in circuit dissection, manipulation, and neural network recordings will provide the necessary information to extend the brain atlas to the periphery.

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