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THE NEUROPHYSIOLOGICAL SUBSTRATES OF STRESS RESPONSE AND THEIR RELEVANCE TO RESILIENCY IN TRAUMA

A Thesis in

Neuroscience

by

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ABSTRACT

This thesis begins as a literature review of the neurophysiological substrates of stress response, with a particular focus on the autonomic and endocrine components of stress response and the brain regions thought to subserve their function. After defining the physiology and neural networks implicated in stress response and the hierarchy along which stress responses are believed to occur, we will depart from the literature review format of this thesis to examine the author's own work developing a novel measure for quantifying fear learning in fear extinction training and testing paradigms. The development of this measure may help avoid the procedure of fasting for the FC/FE experiment paradigm currently used in multiple labs, which by itself can introduce neurophysiological confounds into stress studies. Finally, the application of this measure to existing data has led to interesting observations into possible physiological substrates of vulnerability to posttraumatic stress disorder. These observations will be discussed in the context of the initial literature review. We will argue that stress is experienced in the body as a variety of orchestrated and dynamic physiological states that may become pathogenic when homeorhesis between the endocrine and autonomic systems that subserve them becomes compromised.

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Chapter 1
Historical perspectives: A psychophysiological view of stress

"*It is the fixity of the milieu intérieur which is the condition for all free and independent life. All vital mechanisms, however varied they may be, have only one object, that of preserving constant the conditions of life in the internal environment."*

- *Claude Bernard, 1878*

Historical investigations into the stress response were investigations into compensatory physiological response to bodily injury. Claude Bernard (1878) coined the concept of a *milieu intérieur*, a fluid matrix produced and maintained at optimal set points through compensatory reactions to challenge. Bernard's words defined the concept and the importance of homeostasis for the first time. Walter Cannon was the first to define a compensatory reaction in the adrenal glands in response to both emotional and systemic challenge. This response, he explained, lessened muscle fatigue and facilitated muscular effort, while enhancing coagulation. Cannon defined a non-specific stress response that prepared the body for extraordinary physical effort in spite of hemorrhagic injury, a response that is now referred to as fight-or-flight (1919).

In 1946, Hans Selye elucidated his model of the adaptive course of stress response on the body, going so far as to define a 'General Adaptation Syndrome' of multi-system pathology and exhaustion following an overwhelming of the body's homeostatic resources. This model was not so much a theoretical extrapolation of Cannon's findings regarding fight-or-flight response as it was a systemic characterization of the effects of overwhelming stress on every physiological system in the body. Important, the brain was missing from the model of the general adaptation syndrome, in which it is defined as an "unknown pathway" controlling the pituitary gland.

Selye defined the general adaptation syndrome as a so-called "disease of adaptation" emerging from a tripartite "alarm response reaction to acute or prolonged exposure to sublethal doses of a stressor". This work characterizes pathology emerging from non-specific stress as a syndrome in which the cost of adaptation to stress eventually becomes too high for the body to

pay. The later stage, which Selye called the “stage of exhaustion”, was described as lethal. General adaptation syndrome included impaired cardiovascular, gastrointestinal, renal, reproductive, immune, metabolic, and endocrine function, as well as in the depletion of a nebulous concept that Selye coined “adaptation energy”. Much in the same way that Bernard’s description of the *milieu intérieur* elucidated a governing principle of health physiological function, Selye’s description of the dynamics of physiological breakdown in the general adaptation syndrome stands on its own as a framework in which to understand stress-related pathology:

> “It has been found that if an animal is continuously treated with the same, sublethal, daily dose of one alarming stimulus, its resistance to an additional minimum lethal dose of that same stimulus decreases during the shock phase and gradually returns to, or above normal, in the counter-shock phase of the alarm reaction. During the subsequent stage of resistance, a considerable amount of specific adaptation to this same alarming stimulus becomes evident; yet if exposure is continued. over a period of many weeks or months, eventually this acquired adaptation breaks down. Apparently, even a fully inured organism cannot indefinitely maintain its adaptation when continuously exposed to a great amount of stress. It is this observation which led to the concept of "adaptation energy." Apparently, under the influence of continuous adaptive work, the adaptability or "adaptation energy" of the organism is eventually exhausted. The time at which this breakdown of adaptation occurs, is referred to as the "stage of exhaustion." (1946)

This characterization of stress-induced pathophysiology introduces an important idea that has been somewhat lost in modern investigations into the neurological substrates of psychopathology: By Selye’s characterization, it is not so much the activity of any organ system under stress that induces the lethal stage of exhaustion, but the failure of adaptations within those systems to maintain homeostasis over the long term. This notion of adaptation energy has been brought into modern light by Bruce McEwen’s conceptualization of allostasis and allostatic load (McEwen, 1998). Allostasis is defined as the body’s ability to maintain homeostasis through change, closely linked to the concepts of homeostasis and homeorhesis. For this thesis, homeostasis will be defined as a static state in which a physiological variable is maintained within a range optimal for maintaining healthy function. Homeorhesis, by contrast, describes the

dynamic adaptations a physiological system will make to respond to environmental challenges in a way that moves that system toward homeostasis. In this way, homeorhesis may be understood as the process by which allostasis may occur.

Allostatic load offers a quantifiable conceptualization of what Selye describes as "exhausted adaptation energy" (1946). McEwen describes allostatic load as the consequence of a dysfunctional stress response or from inadequate recovery from stress, characterized in part by excess stress hormone levels in serum. For reasons that will become more apparent throughout this thesis, allostatic load can also be measured by indicators of cardiovascular dysfunction, like blood pressure and HDL cholesterol, and dysregulated metabolic function, such as waist-to-hip measurements and serum levels of glucose-bound hemoglobin (McEwen 1999). Taken together, these variables are used as a measurement of the tax of unreconciled stress on the body, the tax of overwhelming stress on Selye's adaptation energy.

Extending the general adaptation syndrome to include the neurological substrates of stress-related pathology, especially psychopathology, has proven to be extraordinarily difficult. The central nervous system (CNS) controls threat detection and perception, orchestrates autonomic and endocrine stress response, and demonstrates widely-distributed plasticity in response to psychogenic and systemic stress (Gonzalez-Lima & Schiech 1984, 1986). While much progress has been made since the time of Selye in elucidating the neurological substrates that control stress response, the functional role of these networks in psychiatric illness, particularly in psychiatric illness linked to trauma, is less clear. Most efforts to elucidate the neurological substrates of psychological trauma focus on those brain regions most implicated in the behaviors that characterize cognitive and affective components of disorder; for example, the medial prefrontal cortex-amygdala (mPFC-Amyg) circuit in impaired fear learning. This research has been extremely useful in elucidating how these regions subserve affective and cognitive phenomena, but in limiting our investigation to these regions in pathology, trauma researchers

risk losing site of the most fundamentally important aspect of any illness: disrupted homeostasis. We risk losing our appreciation for how fundamental homeostatic disruption as a consequence of adaptation to stress must be, by definition, to stress-related pathology.

Dysregulation of homeostasis within the autonomic nervous system has been hypothesized as a substrate of psychological trauma. Robert Scaer hypothesizes that dysregulated autonomic oscillations represent the underlying neurological substrate of trauma in humans (2001). A human freeze response to inescapable and potentially lethal stress has been characterized as an extreme state of altered consciousness, a cessation of interoception and somatosensation, and a loss of motor control (Levine 1997). Based on his own clinical experience, Levine speculates that traumatized humans are psychologically sick because they are effectively still trapped in that freeze state, though this speculation lacks the physiological specificity necessary to generate testable hypotheses. In animals, however, different behavioral responses to stress have been linked to specific autonomic and endocrine profiles (Porges 1995, Koolhaas et al 1999, Hagenaars et al 2014). Inescapable and lethal stress has elicited a state of tonic immobility in animals in which autonomic regulation of the cardiovascular systems seems particularly vulnerable (Hofer 1970). Together, these observations suggest that impaired homeorhesis within the extraordinary demands of extreme stress, manifest in a dysregulation of the autonomic and endocrine substrates that subserve that homeorhesis, may be critical to the etiology of trauma.

This thesis represents an effort to provide a more holistic view of the psychopathology associated with stress exposure by first examining the autonomic and endocrine components of a stress response in literature review format. We will argue based on the literature that a stress response is a dynamic and in constant, feedbacking communication with each other. It is an attempt to reconcile the spirit of Selye's original general adaptation syndrome with anxiety in animals and trauma in humans following exposure to overwhelming stress. For the purposes of

this thesis, stress will be defined as either a challenge or the threat of a challenge to homeostasis. This definition treats systemic stressors, a challenge within the body such as hypoxemia or pain, as equal to psychogenic stressors, a challenge in the environment. This is because, as Cannon and Selye demonstrated, the non-specific physiological consequences of systemic stress are the same as non-specific physiological consequences to psychogenic stress. When a systemic or psychogenic stressor is known to differentially affect the function of any component of autonomic or endocrine stress response, that will be stated explicitly.

We will examine existing literature to characterize stress response as a dynamic hierarchy of autonomic and endocrine processes. We will begin with fight-or-flight response and characterize brain structures responsible for neural control of the sympathetic nervous system and HPA axis, and the action of the two systems on effector tissues. We will then do the same for the parasympathetic nervous system, with a special focus on Stephen Porges' Polyvagal Theory as it contributes to a more nuanced understanding of the stress response. We will finally provide a model of neurogenic hypertension in sleep apnea to describe how pathology following overwhelming stress may stem from disrupted homeostasis within physiological systems that subserve stress response.

After examining the autonomic and endocrine components of the stress response and the brain regions that control them most directly, we will discuss brain regions implicated in the neural control of the autonomic nervous system more broadly and how their function may become dysregulated in trauma with respect to peripheral autonomic activity. Finally, we will assert that stress responses tend to fall on a hierarchy, wherein some responses are more extreme and physiologically vulnerable states than others.

In Chapter 5, we will depart from the literature review format of this thesis to first examine the author's own work developing a novel measure for quantifying fear learning in fear extinction training and testing paradigms. The development of this measure might help avoid the

procedure of fasting for the FC/FE experiment paradigm currently used in multiple labs, which by itself can be a confounder for stress studies. Finally, the application of this measure to existing data led to interesting observations into possible physiological substrates of vulnerability to PTSD. These observations will be discussed in the context of the initial literature review.

Chapter 2

Fight or Flight: The Sympathetic Nervous System and HPA Axis

In the conventional characterization of stress as arousal, the combined action of the Sympathetic Nervous System (SNS) and Hypothalamic-Pituitary-Adrenal Axis (HPA Axis) is credited with fully contributing the autonomic and endocrine components of a stress response. While this thesis will argue in subsequent chapters that the classical characterization of autonomic stress response as a purely sympathetic and endocrine phenomenon neglects important parasympathetic contributions (Porges 1995, 2007), there is no question of the importance of the contributions of the SNS and HPA Axis in orchestrating response and adaptation to both systemic and psychogenic stress. Though the sympathetic component of the stress response occurs along neural pathways and endocrine signals are circulated systemically via the blood stream, these two aspects of the classical stress response feedback on each other both peripherally and in the brain. The sympathetic component of the classical fight-or-flight response is almost immediate and can, but need not, be followed by more long-term endocrine stress response via the HPA axis, both of which will be discussed in more detail below.

Actions of the Sympathetic Nervous System on Effector Tissues

The Sympathetic Nervous System (SNS) is the branch of the autonomic nervous system responsible for the mobilization of resources necessary to meet the increases in metabolic demand associated with fight and flight. Pre and postganglionic sympathetic nerves use norepinephrine as their primary neurotransmitter (Tortora & Derrickson 2012). Preganglionic fibers of the SNS emerge from the thoracic and lumbar segments of the spinal cord, giving the SNS its alternative

name of the “thoracolumbar division” of the autonomic nervous system. Sympathetic ganglia form close to the spinal cord and postganglionic fibers widely distribute to a variety of effector tissues, including the chromaffin cells of the adrenal medulla, which then release catecholamines -- 80% epinephrine, 20% norepinephrine, trace amounts dopamine -- into circulation. The SNS innervates the skin hair follicles, pupillary muscles, vasculature, heart, lungs, liver, digestive organs, thymus, sweat glands, adipose tissue, bladder, genitalia, and rectum. The gross actions of SNS mobilize metabolic resources and facilitate the redirection of blood supply away from organs whose functions are not critical components to fight and flight. Both fight and flight constitute intense aerobic activity conditions in which peak muscle perfusion must increase dramatically. Ultrasound Doppler measurements of blood flow in the femoral artery and vein during isolated knee-extensor exercise have demonstrated that individual muscle perfusion in exercise can increase by up to 100-fold that of rest (from 0.03 liters at rest to 2-3 liters/minute at peak intensity) to meet metabolic need (Saltin et al 1998). In whole-body aerobic exercise, which is a reasonable model of both fight and flight responses to psychogenic stress, about 90% of the body’s blood supply must be redistributed to skeletal muscle, and cardiac output must at least quadruple (Joyner & Casey, 2015. See Figure 2-1).

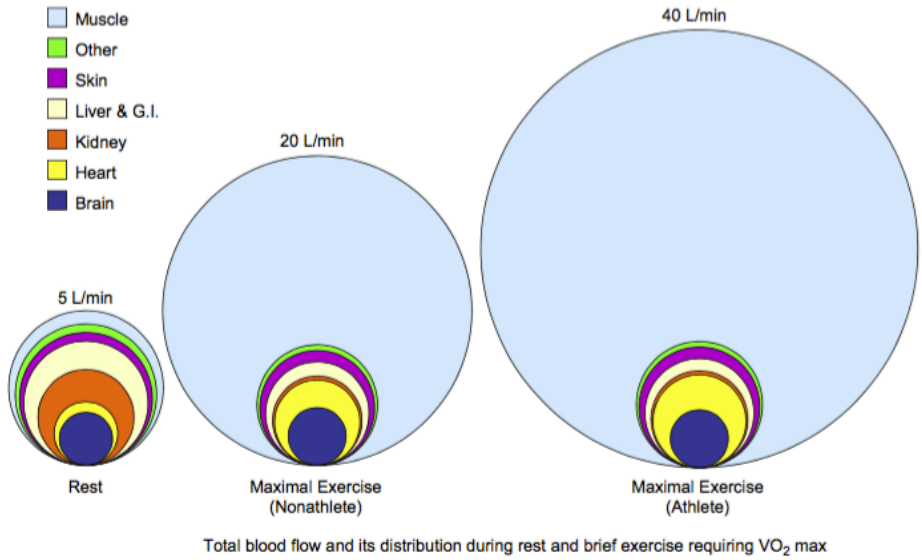

Figure **2-1**: Idealized distribution of blood flow at rest, with cardiac output at 5 liters/minute, and at maximal aerobic intensity for a nonathlete, with cardiac output at 20 liters/minute. In elite endurance athletes, cardiac output at maximal intensities can be up to 8x that of rest, at 40 liters/minutes (Joyner & Casey, 2015). For the purposes of this thesis, the demands of maximal aerobic exercise can be understood as the same demands encountered in executing either a fight or flight response to stress.

To allow this extraordinary redistribution and mobilization of resources, the SNS halts unnecessary bodily functions, like digestion, and makes oxygen and blood more readily available. The airways dilate, allowing faster movement of air in and out of the lungs to increase the amount of oxygen diffusion across its partial pressure gradient into the blood supply (In aerobic exercise, oxygen consumption can increase by 10 to 15-fold that of rest). SNS activity on B adrenergic receptors in the atrial and ventricular muscle fibers of the heart increase the heart's contractile force, and innervation of the SA/AV nodes drives up heartrate, with the net effect of increasing cardiac output. The SNS stimulates the release of vasopressin, a vasoconstrictor and antidiuretic hormone, and directly causes systemic vasoconstriction, allowing mean arterial blood pressure to remain within a homeostatic range and ensuring continuous perfusion of vital organs, including the brain (See Figure 2-1), during high intensities of physical effort. There are conflicting ideas about the effect of sympathetic activity on the vasculature supplying skeletal muscle. In exercise,

it is now thought that the SNS effects system-wide vasoconstriction, which is overwhelmed by the vasodilatory action of additional factors released by physical activity. However, there is evidence of a sympathetically-mediated increase in hindlimb blood flow from hypothalamic stimulation alone (Hagenaars et al 2014).

The mobilization of metabolic resources in anticipation of or in reaction to high demand is the primary task of the SNS. Increased blood supply and oxygen availability are necessary but insufficient to meet this goal – fuel, in the form of glucose, must be mobilized. To this end, the SNS innervates the liver, pancreas and adipose tissue. Sympathetic stimulation of the liver initiates hepatic glycogenolysis, the conversion of stored glycogen into glucose, and gluconeogenesis, the conversion of noncarbohydrates (e.g. amino acids, lactic acid) into glucose, which are released into the blood stream. The pancreas releases glucagon, a peptide that stimulates glycogenolysis, and suppresses insulin secretion from the islets of Langerhan, preventing glucose from being reabsorbed into tissue.

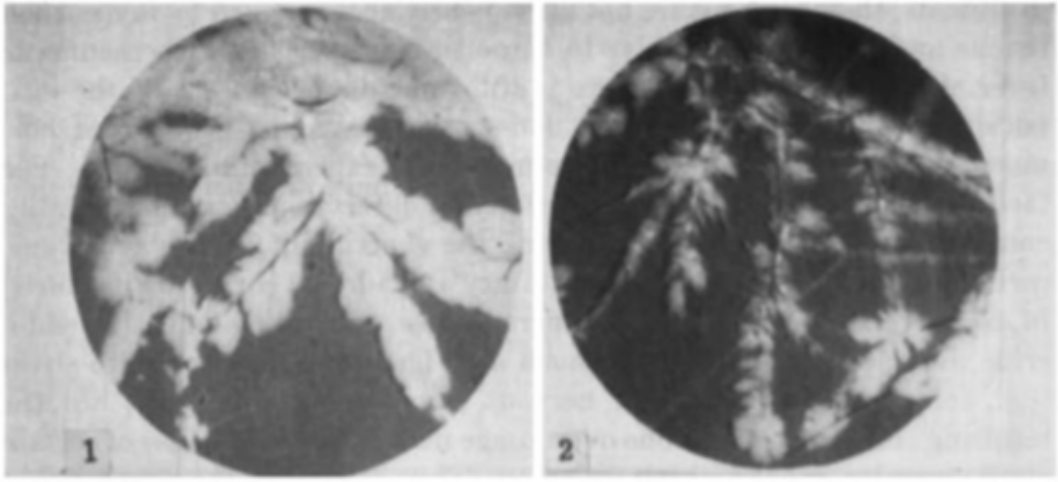

Figure **2-2**: Selye's macroscopic documentation of a normal rat pancreas (1) and stressed pancreas (2) showing atrophy of the insulin-secreting islets of Langerhan after exposure to toxic doses of mAChR antagonist Atropine (1946).

Sympathetic stimulation of B1 adrenergic receptors in adipose tissue initiates lipolysis, the breakdown of stored triglycerides and the release of long chain fatty acids into the blood stream.

While the SNS is anatomically designed to facilitate a more widely distributed activation than the PNS (Chapter 3), there is still evidence of targeted sympathetic function in response to stress and pathology. For example, hypoglycemia, a systemic stressor, results in an isolated sympathetic response to the adrenals and liver, without affecting the heart. Metabolic syndrome in humans (e.g. diabetes) is related to sympathetic overactivity in skeletal muscle, but not in skin (O'Hare & Zsombok 2016). For a more detailed description of the peripheral effects of Sympathetic activation, see table 2-1.

Table **2-1**: Sympethetic actions, the neural reflexes that control them, and associated symptoms in trauma (Adapted from Tortora & Derrickson 2012, pgs. 585-599).

Sympathetic Ganglion or relevant plexus	**Postganglionic effector tissues and receptor type**	**Function (bold functions most classically associated with acute SNS-mediated fight-or-flight response)**	**Mechanism of feedback for key actions (to CNS where appropriate)**	**Associated symptoms in trauma (Scaer 2001)**
Superior Cervical Ganglion	1. Atrial muscle fibers of the Heart (β1) 2. Ventricular muscle fibers of the Heart (via Cardiac Plexus, β1) 3. Pineal Gland (following PVN-driven activation of the supraoptic nucleus of the hypothalamus) (β) 4. Eye (α, β2, CN III efferents via ciliary ganglion, CN V: V1 efferents via SCG and	**1,2. Increased force of heart contraction** **3. Secretion of AVP (vasopressin), a vasoconstrictor that increases arterial blood pressure** **4. Pupil dilation**, ciliary muscle relaxation to adjust shape of lens for distant vision 5. Slight secretion of tears 6. Inhibition of saliva secretion 7. Constriction of mucosal arterioles 8. Sympathectomy in rats reduces thymic weight and cellularity, increases apoptosis, and attenuates number of proliferating T	**1,2, 3. Baroreceptor reflex:** Baroreceptors primarily in the carotid sinus and aortic arch respond to increase in blood pressure by increasing firing rate. Baroreceptors in the carotid sinus are innervated by CN IX. Aortic Arch baroreceptors are innervated by CN X. Afferents from CN IX and CN X travel to NTS, which activates CVLM, inhibiting RVLM, resulting in sympathetic withdrawal. NTS also activates NA and DMNX, increasing efferent CN X activity and increasing parasympathetic tone. (Alloway & Pritchard, 2007).	1, 2, 3. Hypertension, atherosclerosis 4. Ocular divergence

	carotid plexus to iris dilator, Gray 1918 plate 840) 5. Lacrimal Gland (α) 6. Parotid Gland (α1) 7. Mucous membrane of nose and palate (α1) 8. Thymus (T1-T7, β, Nance & Sanders 2007, Trotter et al. 2007)	lymphocytes (β, nAChR, Bellinger et al. 2013).	4. Parasympathetic-mediated pupil constriction (See Table 2)	
Middle Cervical Ganglion	1. Sinoatrial and Atrioventricular nodes (β1)	**1. Increased heart rate**	**1. Baroreceptor reflex**	1. Hypertension, tachycardia
Inferior Cervical Ganlion, Cardiac Plexus	1. Ventricular muscle fibers of the heart (via Cardiac Plexus, β1)	**1. Increased force of heart contraction**	**1. Baroreceptor reflex**	1. Hypertension, atherosclerosis
T1, T2 ganglia and Pulmonary Plexus	1. Trachea, bronchi, lungs (β2)	**1a. The airways dilate, allowing faster movement of air into and out of the lungs** **1b. Sympathetic-mediated increase of inspiratory rate and onset of active exhalation in response to hypoxia**	1a**. Hering–Breuer inflation reflex**: Stretch mechanoreceptors in lungs project through CN X to inspiratory area of medulla and apneustic canter of the pons to inhibit further inspiration 1b. **Hypoxic chemoreceptor reflex (Chapter 3)**: Reduced blood PO2 or increased presence of reactive oxygen species produces CO-mediated disinhibition of excitatory hydrogen sulfide production in Glomus Type 1 chemoreceptors in the carotid body, increasing inspiratory rate, active exhilation, heart rate, blood pressure	1b. In Chronic Intermittent Hypoxia (an animal model of apnea, see Chapter 3) Hypoxia Inducible Factor HIIF-1α/1β signaling increases the production of reactive oxygen species (ROS), signaling chronic catecholamine release from adrenal medullae, inducing hypertension. The increase of ROS changes

			and sympathetic tone in abdominal muscles. The pons and multiple brainstem areas, including Pre-Bötzinger (Moore et al 2013) the Retrotrapezoidal-Parafacial Respiratory Group and the Bötzinger Complex are implicated (Mores 2012).	the redox state at the carotid body, likely 'reprogramming' the homeostatic set point for optimal blood pressure.
Celiac Ganglion via Greater Splanchnic Nerve	1. Liver, gallbladder and bile ducts (α, β2) 2. Stomach, spleen, pancreas (α1, α2, β2) 3. Chromaffin cells of the adrenal medullae (nAChR) 4. Superior mesenteric ganglion 5. Abdominal vasculature (α1, β2)	1. **Glycogenolysis** (conversion of glycogen to glucose) and **release of glucose into the blood stream**, **gluconeogenesis** (conversion of noncarbohydrates to glucose), decreased bile secretion 2. **Decreased motility and tone of stomach and GI tract**, **contraction of sphincters**, blood stored in spleen put back into circulation and **splenic macrophage secretion of TNF-α dramatically attenuated,** Il-1β somewhat decreased **(Nance & Sanders 2007)**, **inhibition of insulin production** and **increase in glucagon production** to initiate hepatic glycogenolysis **3. Secretion of catecholamines (80% epinephrine, 20% norepinephrine, trace amounts of dopamine)** **5. Abdominal viscera arterioles constrict**	**1. Ventromedial hypothalamic nucleus — splanchnic nerve system** **Glucosensitive neurons have been found in the Lateral Hypothalamic Area, an orexic (i.e. hunger-stimulating) area of the hypothalamus, and ventromedial hypothalamic nucleus. Hepatic glucose injection inhibits glucosensitive neurons in the LHA, possibly due to noradrenergic modulation. Hepatic vagal glucosensory afferents facilitate LHA GS neuron firing, possibly via the NTS and parabrachial nucleus. GS neurons have also been found in the NTS,** **2. NE release in rat spleen self-inhibits via action on prejunctional α receptors**	1. High blood sugar and insulin resistance, Type II diabetes

Aorticorenal Ganglion via the Lesser splanchnic nerve (T10-T11)	1. Ureter (α1) 2. Kidney (β1)	1. Increases motility 2. Secretion of renin		
Superior Mesenteric Ganglion via Aorticorenal ganglion (T10-T11)	1. Small intestine, ascending colon, transverse colon (α1, α2, β2)	**1. Decreased motility and tone of stomach and GI tract**, **contraction of sphincters**		
Renal Ganglion via the Least splanchnic nerve (T12)	1. Ureter (α1) 2. Kidney arterioles (α1)	1. Increases motility 2. constriction of renal arterioles , **release of renin**		
Inferior Mesenteric Ganglion vi a the Lumbar splanchnic nerve (L1-3)	1. Urinary bladder (α1, β2) 2. External genitals (α1) 3. Uterus (α1, β2) 4. Rectum (Alloway & Pritchard, 2007)	1. Relaxation of muscular wall, contraction of internal urethral sphincter 2. Contraction of smooth muscle 3. Promotes contraction in pregnant women, inhibits contraction in nonpregnant women 4. Sphincter contraction		
All	1. Skin hair follicles, arrector pili muscles 2. Systemic veins 3. Skeletal muscle arterioles 4. Adipose tissue 5. Sweat glands	1. Erection of hairs, resulting in goosebumps **2,3. Systemic vasoconstriction** **4. Lipolysis (breakdown of triglycerides into fatty acid and glycerol, β1), release of fatty acids into blood stream (β1, β3)** and **thermogenesis (**brown adipose tissue, β3) 5. Increased sweating (mAChR)	**2,3. Baroreflex** **4. Thermoregulatory neurons sensitive to body temperature are located in the Preoptic Area of the Hypothalamus (POA)**	2,3. Hypertension and artherosclerosis 4. Impaired thermoregulation (Natarajan et al 2015)

The HPA Axis and the endocrine stress response

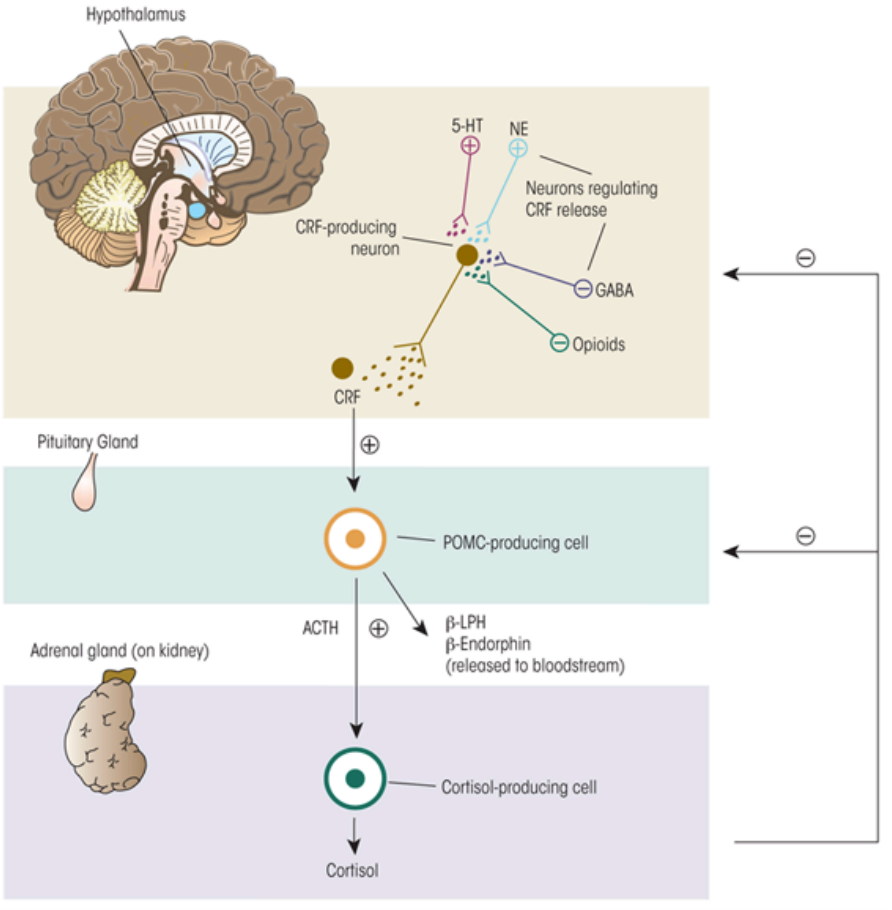

Figure **2-2**: The HPA Axis (Stephens & Wand, 2012). Excitatory seretonergic (5-HT) and noradregerneric (NE) projections onto Corticotrophin Releasing Hormone (CRH or CRF)-producing neurons in the Periventricular Nucleus of the Hypothalamus (PVN) stimulate the release of CRH, which in turn signals corticotrope cells in the Anterior Pituitary to release Adrenocorticotropic Releasing Hormone (ACTH) and increase transcription of its precursor,

proopiomelanocortin (POMC) (Aguira 2012). Once in the blood stream, ACTH travels to the adrenal cortex, where it stimulates the release of CORT (in humans, Cortisol).

The HPA Axis constitutes the body's endocrine response to stress. Here again, stress may be thought of as any psychogenic or systemic stressor, including low serum levels of cortisol (Tortora & Derrickson 2012). An endocrine stress response causes glucocorticoid secretion release into systemic circulation, altering metabolism and immunity in ways that complement the action of the sympathetic nervous system (SNS) and facilitate recovery over the long-term (Sapolsky et al 2000). First, the paraventricular nucleus of the hypothalamus (PVN) secretes corticotrophin releasing hormone or factor (CRH/CRF are used synonymously in the literature) and vasopressin into pituitary portal vessel circulation, where they travel to the pituitary and bind to CRH1 and V1b receptors, respectively. The release of CRH suppresses gonadotropic releasing hormone from the median preoptic area of the hypothalamus and the systemic circulation of luteinizing hormone and follicle-stimulating hormone. The release of prolactin and growth hormone from the pituitary is stimulated. Binding of CHR1 receptors upregulates the transcription of proopiomelanocortin, the precursor to adrenocorticotropic releasing hormone (ACTH). After ~10 seconds (Sapolsky et al 2000), CRH and vasopressin binding in the pituitary work in parallel to stimulate the release of ACTH into circulation, which targets the zona fasciculata of the adrenal cortex, stimulating the secretion of glucocorticoids (Aguira 2012), 95% cortisol in humans, after approximately 10 minutes following stress exposure (Sapolsky et al 2000).

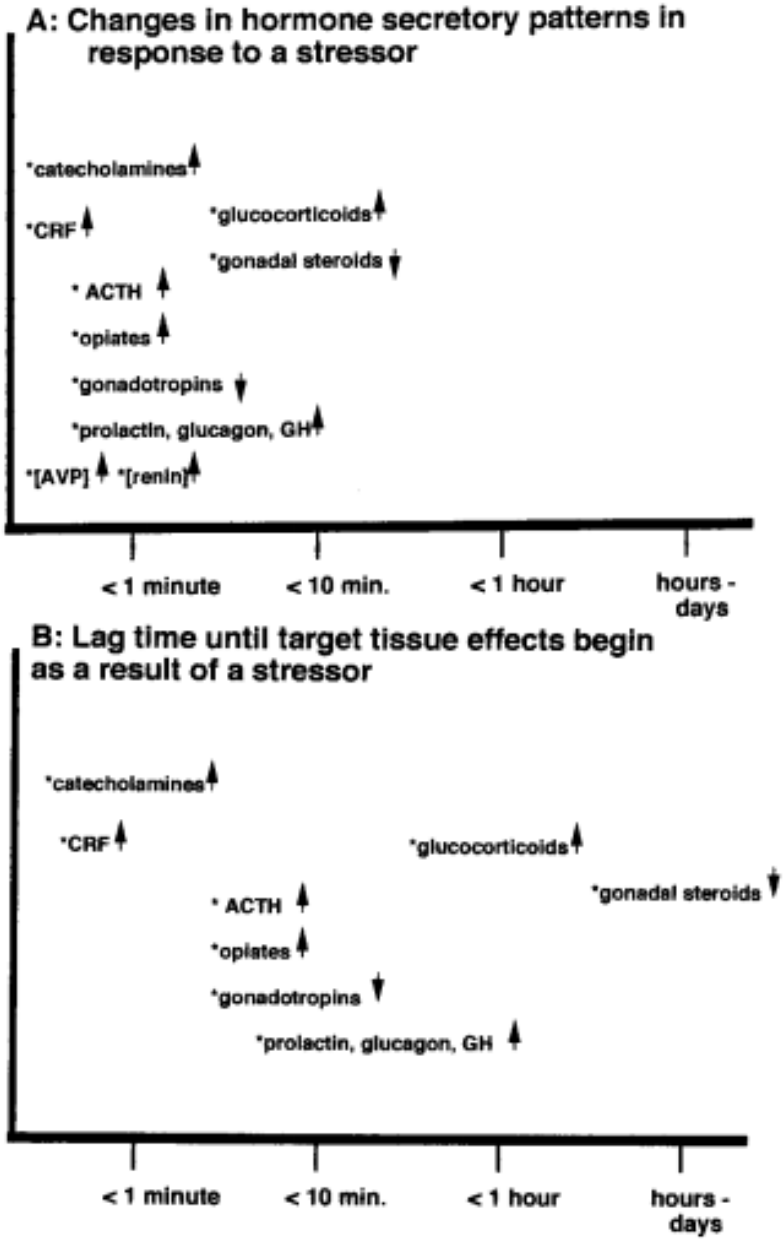

Figure **2-3**: Time course of hormonal release in response to stress and lag time until target tissue becomes effected (Sapolsky et al 2000).

CRH and Glucocorticoids (GCs) show a wide range of physiological effects, especially on the heart, on metabolism and on immunity. CRH acts as a neurotransmitter within the brain to modulate the function of the sympathetic nervous system (SNS). Therefore, on an almost

immediate time scale, CRH works within the brain to enhance the release of catecholamines from the adrenal medullae and potentiate the cardioexcitatory and vasoconstrictive action of the SNS. GC action on the cardiovascular system has an intensifying effect on a longer time scale, increasing the sensitivity of vasculature to catecholamines by enhancing the binding capacity of epinephrine to B-adrenergic receptors. GCs also show a permissive action on sympathetic action in the pulmonary system, enhancing the airway dilation and increase in respiratory rate. This generally permissive effect of GCs on catecholamine action is not ubiquitous; GCs inhibit catecholamine action in response to some stressors. In the high concentrations released after stress exposure, on the time frame of hours after the fact, GCs inhibit the release of vasopressin, ensuring perforation of vital organs (Sapolsky et al 2000).

GCs upregulate protein catabolism, or breakdown, especially in skeletal muscle, releasing amino acids into circulation for ATP production or for the synthesis of new proteins. Protein catabolism likely facilitates muscle recovery after a fight or flight response. Like catecholamines, in lower concentrations GCs stimulate the release of glucagon from the liver (Sapolsky et al 2000). They stimulate hepatic gluconeogenesis and trigger the breakdown of triglycerides in adipose tissue, releasing glucose and fatty acids into circulation, again for ATP production (Tortora & Derrickson 2012). Lambillotte et al. have shown dose-dependent and only slowly reversible (on the time scale of hours) GC inhibition of insulin secretion from mouse islets (1997), mimicking the macroscopic effect of catcholamines on the pancreas (Selye 1946). It is not the case, however, that GCs serve only to accelerate the effects of an increase in sympathetic tone on metabolism; GCs show an antagonistic action to catecholamines on hepatic glycogen stores. While catecholamines stimulate glycogenolysis, GCs stimulate glycogenesis and the deposition of glycogen in tissue in a protein-synthesis dependent manner (Hers 1985), consolidating glycogen stores in hepatic and muscle tissue, important for recovery from aerobic activity (e.g. fight or flight, Duclos 2010). On a molecular level, GCs are able to maintain glycogen synthase in

its active form even in conditions where it would normally be inactive, e.g. when blood sugar is low. The gluconeogenolytic action of GCs is a permissive effect driven by CRH release and low levels of GCs already present in serum before the stressor. It therefore occurs less than one minute after stress exposure (Sapolsky et al 2000). The effect of glycogen store consolidation can be attributed to the high levels of GCs present after stress exposure. This effect takes place on the time scale of hours, experimentally it requires around 3 hours to become apparent in the liver tissue of rats and mice (Hers 1985). This makes physiological sense; While gluconeogenesis is necessary for immediate energy mobilization during fight or flight, glycogenesis is necessary to replenish glycogen stores in the liver and skeletal muscle during a recovery period.

The HPA axis also exerts an immunosuppressive and anti-inflammatory effects on immune function. Endocrine stress response has been associated with thymus inversion (see Figure 2-3) corresponding to an immediate, massive immune response followed by immunosuppression (Selye 1946). Peripherally, for example, GCs may bind to glucocorticoid receptors in target cell cytoplasm, which then dimerize, forming a complex with two molecules of heat shock protein 90, and translocate into the cell nucleus. There, they further bind to glucocorticoid recognition sequences (GREs), increasing the transcription of anti-inflammatory proteins (e.g. IL-10, IL-1RA) and pro-inflammatory cytokines (e.g. IL-1, IL-6, TNF-a) (Barnes 1998). This suppression of cytokine release is coupled with the suppression of histamines, nitric oxide and other inflammatory mediators. There is some evidence that *in vivo* this suppression is targeted, allowing GCs to facilitate an immune response against a specific pathogen while suppressing extraneous factors that may, if left unchecked, provoke an autoimmune reaction (Sapolsky et al 2000).

In addition to immune and metabolic recovery, high levels GCs after stress response have been shown to induce genomic changes thought to be responsible for specific adaptations to stress (Joëls & Baram 2009).

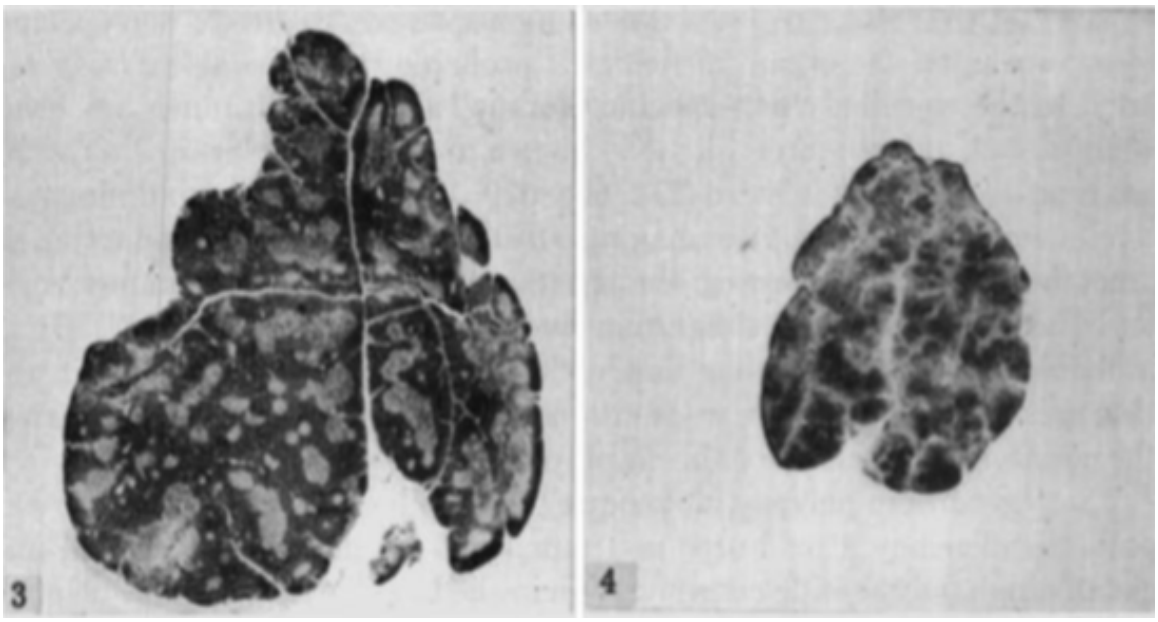

Figure **2-3**: Selye's documentation of a normal rat thymus gland (3) and thymus gland after acute stress (4) showing thymal inversion response (1946).

Neural Control of the Sympathetic Nervous System and HPA Axis

The Sympathetic Nervous System (SNS), working in concert with the Hypothalamic-Pituitary-Adrenal Axis (HPA Axis), has been discussed above as a massive mobilizer of energy in effector tissues in order to prepare the body for the extraordinary demands of fight and flight stress responses. It is important to note, as is well-exemplified by Fig 2-1, that the physiological demands of fight and flight result in marked divergences from homeostatic norms. In his theory of allostasis, Bruce McEwen discusses the importance of a recovery period following stress in preventing the pathogenic effects of allostatic load for this reason; After stress, the effector tissues of the sympathetic nervous system must be allowed to recover. This includes recovery of

respiratory rate, airway dilation, cardiac output, metabolism, and glucocorticoid levels to within a homeostatic range.

There are feedback mechanisms for restoring homeostasis after a stress response built into the architecture of the CNS. Reflexive antagonism from the Parasympathetic Nervous System (PNS), cortical structures, and the hypothalamus all play important roles in modulating sympathetic and endocrine stress response and facilitating recovery. Glucocorticoid receptor (GR), Minerocorticoid receptors (MR) and Corticotropin-Releasing Hormone Receptors (CRHR) are expressed in many brain regions whose activity can modulate HPA-Axis function, providing a mechanism for negative feedback. Many of these regions provide both systemic and molecular platforms for cross-talk between the HPA-Axis and the SNS, both via connections to primary regions for sympathetic control and the co-expression of β1-adrenoreceptors (β1Rs). (Joëls & Baram 2009). Peripheral afferents, including baroreceptors sensitive to stretch and chemoreceptors sensitive to metabolite concentrations and pH stimulate reflexive arcs involving the hypothalamus and parasympathetic antagonism to help "reset" cardiac output, blood pressure respiratory rate, and metabolism.

Neural control of the Sympathetic Nervous System

The Sympathetic Nervous System (SNS) receives and provides modulatory feedback to several structures in the brain, including the Nucleus of the Solitary Tract (NTS), Locus Coeruleus (LC), ventral Septum (vSept), prefrontal cortex (PFC), amygdala (AMYG), and the Dorsomedial and Paraventricular nuclei of the Hypothalamus (DMH and PVN, respectively). It is peripherally driven by the locus coeruleus (LC), a pontine structure, the ventrolateral medulla (VLM), and the hypothalamus, all of which directly innervate preganglionic sympathetic fibers. Preganglionic sympathetic fibers travel to postganglionic sympathetic ganglia through the

intermediolateral column of the spinal cord (Urich-Lai & Herman 2009). Altered communication in these neural centers of autonomic control are implicated in the localized autonomic imbalances frequently observed in pathology related to stress (O'Hare & Zsombok 2016).

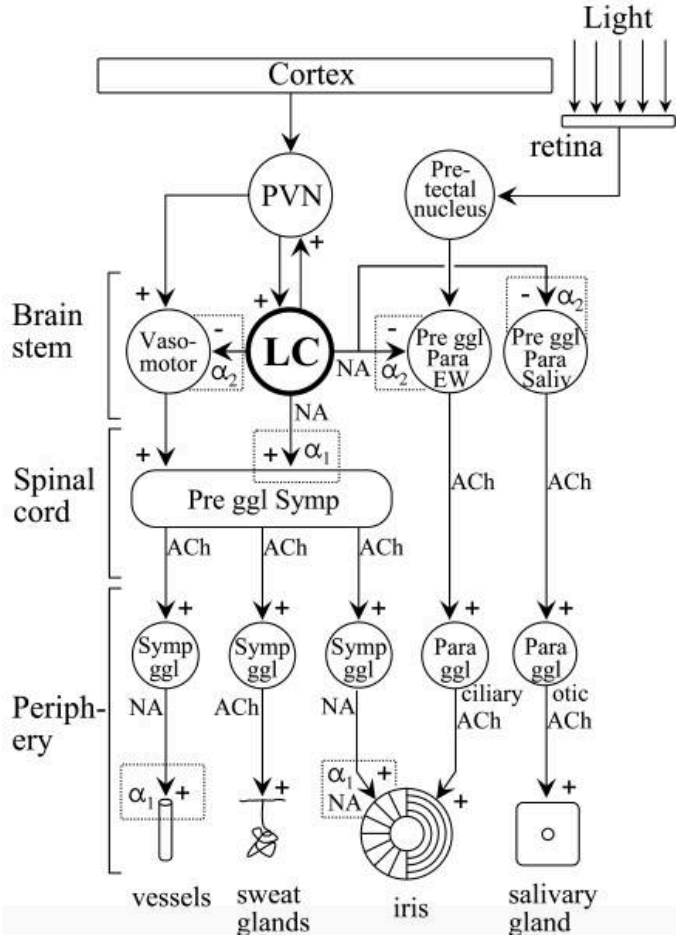

Figure **2-4**: Differential control of the locus coeruleus on sympathetic and parasympathetic ganglia Samuels & Szabadi, 2008)

A decade after von Euler identified norepinephrine as the primary neurotransmitter of the peripheral SNS (1946), Vogt provided evidence that norepinephrine had functional significance in the CNS as well. Soma for noradrenergic or norepinphrinergic (NE) neurons are localized primarily in the LC, where they synthesize NE for distribution as a modulatory neurotransmitter

throughout the brain (Sara 2009). Noradrenergic modulatory tone, and therefore LC activity, has a wide range of implications for cognitive function. The LC is a constituent of the "ascending reticular activation system" and has therefore been studied extensively for its role in arousal and focused attention. There are approximately 1,500 soma in the LC, and each of these neurons has the potential to extend projections to a broad range of targets (See Figure 2-4, Sara 2009). Noradrenergic projections emerging from the LC constitute the only noradrenergic neurons innervating the forebrain and project to nearly all brain regions (with the exception of the basal ganglia), including the forebrain, thalamus, sensory sites, and limbic areas (Sara 2009). In the 1980's, the first electrophysiological recordings in LC in awake rats showed phasic and tonic behavioral responses to salient environmental stimuli and vegetative behaviors (e.g. resting, eating), respectively. It was suggested that the LC was responsible for shifting systemic neural activity in a way that permitted attending to environmental stimuli and extracting salient features from it, at the expense of maintaining vegetative function (Svensson 1987). This idea expands the effects of the SNS to include altering attentive processes in a way that promotes survival and learning. LC provides dense excitatory and inhibitory tone to the ventral subiculum, an area involved in processing contextual information and inhibition of the psychogenic endocrine stress response (Ulrich-Lai & Herman 2009, Lipski & Grace 2013). It also extends projections to the hippocampus, and the basolateral nucleus of the amygdala (BLA), which is involved in fear learning and modulates endocrine components of stress response. NE acting within the BLA produces an inhibitory effect when binding to a-2 receptors and an excitatory effect when binding to B receptors (Ulrich-Lai & Herman 2009, Buffalari & Grace, 2007). The LC has been shown to be particularly active upon exposure to reinforcement, whether positive or aversive. It has been shown to respond strongly to the reversal of learning processes, and its firing in monkeys and in rats precedes behavioral changes that indicate a reversal learning paradigm is successful

(Svensson 1987). Chronic stress potentiates LC projections to the cortex and hippocampus, resulting in increased excitability and neurotransmitter release (Urich-Lai & Herman 2009).

The LC exerts an sympathoexcitatory influence via a1-receptiors on sweat glands and pupillary dilators, but interestingly, can inhibit rVLM via action on a2 receptors, blocking the vasoconstrictive action of rVLM activity. This is one possible systemic neural mechanism of differential sympathetic activity. LC also inhibits preganglionic parasympathetic fibers in the Edinger-Westphal nucleus innervating the iris and pregangionic parasympathetic fibers innervating the parotid gland via a2 receptors (Samuels & Szabadi, 2008).

In addition to its roles in cognition, affect regulation, and differential autonomic control, LC also plays a role in monitoring the internal milieu. In the 1980's, it was observed that exposure to noxious stimuli elicited parallel and identical reactions in the LC and in peripheral sympathetic nerves (Svensson 1987). LC had been found to show a transient increase in response to noxious systemic stimuli (e.g. moderate increase in blood pressure) and a pronounced, enduring increase in response to potentially lethal stimuli (e.g. hypoxemia, blood loss). LC receives innervation from the nucleus of the solitary tract (NTS), a major site of termination for vagal afferents (Ulrich-Lai & Herman 2009). Svensson found that, in response to hypertension, an LC activity was exclusively inhibited by cardiopulmonary vagal afferents (1987). In response to both psychogenic and systemic stress, the LC increases sympathetic tone via direct stimulation of preganglion sympathetetic cells fibers in the intermediolateral cell column of the spinal cord, driving peripheral sympathetic response (Urich-Lai & Herman 2009).

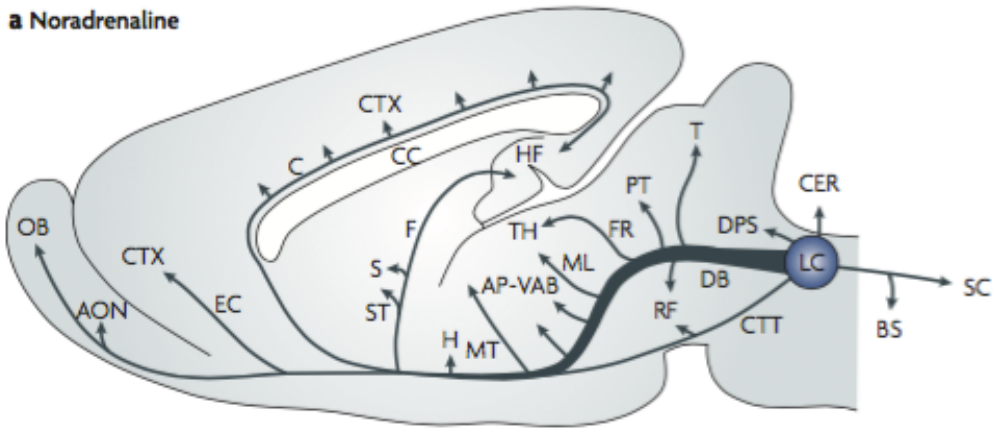

Figure **2-5**: The reach of the noradrenergic modulatory system emerging from the locus coeruleus (LC,) extends to the entire forebrain, less the basal gangla, the **brain stem (BS)** and the intermediolateral column of **the spinal cord (SC)**. **amygdala (A)**, **cortex (CTX)**, corpus collosum (CC), **cingulum (C)**, central tegmental tract (CTX), olfactory bulb (OB), anterior olfactory nucleus (AON), **entorhinal cortex (EC)**, **stria terminalis (ST)**, septum (S), **fornix (F)**, **hippocampal formation (HF)**, **hypothalamus (H)**, mammilothalamic tract (MT), **ansa peduncularis–ventral amygdaloid bundle system (AP-VAB, amygdalofugal projections to the mediodorsal nucleus of the thalamus), thalamus (TH),** fasciculus retroflexus (FR), pretectal area (PT), tectum (T), **dorsal periventricular** system (DPS), dorsal bundle (DB), **cerebellum** (CER), central tegmental tract (CTT) (Sara 2009).

In primates, rats and mice, the LC has been shown to receive innervation from cortical areas, the central nucleus of the Amygdala (cAmyg) and the hypothalamus, including the paraventricular nucleus (PVN). Anterograde tracer experiments in mice, made public by the Allen Brain Institute (http://connectivity.brain-map.org/), have shown sparse innervation from the primary somatosensory homunculus (S1) to the LC and peri-LC area. Ideas relating this finding to cases of human psychopathology are purely speculative. That said, neuroimaging experiments using MRI to assess cortical grey matter volume have found reductions in S1 of medically healthy adult survivors of child sexual abuse (Heim et al 2013), a possible adaptation to the abuse that may have contributed to their resiliency. Direct non-GABAergic connections between S1 and LC may hold implications for altered autonomic stress response in those survivors as a direct consequence of somatosensory stimulation. The cAmyg sends both inhibitory GABA-ergic (an estimated 30-40%) and nonGABA-ergic projections to LC and the peri-LC area. PVN projections

to LC are likely excitatory, as they typically do not show antibodies for glutamate decarboxylase, a catalyst in the synthesis of GABA, or enkephalin, another inhibitory neurotransmitter (Dimitrov et al 2013).

The ventrolateral medulla (VLM), the medullary center that gates sympathetic and parasympathetic tone to control respiration, vascular resistance and cardiac output, sends direct projections to the intermedialateral column and therefore directly controls sympathetic activity in the periphery. The historic focus of research into the VLM as a major center of autonomic control has been on respiration, and on the vasodepressor function of the caudal VLM (cVLM) and vasopressor function of the rostral VLM (rVLM, see Fig. 2-5) in the sympathoinhibitory pathway of the arterial baroreflex (Zagon et al 1994, Bennaroch 2008). It has also been implicated in modulating somatosensation, nociception, and arousal. This characterization of VLM as a cardioregulatory center is important, but it is also important not to overlook the region's direct modulation of brain regions involved in affect and cognition. VLM sends projections to the prefrontal cortex and limbic areas. A 1994 study using anterograde and retrograde tracers found evidence of direct rVLM innervation of the hippocampus, and efferents from cVLM and rVLM were found in the septum and anterior cingulate cortex (Zagon et al 1994)

As mentioned above, the vagally-mediated inhibitory effect of hypertension on LC occurs in parallel to the arterial baroreflex, the canonical reflex responsible for medullary control of autonomic output in response to changes in arterial blood pressure. As we have seen, the SNS increases arterial blood pressure in two ways: First, it increases total cardiac output. Second, sympathetic output causes systemic vasoconstriction, with the effect of increasing vascular resistance. Baroreceptors, primarily in the carotid sinus and aortic arch, respond to this increase in blood pressure by increasing firing rate. Baroreceptors in the carotid sinus are innervated by the Glossopharyngeal Nerve (CN IX). Aortic Arch baroreceptors are innervated by the Vagus Nerve

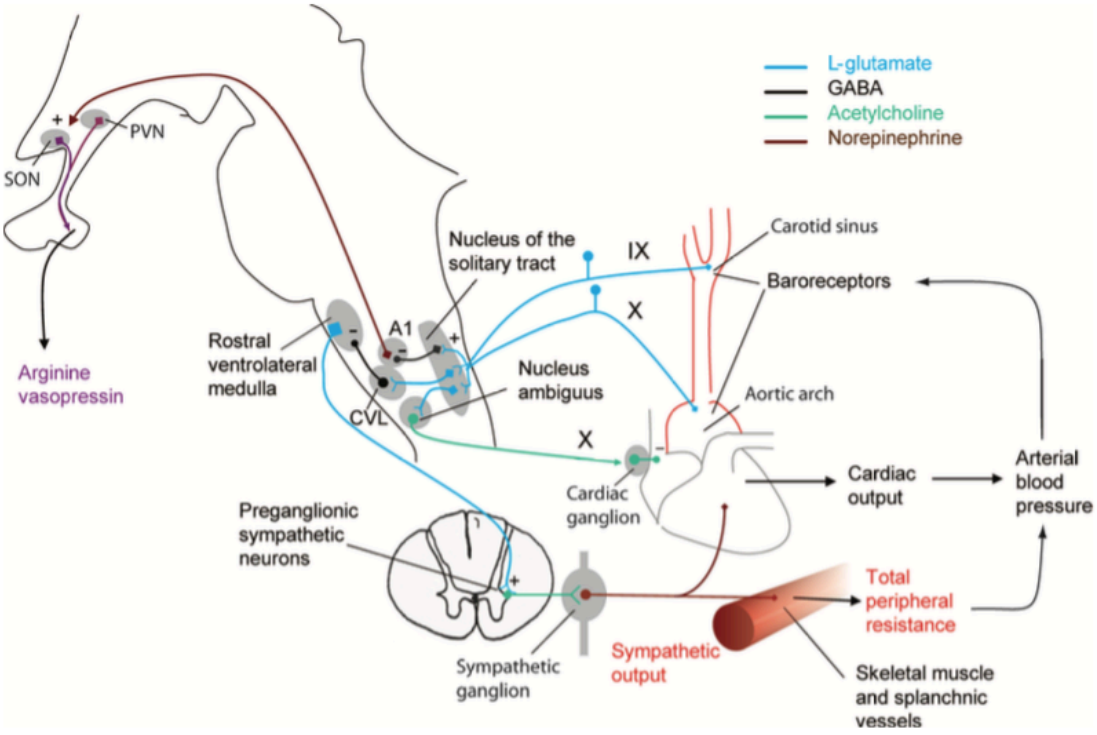

Figure **2-6**: Arterial baroreflex circuit (Bennaroch 2008).

(CN X). Primarily glutamatergic afferents from CN IX and CN X excite the NTS via NMDA and non-NMDA receptors. The NTS activates caudal venrolateral medulla (cVLM) inhibitory interneuron control of the rostral venrolateral medulla (rVLM), reducing sympathetic tone with the primary effect of reducing vascular resistance (Bennaroch 2008). In addition, NTS activation inhibits A1, a noradrenergic group of cells in the brainstem, inhibiting the PVN and supraoptic nucleus (SON) of the Hypothalamus. This action on the hypothalamus inhibits vasopressin release from the pituitary. NTS excitation also activates the primarily cardioinhibitory pathway of the baroreflex via excitation of the Nucleus Ambiguus (NA) and the Dorsal Motor Nucleus of the Vagus (DMNX), increasing efferent parasympathetic activity to the heart and inducing both a relaxation of cardiac muscle and a reduction in heart rate, causing a decrease in cardiac output

(Bennaroch 2008, Alloway & Pritchard, 2007). This reflexive vagal control of the heart, and the differential roles of the NA and DMNX in modulating cardiac function, will be discussed further in Chapter 3.

While the hypothalamus is typically associated only with the endocrine component of a stress response, the paraventricular and dorsomedial nuclei also regulate autonomic response. The preautonomic area of the paraventricular nucleus of the hypothalamus (preautonomic PVN) sends direct projections to preganglionic parasympathetic fibers in the brain stem and to sympathetic preganglionic fibers in the intermediolateral column of the spinal cord. In hypertension, the subfornical organ releases angiotensin II into the PVN. Angiotensin II excites these preautonomic hypothalamic cells, stimulating the SNS and increasing cardiac output (Ulrich-Lai & Herman 2009). The preautonomic hypothalamus has also been identified for its role in the control of hepatic metabolism, which we recall has been shown to function independently of the rest of the sympathetic nervous system in hypoglycemia. The PVN in densely innervated by GABA-ergic (gamma-amino-butyric acid) inhibitory neurons (Ulrich-Lai & Herman 2009) and bicuculline (GABA antagonist) injection into the PVN has been shown to stimulate hepatic glycogenolysis and gluconeogenesis in a sympathetic-dependent manner (O'Hare & Zsombok 2016).

The dorsomedial nucleus of the hypothalamus (DMH) is a component of the peri-PVN area that deserves special attention. It may exert modulatory tone on the PVN. Interestingly, the DMH may serve as a hypothalamic classifier between psychogenic and systemic stress; its inhibition attenuates HPA reactivity to psychogenic, but not systemic, stress. The DMH also exerts a stressor type-dependent sypmpathoexcitatory control of cardiac output: Its inhibition attenuates increases in cardiac output in response to psychogenic stress but not to hypovolemia, a systemic stressor (Ulrich-Lai & Herman 2009). The DMH has been coined the "defensive hypothalamus", as in rabbits its electrical stimulation produces a range of physiological and behavioral responses associated with fight or flight: tachycardia, hyperventilation, inhibition of

the cardioinhibitory pathway of the baroreflex, increases in hindlimb bloodflow, and aberrant running (Hagenaars et al 2014). It is likely that these responses are mediated by connections between the DMH and the preautonomic PVN.

The LC, VLM and PVN of the hypothalamus are all involved in the direct control of the sympathetic nervous system via projections to preganglionic sympathetic fibers in the spinal cord. The LC appears to function as a direct modulator of both preconscious physiological components of the stress response (e.g. blood pressure) and of cognitive and affective components of stress (e.g. focused attention). It responds to both psychogenic and systemic stress, is capable of inhibiting parasympathetic activity and rVLM, and differentially exerts inhibitory or excitatory modulation on high-order limbic structures involved in stress and fear learning, including the amygdala and vSub. Its own firing patterns allow it to discriminate the lethality of a stressor, and its functional connectivity, especially with vSub, may allow further differentiation between psychogenic and systemic stress. The VLM also projects to preganglionic sympathetic fibers and is primarily understood as a controller of respiratory and cardiac function, however, it too sends direct projections to higher-order limbic structures, including the hippocampus and anterior cingulate cortex. PVN, usually attributed to the endocrine component of a stress response alone, also directly projects to the intermediolateral column of the spinal cord. These efferents are important in the differential control of hepatic metabolism. Though the SNS is anatomically designed to provide more widespread and less precise activation than the PNS, there is evidence of differential control in response to specific systemic stressors; An uncoupling, for example, of hepatic metabolism and cardiovascular control in the case of hypoglycemia. Overall, examining the connectivity and function of these regions paints a picture of the sympathetic nervous system as a more targeted system, and a system with more potential to alter cognition and affect, than is often described.

Neural control of the HPA Axis

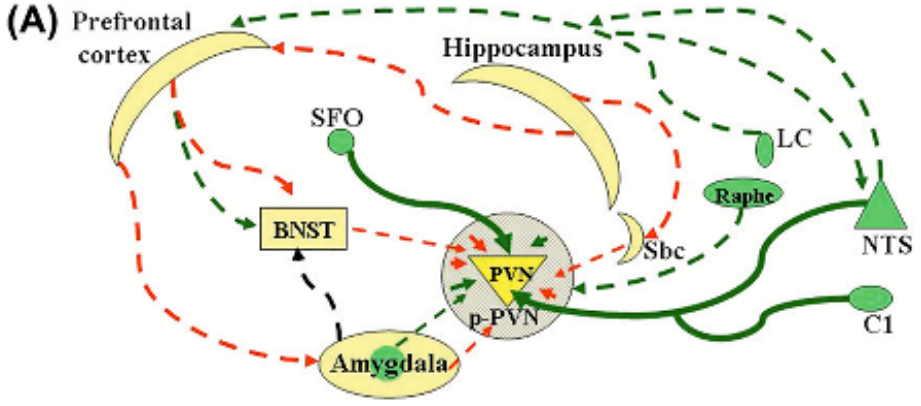

Figure **2-4**: Circuit diagram of Neural Control of the HPA Axis via the PVN (Aguira, 2012).

The HPA Axis is directly controlled by the paraventricular nucleus of the hypothalamus (PVN), which is responsible for the secretion of corticotrophin releasing hormone (CRH). GRs, MRs, and CRHRs are found in the Nucleus of the Solitary Tract (NTS), Locus Coeruleus (LC), Dorsal Raphe Nuclei (DR), prefrontal cortex (PFC), amygdala (AMYG), Hippocampus (HIPP), Bed nucleus of the Stria Terminalis (BnST) and Paraventricular Nucleus of the Hypothalamus (PVN), suggesting that all of these regions contribute to the modulation of HPA axis tone, many through direct or indirect negative feedback to the hypothalamus. Those that provide direct stimulation to the hypothalamus will be discussed here, the rest will be discussed in Chapter 4. These receptors work across multiple time scales. CRHRs work quickly, on the time scale of miliseconds to seconds, to promote synaptic changes. Mineralocorticoids in the CNS are associated with the maintenance of basal serum cortisol levels and with stress response, whereas

glucocorticoid signaling is associated with the stress response alone. GRs and MRs in the CNS work on the order of minutes to hours to upregulate transcription of various relevant proteins and produce long-term genomic changes that promote adaptation.

The PVN is the hypothalamic nucleus that drives endocrine stress response. CRH-producing parvocellular neurons in the anterior and dorsomedial regions of the PVN send axonal projections to the external zone of the median imminence. Studies in rats, mice and humans (CITATIONS) have shown that a portion of CRH-producing neurons neurons in the anterior and dorsomedial PVN co-release vasopressin. Vasopressin is the antidiuretic and vasoconstrictor hormone that has already been discussed for its role in neurogenic hypertension following activation of the sympathetic nervous system (SNS). As mentioned previously, vasopressin further stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary. The co-release of vasopressin in CRH-producing parvocellular neurons is a distinct phenomenon from sympathetic release vasopressin into circulation; Those neurons are magnocellular and their soma are found in the anteromedial and dorsolateral divisions of the PVN. They do not terminate at the median eminence, but travel through its internal zone to the posterior pituitary, where they release vasopressin into circulation, in mild doses in the case of most systemic and psychogenic stress and in massive amounts in the case of hemorrhage (Sapolsky et al 2000). Parvocellular, but not magnocellular, vasopressin release amplifies the CRH-mediated release of ACTH via binding to the V1b receptor in the posterior pituitary.

Type 1 CRH receptors (CRHR1) are the most important CRH receptor in the seconds-to-minute time frame of the stress response, where their central action is generally accepted to either the attenuation or enhancement of the cognitive and physiological aspects of the response. CRHR1 is expressed throughout the brain, including cortical, limbic, cerebellar, brain stem and diencephalic structures. Interestingly, CRHR1 expression in the diencephalon, including the hypothalamus, seems stressor type-dependent. Psychogenic (e.g. social stress) and metabolic

stress (e.g. low blood sugar) promote dramatically increased expression of CRHR1 in parvocellular (anterior and dorsomedial) regions of the PVN, potentiating ACTH release from the pituitary, whereas osmotic stress (e.g. low blood pressure) induces increased CRHR1 expression in magnocellular regions of the PVN, potentiating vasopressin release into circulation. In response to acute stress, CRHR1 expression in the PVN will increase. Upon exposure to chronic repeated stress of a first, same type, CRHR1 expression may show habituation. Regardless of habituation, upon exposure to a second, novel stressor, expression of the receptor will increase dramatically, suggesting enhanced HPA reactivity in response to novel stress after repeated exposures to a first stressor. The response curves of neuropeptides like CRH and ACTH to any individual stressor varies considerably and depends in part on the intensity of the stress, its chronicity, and whether or not the animal has any control over aspects of the stress's presentation (Figure 2-5, Aguira 2012).

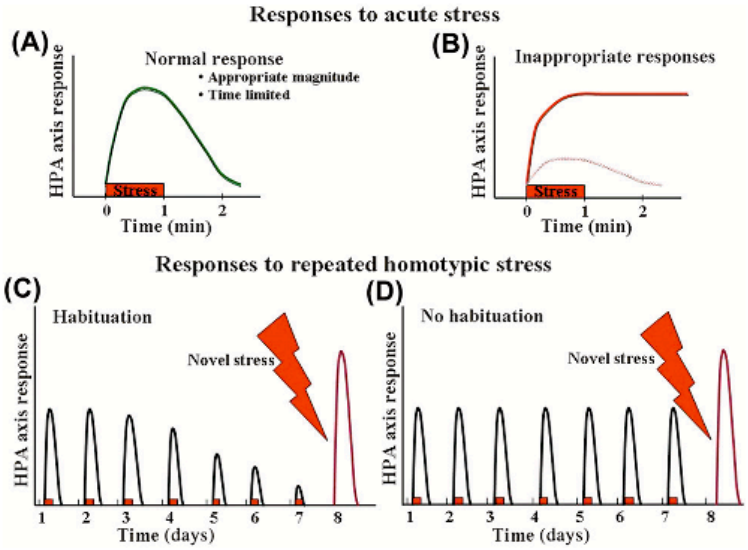

Figure **2-5**: Idealized time response for pituitary ACTH secretion for normal and abnormal stress responses (A,B, respectively), as well as the effects of repeated homotypic stress on ACTH secretion in response to a novel stressor (C,D) (Aguira, 2012).

PVN synthesis and release of CRH is modulated by dense inhibitory GABAergic projections and opioid signaling into the region, and by excitatory afferent norepinephrinergic and seretonergic projections (Fig 2-2). Inhibitory enkephalinergic projections to the PVN emerge from the brainstem, demonstrating a role of opioids in the modulation of stress response (Dimitrov et al 2013). The GABA-ergic modulation of PVN activity is supplied primarily by efferent fibers from the peri-PVN area, including the arcuate nucleus and the dorsomedial DMH, which we recall has a gating role on heart rate increase in psychogenic stress (Aguira 2012, Ulrich-Lai & Herman 2009). Amygdalar excitation of the PVN is thought to occur indirectly through modulation of these neurons; The amygdala sends inhibition to the GABA-ergic interneurons in the peri-PVN area, disinhibiting the PVN (Aguira 2012). Likewise, the peri-PVN area and PVN receive both GABA-ergic inhibition and excitation from the bed nucleus of the stria terminalis (BnST), which can either activate an endocrine stress response or implement forebrain-mediated inhibition (Urlich-Lai & Herman, 2009, Aguira 2012).

The peri-PVN region, including the ventrolateral DMH, also supplies excitatory glutamatergic tone to the PVN (Aguira 2012). This excitation is stimulated by seretonergic cells of the raphe nuclei (Aguira 2012, see Fig 2-2, Fig 2-4). Norepinephrine secreted by cells in the NTS and C1 cells in the rVLM directly excite the PVN (the same rVLM cell type that innervates preganglionic sympathetic and parasympathetic fibers, Guyenet et al 2013). In addition to receiving direct excitatory modulation from neighboring nuclei and the sympathetic nervous system, the PVN is directly innervated by efferents from the subfornical organ, a circumventricular organ outside of the blood-brain barrier that is able to transduce peripheral endocrine signals (e.g. low circulating cortisol levels), changes in serum electrolyte levels and blood pressure. In response to low blood pressure, the subfornical organ release of angiotensin II on the PVN stimulates an endocrine stress response.

Chapter 3

Immobility: Polyvagal Theory and the Parasympathetic Nervous System in Stress

"*The switch from anxiety to the catatonoid response is the subjective evaluation of impending danger as one that cannot be avoided or modified. With the perception of fatal helplessness in the face of destructive danger, one surrenders to it.*"

- Henry Krystal (1988)

Peripheral effects and neural control of Parasympathetic Activity

The Parasympathetic Nervous System (PNS) is classically described as antagonizing the Sympathetic Nervous System (SNS) and thus with physiological functions of "rest and digest", or energy conservation. The effector tissues of the PNS are innervated by cranial nerves emerging from the brainstem and splanchnic nerves emerging from the sacral spine. The actions of the PNS may be thought up as generally opposite and opposing the action of the SNS; For example, where activation of the SNS will induce tachycardia and increase cardiac output, the PNS serves as a "break", keeping heart rate down and, in some cases, inducing bradycardia.

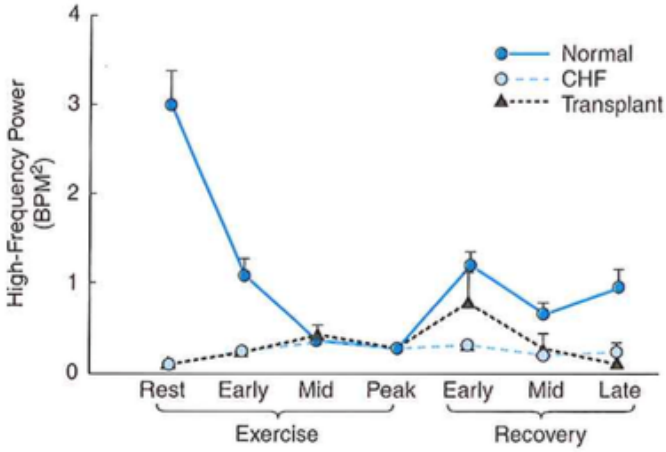

Figure **3-1**: High frequency power from spectral analysis of heart rate, a surrogate measure of ventral vagal tone, declines after onset of aerobic activity in healthy individuals, demonstrating the withdrawal of the ventral vagal "break" necessary to allow fight or flight behaviors. Other conditions are congestive heart failure and transplant patients (Seals 2005).

In stress research, the role of PNS function is recognized to be more complicated than simply antagonistic to the SNS (Porges, 1995, 2009, Scaer, 2001). In particular, the Vagus Nerve (CN X), a complex and far-reaching effector of PNS activity, has been the subject of extensive study for its role in the etiology of stress-born psychiatric illness. The role of the PNS in mediating a potentially pathogenic freeze state in response to extreme, inescapable and overwhelming stress has been hypothesized to play a causal role in the development of trauma, and in dissociative disorders, such as the dissociative subtype of Post-Traumatic Stress Disorder (PTSD). The peripheral action of the PNS in a healthy, "rest-and-digest" state and in a putative "freeze" state will be discussed here.

In addition to its opposite and inhibitory action, the PNS differs from the SNS in two important ways: First, where with only a few exceptions, postganglionic sympathetic neurotransmission relies on norepinephrine, the PNS used acetylcholine as its primary postganglionic neurotransmitter. Second, where the anatomy of the SNS facilitates more widely distributed function throughout the body, the anatomy of the PNS allows its action to be more

localized. Preganglionic fibers of the SNS synapse close to the spinal cord and postganglionic fibers widely distribute to a variety of effector tissues; Preganglionic fibers in the PNS, in contrast, synapse onto postganglionic fibers in terminal ganglia close to effector tissues, with postganglionic fibers typically targeting only a single tissue. In this way, while the PNS and SNS are mutually antagonistic, because the PNS is so much more localized, it does not necessarily follow that increasing parasympathetic tone will have an equal and opposite functional effect to increasing sympathetic tone on the body as a whole. This nuance is important to bear in mind when discussing a role of pathogenic autonomic function in the etiology of stress disorders.

Generally, the peripheral actions of the PNS are best remembered by the acronym SLUDD — salivation, lacrimation, urination, digestion and defecation (Tortora & Derrickson 2012, for more detail, see Table 3-1). The SLUDD actions are all important for preventing unnecessary energy expenditure. For example, the PNS, acting on the SA node of the heart and ventricular muscle (Seals 2005) via the vagal nerve, serves as a cardiac "break", establishing a metabolically efficient resting heart rate. In aerobic exercise, a good analogue for a fight or flight state, the metabolic demands of skeletal muscle dramatically increase, and the PNS withdraws its control of the heart to allow heart rate to rise, and ultimately to allow the SNS to increase cardiac output and oxygen supply. In a healthy human, this vagal "break" is necessary to keep the heart rate below around 100 bpm at rest, and the SNS must engage to elevate the heart rate above approximately 130 bpm. Though the PNS is necessary to maintain appropriate cardiac function at rest, and while in fight or flight the SNS must engage to mobilize resources and meet metabolic demand, the PNS is not necessarily "turned off" in conditions of submaximal aerobic effort. Blockade treadmill studies in humans using atropine, an mAChR antagonist, suggest that the PNS only completely withdraws its influence on heart rate in conditions of absolute maximal physical effort (Seals 2005).

Table **3-1**: The action of the parasympathetic nervous system on effector tissues (Adapted from Tortora & Derrickson 2012, pgs. 585-599)

Effector tissue	**Consequence of Parasympathetic activity**	**Implicated pathology in prolonged freeze response/parasympathetic dominance (Scaer 2001)**
Larynx, Trachea, Bronchi, Lungs	Constriction of the airways	Apnea
Liver	Increased glycogen synthesis	
Bile ducts	Increased bile secretion	Indigestion
Stomach	Secretion of digestive enzymes	Indigestion
Pancreas	Secretion of insulin	
Increased motility and tone of the intestines, relaxation of sphincters	Facilitates digestion, defecation	Indigestion
Contraction of the muscular wall of the urinary bladder and relaxation of the internal urethral sphincter	Urination	Incontinence
External genitals	Vasodilation and erection of the clitoris or penis	
Atrial muscle fibers, SA node	Decreased heart rate and cardiac output	Bradycardia, arrhythmia
Parotid gland	Increased secretion of saliva	
Lacrimal gland	Secretion of tears	
Iris circular muscle	Constriction of the pupil	

While parasympathetic efferents exit the CNS in multiple cranial nerves, the most important of them with regard to stress is the Vagus (CN X). In establishing his Polyvagal Theory, Porges (1995) describes the Vagus as "a family of neural pathways originating from several areas of the brainstem" consisting of roughly 80% afferent, 20% efferent neurons with lateralized function. Specifically, the right side of the Vagus is responsible for effecting the most control over the heart (RSA). Further, the peripheral action of the Vagus nerve can be differentiated into two phylogenetically and functionally distinct parts: That of the dorsal vagal complex (DVC) and ventral vagal complex (VVC) (Porges 1995). The differential function of these two vagi is a critical concept in understanding the role of the PNS in modulating prosocial, non-defensive behaviors on one hand, and extreme stress response on the other.

The VVC, emerging from the Nucleus Ambiguus, is mylenated and considered the most phylogenetically recent and specifically mammalian of the two. It plays an important role in cardiac and respiratory function, and there is anatomical evidence to support a role for the VVC in regulating prosocial mammalian behavior (Porges 1995). In healthy humans, the VVC is primarily responsible for the control of heart rate at rest, functioning as the vagal "break" mentioned in the above example of aerobic exercise, and is especially noteworthy for its role in establishing respiratory sinus arrhythmia (RSA), the irregularity of the time interval between heart beats synchronized to respiration (Porges 1995). The VVC plays an important role in human infant suckling behaviors and in the synchronization of willful swallowing and respiration (Porges 1995). VVC efferents innervate the trachea, pharynx, soft palate, larynx, and esophagus and controls vocal intonation, which importantly links the VVC to affective expression.

Anatomical connections between the Nucleus Ambiguus (NA) and the source nuclei of special visceral efferent neurons controlling the striated muscles of the face and head present structural evidence for a modulatory role for the VVC in turning the head and controlling muscles of facial expression, representing what, for Porges, is a critical role of the Vagus nerve in an "integrated social engagement system" that must be withdrawn for defensive behaviors, mediated by either the Sympathetic Nervous System or the DVC, to occur (Porges 2009).

The Dorsal Vagal Complex (DVC), acting via the Dorsal Motor Nucleus of the Vagus (DMNX), is the unmylenated and most conserved of the two vagal branches, representing a phylogenetically distinct vestige of reptilian ancestry that is referred to in Porges' Polyvagal theory as the "vegetative" vagus. The DVC innervates the subdiaphragmatic organs to mediate digestion and asserts a secondary influence on heart and respiratory rate. While its influence on RSA is minimal, the DVC is credited with vagal modulation of heart rate in the classical baroreflex (Alloway & Prichard, 2007, see Table 1) and with the induction of neurogenic bradycardia observed during vagal stimulation and during the orienting reflex (Porges 1995). This DVC-mediated phenomenon, called "behavioral orienting", can be thought of as a conserved, precognitive precursor to focused attention in humans. An orienting response involves sensory focusing, a cessation of gross motor movement, and transient bradycardia (Porges 1995). Bradycardia, associated with DVC dominance, has been reported as a component of fear response in iguanas and as a component of death-feigning in hog-nosed snakes, establishing a phylogenetic pretext for a role of the DVC in mammalian fear response (Scaer 2001).

In the polyvagal characterization of the human repertoire of stress responses (See Chapter 4), the DVC is believed to assume dominance in states of helplessness, when the sympathetically-mediated strategies of fight or flight are no longer viable (Porges 1995, Scaer 2001). This "last resort" physiological state is not unlike the reptilian death-feigning mentioned above. It is often referred to in literature as a freeze response but should not be confused with the behavioral

freezing seen in mildly stressed laboratory rats. Allowing this response to terminate on its own has been shown to prolong the time chicks were able to resist drowning (Ginsberg 1974), whereas artificial termination or prolongation resulted in the animals succumbing more quickly than an unstressed control. While this is evidence that the successful termination of this response may enhance resiliency to lethal stress, the state itself is a dangerous one, associated with hypotension. Laboratory rats who die during induction of this response suffer cardiac arrest during diastole, "in a state of complete cardiac flaccidity and engorgement with blood" (Scaer 2001), pointing to the vulnerability of the mammalian heart in a cardiac state of stress response that, by Porges' model, should be controlled by the DVC (Porges 1995, Hofer 1970). Scaer attributes parasympathetic dominance in humans to bradycardia, heart palpitations and arrhythmias, apnea, nausea, dizziness, indigestion, abdominal cramps, diarrhea, incontinence, and behavioral withdrawal (2001).

Neurogenic hypertension in sleep apnea: A model for stress pathology as a consequence of disrupted homeostasis between autonomic systems

Dynamic, localized, reflexive control between the parasympathetic and sympathetic nervous system is totally omitted from arousal-based conceptualizations of stress, but it also lacking from a characterization of stress pathology as a vagally-driven phenomenon. There is an implicit suggestion that a symptom of parasympathetic dominance, for example apnea, occurs in the absence of sympathetic response. To illustrate the importance of challenging this notion, consider a more detailed description of the effects of apnea. The systemic and molecular substrates of hypertension as a consequence of sleep apnea are relatively well characterized. This

etiology serves as a nuanced model of how autonomic dysfunction in chronic stress may become pathogenic, and as Scaer mentions, apnea itself is relevant to trauma. Clinicians have speculated a link between peritraumatic sleep fragmentation and exacerbated collapsibility of the airway, leading to Sleep-Disordered Breathing (SDB), including Obstructive Sleep Apnea (OSA) (Krakow et al 2002). As many as 90% of PTSD patients seeking treatment for insomnia meet the diagnostic criteria for SBP, and half of those have OSA (Krakow et al 2002. In addition, apnea is considered a possible consequence of parasympathetic dominance over pulmonary tissues. Apnea is a condition that presents considerable systemic stress on the body, and induces hypoxemia. The problem with characterizing apnea as a parasympathetic phenomenon is that low blood oxygen is a systemic stressor that triggers well-defined, VLM-mediated sympathetic reflex called the hypoxia reflex.

The body's reflexive response to the hypoxemia is controlled by the carotid bodies, located bilaterally at the carotid bifurcation in the neck. In the carotid bodies, blood PO2 levels are primarily transduced via type 1 afferent chemoreceptors (Kumar & Prabhakar 2012), which increase firing rate in response to decreases in blood PO2 and signal to the nucleus of the solitary tract (NTS) via the glossopharyngeal nerve (CN IX). The carotid bodies directly innervate the VLM. Through these parallel pathways, the VLM responds to hypoxemia by signalling catecholamine release from the adrenal medullae. In acute hypoxemia as in any other stress response, this increase in sympathetic tone is homeorhetic; Systemic vasoconstriction increases blood pressure, ensuring adequate perforation, increased cardiac output, respiratory rate, active exhalation, and dilation of the airways all help increase the amount of available oxygen. But here, the physiological consequences of apnea on the carotid bodies themselves complicate the capacity of the SNS and PNS to reflexively recover from hypertension.

There are parallel mechanisms for chemotransduction of blood PO2 levels in the carotid body. First, in the Type 1 chemoreceptor, blood PO2 is used as a substrate by the enzyme heme

oxidase (HO2), resulting ultimately in the phosphorylation of cystathionine-gamma-lyase (CSE), inhibiting its catalytic action in converting homocysteine to hydrogen sulfide (H2S). In neurons, K+ leak channels, which allow K+ to move out of the cell along its concentration gradient, are typically associated with either maintaining cell membrane potential at rest, or repolarizing the membrane after the cell fires. H2Sinhibits these channels, meaning that in its absence, K+ channels are functional and the cell is less likely to depolarize and fire. If blood PO2 drops, H2S levels will increase and K+ channels will close, inhibiting the outflow of K+, and the cell will be more likely to depolarize, signaling to the NTS and VLM.

Secondly, Reactive Oxygen Species (ROS), which will be discussed below as an important byproduct of Hypoxia Inducible Factor (HIF) signaling, interact with the HO2 molecule at the Cysteine 265 residue to inhibit it, thus depolarizing carotid body chemoafferents using the same H2S-dependent mechanism as blood PO2. In both of these scenarios, the SNS will be activated and catecholamines will be released in a chronic and unpredictable manner. This catecholamine release, through its action on vasculature (Chapter 2), is responsible for the increase in blood pressure (Samanta et al 2017).

As implicitly assumed by Selye's characterization of the stage of exhaustion (Selye 1946), and as explicitly described in McEwen's theory of Allostasic Load (McEwen 1999), the hypoxemic reflex becomes dysregulated in chronicity. Chronically elevated catecholamine levels induce systemic hypertension, which in turns leads to myocardial hypertrophy, interstitial fibrosity, decreased microvessel density, and heart failure (Samanta et al 2017), but the effect on neural control of blood pressure is far more complicated, and frightening, than that. Studies examining the mechanisms of systemic hypertension in chronic intermittent hypoxia (CIH), an animal model of apnea, have implicated signaling pathways dependent on two isoforms of HIF-alpha, HIF-1a and HIF-2a that result in an increase of reactive oxygen species (ROS) in the

carotid body, as critical for this long-term dysregulation of homeostasis. In CIH, increased [Ca2+]i causes a PKC and MTOR-mediated increase in HIF-1a, and an increase in calpains, calcium-decedent proteases that degrade HIF-2a. Increased levels of the HIF-1a transcription factor increases expression of Nox2, interfering with the mitochondrial electron transport chain and increasing levels of ROS. HIF-2a activates transcription of the Sod2 gene, which encodes for the antioxidant superoxide dismutase. Upregulating HIF-2a degradation causes a decrease in antioxidant transcription. The net effect is an increase in ROS production, which leads to a vicious cycle, increasing HIF-1a levels and decreasing HIF-2a, depolarizing chemoafferents, increasing ROS concentrations at the carotid body and ultimately, changing its redox state.

HIF-1a or HIF-2a knockdown mice show altered SNS reactivity in response to normoxia and CIH, but compound heterozygotes show intact responses, indicating that it is the balance of HIF-1a or HIF-2a, not the absolute concentrations of either, that affect SNS reactivity to hypoxemia. This altered redox state appears to play a role in maladaptively recalibrating the SNS: The redox state in the carotid body has been shown to be critical for calibrating the set point for blood pressure within the SNS (Semenza et al. 2014). This means that over the long term, chronic changes in HIF signaling will not just induce hypertension via over-activation of the sympathetic nervous system, but could actually change the set point at which VLM-mediated sympathetic reflexes 'believe' optimal blood pressure exists.

Interestingly, this imbalance in HIF 1/2a levels extends from the peripheral carotid body to regions of the CNS involved in the hypoxia reflex in a manner thought to be [Ca2+]i dependent. Carotid-body ablation experiments have shown that this phenomenon within the CNS is carotid-body dependent, so the CNS is not detecting hypoxemia itself (Semenza & Prabhakar, 2015). These regions are, however, plastic to hypoxemia and demonstrate the same molecular changes associated with pathology in the carotid body. We see here a mechanism by which a

symptom attributed to pathological parasympathetic dominance, apnea, will result in the chronic activation and dysregulation of sympathetic reflexes, ultimately resulting in a pathogenic obfuscation of the set points necessary to maintain autonomic control over blood pressure homeostasis. In addition to this, the function of brain regions (e.g. NTS, VLM) necessary for communicating the state of the *milieu intérieur* to structures involved in orchestrating integrated autonomic response, will be affected. This systemic dysregulation has implications for psychological function in apnea as well; First, it is hard not to interpret repeated, unpredictable hypoxemia as a sort of interoceptive learned helplessness (Maier & Seligman, 1976). Second, via HPA response, chronically elevated glucocorticoid levels could render the hippocampus vulnerable to the same morphological and functional impairments seen in chronic psychogenic stress (McEwen, , see Chapter). Apnea in trauma may be taken as a state of parasympathetic dominance, but it is a state that recruits and, over the long term, recalibrates the sympathetic nervous system, as well. There is also a precedent by which to expect impaired homeorhesis as a consequence of psychogenic stress: Through an unknown mechanism acting on the median preoptic nucleus of the hypothalamus, chronic unpredictable stress has been shown to induce a long-term elevation in body temperature and impaired thermoregulatory response, phenomena which are attributed to a skewed hypothalamic set point for optimal body temperature (Natarajan et al 2015).

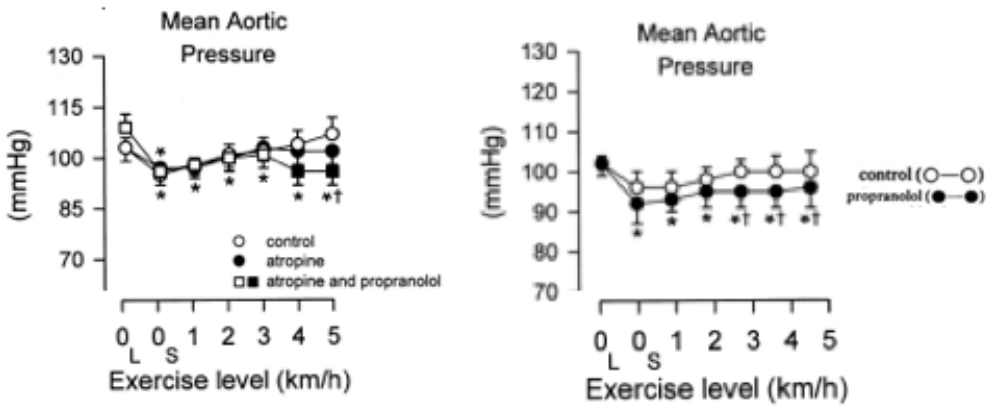

Figure **3-2**: Mean aortic pressure (MAP) measured in swine during mAChR antagonist atropine and beta-blocker Propranolol blockades conducted on treadmills. MPA is plotted across exercise intensities from laying (OL), standing (Os) and multiple workloads on the treadmill. On the left, the atropine + propranolol condition shows a decline in MAP at high intensities of exercise not captured in a propranolol condition alone, suggesting that autonomic dysregulation of the cardiovascular system similar that which is observed in tonic immobility depends on the balance between the parasympathetic and sympathetic nervous systems, not on the absolute tone of either system (Stubenitszky et al 1998).

Figure 3-2 shows more evidence that the balance between autonomic systems is important for protection of the heart in physiologically extreme states. Mean aortic pressure (MAP) was measured in swine during mAChR antagonist atropine and beta-blocker propranolol blockades conducted on treadmills. MPA is plotted across exercise intensities from laying (OL), standing (Os) and multiple workloads on the treadmill. On the left, the atropine + propranolol condition shows a decline in MAP at high intensities of exercise not captured in a propranolol condition alone, suggesting that autonomic dysregulation of the cardiovascular system similar that which is observed in tonic immobility depends on the balance between the parasympathetic and sympathetic nervous systems, not on the absolute tone of either (Stubenitszky et al 1998).

Given the reflexive communication between the autonomic systems necessary to maintain peripheral homeostasis, as well as the specificity with which these systems can respond to systemic stressors, arousal-based theories of stress seem insufficient in scope. It cannot be enough to think of pathology in chronic stress as phenomena fully captured or driven by an overactive HPA axis, or as the outcome of a zero-sum game between the sympathetic and parasympathetic divisions of the autonomic nervous system on any organ system. As illustrated with blood pressure in the case of apnea and thermoregulation in chronic stress, it may be more panoptic to conceptualize stress-related pathology as a consequence of an impaired capacity within and between the endocrine and two autonomic systems to regulate homeorhesis, in part due to a long-term obfuscation of homeostatic set points. In his review of the autonomic

substrates of dissociative phenotypes of trauma, Scaer describes a dynamic autonomic model for psychological trauma that seems aligned to this idea (2001):

> "In PTSD, through unresolved peritraumatic dissociation[1], internal and external stimuli impacting the central neural circuits mediating memory and arousal will contribute to kindling, leading to internally-based stressors of associated neural subsystems, especially the autonomic nervous system. By this model, cyclical autonomic dysfunction will result, leading to many of the divergent but dramatic autonomic symptoms of the traumatized victim. Thus periods of sympathetic arousal will include symptoms of muscle bracing, bruxism, ocular divergence, tachycardia, diaphoresis, pallor, tremor, startle, hypervigilence, panic, rage and constipation. These states will alternate with parasympathetic dominance, including symptoms of palpitations, nausea, dizziness, indigestion, abdominal cramps, diarrhea and incontinence. Although many of these symptoms are often attributed to somatization disorder, they in fact represent the extremes of the cyclical autonomic dysfunction seen in trauma, are inherently self-perpetuating, and contribute to continued abnormal autonomic oscillation. The syndrome of trauma has now literally taken control of the body."

[1] Evidence suggests that peritraumatic dissociation is an important possible predictor for PTSD (e.g. Vasquéz et al 2012), but it is not the only one. In one study of child burn victims, two distinct pathways, one involving peritraumatic dissociation and the other involving pain perception and attachment to caregivers were shown to account for 60% of the variance in PTSD outcomes (Saxe et al 2005).

Chapter 4

Towards an integrated hierarchy of stress response

Understanding the sympathetic, parasympathetic and endocrine substrates of fight, flight and immobility responses to stress is critical, but it isn't the whole picture. In reality, as we have seen, these systems work in concert, and appear capable of orchestrating more specific responses to any given stressor than has historically been appreciated. Much of this orchestration is believed to occur in the central nervous system (CNS), through the integration of information regarding systemic and psychogenic stressors and the feedbacked control of both autonomic and endocrine components of the stress response. For an illustration of how systemic (stress response triggers) and psychogenic (top-down) stress may influence the HPA Axis and Sympathetic Nervous System (SNS), see figure 4-1.

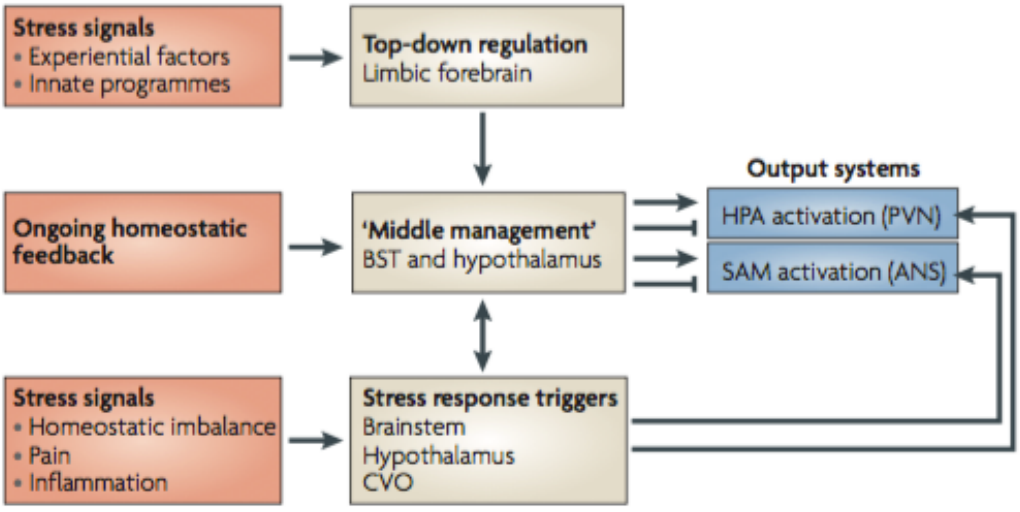

Figure **4-1**: Model for bottom-up and top-down control of sympathetic and endocrine stress response (Ulrich-Lai & Herman 2009).

Central Autonomic Network and Endocrine Control

The Central Autonomic Network (CAN) consists of limbic and higher-order autonomic processing centers essential for integrating environmental and interoceptive signals of stress with affect and cognition, and for orchestrating complex and dynamic autonomic states in response. The CAN is classically defined as consisting of many regions already discussed in depth, including the ventrolateral medulla (VLM), the periaqueductal grey (PAG), the hypothalamus, and the nucleus of the solitary tract (NTS). Bennaroch defines a model of the CAN that also includes the parabrachial complex (PB), the amygdala (Amyg), the ventromedial prefrontal cortex (vmPFC), the anterior cingulate cortex (ACC), the insula, and the thalamus (1993). Respectfully, it does not make sense to define a network of autonomic control that excludes brain regions that directly activate both SNS and PNS pre and post-ganglionic fibers, and so here, we will add the locus coeruleus (LC), the nucleus ambiguus (NA) and the dorsal motor nucleus of the vagus (DMNX) to this list. For the sake of readability, the list remains incomplete, and excludes other regions of higher-order autonomic processing found in the brainstem and the pons, including the A1 cell group, respiratory areas that in mice and rats have been found to control upper airway resistance (Kölliker-Fuse nucleus, see the example of apnea in trauma from Chapter 3), inspiratory rate, the synchronization of inspiration with other tasks (e.g. chewing), and active expiration (Dutschmann & Herbert 2006, Moore et al 2013, Mores et al 2012, respectively). Many of the higher-order brain regions listed here play a role in endocrine regulation, or are functionally associated with regions that do (e.g. hippocampus, bed nucleus of the stria terminalis). For a more comprehensive review of the role these high-order structures play in orchestrating both autonomic and endocrine stress response, see Table 4-1.

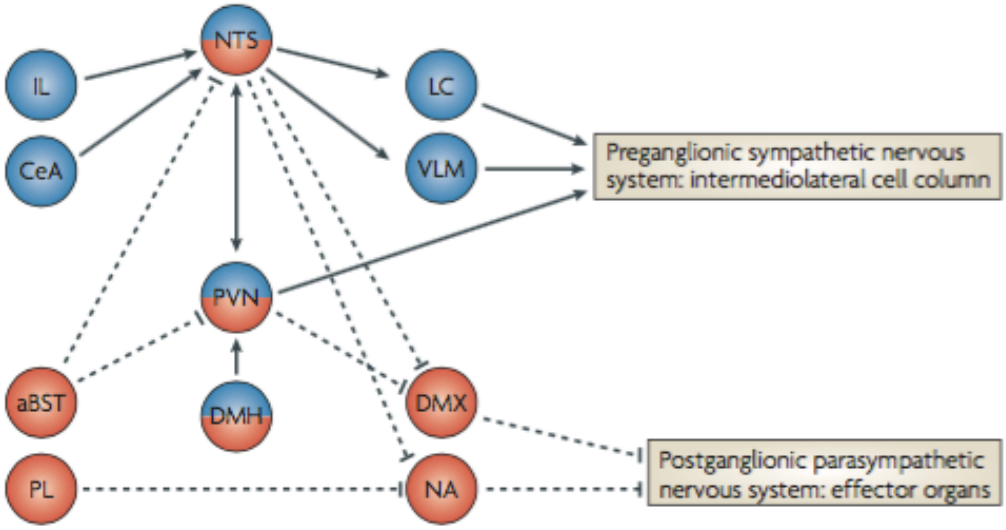

Figure **4-2**: Central autonomic network including the Locus Coeruleus (LC) ventrolateral medulla (VLM), dorsal motor nucleus of the vagus (DMX) and nucleus ambiguus (NA) (Ulrich-Lai & Herman 2009).

The brain is, however, arguably more than the sum of its parts, and a whole brain perspective of the functional connectivity between these regions is missing from Table 3. To gain a whole-brain perspective on the CAN, we consider a 2017 fMRI neuroimaging paper by Thome et al. This paper examines the topographical structure of the CAN network and the correlation between functional connectivity of the CAN and heart rate variability (HRV) in subjects with PTSD and in healthy controls. First, Thome et al. describe HRV as a "promising index of autonomic activity"; this is inaccurate. Only the high frequency component of heart rate variability has a clear physiological interpretation, which is as an index of ventral vagal tone emerging from the nucleus ambiguus (Porges 1995). The low frequency component of heart rate variability has no clear interpretation, and therefore cannot be used as an index of sympathetic or of dorsal vagal complex tone. Futhermore, as we have seen, the ANS is capable of distributing

differential control to different organ systems. The analysis of HRV extends to the heart; it is misleading to extrapolate it to the body as a whole.

This study included 57 participants with PTSD and 41 controls. The mean blood-oxygen-level-dependent (BOLD) time course was extracted from the voxels representing each seed region (medial prefrontal cortex (mPFC), amygdala (Amyg), and the periaqueductal grey (PAG)), and functional connectivity (FC) was taken as the correlation between the BOLD time course of the seed region and other areas in the brain. High frequency HRV (HF HRV) scores were regressed against the correlation coefficients calculated between CAN regions. Higher HF HRV correlated positively with FC between the mPFC and insula. PET studies have shown that the insula is important for feedforward control of increased cardiac output on the onset of exercise (Nowak et al 2005, Table 3), suggesting a possible role for the insula in mediating the cardiac component of a fight-or-flight response as well. This correlation was absent in the PTSD group. Higher HF HRV also predicted higher FC between the right Amyg and PAG and right insula. The Amyg can drive both autonomic and endocrine stress response and is important for associative fear learning (Table 3, Ulrich-Lai & Herman 2009), while the PAG is capable of stimulating aberrant running as well as distinct pathways eliciting freezing behavior (Hagenaars et al 2014). Positive correlations were also found between HR HRV and FC between the PAG and the right dorsal cingulate cortex, which is thought to be involved in incongruence and error detection, the right mPFC, and the left thalamus, though no specific thalamic nuclei were mentioned. None of the FC between these regions correlated with HF HRV in the PTSD group.

It is impossible to speak to the directionality or inhibitory/excitatory content of FC from fMRI data alone, and the thalamus and mPFC are such complex areas that speculating about their effect on the vagal control of the heart without more information may not be useful (See Table 3). What this finding does seem to suggest, however, is that in healthy subjects, functional connectivity between cortical CAN regions appears to exert a tighter control over ventral vagal

modulation of the heart than it does in PTSD. HR HRV was also significantly lower in the PTSD group, suggesting that the VVC is also more withdrawn in PTSD than in healthy controls. Therefore, these circuits may be important for the normal, "prosocial" VVC function (Porges, 1995). In this study, the hypothalamus, LC and brainstem regions were not examined, suggesting that in trauma, parasympathetic control of the heart may become more tightly associated with networks involving these regions.

Table **4-1**: Brain regions involved in the orchestration of autonomic and endocrine response, and their function. Information from the review by Ulrich-Lai & Herman, 2009 unless otherwise stated.

Brain Region	**Autonomic or Endocrine control**	**Function**
vmPFC IL	Sympathetic (+) and endocrine (+)	Inhibits cardioinhibitory pathway of the baroreflex in conditioned autonomic response
vmPFC PL	Sympathetic (+/-) and endocrine (-)	Controls duration, not peak of GC levels, thought to control termination of response. Inhibition and injection of NE induce tachycardic response.
Amygdala CeA	Sympathetic (+/-)	Preferentially activated by systemic stressors. Inhibits bradycardic responses to psychogenic stress.
Amygdala BLA	Endocrine (+)	Major input nucleus of the thalamus and sensory cortices (Calhoon & Tye 2015). Preferentially activated by psychogenic stressors, lesions attenuate HPA response to restraint stress. Stores simple A+B associations in fear learning (Philips & LeDoux 1992).
Amygdala MeA	Endocrine (+)	Preferentially activated by psychogenic stressors.
Hippocampus	Sympathetic (-) and endocrine (-)	Controls duration, not peak, of GC time response. Plays a role in the use of temporal, spatial contextual information

		to inhibit stress response. Subserved by vSub.	
Bed nucleus of the stria terminalis	Autonomic (?) and endocrine (+/-)	Important for cardiac inhibition, excitation *in vivo* produces rapid tachycardia followed by bradycardia. Anteroventral subregions excites HPA axis, posteromedial BnST provides inhibition of HPA axis.	
Insula	Autonomic (+/-)	Involved in interoception and emotional experience (Zaki et al 2012), resection following tumor extraction results in bradycardic arrhythmias in one case study (de Morree et al 2004). Bradycardia, tachycardia and increases in blood pressure have also been attributed to insula stimulation. The insula is considered to be a necessary component for feedforward central autonomic control of the heart during initiation of exercise (Nowak et al 2005).	
Anterior Cingulate Cortex	Parasympathetic (+)	Response inhibition in response to incongruence, error, the left dACC and a part of the left vACC show BOLD activity in response to incongruent stimuli that is positively correlated with the HF component of HRV, suggesting a role of the ACC in VVC control (Mathews et al 2004).	
Paraventricular nucleus of the thalamaus	Autonomic	Lateral shell important relay station for the processing of afferent pain information, medial shell for visceral information. Both regions project to the insular cortex (Saper 2002) and will be subject to modulation by corticothalamic projections acting on the reticular thalamic nucleus (Sherman 2000).	

Mediolateral nucleus of the hypothalamus	Autonomic	Associated with vigilance; Stimulation associated with bradycardia, apnea, decreased hind limb blood flow, crouching, phasic immobility, increased muscle tone, head trembling (Hagenaars et al 2014).	
Dorsomedial nucleus of the of hypothalamus	Autonomic, endocrine	Associated with proactive coping; tachycardia, hyperventilation, inhibition of cardioinhibitory component of the arterial baroreflex, aberrant running (Hagenaars et al 2014).	
Paraventricular nucleus of the of hypothalamus	Parasympathetic (-), sympathetic (+) and endocrine (+/-)	Recieves inhibition for mPOA involved in thermoregulation, gonadal steroid signaling, and sleep. Innervation from arcuate nucleus impacts metabolism. Circadian control is mediated by SCN innervation of the peri PVN area. PVN activates HPA axis; preautonomic area of PVN inhibits PNS, may play special role in hypoglycemic reflex (O'Hare & Zsombok 2016), cardioexcitatory sympathetic response, and agitated running (Hagenaars et al 2014).	
Locus Coeruleus	Autonomic (+)	Receives excitatory and inhibitory input from the amygdala and excitatory input from PVN (Dimitrov et al 2013). Directly excites preganglionic sympathetic fibers. Inhibits parasympathetic pupillary constriction and salivation. Increases cardiac output and exerts inhibitory tone on VLM-mediated vasoconstriction. Involved in attentional changes to novelty, extraction of salient environmental cues, and	

		conditioned fear response.	
Periaqueductal Grey	Autonomic	BOLD activity has been shown to shift from the PFC to the PAG in response to a virtual threat passing from a distal to proximal position, where its activation was related to dread and helplessness, suggesting a role in tonic immobility (Hermans et al 2012). The dorsolateral PAG is attributed with controlling fight and flight behavior, and its electrical stimulation initiates aberrant running. The dorsal PAG may also indirectly activate freeze responses via connections to forebrain structures. Stimulation of the ventrolateral PAG initiates freeze and tonic immobility and may have a depressor action on cardiac output via direct projections to parasympathetic preganglionic fibers (Hagenaars et al 2014).	
Parabrachial Nucleus	Autonomic	Second order relay for sympathetic visceral afferets and possible first order relay for parasympathetic visceral afferents, important for perception of pain and systemic stressor information.	
Nucleus Ambiguus	Parasympatheic (+)	Receives and provides modulatory feedback to facial, glossopharyngeal, trigeminal nuclei, influencing prosocial facial expression and affective vocal tone. Regulates respiratory sinus arrhythmia and provides vagal break to the heart (Porges 1995).	
Dorsal motor nucleus of the vagus	Parasympathetic (+)	Innveration of subdiaphragmatic organs, bradycardic influence on the heart (Porges 1995).	

Ventrolateral medulla	Sympathetic (+)	Sympathoexcitatory component of the baroreflex mostly involved in vasoconstriction in the regulation of blood pressure (Bennaroch 2008).	

Hierarchy of Stress Response and relevance to trauma

The notion of a hierarchy in stress response is an important aspect of many models of stress reactivity (Porges, 1995, Hagenaars et al 2014). Porges aligns the hierarchy of stress response in his theory with that of Jacksonian dissolution, which suggests that phylogenetically newer coping strategies, in this case executed via the mylenated nerves of the ventral vagal complex (VVC) and the sympathetic nervous system (SNS), should be recruited before those responses that are more conserved, which here should be executed by the unmyelinated projections emerging from the dorsal vagal complex (DVC). In this conceptualization, after an orienting response to bovelty, reactions to stress should first be mediated by the coupled VVC and special visceral efferent pathway regulating the striated muscles of the face and head, including muscles of facial expression. If this response is insufficient to neutralize the source of alarm, the stress response should then be subserved by the withdrawal of the VVC and activation of the SNS. If the stress is so extreme that neither flight nor fight are viable, the stress response should then move to the DVC, manifesting in tonic immobility, a death-feigning response characterized by altered heart rate, hypotension, cardiac vulnerability and immobility (2011).

While this model is appealing and likely holds valuable insight into factors modulating the recruitment patterns of autonomic subsystems, it is insufficient. In fear conditioning, animals

exhibit conditioned immobile responses to mild stress that are not orienting responses. While there is no way to differentiate this freezing from tonic immobility based on behavioral data alone, human accounts give reason enough to suspect that this freezing is not tonic immobility, either. Mild shock, an aversive stimulus used in both human and laboratory animal fear conditioning paradigms, is sufficient to provoke a conditioned cessation of locomotion, but not true tonic immobility in a physiological sense. The state that is described by human trauma survivors and clinicians (Levine 1997) during an actual assault is described as producing profound alterations in consciousness, a loss of body sensation and an avolitional paralysis that is inconsistent with the freeze response elicited in humans by mild stress in laboratory conditions. In animals, a state characterized by immobility in response to genuinely potentially lethal stress alone is attributed with the cardiac vulnerability and sudden death discussed in Chapter 3. It is thus likely that tonic immobility, not freezing per se, is most analogous to the state clinicians implicate in the etiology of traumatic stress disorders.

While the orienting response, freezing responses to mild stress, and tonic immobility in response to overwhelming, life-threatening stress all result in a cessation of locomotion and usually in bradycardia, they appear dissimilar enough in human experience that it is unlikely they can all be attributed to the same neurophysiological state or pathway. Unfortunately, this important distinction is often lost in the literature, and the word “freeze” is often used ubiquitously, to describe all immobile behavioral states elicited by any level of stress exposure.

The orienting response is defined by Porges as a conserved, DMNX-mediated precursor to shifted attention and is characterized behaviorally by a cessation of gross locomotion, with a shift of sensory organs toward the stimulus. This is a transient state connected to the experience of novelty in general and to the evaluation of a presenting threat, which is very similar to the role ascribed to the locus coeruleus by researchers in the 80’s (Chapter 2). This state is typically associated with parasympathetic dominance over the heart, manifest in transient bradycardia. it

has also been associated with physiological markers of increased sympathetic tone, including pupillary dilation and increased muscle tone.

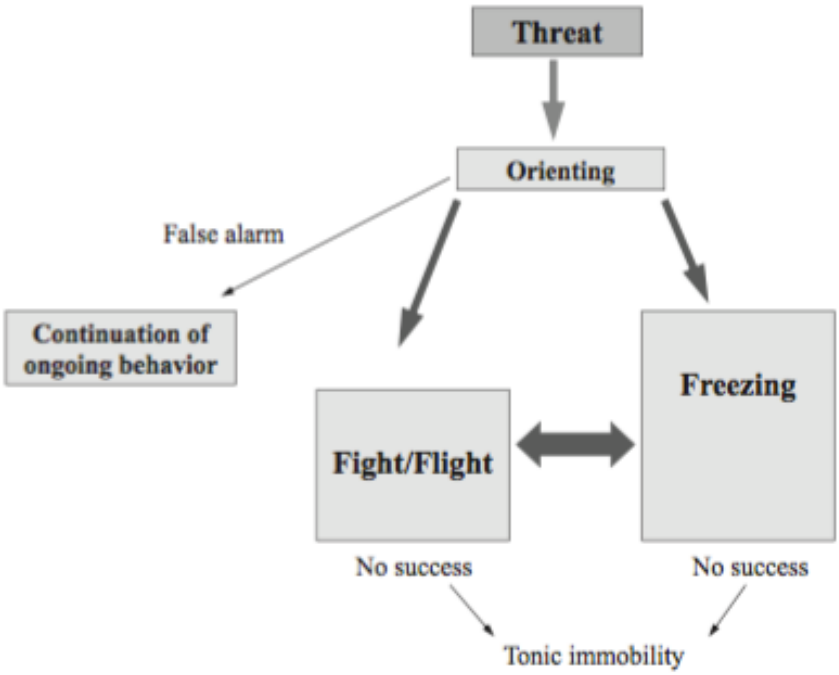

Figure **4-3**: Hierarchy of stress response (Haagenars et al 2014)

Freeze responses to mild stress or to the anticipation of mild stress are thought to be a more flexible, prolonged and stressor-specific reaction to threat than is an orienting response. They optimize the likelihood that a nearby predator drawn to movement will fail to locate an animal, and also may allow the optimization of the same attentive selection for salient cues mentioned in Chapter 2. Freezing behaviors in response to mild stress or to threat may also help the body prepare to execute a fight or flight response. Freezing behavior has been associated with bradycardia, and possibly with tachycardia, but also with increases in muscle tone. Freezing behaviors are seen through stimulation of the dorsal and ventrolateral PAG, vigilance-associated behaviors including bracing are seen in stimulation of the mediolateral nucleus of the hypothalamus. Compellingly, cessation of stimulation of the ventrolateral PAG causes an

immediate cessation of freezing behavior, while freezing initiated by stimulation of the dorsolateral PAG continues even after stimulation ends. It is thought that this is because the dorsolateral pathway routes first to cortical structures, including the PFC. There appear to be separate systemic neural substrates for volitional and preconscious freezing behavior (Hagenaars et al 2014). Of all possible freezing behaviors, the state thought most homologous to that of the avolitional paralysis reported by trauma survivors is tonic immobility, a state thought to occur when fight, flight, and avoidance are impossible, and an overwhelming injury is imminent. Levine (1997) speaks of the death-feigning paralysis observed in chickens when a fox enters the henhouse, from which those who survive emerge shaking. Hofer demonstrated a state of tonic immobility in extreme stress in which the heart shows vulnerability not reported in freezing to mild stress (1970), occasionally resulting in the death of the animal. Tonic immobility has been associated with tachycardia, bradycardia. However, unlike other freezing behaviors associated with mild stress, tonic immobility has also been characterized with hypotension (Hagenaars et al 2014).

For a detailed discussion of the physiology of a fight or flight response, see Chapter 2. Both conditions are associated with hypertension and tachycardia, necessary for maintenance of mean arterial blood pressure and the mobilization of resources. Porges (1995) characterizes fight and flight as responses to stress that come after orienting and that precede freeze, but there is reason to question this model. Freezing behavior is a canonical and robust behavioral response to mild stress seen in laboratory animals, however, if given the opportunity to escape from a mild stressor, laboratory animals will typically prefer to flee than to freeze. Furthermore, aberrant running, or directionless fight-or-flight, is a phenomenon we report here seen in response to predator odor and in the intertrial intervals of a conditioned fear response paradigm (Hagenaars et al 2014, see Chapter 5 for discussion of data). This phenomenon has also been observed upon stimulation of the dorsal PAG and dorsomedial hypothalamus, and described as a behavioral

indication of panic analogous to panic attacks in humans (Siqueira et al 2010). While freezing, fight and flight all may serve as appropriate responses to mild stress (See Figure 4-3), directionless flight may indicate a more extreme state of panic.

Orienting, freezing and tonic immobility are all states conventionally ascribed to parasympathetic control and fight and flight, conversely, are associated with sympathetic activation. This is a model that risks abstracting something fundamental out of our understanding of the autonomic substrates of stress response. In exercise physiology, atropine treadmill studies in humans (Chapter 2) were designed to chart the time course of vagal withdrawal from the heart after the onset of aerobic exercise. Conventional wisdom states that the vagal break must withdraw to allow heart rate to climb above baseline, and the SNS must take over to bring heart rate up to levels where aerobic exercise is feasible. It was thus a shock when the data demonstrated some degree of parasympathetic control over the heart remained at all intensities of aerobic exercise except at absolute maximum. Likewise, investigations into the physiological markers of a variety of reactive stress responses associated with cessation of locomotion have shown reactive coping strategies to be dynamic, mixed autonomic states. Orienting in response to an adverse stimulus is associated with transient bradycardia but attenuated pupillary constriction. Freezing behaviors and tonic immobility have also been characterized as bradycardic responses, but involve mild increases in muscle tone. Some studies have shown that these states may sometimes also be associated with tachycardia. This suggests that perhaps none of these states should be purely defined in terms of sympathetic or parasympathetic activation, but instead understood as a collective of innate and learned responses that reflect a dynamic and targeted reflexive balance between the two autonomic systems intended to respond with some specificity to the demands of any individual stressor. In this characterization, it is perhaps not so much the state that requires the activation of one system over the other that is interesting to pathology, but the states that renders control of the balance between them most vulnerable. This idea is captured

in the model of chronic hypoxemia as a cause of hypertension (Chapter 3). Thome at al present evidence of an apparent functional uncoupling of cortical circuits in the CAN and peripheral control of heart rate variability in PTSD patients that may also speak to the importance of this kind of vulnerability to psychopathology.

Interestingly, though it may correspond to either tachycardia or bradycardia, tonic immobility alone has also been associated with hypotension, indicating a failure of the impressive and parallel central autonomic and endocrine reflex circuits discussed in Chapter 2 to successfully regulate vascular resistance in response to autonomic action on the heart. Indeed, tonic immobility, the most hierarchically extreme and perhaps most physiologically expensive (Hagenaars et al 2014, Porges 2009) response, and not mild freezing, flight, fight or orienting behaviors, has been characterized as a state of cardiac vulnerability in mammals, resulting in cardiac arrest during diastole, perhaps because of this dissociation between cardiac output and vascular reflexes (Hofer 1970).

Clinicians speculate that trauma patients develop pathology because they are "stuck" in pathogenic vestiges of an extreme "freeze" state (Levine 1997). This state appears homologous to the state of tonic immobility, a state that Porges' polyvagal hierarchy associates with the withdrawal of the ventral vagal break from the heart. This idea, interpreted through polyvagal theory, is supported by the repeated finding of decreased HRV in PTSD patients (Thome et al 2017). However, polyvagal theory would also assert that being stuck in a "freeze" would be a condition of unopposed, bradycardia-inducing DMNX control over the heart. While clinicians have observed bradycardia in PTSD (Scaer 2001), PTSD has also been associated with elevated resting heart rate attributed to an overactive SNS (Bedi & Aurora 2007). The idea of ventral vagal withdrawal appears important in understanding autonomic stress contributions to trauma pathology, but it fails to adequately capture the dynamic, reflexive nature of the autonomic nervous system. Taking that into account, we may move to a speculation that may produce more

testable hypotheses (see Future Directions). It is perhaps tonic immobility that we might suspect in the etiology of trauma-related (psycho)pathology in humans, not necessarily because of ventral vagal withdrawal and DVC-mediated cardiac control per se, but because it is here within the repertoire of stress response that the homeorhetic integration of autonomic reflexes appears most vulnerable to immediate dysregulation. It may not be tonic immobility per se, but a breakdown of centrally-mediated homeorhetic reflexes therein, that is relevant to the etiology of trauma.

Heterogeneity in Mammalian Autonomic Profiles and Behavioral Repertoires to Stress

To investigate the idea that autonomic integrity may present a resiliency factor in trauma, we note first that there are individual differences in the coping styles animals will exhibit to any homotypic stressor. In the model of apnea used in Chapter 3 to illustrate the emergence of pathology in chronic stress, obstructive sleep apnea was discussed as a chronic consequence of a normal phenomenon: peritraumatic sleep fragmentation. A loss of airway tone will not affect all trauma survivors equally, as the morphology of some airways makes them more vulnerable to exacerbated collapsibility than others (Krakow et al 2002). Much as a screening of morphological vulnerability to airway collapse could enable clinicians to better protect trauma survivors from apnea, elucidating biomarkers for susceptibility to trauma could allow clinicians to better protect psychologically vulnerable populations. The vulnerability of the autonomic nervous system both in post-traumatic stress disorder (PTSD) and in tonic immobility suggests that perhaps individual differences in the function of the ANS or in the central autonomic network (CAN) may contribute to vulnerability in exposure to potentially traumatic stress. If this is the case, it makes sense to examine individual differences in stress response.

Heterogeneity in mammalian stress response has already been examined. In their 1999 review, Koolhas et al. discuss the behavioral, autonomic and endocrine profiles of animals who

exhibit reactive (i.e. immobile) and proactive (e.g. fight or flight) coping strategies to a homotypic stressor. Animals more prone to react to novelty and mild stress with immobility tend to show parasympathetic dominance over the heart and low sympathetic cardiac reactivity, manifest as normotropic or bradycardic response to novelty and mild stress. They show normal HPA axis function, measured by serum CORT levels, and high levels of HPA reactivity. By contrast, more proactive animals tend to show spikes in heart rate upon exposure to mild stress or novelty indicative of either vagal withdrawal and or vagal withdrawal and increased sympathetic tone. These animals demonstrate low levels of both HPA baseline activity and reactivity.

Chapter 5

Assessing fear extinction learning, heterogeneity in stress response and vulnerability to trauma

Trauma survivors often experience with fear extinction learning (Milad et al 2009). Studies demonstrating differences in fear extinction learning between post-traumatic stress disorder (PTSD) patients and healthy controls have led to the speculation that PTSD constitutes an impaired ability to effectively consolidate fear extinction memory after exposure to overwhelming stress. Through Pavlovian conditioning (Pavlov, 1927), autonomic and endocrine physiological stress responses can become conditioned to environmental and systemic cues, known as triggers in PTSD literature. In PTSD, these triggers appear to endure in spite of a lack of reinforcement. In this chapter, we will depart from the literature review format of this thesis to first examine the author's own work developing a novel measure for quantifying fear learning in fear extinction training and testing paradigms. The development of this measure might help avoid the procedure of fasting for the FC/FE experiment paradigm currently used in multiple labs, which by itself can be a confounder for stress studies. Finally, the application of this measure to existing data led to interesting observations into possible physiological substrates of vulnerability to PTSD. These observations will be discussed in the context of the initial literature review (Chapters 2-4).

A New Measure of Reactivity in Pavlovian Fear Conditioning

Fear extinction paradigms depend on a single behavioral measure, freeze time, to provide quantified assessments of anxiety and learning. While this measure has been accepted as

noncontroversial and robust, it is not without its limitations as a behavioral assay. Fear conditioning paradigms teach an association between an unconditioned, aversive stimulus (US, e.g. shock) and a conditioned stimulus (CS, e.g. single-frequency tone). In fear extinction, the CS is repetitively presented without the US, which results in the development of a new, "overriding" fear extinction memory (Bouton & Bolles 1979). Associative fear learning is quantified by an increase in freeze time in response to CS exposures across time, and fear memory retention is quantified as a maintained increase in freeze behavior after a 24-hour consolidation period. Similarly, the efficacy of fear extinction is quantified by a decrease in freeze time. Fear extinction is typically broken down into two phases; During the first phase, fear extinction learning, it is expected that subjects will show an initially high freeze response upon exposure to the CS that decreases over time (15-20 trials). The second phase is called fear extinction testing and occurs after at least 24 hours have been allotted for memory consolidation. Some spontaneous recall is expected during the initial exposures to the CS, followed by a return to low levels of freezing across CS exposures. Idealized freeze time profiles for a successful fear conditioning and extinction paradigm can be seen in Figure 5-1. Of note here is the monotonic increase in freeze time across tone exposures during fear conditioning. For fear extinction, the freeze time across the first few exposures of the unpaired tone reflects the elevated levels of freezing that occurred during conditioning and then decreased as the fear extinction memory is acquired. After a consolidation period of 24 hours, some spontaneous recall is expected, reflected by the green bar in Fig 5-1. Freeze times are then expected to taper off as during extinction acquisition.

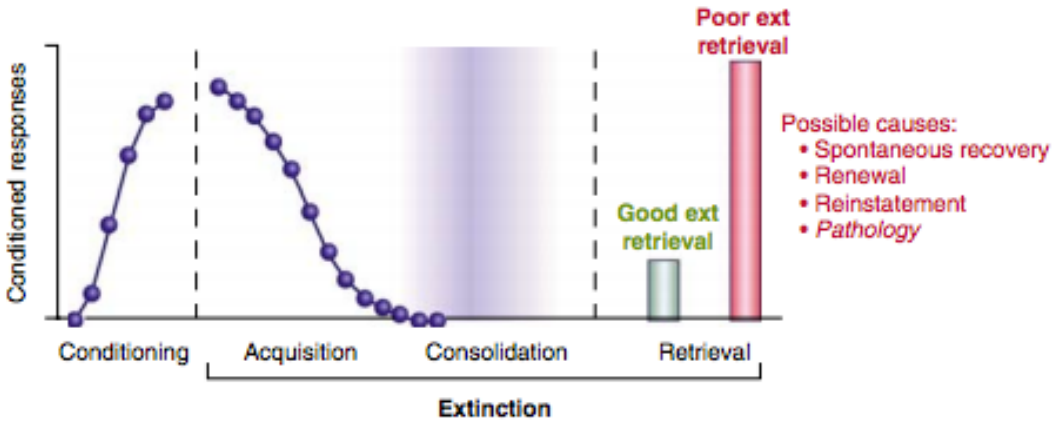

Figure **5-1**: Idealized fear conditioning and extinction data (Quirk & Meuller 2008)

Behavioral freezing in response to stress (See Chapter 4) is, in isolation, impossible to distinguish from immobility indicative of fatigue or boredom. Despite visible differences in crouching, respiration and muscle tone, an automated scoring program will score sleep, off-camera exploration, and immobility associated the boredom as equitable to freezing induced by fear. Fatigue and boredom are not particularly concerning phenomena during fear conditioning, but they present significant confounds in interpreting automatically scored fear extinction acquisition and especially testing. Effective fear extinction protocols easily require more than an hour to expose animal to the 15-20 trials of required CS/no-US exposure. In the later exposures, animals will become immobile for up to the entire duration of the tone but will subjectively appear to be sleeping. To circumvent this, it is common practice to fast animals beforehand, as fasting will incentivize them to move to acquire food whenever they're not too afraid to do so. However, food deprivation is itself a stressor, which results in a CRH-mediated potentiation of the sympathetic nervous system and in a blunted release of GCs released during exposure to a second stressor (Sapolsky et al 2000, Kirschbaum et al 1997). Because of the permissive and suppressive effects of GCs released in the time course of hours after stress exposure, fasting likely alters the time course of GC effect on peripheral tissues (Chapter 2). Fasting has also been

shown to reduce the sensitivity of vascular vasopressin receptors, attenuating vasoconstriction in apparent preparation for hemorrhage (Sapolsky et al 2000). In addition, we have also seen that hypoglycemia provokes a differential activation of the ANS vis-à-vis the liver than do other stressors. While blood glucose may be maintained within a euglycemic range during fasting (Kirschbaum et al 1997), this may require specific sympathetic action on the liver, suggesting that the metabolic component of the stress response may be altered. Altered HPA axis and SNS function due to fasting stress introduce new physiological confounds in an effort to eliminate behavioral ones. This makes a different approach to parceling out fatigue and boredom-related immobility desirable. Here, we present a simple approach compatible with automated scoring protocols for parceling out stress-induced freezing from boredom and sleep.

This technique was developed as part of a broader neuroimaging study, designed by Dr. Pablo Perez, using a battery of behavioral testing including fear conditioning and extinction to examine resiliency after exposure to a potentially traumatic stressor. For the sake of this thesis, only the behavioral data relevant to heterogeneity in fear response will be discussed.

First, baseline acoustic startle data was collected on adult male Long-Evans rats. Startle response is a reflexive reaction and so an increase in startle response was thought to be a good indication of increased arousal. Animals were placed in a Plexiglas tube sufficiently large to allow them to fully turn without restraint. The tube was placed in a dark soundproof chamber equipped with a speaker and pressure-sensitive floor. The animal was habituated to the chamber with a 5-minute exposure of continuous white noise at 68 dB. Each animal was subjected to 30 successive exposures of 0.4 second 110 dB bursts of white noise spaced apart with randomized ITIs ranging between 30-45 seconds.

Second, animals were exposed to fox urine as an overwhelming stressor. Each animal was acclimated to an empty, plastic cage for thirty minutes the day before exposure. On the day of exposure, a cotton pad soaked with red fox urine from the Wildlife Research Center was

placed in the cage and the animal was left alone for 10 minutes. Video was recorded and hand-scored for freezing time.

One week after fox urine exposure, elevated plus maze (EPM) and a second round of ASR were conducted to assess basal anxiety levels and fear, respectively. Because navigating the EPM requires decision-making not assessed by ASR, EPM is a more reliable indicator of affective anxiety than is a startle response per se. Animals were placed on the center of the EPM in a lit room and allowed to roam freely for 5 minutes. Time spent in the open arms was scored as time spent with the animals' entire body outside of the center of the maze, time in the closed arms likewise required the animals' body to be outside of the maze center. Time spent in the center of the maze was omitted from the final EPM score to facilitate interpretation of the results.

Ratios of averaged first 10 ASR scores [ASR after FU exposure/ASR before FU exposure] and EPM scores [Time on open arms/(Time on open + closed arms)] were used to group the animals into resilient and vulnerable categories. Rats with an ASR ratio was greater than 1 and whose EPM ratio was above median were classified as vulnerable, other animals were labeled resilient.

Vulnerable, resilient and control rats were put through a classical fear conditioning and extinction paradigm. The animals were conditioned on day 1 and given a 24-hour consolidation period to accommodate imaging. Extinction acquisition and testing were carried out on days 3 and 4. Fear conditioning and extinction for each animal was carried out in the same soundproof chamber, under dim light (20 lumen) and on a mesh wire floor. For fear conditioning, a 0.6 mA shock (CS) that lasted for a duration of 0.5 seconds co-terminated with a 30-second 4000 Hz tone at an intensity of 70 dB. The animal was exposed to one acclimation tone and then seven CS/US pairings. Each tone presentation was separated by an intertrial interval (ITI) randomly selected from a duration of 2-4 minutes. A laminated checkerboard pattern was placed in the cage floor for fear extinction and testing periods to reduce contextual freezing. Fear extinction acquisition and

training consisted of 20 and 10 tone presentations, respectively, without presentation of the shock. Freeze times were scored automatically with Anymaze software.

Because freeze time during fear conditioning and extinction was automatically scored without fasting the animals first, the rats dramatic increases in freeze time in the last ~10 trials of fear extinction training and after the first few extinction testing trials that were thought to correspond to sleeping and boredom, not to stress-related freezing. This trend was particularly concerning in fear extinction testing, as freeze scores show the opposite trend to what is expected and, because of that, sleep and boredom risked obfuscating evidence of extinction learning. To demonstrate that this was owed to sleep and boredom, not to a tone-conditioned stress response, the freeze times for each animal during the ITIs were scored and normalized to 30 seconds. The averaged freeze time during tone exposure was then subtracted from the normalized ITI time as an indicator of the animals' reactivity to the tone, with the reasoning that if the animal was afraid, it would freeze more during tone exposure than during the ITI, and if it was asleep, it likely would not. Note that this measure only makes sense during fear extinction training and testing; in fear conditioning, immobility during the intertrial intervals is associated with stress and contextual fear learning (Philips & LeDoux 1992). Because high levels of freezing during tone exposure were robustly demonstrated by all groups during fear conditioning and the beginning of fear extinction, negative scores, indicating that the animal moved more during than the tone than during the ITI, arguably reflect an "alarm clock" effect of the animal being woken up by the tone, and not stress-induced, aberrant running.

Using this metric, we found that the control animals showed reactions to the tone consistent with expected freeze curves for successful extinction learning (See Fig 5-1), without the physiological confounds inherent in fasting. To demonstrate the efficacy in this measure, see Figure 5-2 and Fig 5-3.

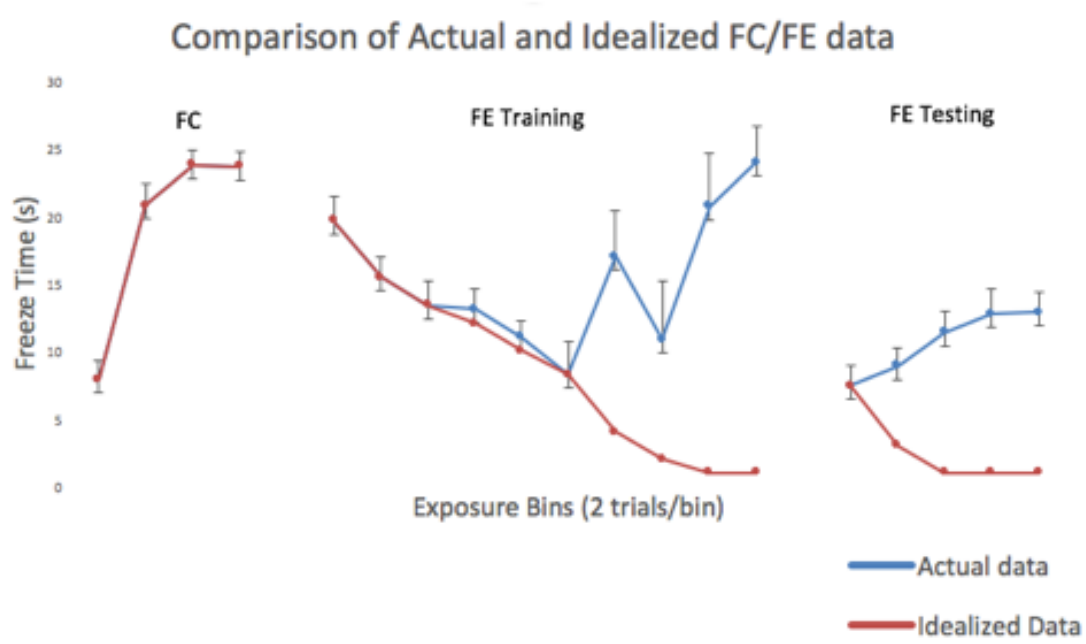

Figure **5-2**: Comparison of actual fear extinction data (blue) and idealized data (red). Bars represent standard error. N=18 rats for fear conditioning and fear testing. For fear extinction (FE) training, the last five bins represent 10 CS exposures that were added into the protocol to facilitate fear extinction learning. Only 8 of the 18 rats received these exposures, and therefore the variance for these bins is higher than for the others. Actualy fear conditioning data here is consistent with what would be expected, but of note here are the opposite trends to idealized data in the last 10 CS exposure of FE training and during the whole of FE testing.

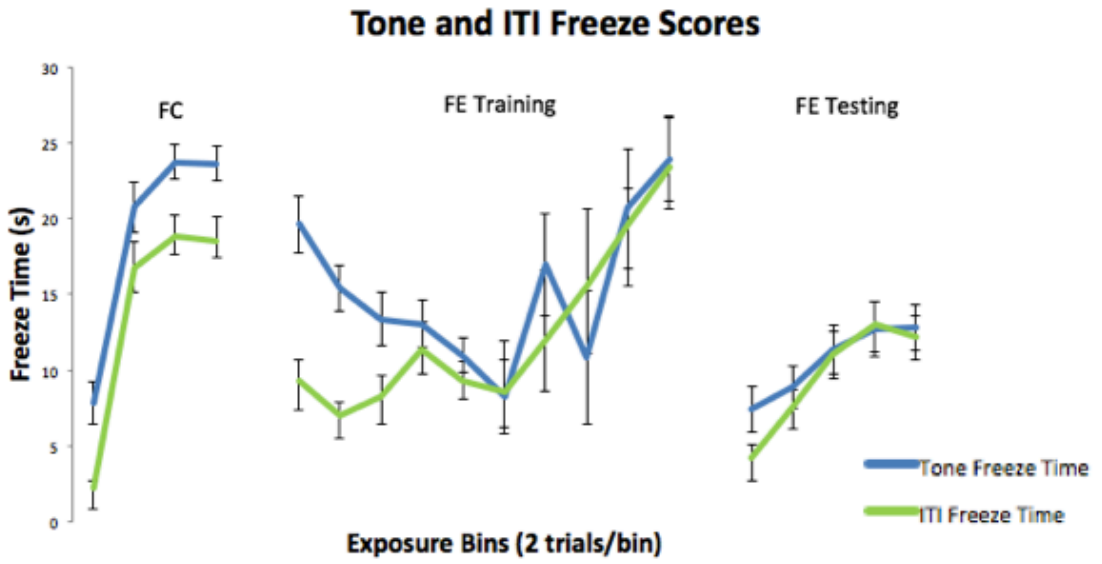

Figure **5-3**: The same actual freezing data scored during tone exposure from Fig 5-2 plotted against ITI freezing scores normalized to 30 seconds. Note that while during fear conditioning (FC) ITI freeze time is indicative of fearful behavior (e.g. contextual fear learning), during fear extinction (FE) it is a good indicator of immobile behaviors that are not attributed to fear.

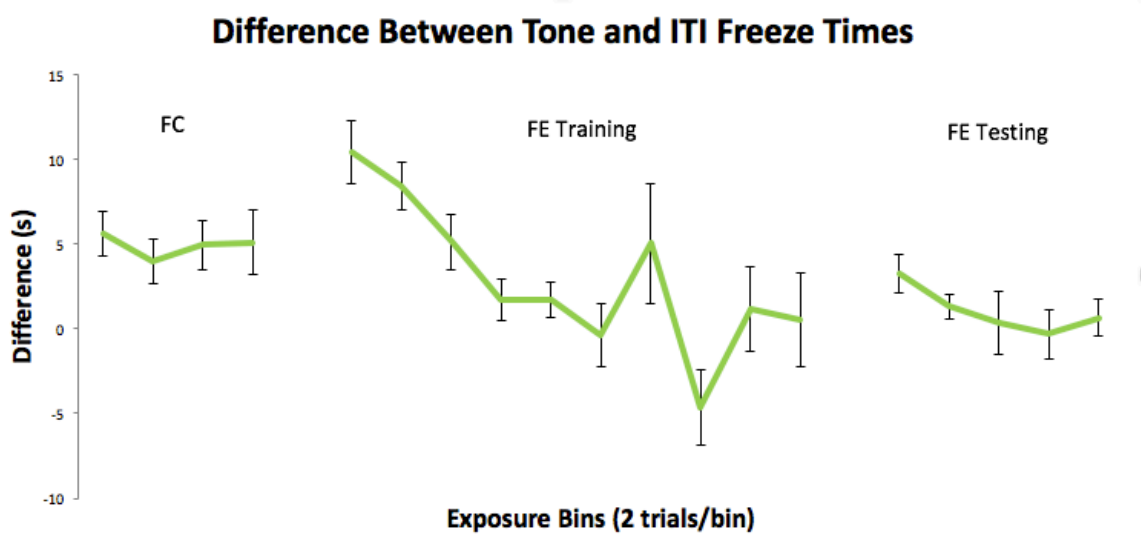

Figure **5-4**: The difference between tone and ITI freeze times shown in Fig 5-3, showing evidence of a cessation of fear-induced immobility during all fear extinction training trials. This measure also shows spontaneous recovery of the fear memory in the initial 2 exposures of fear extinction testing, as expected, followed by a taper of fear-induced freezing. This measure recovers evidence of successful fear extinction training and testing without fasting and with an automated scoring protocol.

The development of this metric led to the discovery that vulnerable rats tended to freeze less during the fear conditioning ITIs, though the difference was only significant with controls (Fig 5-4, $p<.05$). Further investigation of freeze times show a robust and unexpected finding that resilient rats froze significantly more during fox urine exposure than did vulnerable rats or unexposed controls (Fig 5-5). This difference was importantly observed from the onset of fox urine exposure, suggesting that this heterogeneity in response is representative of a preexisting difference between groups. During fox urine exposure, this effect was robust across binned 2 minute intervals ($p<.05$ for bins 2 and 4, $p<.01$ for bin 3, $p<.001$ for bin 5, see Fig 5-5). This result gives a surprising indication that heterogeneity in behavioral response during overwhelming stress exposure may represent a physiological resiliency factor to trauma or anxiety-related psychopathology thereafter.

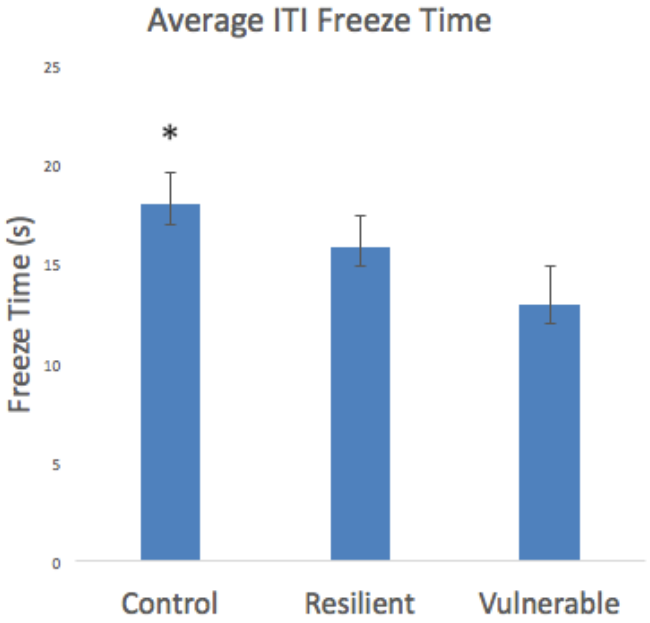

Figure **5-5**: Difference in averaged ITI freeze times for control, resilient and vulnerable rats. * = significant difference with vulnerable rats, $p < 0.05$

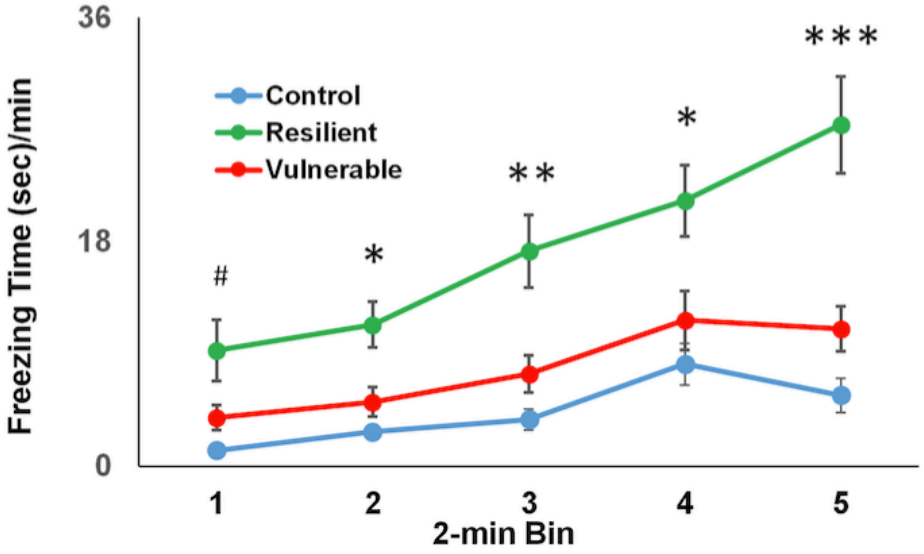

Figure **5-6**: Difference in freeze time during fox urine exposure for resilient, vulnerable, and control rats. * = significant from controls $p < 0.05$, ** = significant from controls $p < 0.01$, *** = significant from controls, $p < 0.001$

The study of physiology encourages the constant question, what is the homeostatic benefit of this response? In the case of a threatening fox odor within an enclosed environment, a more vigilant response may help the rat avoid detection, and conserve metabolic resources to better the chance of escape in spite of a possibly severe injury. Aberrant running without an escape route, which is considered indicative of a panicked state, may unnecessarily deplete metabolic resources and draw the attention of a nearby predator. While panic and tonic immobility are not equivalent experiences, prolonged panic across a 10-minute interval may be reflective of a dysregulated state. In this way, it is possible that vulnerable rats are more prone to reach dysregulated autonomic and extreme physiological states when threatened. Another, not mutually exclusive, possibility comes from the observation that mammals who employ more proactive strategies tend to have less a less reactive HPA axis than do reactive animals (Koolhas et al 1999). As we have seen (Chapter 2), a less reactive HPA axis may contribute over the short-term to autonomic dysregulation in the CNS through insufficient CRH production and feedback on autonomic centers. Over the long-term, high levels of glucocorticoids released from the adrenal cortex within hours of a stress response are supposed to facilitate metabolic and immune recovery and are responsible for genomic changes that facilitate long-term adaptation to future exposures of the same stressor. While without serum CORT measurements it is impossible to know if these vulnerable animals had attenuated HPA reactivity, insufficient recovery and adaptation after fox urine exposure could contribute on its own to increased baseline anxiety levels after exposure.

Future Directions

Scaer hypothesizes that autonomic dysregulation owed to peritraumatic dissociation represents the underlying neurological substrate of the syndrome of trauma (2001). A human freeze response to inescapable and potentially lethal stress has been characterized as an extreme state of altered consciousness, a cessation of interoception and somatosensation, and a loss of motor control (Levin 1997). In animals, inescapable and lethal stress has elicited a state of tonic immobility in which autonomic regulation of the heart seems particularly vulnerable (Hofer 1970, Scaer 2001, Porges 1995). These characterizations of extreme stress as etiological factors for pathology, coupled with the observation of a robust difference in stress response between vulnerable and resilient rats when exposed to predator odor, led to the creation of this thesis. The question was, could individual differences in the autonomic and endocrine components of a peritraumatic stress response predispose an animal to pathology? How?

The model of neurogenic hypertension in sleep apnea (Chapter 3) and the Thome paper demonstrating an uncoupling of central autonomic network (CAN) functional connectivity from ventral vagal control of the heart (2017, Chapter 4) allow us insight into how the autonomic nervous system may become pathogenically dysregulated following traumatic stress exposure. An immediate step can be taken to investigate whether or not FC between cortical regions of the CAN represents a vulnerability to or a consequence of PTSD. If this relationship exists, it is likely not the FC value itself but the correlation of that FC with ventral vagal control of the heart. From Fig 3-1, we know that the ventral vagal complex must withdraw to permit aerobic activity, and this would include aberrant running. The neuroimaging data from Dr. Perez's study may reveal if FC between the same CAN regions identified in Thome's 2017 study before and after fox urine exposure correlate with the freeze time, or with the speed or duration of aberrant running, during

exposure. This would be a hypothesis-driven indication that FC within the CAN network represents a vulnerability to PTSD, and may serve as a biomarker for vulnerable populations.

Furthermore, while descriptions of trauma as a freeze state (Levin 1997), or a condition of structural dissociation of personality (Hart et al 2006), are clinically useful and are likely grounded in a meaningful intuition, they are not helpful in generating testable neurophysiological hypotheses. Physiological markers indicative of states of extreme stress in which the autonomic nervous system is vulnerable to homeostatic dysregulation are. Together, disrupted heartrate and hypotension are physiological markers of an extreme and vulnerable state in animals that, if assayed by a measure of immobility alone, may be overlooked or confused with other freeze responses. Taking physiological measurements as descriptors of autonomic response to stress may allow a more precise understanding of what physiological parameters indicate that an animal more vulnerable to pathology. For example, actual predator exposure could be implemented while an animal is restrained, allowing its heart rate and blood pressure data to be tracked. If a failure of the autonomic nervous system to integrate control over the heart and vasculature is important in the etiology of PTSD, that should become evident there.

Finally, interventions targeting the autonomic nervous system are easier to design and implement than purely psychiatric or neurological interventions. If autonomic biomarkers for PTSD vulnerability can be identified involving cardiac function, then at-risk populations can be taught exercise-based interventions and biofeedback interventions either to protect themselves or to use in conjunction with therapy. Aerobic exercise has already been developed and explored for decades as an intervention for cardiovascular disease in part because of its health-promoting effects on autonomic function (see Rosenwinkle et al 2001 for review). Biofeedback interventions targeting the integration of cognitive control and physiological output variables also already exist. These could be further developed for this purpose. Biofeedback focusing on autonomic control of the heart could be cheaper and easier to implement than interventions focused solely on neural

activity: Neurofeedback interventions require access to an EEG, but rudimentary biofeedback involving heart rate variability could be developed and implemented on a smart phone with already-existing aps that use the phone's flashlight and camera as an optic sensor of heart rate.

REFERENCES

Alloway, K. D., & Pritchard, T. C. (2007) *Medical Neuroscience*. Hayes Barton Press. Raleigh, NC.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders (5th ed.)*. Washington, DC.

Antelman, S., Caggiula, A., Gershon, S., Edwards, D., Austin, M., Kiss, S., Kocan, D. (1997). *Stressor-induced oscillation: A possible model of the bidirectional symptoms of PTSD*, New York Academy of Sciences, 21:296- 305

Aquira G. (2012) *Chapter 8: The Hypothalamic–Pituitary–Adrenal Axis and Neuroendocrine Responses to Stress.* In Handbook of Neuroendocrinology, Fink G., Pfaff D. W., and Levine J. E., eds. Academic Press, San Diego, 2012, Pages 175-196, ISBN 9780123750976

Barnes, P. J. (1998) *Anti-inflammatory actions of glucocorticoids: Molecular mechanisms*. Clinical Science 94, 557-572

Bernard C. (1878*): Lectures on the phenomena common to animals and plants* Hoff HE, Guillemin R, Guillemin L, trans. Springfield (IL): Charles C Thomas; 1974.

Benarroch EE (1993): *The central autonomic network: Functional organization, dysfunction, and perspective.* Mayo Clin Proc 68: 988–1001.

BenarrochE EE (2008): *The arterial baroreflex: Functional organization and involvement in neurologic disease*. Neurology 71

Bouton ME, Bolles RC (1979). *Role of conditioned contextual stimuli in reinstatement of extinguished fear.* J Exp Psychol Anim Behav Process 5: 368–378.

Buffalari, D. & Grace, A. (2007) *Noradrenergic modulation of basolateral amygdala activity: Opposing influences of a-2 and B receptor activation.* J Neuro. 27(45):12358 –12366

Cannon, W. B. (1919). *Studies on the Conditions of Activity in Endocrine Glands: The Isolated Heart as an Indicator of Adrenal Secretion Induced by Pain, Asphyxia and Excitement. V.*

De Morree, H. M., Rutten, G. J., Szabó, B. M., Sitskoorn, M. M., Kop, W. J. (2016) *Effects of insula resection on autonomic nervous system activity.*
J Neurosurg Anesthesiol. Apr;28(2)

Dimitrov, E., Tanagawa, Y., Usdin, T. (2013) *Forebrain GABA-ergic projections to locus coeruleus in mouse*. J Comp Neurol. 521(10): 2373–2397.

Duclos, M. (2010) *Glucocorticoids: A doping agent?* Endocrinol Metab Clin N Am 39 (2010) 107–12

Dumbbell R., Matveeva O., Oyster H. (2016). *Circadian Clocks, Stress and Immunity*. Front. Endocrinol. May

Dutschmann M., Herbert H. (2006) *The Kölliker-Fuse nucleus gates the postinspiratory phase of the respiratory cycle to control inspiratory off-switch and upper airway resistance in rat*. Eur J Neurosci. 1071-84.

Felitti, V., Anda, T., Nordenberg, D., Williamson, D., Spitz, A., Edwards, V., Koss, M., & Marks, J. (1998). *Relationship of childhood abuse and household dyxfunction to many of the leading causes of death in adults: The adverse childhood experiences (ACE) study.* American Journal of Preventive Medicine, 14:245-257

Ginsberg, H. (1974). *Controlled vs noncontrolled termination of the immobility response in domestic fowl (Gallus gallus): parallels with the learned helplessness phenomenon, as quoted in Seligman, M. (1992)* Helplessness: On depression, development and death, New York:W.H. Freeman

Gonzalez-Lima, F & Scheich, H. (1984) *Neural substrates for tone-conditioned bradycardia demonstrated with 2-deoxyglucose. I. Activation of auditory nuclei.* Behav Brain Res 14(3):213-233

Gonzalez-Lima, F & Scheich, H. (1986) *Neural substrates for tone-conditioned bradycardia demonstrated with 2-deoxyglucose. II. Auditory cortex plasticity.* Behav Brain Res 20(1):281-293

Hagenaars, M. A., Oitzl, M., Roelofs, K. (2014) *Updating freeze: Aligning animal and human research*. Neuroscience and biobehavioral reviews 47

Hart, O., Nijenhuis, E. R. S., & Steele, K. (2006). *The haunted self: Structural dissociation and the treatment of chronic traumatization*. New York: W.W. Norton.

Heim, C., Mayberg, H. A., Mletzko, T., Nemeroff, C. B., Pruessner, J. C., (2013): *Decreased Cortical Representation of Genital Somatosensory Field After Childhood Sexual Abuse.* Am J Psychiatry 170(6)

Hessing, J. C. M., Hagelsø, A. M., Schouten, W. G. P., Wiepkema, P. R., Beek, J. A. M. (1994) *Individual behavioral and physiological strategies in pigs.* Physiology & Behavior 55

Hers, H.G. (1985) *Effects of glucocorticoids on carbohydrate metabolism.* Agents and Actions, vol. 17, 3/4

Hofer, M. (1970). *Cardiac and respiratory function during sudden prolonged immobility in wild rodents*, Psychosomatic Medicine, 32:633- 647

Joëls, M. & Baram, T. Z., (2009) *The neurosymphony of stress.* Nature Reviews Neuroscience 10

Joyner, J. M. & Casey, D. P. (2015) *Regulation of increased blood flow (hyperemia) to muscles during exercise: A hierarchy of competing physiological needs.* Physiol Rev 95: 549–601

Kirschbaum, C., Bono, E. G., Rohleder, N., Gessner, C., Pirke, K. M., Salvador, A., Hellhammer, D. H. (1997): *Effects of fasting and glucose load on free cortisol response to stress and nicotine.* Journal of clinical endocrinology and metabolism 82(4)

Koolhaas J. M., Korte, S. M., de Boer, S. F., van der Vegt, B. J., van Reenen, C. G., Hopster H., de Jong, I. C., Ruis, M. A. W., Blockhuis H. J. (1999) *Coping styles in animals: current status in behavior and stress physiology.* Neuroscience and Biobehavioral Reviews 23 925–935

Krakow, B., Melendrez, D., Warner T., Dorin, R., Harper, R., Hollifield, M. (2002) *To breathe, perchance to sleep: Disordered breathing and chronic insomnia amoung trauma survivors.* Sleep and breathing (6)4

Krystal, H. (1988). *Integration and self-healing: Affect, trauma, alexithymia*, Hillsdale:Lawrence Erlbaum

Kumar, P. & Prabhakar, N. (2012*). Peripheral Chemoreceptors: Function and Plasticity of the Carotid Body*. Compr Physiol 2(1): 141-219

Lambillotte, C., Gilon, P., & Henquin, J.C. (1997). *Direct glucocorticoid inhibition of insulin secretion. An in vitro stufy of dexamethasone effects in mouse islets*. J Clin Invest Vol. 99, No. 3, pp. 414-423.

Levine, P. (1997). *Waking the Tiger*, Berkeley:North Atlantic Books

Lipski, W. & Grace, A. (2013) *Activation of neurons in the hippocampal ventral subiculum by norepinephrine and the locus coeruleus*. Neuropsychopharmacology. 2013 Jan; 38(2): 285–292.

Matthews, S., Paulus, P. M., Simmons, A. N., Nelesen, R. A., Dimsdale J. E. (2004) *Functional subdivisions within anterior cingulate cortex and their relationship to autonomic nervous system function*. Neuroimage 22 (2004) 1151–1156

McEwen, B. (1999) *Allostasis and Allostatic Load: Implications for Neuropsycholopharmacology*. Neuropsychophramacology 22(2)
McEwen, B. S. (1999) *Stress and hippocampal plasticity.* Annual Review of Neuroscience. Vol. 22:105-122

Milad, M. R., Pitman, R. K., Ellis, C. B., Gold, A. L. Shin, L. M., Lasko, N. B., Zeidan, M. A., Handwerger K., Orr S. P., Rauch S. L. (2009) *Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder.* Biol Psychiatry. 2009 66(12):1075-82

Mores, D. J. A., Mirela, D. B., Cavalcanti-Kwiatkoski, R., Machado, B. H., Zoccal, D. B. (2012) *Contribution of the retrotrapezoid nucleus/parafacial respiratory region to the experiatory-sympathetic coupling in response to peripheral chemoreflex in rats*. *J Neurophysiol* 108: 882–890

Moore, J.D., Deschênes, M., Furuta, T., Huber, D., Smear, M., Demers, M., Kleinfield, D. (2013) *Hierarchy of orofacial rhythms revealed through whisking and breathing.* Nature 497: 205-212

Natarajan, R., Northrop, N., Yamamoto, B. K. (2015) *Protracted effects of chronic stress on serotonin dependent thermoregulation*. Stress. 2015; 18(6): 668–676.

Nijenhuis, E., Vanderlinden, J. & Spinhoven, P. (1998), *Animal defensive reactions as a model for trauma-induced dissociative reactions. Journal of Traumatic Stress*, 11(2), 243-260

Nowak, M., Holm, S., Biering-Sørensen, F., Secher, N. H., Friberg, L. (2005*): "Central command" and insular activation during attempted foot lifting in parapalegics.* Hum Brain Mapp. 25(2):259-65.

O'Hare, J. D. & Zsombok, A. (2016) *Brain-liver connections: role of the preautonomic PVN neurons.* Am J Physiol Endocrinol Metab.; 310(3): E183–E189.

Phillips, R. G.; LeDoux, J. E. (1992) *Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning.* Behavioral Neuroscience, Vol 106(2), 274-285

Porges, S. (1995). *Orienting in a defensive world: Mammalian modifications of our evolutionary heritage. A polyvagal theory*, Psychophysiology, 32:301-318

Porges, S. (2009) *The Polyvagal Theory: New Insights into Adaptive Reactions of the Autonomic Nervous System.* Cleve Clin J Med. April ; 76(Suppl 2): S86–S90
Quirk, G. J. & Meuller, D. (2008): *Neural mechanisms of extinguished learning and retrieval.* Neuropsychopharmacology 33 pg. 56-72

Rosenwinkle, E. T., Bloomfield, D. M., Arwady M. A., Goldsmith R. L., (2001): *Exercise and autonomic function in health and cardiovascular disease*. Cardiology Clinics 19(3)

Saltin, B., Radegran, G., Lou, M. D., & Roach, R. C. (1998). *Skeletal muscle blood flow in humans and its regulation during exercise*. Aeta Physiol Scand 1998, 162, 421-436

Samanta, D., Prabhakar, N., Semenza, G. (2017) *Systems biology of oxygen homeostasis.* WIREs Syst Biol Med 1382

Sapolsky R. M., Romero L. M., Munck A. U. (2000) *How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions.* Endocr Rev. (1):55-89.

Saxe, G. N., Stoddard, F., Hall, E., Chawla, N., Lopez, C., Sheridan, R., King, D., King, L., Yehuda, R. (2005) *Pathway to PTSD, Part 1: Children with burns.* Am J Psychiatry 162(7)

Scaer, R. (2001) *The Neurophysiology of Dissociation and Chronic Disease.* Applied Psychophysiology and Biofeedback *26(1), 73-91,*

Seals, D. R. (2005) *Chapter 9: The Autonomic Nervous* System. In American College of Sports Medicine Advanced Exercise Physiology, Sawka M. N., Tipton C. M., eds. ACSM, Baltimore, MD pgs 197-246.

Selye, H. (1946) *The General Adaptation Syndrome and the disease of adaptation.* The Journal Clinical Endocrinology 6(2)

Semenza, G. (2014) *Hypoxia-inducible Factors and Disease Pathology*. Annu Rev Pathol Mech Dis 9:47-71

Semenza, G. and Prabhakar, N. (2015) *Neural regulation of hypoxia-inducible factors and redox state drives pathogenesis of hypertension in a rodent model of sleep apnea*. J Appl Physiol 119: 1152-1156

Sephens, M. A. & Wand, G. (2012). *Stress and the HPA Axis: Role of Glucocorticoids in Alcohol Dependence*. Alcohol Research: Current Reviews (34) 4

Stubenitsky R., Verdouw P.D., Duncker D.J. (1998) *Autonomic control of cardiovascular performance and whole body O2 delivery and utilization in swine during treadmill exercise.* Cardiovasc Res. 1998 Aug;39(2):459-74.

Thome, J., Densmore, M., Frewen, P. A., McKinnon, M. C., Théberge, J., Nicholson, A. A., Koenig, J., Thayer, J. F., Lanius, R. A. (2017): *Desynchronization of the Autonomic Response and Central Autonomic Network Connectivity in Posttraumatic Stress Disorder.* Human Brain Mapping 38 pg. 27-40

Tortora, G. J., & Derrickson, B (2012). *Principles of anatomy and physiology*. New York, NY: HarperCollins College.

Ulrich-Lai, Y. M. & Herman, J. P. (2009): *Neural regulation of endocrine and autonomic stress response.* Nature Reviews Neuroscience 10

Vasquéz, D. A., de Arellano, M. A., Reid-Quñones, K., Bridges, A. J., Rheingold, A. A., Stocker, R. P. J., Danielson, C. K. (2012): *Peritraumatic dissociation and peritraumatic emotional predictors of PTSD in Latino Youth: Results from the Hispanic Family Study*. J Trauma Dissociation. 2012; 13(5): 509–525.

Wantanabe Y., Gould E., McEwen B. 1992. *Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons.* Brain Research,588

Zagon, A., Totterdell, S., & Jones, R. S. G. (1994): *Direct projections from the ventrolateral medulla oblongata to the limbic forebrain: Anterograde and retrograde tract-tracing studies in the rat*. Journal of Comparative Neurology 340(4)

Zaki, J., Davis, J. I., Ochsner, K. N. (2012) *Overlapping activity in anterior insula during interoception and emotional experience.* Neuroimage 62(1) pg. 493-499