Reciprocal Autonomic Regulation in Mother-Infant Dyads during Social Interaction

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Dissertation

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<tr>
<td>ANS</td>
<td>Autonomic Nervous System</td>
</tr>
<tr>
<td>BSI</td>
<td>Brief Symptom Inventory</td>
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<tr>
<td>DMNX</td>
<td>Dorsal Motor Nucleus of the Vagus (cranial nerve X)</td>
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<td>FFSF</td>
<td>Face-to-Face Still Face</td>
</tr>
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<td>GSR</td>
<td>Galvanic Skin Response</td>
</tr>
<tr>
<td>HF-HRV</td>
<td>High-frequency Heart Rate Variability</td>
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<tr>
<td>HP</td>
<td>Heart Period</td>
</tr>
<tr>
<td>HRV</td>
<td>Heart Rate Variability</td>
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<tr>
<td>IBQ-R</td>
<td>Infant Behavior Questionnaire-Revised</td>
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<tr>
<td>LF-HRV</td>
<td>Low-frequency Heart Rate Variability</td>
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<tr>
<td>NA</td>
<td>Nucleus Ambiguus</td>
</tr>
<tr>
<td>NPY</td>
<td>Neuropeptide Y</td>
</tr>
<tr>
<td>NTS</td>
<td>Solitary Tract Nucleus</td>
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<tr>
<td>PNS</td>
<td>Parasympathetic Nervous System</td>
</tr>
<tr>
<td>PSI</td>
<td>Parental Stress Index</td>
</tr>
<tr>
<td>SA node</td>
<td>Sinoatrial node</td>
</tr>
<tr>
<td>SCG</td>
<td>Superior Cervical Ganglion</td>
</tr>
<tr>
<td>SNS</td>
<td>Sympathetic Nervous System</td>
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<td>THM</td>
<td>Traube-Hering-Meyer</td>
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SUMMARY

The concept of emotional and behavioral self-regulation is part of a larger discussion of human behavior, which relates to social functioning. Appropriate social functioning is, of course, essential to quality of life and even to longevity. Healthy social functioning, for example, is essential to finding and maintaining employment and to creating social relationships. Good social support has been cited as important when predicting health outcomes in cancer patients and patients with other serious illnesses. And, of course, poor social functioning is associated with increased risk of psychiatric disorders and suicide. Research has demonstrated that intervention to improve social behaviors is most effective when begun as early as childhood, when specific patterns of social interaction first emerge. Recognizing dysfunctional social behavior early in life, then, is important.

The Mother-Child Play Project, described in the final chapter of this dissertation, was designed to demonstrate the importance of integrating essential subdisciplines within psychology, psychophysiology and neuroscience in order to create a comprehensive model to fully examine the neurophysiologic mechanisms that might underlie emotional and behavioral regulation in infants during the first year of life. The Project results demonstrate that development, individual differences, and social context are integrally related, and isolated study of only one or two of these dimensions is insufficient to achieve meaningful understanding of the underlying processes that guide regulation in infants. To understand the results and discussions presented in this last chapter, the first three chapters were written to offer an introduction to the structure and function of the autonomic nervous system; to present experimental data, which emphasizes the importance of employing accurate, reliable methods of measuring autonomic, specifically parasympathetic, activity in humans; and to detail the historical research and literature focused on characterizing emotional and behavioral regulation in early life.
1. THE AUTONOMIC NERVOUS SYSTEM

1.1 General Functions of the ANS

The autonomic nervous system (ANS), formally termed the “vegetative nervous system,” provides motor control to and receives sensory input from visceral structures throughout the body, including cardiac muscle, smooth muscle, and glands. It is comprised of both central and peripheral nervous system components, and its primary function is to maintain physiological homeostasis within the body. The ANS is, therefore, capable of sensing changes in both internal and external environments and responding accordingly by changing patterns of cardiac or smooth muscle contraction or in gland secretion. Coordinated ANS responses to internal and external environmental stimuli can be fairly complex or relatively simple and proceed without conscious control. The baroreflex response, for example, regulates blood pressure, which is an important factor in maintaining internal homeostasis. In response to an internal signal such as a drop in arterial blood pressure upon standing, the ANS coordinates a response to temporarily increase blood pressure to counteract gravity and maintain cerebral blood flow. This is accomplished by increasing total peripheral resistance (i.e., increasing blood vessel constriction) and by increasing cardiac output (e.g., increasing heart rate or increasing force of cardiac contraction). Once normal blood pressure is restored in the standing position, the ANS must, again, adjust blood vessel constriction and cardiac output to maintain a healthy blood flow. These changes are tightly controlled and occur very quickly so that throughout the reflex, blood pressure is maintained within healthy parameters, and healthy individuals are able to alternate between standing and sitting positions without experiencing the uncomfortable symptoms of hypo- or hypertension. During pathological situations, such as that which occurs during the alternating vasoconstriction and vasodilation of blood vessels in the head during migraine headaches, the ANS fails to maintain homeostasis, and pain ensues. Migraine sufferers often report difficulty changing vertical positions
during migrainous episodes due to dizziness and syncope (or fainting). They can also experience marked increases in heart rate upon standing (postural orthostatic tachycardia) along with drops in blood pressure, which may intensify the headache pain (Piovesan et al., 2008; Seçil et al., 2010; Vuković et al., 2007).

Responses to external stimuli, such as changes in pupil diameter to accommodate changes in the intensity of ambient light, are also controlled by the ANS. Again, the pupil constriction, which occurs when walking from a dark building into sunlight, and the compensatory pupil dilation, when walking back into a dark building from outside, occur so automatically that a healthy person barely notices the change. ANS responses to extreme external conditions, while often appropriate, can be more complex than simple changes in pupil size and are certainly noticeable, especially in situations that endanger or threaten survival. For example, in situations of danger or life-threat, the ANS coordinates fight-flight or what I will call “freeze-faint” behaviors, respectively. Core features of the fight-flight response include shunting of blood flow away from abdominal visceral organs and toward skeletal muscles throughout the body to allow increased strength or speed, which might be necessary to contend successfully with the environmental dangers. Pupils are dilated to allow better vision. Cardiac muscle contracts faster and with more force along with coordinated smooth muscle activity in the lungs to increase bronchiole diameter, to allow more oxygen exchange with the increased pulmonary blood flow. The freeze-faint response, in contrast, occurs in situations of life-threat in which escape options are limited or absent. In this case, the ANS prepares the body by first causing it to freeze, which may either help avoid detection by a predator or help delay attack by seeming unthreatening or uninteresting to a predator. This response also includes raised pain thresholds and decreased metabolic functioning, including extremely depressed heart and breathing rates along with fainting, which lowers the head and allows continued cerebral perfusion under such extreme conditions. This response to life threat where one is “scared stiff” or “scared to
"death" is also often associated with involuntary excretory activity, another set of functions controlled by the ANS.

1.2 General Organization and Anatomical Distribution of ANS Components

In order to appreciate how the ANS accomplishes its myriad functions, it is necessary to understand the organization and anatomical distribution of its components. First, the ANS is divided into two main divisions—the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). A complete description should also include the intrinsic cardiac and enteric nervous systems as part of the ANS, but for the purposes of this more general discussion, these two systems will be left out. The motor components of both the SNS and PNS consist of a basic, two-neuron configuration—one preganglionic neuron originates in a central autonomic nucleus and synapses with a second, postganglionic neuron with a cell body located in a peripheral autonomic ganglion. The central nuclei of the SNS reside in the intermediolateral cell column in the lateral horn in the thoracic and lumbar segments (T1 – L2) of the spinal cord. These thoraco-lumbar neurons have relatively short axons, which synapse in interconnected paravertebral (thorax) and prevertebral (abdominal) ganglia. Postganglionic axons from these ganglia then travel long distances to reach their final target tissues. The central nuclei of the PNS are located in the brainstem and in the sacral segments of the spinal cord. Preganglionic neurons in the cranial PNS travel in cranial nerves III (oculomotor), VII (facial), IX (glossopharyngeal), and X (vagus)—the cranial nerves of the branchial, or pharyngeal, arches. Sacral preganglionic neurons travel in the pelvic splanchnic nerves. Unlike sympathetic preganglionic axons, parasympathetic preganglionic axons are long and synapse in visceral ganglia located very near or within the walls of their target organs. The preganglionic neurons of both the sympathetic and parasympathetic systems are cholinergic, and the majority of these neurons synapse at nicotinic cholinergic receptors in peripheral ganglia. Postganglionic, sympathetic fibers are largely adrenergic with some exceptions
of cholinergic neurons innervating salivary glands, for example. The adrenergic neurons synapse at different subtypes of beta adrenergic and at pre- and post-synaptic alpha adrenergic receptors depending on the target tissue. Postganglionic parasympathetic fibers, on the other hand, are cholinergic and synapse at all subtypes of muscarinic receptors, again, depending on the receptors present in the target tissue.

Traditional and popular descriptions of the two ANS divisions tend to characterize them as accomplishing opposite, antagonizing functions—SNS leads to fight-flight, while PNS leads to rest-digest. This is true in many cases, especially when both systems innervate the same organs. For example, PNS activity slows heart rate and decreases cardiac output, while SNS activity at the heart increases cardiac output. The systems do not always innervate the same structures, however, and since they are each always active at some level of resting “tone” at each organ it is much more useful and accurate to use cooperative descriptions when describing their ultimate, ensemble function, which is to maintain homeostasis in the midst of changing internal and external environments. The baroreceptor reflex described earlier is, again, a good example, because it coordinates both SNS and PNS activity to maintain the most important vital sign—mean arterial pressure—within a narrow physiological range. Baroreceptors are innervated by the parasympathetic nervous system: Receptors located in the carotid sinus are innervated by the sinus nerve of Hering, a branch of the glossopharyngeal nerve (CN IX). Arterial stretch receptors in the aortic arch arterial are innervated by the aortic nerve, a branch of the vagus nerve (CN X). Afferent signals regarding the status of blood pressure travel in afferent fibers within these nerves, which are each tonically active. Sensory fibers in both nerves synapse in the medulla in dorsal medial portions of the caudal solitary tract nucleus (nucleus tractus solitarius, NTS), a major sensorimotor relay station with reciprocal connections to several brainstem nuclei and subcortical structures (Czachurski, Lackner, Ockert, & Seller, 1982; Mendelowitz, Yang, Andresen, & Kunze, 1992). When blood pressure increases, the baroreceptor-to-NTS afferents increase their firing
frequency. The NTS, in turn, stimulates cardioinhibitory activity via its projections to both the nucleus ambiguous (NA, the ventral nucleus of the vagus) and the dorsal motor nucleus of the vagus (DMNX), which both slow heart rate and decrease cardiac output. NTS neurons also stimulate the caudal ventrolateral medulla, which inhibits premotor sympathetoexcitatory neurons in the rostral portions of the ventrolateral medulla, ultimately inhibiting sympathetic output to the heart and blood vessels. Reduced sympathetic tone contributes to lowered peripheral vascular resistance and decreased cardiac output, all helping to lower elevated blood pressure. This reflexive process is very dynamic and fast-responding (Kunze, 1972). It begins with parasympathetic stretch receptors and ends with both PNS activation and SNS inhibition that is coordinated by the central nervous system in order to achieve a single goal.

When (re)conceptualizing the SNS and PNS as cooperative systems working together to limit deviations from homeostasis, it is also useful to appreciate that not all pathways within each system are similar in terms of their effects on global physiological processes. For example, during the fight-flight response, largely accomplished through diffuse activation of the SNS and dampening of PNS activity, blood is shunted away from the gut, and digestion is inhibited. SNS innervation of salivary glands, however, acts in concert with parasympathetic digestive activity by increasing the rates of salivary enzyme secretion thereby increasing the amount of oral digestive proteins released from salivary glands during ingestion. In the PNS, parasympathetic innervation of the heart via vagal fibers originating in the nucleus ambiguous (NA) result in the healthy pattern of respiratory sinus arrhythmia at rest, while vagal fibers from the dorsal motor nucleus of the vagus (DMNX) can lead to life-threatening bradycardias. In other words, the PNS is responsible for both rest-digest and freeze-faint patterns of functioning. Last, the SNS and PNS also innervate each other directly in certain organs and can directly inhibit or potentiate each other’s effects on target tissue function.
Since the global focus of this dissertation with respect to the ANS is social engagement in humans as measured by changes in cardiac rhythms, the next two sections will outline autonomic innervation of the face, head, and neck, and the heart. While not discussed in detail, it is important to note that the ANS, especially vagal nerve fibers from the DMNX, also innervates the gut and regulates the enteric nervous system. Not surprisingly, many disorders associated with cardiac abnormalities or difficulties in social engagement or self-regulation also have gastrointestinal problems. For example, children with familial dysautonomia, selective mutism, autism spectrum disorders, social phobia, and a variety of other deficits in psychosocial functioning often have gastrointestinal disorders, including dysmotility, gastroesophageal reflux disease, diarrhea, and constipation (Adams, Johnansen, Powerll, Quig, & Rubin, 2011; Hommel et al., 2010; Horvath & Perman, 2002; Ming et al., 2011). The specific character of these aspects of disorders of social engagement are highly variable, difficult to quantify, and, thus, difficult to study systematically. However, the frequent presence of gastrointestinal dysfunction in children with emotional and behavioral regulation difficulties supports the larger perspective of this dissertation, which is that the ANS is central to the etiology of healthy, physiological and pathological processes underlying appropriate and disordered self-regulation in development.

1.3 Autonomic Innervation of the Face, Head, and Neck

Autonomic fibers that innervate the face, head, and neck originate from both parasympathetic and sympathetic nuclei, including several parasympathetic cranial nerve nuclei in the brainstem and upper sympathetic thoracic segments which innervate cutaneous, glandular, vascular, and smooth and cardiac muscle targets via the stellate and superior cervical ganglia (SCG). The cell bodies of postganglionic sympathetic fibers innervating the face are located between the second and third cervical vertebrae in the SCG at the most rostral end of the cervical sympathetic chain. Unlike thoracic paravertebral ganglia, the SCG develops from the fusion of several cervical
sympathetic ganglia; does not receive preganglionic projections from the corresponding vertebral level, and it is the largest of the paravertebral ganglia. The SCG contains the upper two or three cervical spinal nerves and receives central, myelinated, preganglionic fibers originating in the lateral horns of the first through third thoracic spinal segments. Postganglionic efferent axons from the SCG are, like most other postganglionic sympathetic fibers, unmyelinated (Jänig, 2006). Lateral branches from the SCG extend to join cranial nerves IX, X, and XII and the upper cervical spinal nerves, as well as the posterior cranial fossa, and the jugular bulb. Medial branches innervate the carotid body, pharynx, and cardiac plexus. Almost all anterior branches course along the external carotid arteries, including vasomotor and sudomotor neurons to the face. Some anterior branches also extend along the internal carotid artery to the nose, middle forehead, eyeball, eyelid, and orbit. Motor branches to smooth muscle in the eye—the superior tarsal muscle of the upper eyelid and the dilator papillae muscle—pass through the cavernous sinus medial to the trigeminal ganglion where they leave the internal carotid artery and join the oculomotor nerve (Amonoo-Kuofi, 1999; Wasner et al., 2005).

Some clues about the function of SCG efferents to the face and neck come from observations in patients with Horner's syndrome, which is caused by interruption of normal sympathetic innervation to the face, most often resulting from surgical damage or pathological compression of the SCG. Symptoms include ipsilateral ptosis (drooping of the eyelid due to reduced activity of the superior tarsal muscle), miosis (narrowed pupil due to loss of innervation to the dilator papillae muscle), and anhidrosis (lack of sweating due to loss of sudomotor activity to sweat glands in the face). Horner's syndrome is sometimes referred to as Harlequin syndrome when asymmetrical flushing occurs. The affected side of the face (with ptosis, miosis, and anhidrosis) remains pale and dry while the contralateral side of the face flushes and sweats normally. This finding is unexpected at first, because in the rest of the body, the sympathetic nervous system is thought to cause vasoconstriction in cutaneous blood vessels due to norepinephrine release at α-adrenergic
synapses. However, sympathetic activity is also known to cause vasodilation in blood vessels found in skeletal muscle of the upper and lower limbs due to sympathetic cholinergic transmission. In the face it is unclear whether sympathetic vasomotor fibers lead to active vasodilation or vasoconstriction, and the mechanisms underlying either of these possibilities is probably complicated (Izumi, 1999). There are distinct populations of both catecholaminergic and cholinergic neurons originating in the SCG (Ernsberger & Rohrer, 1999). However, it is now thought that neuropeptides and other neuromodulators that are co-released with the classic neurotransmitters (Vasointestinal Peptide and Neuropeptide Y, in particular) play an important role in regulation of vasomotor activity in orofacial tissues. Some evidence of this comes from the parasympathetic system, which causes vasodilation in the face via non-cholinergic mechanisms (i.e. vasodilation is not attenuated with the anticholinergic agent, atropine). Vasointestinal Peptide is currently the most likely candidate (Izumi, 1999).

In addition to sympathetic and parasympathetic vasomotor control, trigeminal nociceptive C-fibers are also important in facial flushing and sweating (Drummond, 1995). Izumi (1999) provides a thorough discussion of what is identified as four vasomotor responses in the face of cats and humans: sympathetic vasoconstriction, sympathetic vasodilation, parasympathetic vasodilation, and trigeminal nociceptive nerve-mediated vasodilation. The mechanisms and distribution of these responses varies even within each category, and is thus, complicated. Overall, however, sympathetic activation in the SCG is still considered to be responsible for generalized facial flushing and sweating in humans (Amonoo-Kuofi, 1999; Wasner et al., 2005).

Not much is known about DMNX vagal fiber contribution to facial structures. Other parasympathetic cranial nerves, however, including the myelinated vagus from the NA, are extensively involved in both efferent and afferent innervation of all the vasculature and striated musculature of the face, head, and neck in humans. Cranial Nerves V (trigeminal), VII (facial), IX (glossopharyngeal), and X (vagus from NA) contain special visceral efferent and special visceral
afferent fibers that innervate the muscles of mastication, the muscles of facial expression, and pharyngeal and laryngeal muscles, respectively. Most sensory information—including nociception, pressure, and light touch—from the face is also relayed through the trigeminal nerve. Reflexive responses to sensation are complex and thought to be mediated by both sympathetic and parasympathetic pathways (Drummond, 1995; Izumi, 1999).

Postganglionic autonomic fibers also innervate glandular tissue in the face and neck. Preganglionic parasympathetic neurons from brain stem nuclei exit in cranial nerves III, VII, IX, and X to synapse in the ciliary, pterygopalatine, submandibular, lingual and otic ganglia. Postganglionic fibers then project to lacrimal glands, cerebral vasculature, submandibular and parotid glands, tongue, lower lip, and facial skin (Jänig, 2006). Sympathetic postganglionic fibers from the superior cervical ganglion also pass through the cranial parasympathetic ganglia and extend to similar regions. Sweat glands receive cholinergic innervation from sympathetic neurons. Salivary glands are innervated by noradrenergic sympathetic neurons, which cause weak mucous secretions via $\alpha_1$ receptors. Parasympathetic cholinergic innervation of salivary glands results in increased blood flow to the glands (Izumi, 1999) and copious serous digestive enzyme secretion, which contains digestive enzymes (Jänig, 2006). Interestingly, salivary amylase secretion has been associated in recent literature with sympathetic activation (Fortunato, Dribin, Granger, & Buss, 2008; Gordis, Granger, Susman, & Trickett, 2008; Nater et al., 2006). In Nater et al (2006), increases in salivary amylase was not found to be related to catecholamine release. However, since salivary amylase is associated with “stress” and with increases in the ratio of low-frequency to high-frequency heart rate variability, both of which are traditionally associated with “sympathetic arousal,” salivary amylase was still considered an index of sympathetic tone (see section on criterion variables below). The presumed association between salivary amylase secretion and sympathetic tone, though it persists in the literature, is inappropriate and not supported by known anatomy and physiology of salivary gland innervation.
1.4 **Autonomic Innervation of the Heart**

Autonomic innervation of cardiac tissue is complicated. In addition to the intrinsic conduction system consisting of modified myocardial cells within the walls of the heart chambers and septa, there is also an intrinsic cardiac nervous system consisting of interneurons, which communicate with each other and with the heart directly. Outside of these two electrical systems, the autonomic nervous system provides extrinsic regulation of cardiac rhythms (chronotropy), forces of contraction (inotropy), and conduction velocities (dromotropy). A more detailed discussion of autonomic regulation of cardiac rhythms is provided in the experiments and discussion in Chapter 2. This section is meant as a brief introduction.

Parasympathetic innervation of the heart is supplied by the vagus nerve, which includes preganglionic, cardioinhibitory fibers from both source nuclei in the brainstem—the DMNX and the NA. NA fibers innervate the cardiac pacemaker tissue and regulate, or slow, specialized potentials, resulting in slower heart rates at rest. The term “vagal tone” is often used to describe the constancy with which the vagus nerve regulates pacemaker potentials, however, the firing pattern of NA fibers themselves are far from static. Firing patterns of NA cardioinhibitory neurons increase during expiration and decrease during inspiration, resulting in increased heart rate during inhalation and decreased heart rate during exhalation. This naturally occurring rhythm maximizes ventilation-perfusion ratio in the pulmonary circulation and, when present, is considered a sign of good health. Respiratory sinus arrhythmia, or RSA, occurs at the frequency of respiration, and its main regulator is the NA vagus (see Figures 1.1 and 1.2 for illustrations heart period and RSA).
**Figure 1.1. Illustration of Heart Period in the electrocardiogram (ECG) waveform.** The R-R interval, or length of time between successive heart beats is referred to as Heart Period (HP). HP and heart rate are inversely related. In this example, the first two heart beats are closer together than the second two heart beats. HP, then, is shorter (and heart rate is faster) in the first interval (HP-1) and longer (slower heart rate) in the second interval (HP-2). HP is typically measured in milliseconds.

**Figure 1.2. Illustration of RSA as high-frequency heart rate variability.** With each exhalation, the heart period increases, indicating a slower heart rate. The reverse is true during inhalation. The frequency of RSA is the same as the frequency of respiration. The amplitude of RSA is determined by the level of vagus nerve activity at the sinoatrial (SA) node. HP, heart period, the
period of time between successive heart beats is measured in milliseconds. RSA, respiratory sinus arrhythmia. HPV, heart period variability. RESP, respiration. BPM, beats per minute.

The coupling of respiration and heart rate patterns has been a research interest for over a century, and several studies have been conducted to characterize all important aspects of the circuit. High variability in brainstem organization between different mammalian animal models and between many animals and humans has limited some aspects of this work, however the basic circuitry has been well-described (Daly, 1991; Gilbey, Jordan, Richter, & Spyer, 1984; Lopes & Palmer, 1978; Spyer & Gilbey, 1988). The NA receives a variety of sensory inputs (mostly via the NTS) from receptors throughout the thorax and brainstem, including carotid chemoreceptors, arterial baroreceptors, cardiac stretch receptors, pulmonary C fibers, and central respiration centers, including the pre-Botzinger complex in the ventral brainstem. NA neurons are regulated by cholinergic interneurons via muscarinic receptors, which powerfully inhibit synaptic input during inspiration, allowing greater pulmonary blood flow when the lungs are filling with oxygen. During expiration, cardioinhibitory NA neurons are disinhibited and the period of time between successive heart beats lengthens until the next respiratory cycle begins. Activation of carotid bodies (signaling hypoxia), the arterial baroreflex (signaling hypertension), and the cardiac receptors (signaling increased pressure) all stimulate cardioinhibitory neurons to accentuate expiratory bradycardia. Within the range of normal, spontaneous changes in lung volumes, however, these inputs are suppressed by central inspiratory drive, which causes vagal inhibition and increased heart rate. The central inspiratory drive does not affect arterial blood pressure (Daly, 1991).

The NA, like most central nuclei, is not a homogenous structure. Massari et al (1995) proposed a cardiotopic model for the NA based on data obtained from retrograde tracing from the cardiac ganglion, which regulates AV conduction. They demonstrated that neurons innervating the AV ganglion originated from both the NA and the DMNX, and the antidromotropic effects of activity
in these neurons were independent of heart rate changes (Massari, Johnson, & Gatti, 1995). Just as preganglionic origins have segregated, specialized functions, postganglionic parasympathetic neurons have also been found to have specific, independent functions. For example, ablating pathways in the right cervical vagus innervating the AV ganglion can interrupt dromotropic effects without affecting heart rate.

Delineation of functional impacts of NA vagal fibers and DMNX vagal fibers has not been well-studied in humans or other primates. In rats and rabbits, it is well-known that DMNX fibers innervate cardiac tissue and can lead to severe apnea and bradycardia (Loewy & Spyer, 1990; Marchenko & Sapru, 2000). In human infants, bradycardia of prematurity has been observed in the presence of depressed RSA (Porges, 1995). It is possible that the NA and DMNX fibers accomplish drastically different heart rhythms associated globally with healthy homeostasis and freeze-faint autonomic responses, respectively. To date, however, no published research has adequately addressed this topic.

In terms of function, sympathetic innervation of cardiac tissue is relatively straightforward. Sympathetic fibers from upper thoracic segments participate in the cardiac plexus and innervate ventricular myocardium (positive inotropy), with some fibers extending to the SA node (positive chronotropy). Vagal fibers directly inhibit sympathetic axons via muscarinic, cholinergic synapses at rest (Rardon & Bailey, 1983; Schmid, Whiteis, Oda, & Lund, 1986). This inhibition is enhanced during increased sympathetic activity (Levy & Zieske, 1969). Sympathetic neurons do not directly synapse on postganglionic vagal projections.

1.5 Development of Autonomic Cardiac Regulation in Humans

It is important to remember that afferent (sensory) fibers in each of the ANS compartments play a vital role in ANS function, however, only development of efferent (motor) fibers are discussed here. The oldest existing autonomic system in phylogeny, which is comprised of
unmyelinated, cholinergic, vagal fibers originating from the DMNX (Porges, 1995; Taylor, 1994), is also, embryologically, the earliest system to develop in utero. An immature, undifferentiated DMNX first appears in the brainstem at 9 weeks gestation (Cheng, Zhou, Qu, Ashwell, & Paxinos, 2004; Nara, Goto, & Hamano, 1991). Magnocellular subdivisions become visible by 13 weeks, and clear demarcation of DMNX subnuclei, including the lateral cardiomotor subnucleus, occurs by 23 weeks. At 28 weeks, all magnocellular subnuclei are considered essentially mature (Cheng et al., 2004). Although, some investigators, including Nara et al, believe there may be some postnatal changes in the DMNX, such as increased nuclear columnar length and volume. Even if present, these postnatal changes are not considered to have much functional significance or physiological consequence in the neonate.

The other major component of the parasympathetic, cardioinhibitory ANS is the phylogenetically newest, myelinated vagal system, which originates in the Nucleus Ambiguus (NA). This system develops last in the fetus, and continues functional development well into the first postnatal year. Mature neurons appear in the rostral NA by 8 or 9 weeks gestation and fill the nucleus by 12.5 weeks (Brown, 1990). Unlike mature neurons in the lateral subnucleus of the DMNX, however, axons of these mature neurons have not yet reached cardiac tissue to exert cardioinhibitory effects. The functional significance of vagal fibers from the NA depends heavily on myelination, which does not begin until 23 weeks gestation, when near mature axon diameter is achieved (Wozniak & O'Rahilly, 1981). Myelination of NA vagal fibers increases linearly from 24 to 40 weeks gestation and, again, continues actively during the first year postpartum (Pereyra, Zhang, Schmidt, & Becker, 1992; Sachis, Armstrong, Becker, & Bryan, 1982).

Development of the sympathetic, cardioexcitatory ANS is less well described. Phylogenetically, this largely catecholaminergic system appears before the mammalian NA vagal system and after the older DMNX vagus. Theoretically, then, this system should begin development in the human fetus sometime between the two parasympathetic systems. Anatomically,
sympathetic innervation of cardiac tissue is complex and difficult to isolate. Postganglionic cardiomotor nuclei lie mostly within the cardiothoracic and middle cervical ganglia, which lie caudal to the sympathetic superior cervical ganglion. Postganglionic cardiomotor axons travel within the ansa subclavia, which connects the two ganglia, and within the vagus nerve to the cardiac plexus and pulmonary tissue. Functionally, sympathetic influence on the heart is also varied. Unlike the parasympathetic systems, which exert mostly chronotropic and dromotropic effects (slowing heart rate and intrinsic conduction), sympathetic activity leads to changes in chronotropy (increasing heart rate) by innervating pacemaker tissue and inotropy (increasing cardiac contractility) by innervating ventricular myocardium.

Investigations using fetal heart rate monitoring to infer sympathetic activity provide potential insight into development of this system. However, published results of these investigations are often confounded by the unlikely assumption in the literature that low frequency heart rate variability (LF-HRV) or its ratio to high frequency HRV is somehow indicative of sympathetic tone. In fact, no plausible physiological mechanism has been presented to support this assumption, and therefore, literature presenting “LF/HF” data is of limited utility (see Chapter 2). Fortunately, the neurobiology literature may provide preliminary answers. Hill (2004) concluded that intact, functional target tissue is required for sustained sympathetic innervations and neuronal survival (Hill, 2004). The heart develops between 5 and 9 weeks gestation, at which point it reaches a maximum heart rate of 175 beats per minute, which limits functional sympathetic innervation to 9 weeks at the earliest. Moreover, Roudenok (2000) found increasing Neuropeptide Y (NPY) expression in developing human sympathetic ganglia from 24 to 41 weeks gestation. NPY is known to modulate norepinephrine release and to potentiate vasoconstriction in mammals (Gibbins, 1992), and NPY expression could be a marker of neonatal maturity (Roudenok, 2000). Gross measurements of overall heart rate changes during fetal activity can also be used to infer sympathetic development. Kinbraia et al (2005) used continuous, 24-hour, fetal heart rate
monitoring in 28 healthy women, who were 16 to 28 weeks pregnant and reported that healthy fetal locomotor activity should increase between 16 and 20 weeks gestation (Kintraia, Zarnadze, Kintraia, & Kashakashvili, 2005). At this stage, increases in activity were accompanied by corresponding increases in heart rate, which returned to normal during “quiet” fetal periods. The authors added that absence of such coordinated heart rate increase with increased locomotion by 24 weeks gestation indicates “developmental retardation”. Given the relative development of DMNX and NA vagal systems during this gestational period, the increases in heart rate seen in this study were most likely due to activity in the sympathetic nervous system.

References


2. NEUROPHYSIOLOGIC MECHANISMS AND MEASUREMENT OF HEART RATE VARIABILITY—
THE IMPORTANCE OF APPROPRIATE CRITERION VARIABLES

2.1 Abstract

Low-frequency (LF) and high-frequency (HF) heart rate variability (HRV) may reflect distinct autonomic mechanisms, which are important both in research, as indices of autonomic activity, and in clinical medicine, as important prognostic indicators of cardiovascular health and disease. Differences in LF and HF HRV patterns are often interpreted in the literature as differences in heart rate responses to opposing sympathetic and parasympathetic processes, and the LF/HF ratio is often used as a measure of “sympathovagal balance”. This study challenges interpretation of LF HRV as representing sympathetic activity by demonstrating its profound, specific response to pharmacologic blockade of parasympathetic activity. Additionally, despite repeated attempts in the literature to standardized methods of quantification of HRV, debate continues. Relative sensitivity and specificity of the three most commonly used quantification methods of LF and HF HRV measures to vagal blockade are also contrasted.

ECG recordings were obtained from fifty-two 18 – 35 year-old healthy male volunteers during baseline, spontaneous breathing conditions before and after administration of either intravenous glycopyrrolate (.006mg/kg bolus) or saline vehicle. Glycopyrrolate is a non-specific, peripheral, muscarinic antagonist and, therefore, interrupts parasympathetic, specifically vagus nerve, neurotransmission to the heart. Power spectral, Peak-to-Trough (Pk-tr), and Porges-Bohrer (PB) time domain analyses were used to quantify LF (0.06 - 0.10 Hz) and HF (0.12 - 0.40 Hz) HRV. Both LF and HF HRV were abolished following glycopyrrolate infusion.

Linear methods of quantifying HRV are most widely used, however, these methods vary significantly in accuracy and appropriateness. In this study, the PB method was most sensitive to glycopyrrolate. Only changes in the spectral and PB methods were specific to cholinergic blockade.
2.2 Introduction

The mechanisms through which the autonomic nervous system (ANS) regulates cardiac rhythms have held scientists' attention since before Loewi (1921) and Loewi and Navratil's 1926 descriptions of “Vagusstoff”—the vagus nerve substance that, when passed through successive frog hearts, slowed heart rate (Bennett, 2000; Loewi, 1921; Loewi & Navratil, 1926; Sourkes, 2009). Less than a decade later, Feldberg and Minz (1934) confirmed that Loewi’s Vagusstoff was, in fact, the chemical known as acetylcholine, which, at the time, was still being established as a chemical transmitter in the nervous system (Feldberg & Minz, 1934; Feldberg, Minz, & Tsudzimura, 1934; Sourkes, 2009). Today, eighty years after Loewi and Feldberg’s discoveries, the impact of the autonomic nervous system, specifically the cholinergic pathways of the vagus, on thoracic viscera continue to interest neuroscientists, physiologists, psychophysicists, and others. Moreover, quantifying the effects of vagus nerve activity on changes in heart rate and rhythm (heart period variability, heart rate variability, or HRV) has proven to have great clinical significance. In 1965, Hon and Lee reported that certain changes in fetal heart rate variability could predict fetal distress (Hon & Lee, 1965). More studies in the 1970s concluded that changes in beat-to-beat variability in fetal heart rate patterns preceded fetal deaths that were not otherwise predictable by clinical evaluation (Cetrulo & Schifrin, 1976; Paul, Suidan, Yeh, Schifrin, & Hon, 1975; Rochard et al., 1976). In adults, heart rate variability was shown to be an important and independent indicator of prognosis following acute myocardial infarction (Kleiger, Miller, & Bigger Jr, 1987). In the 1980s scientists pursued more detailed study of the non-stationary, semi-periodic oscillations occurring in the inter-beat-intervals (R-R intervals from ECG recordings) of awake and anaesthetized animals and humans. Two important kinds of variability were studied: (1) high-frequency (HF) HRV, or respiratory sinus arrhythmia (RSA)—the heart period oscillations occurring at the frequency of spontaneous respiration, and (2) low frequency (LF) HRV related to the Traube-Hering-Meyer (THM) waves of blood pressure.
Since various commercial programs have been developed to make certain techniques accessible to investigators across fields, both HF and LF HRV evaluation in clinical and research settings have expanded to include many disciplines. In anaesthesia, for example, HRV has been used to predict hypotension and bradycardia after induction of spinal anaesthesia in various patient populations (Chatzimichali et al., 2011; Hanss et al., 2006). In gastrointestinal research, HRV is being investigated as a potentially useful variable to further understand the role of “abdominovagal” and “cardiovagal” autonomic physiology in feeding behaviors and gastrointestinal disorders (Chang, Ko, Lien, & Chou, 2010). In addition, investigators who study diabetic neuropathy, neurogenic syncope, pediatric autonomic disorders, cyclic vomiting syndrome, autism spectrum disorders and social engagement, adult psychiatric disorders, sleep apnea, endocrine dysfunction, stress, exercise, and more are increasingly adding HRV measures to their research protocols. Such broad appeal for study is not surprising, because the ANS is extensively involved in nearly all mammalian physiological functions (the word vagus, after all, means “wandering”). Unfortunately, there are divergent views concerning interpretation of HRV measures and the physiological processes they represent. Moreover, different investigators within and between different disciplines currently use different methods of HRV quantification that cannot necessarily be compared to each other. In some cases, the methods might not accurately reflect the autonomic mechanisms of stated interest. These are major problems, which limit both interdisciplinary communication and applicability of potentially important scientific findings.

As more investigators at the bench and in the clinic find novel and innovative ways to use HRV measures, developing insights into the neurophysiological mechanisms underlying HRV becomes more important. Understanding parasympathetic, vagal mechanisms are particularly important. In neurocardiology, the vagus nerve is of clinical significance in two main ways: it is responsible for both RSA, which is generally considered an indicator of good health, and severe bradycardia, which is life-threatening. This “vagal paradox” (Porges, 1995) reflects the functionally
distinct neural pathways within the vagus nerve, which originate from two different source nuclei in the brainstem: Myelinated vagal pathways originating in the nucleus ambiguous (NA) are responsible for RSA; unmyelinated vagal pathways from the dorsal motor nucleus of X (DMNX) can be responsible for severe bradycardia, hypotension, and apnea (Marchenko & Sapru, 2000; Nosaka, Yamamoto, & Yasunaga, 1979; Rogers & Hermann, 1985). Phylogenetically, the unmyelinated DMNX system is oldest, followed by catecholaminergic, sympathetic precursor systems. Last, in mammals, the myelinated NA system emerged as an integral part of a cranial parasympathetic system, which facilitated uniquely mammalian social engagement behaviors (Porges, 1995). In human development, formation of these systems in utero mirror phylogeny. The DMNX develops first between 9 and 23 weeks gestation; sympathetic function starts around 24 weeks; and the myelinated NA vagal system appears at 23 weeks and continues to develop throughout the first postnatal year (Porges & Furman, 2011). Both vagal pathways converge on muscarinic acetylcholine receptors in nodal cardiac tissue. However, the preganglionic cholinergic receptors for NA vagal fibers are nicotinic (ionotropic), and those for DMNX fibers are muscarinic (metabotropic). Distribution of final vagal pathways in the heart also differs. Postganglionic NA fibers predominately innervate SA nodal tissue, and postganglionic DMNX fibers have greater influence at the AV node (Massari, Johnson, & Gatti, 1995; Massari, Johnson, Llewellyn-Smith, & Gatti, 1994). These distinct pathways can also act relatively independently—bradycardia, for example, can occur in the presence of decreased RSA (Porges, 1995).

Since 1910, when preganglionic vagal neurons were first found to have intrinsic respiratory rhythm (Hering, 1910), it has been accepted that RSA is an index of activity in that pathway (Loewy & Spyer, 1990). There is considerably less clarity, however, regarding the mechanisms that are represented in LF HRV. Several published discussions of the mechanisms of LF HRV have concluded that both sympathetic and parasympathetic processes contribute to this oscillation (Cacioppo et al., 1994; Electrophysiology, 1996; Malik & Camm, 1993; Pomeranz et al., 1985; Saul,
Rea, Eckberg, Berger, & Cohen, 1990; Taylor, Carr, Myers, & Eckberg, 1998). However, calculations and interpretations of the “LF/HF ratio” as representing a “sympathovagal balance” persist in the literature. This might be due, in large part, to historical, popular, and now outdated descriptions of the ANS as a system based on paired antagonism (Langley, 1921). This characterization of dueling functions between the sympathetic and non- or para-sympathetic systems has become part of popular culture. Today, even as our understanding of autonomic systems has become more sophisticated, the simple concepts of “fight or flight” versus “rest and digest” have been slow to vanish. Clinical medicine, (anaesthesiology and neonatology, in particular) acknowledges distinct functions of different neural pathways in the vagus nerve. Investigators recognize and continue to explore the neuroanatomical and physiological complexities involving both efferent and afferent pathways within the vagus. This study presents further, novel evidence that LF HRV is not simply anti-RSA or, by extension, anti-parasympathetic by demonstrating that it is profoundly affected by changes in peripheral vagal cholinergic transmission.

To characterize the neurophysiological mechanisms of the LF HRV pattern, the following study employed intravenous infusion of glycopyrrolate, a non-specific, peripheral, muscarinic antagonist, to produce a partial, but significant vagal blockade in 18 to 35 year-old male volunteers. Unlike other studies, the findings here relied on direct, pharmacological intervention rather than on comparisons between normotensive and hypertensive adults or between well-trained and untrained athletes (Dassi et al., 1991; Reiling & Seals, 1988). Also, by using a peripheral cholinergic antagonist that does not cross the blood-brain barrier, potential confounding impacts of chemicals like atropine and scopolamine on central autonomic mechanisms were avoided (Gilbey, Jordan, Richter, & Spyer, 1984). We contrasted the effects of the partial vagal blockade on HRV variables with the effects of a pharmacologically inactive vehicle. We also contrasted the sensitivity and specificity of the three most widely used methods of quantifying HRV to disruption of vagal efferent
activity. To our knowledge, this is the first study to use a peripheral antagonist to evaluate LF and HF HRV and the mathematical strategies used to quantify them.

2.3 Methods

Participants and Screening:

Fifty-two healthy male participants, with a mean age of 25.54 years (s.d. = 4.01), were recruited via flyers, internet advertisements, brochures, or through the University of Illinois at Chicago psychology subject pool. The study groups included self-identified non-Latino Caucasian (58.3%), non-Latino African-American (19.4%), non-Latino Asian or other (12.6%), and Latino (9.7%) participants. Participants were not tested if they had smoked more than one cigarette or used tobacco, consumed more than three alcohol-containing beverages, or used any non-prescription drugs during the 24 hours prior to each experimental session. Participants, who had consumed caffeine in the two hours prior to each session, were also excluded from testing. No participant had a medical history (e.g., cardiovascular, respiratory, or psychiatric disorders) that would put him at risk or confound experimental outcomes. Moreover, no participant was taking any medication that would put him at risk or confound experimental outcomes (e.g., central nervous system depressants or stimulants, hypertension medications, or anti-cholinergic agents, including antihistamines).

Prior to testing in the laboratory sessions, potential participants were screened via a telephone interview. During the interview, potential participants answered questions about their general medical health and health habits. A more detailed screening for cardiovascular and respiratory health, including a 12-lead ECG administered by clinical staff and reviewed by a study physician, was conducted prior to the session that involved administration an intravenous infusion.
Procedures:

This study employed a randomized, controlled, double-blind design. Participants were each tested in an active full-service hospital at the Clinical Research Center (CRC). During the session, participants read and signed consent forms specific to the session procedures. If subjects passed the screenings, they were fitted with a LifeShirt® to monitor continuous heart rate, respiration, and activity data throughout the testing session.

After screening, prior to testing, all participants were instructed to sit quietly and maintain an alert state for five minutes to collect pre-testing baseline spontaneous heart rate and respiration data. Following the initial pre-testing baseline, clinical staff set up an intravenous apparatus to deliver an infusion of either saline (vehicle) or glycopyrrolate, a peripheral muscarinic blockade. Following the infusion, participants continued to sit for a three-minute post-infusion baseline before behavioral testing began. LifeShirt® activity recordings and researcher observation confirmed participants were compliant with instructions for completing all baseline measurements. The five-minute pre-test baseline and the three-minute post-infusion baseline at the CRC were used to evaluate the effect of drug versus vehicle on HRV variables.

Intravenous Infusion of Glycopyrrolate:

All participants in the CRC session received an intravenous bolus infusion of either saline (vehicle) or glycopyrrolate (.006mg/kg) following the initial five-minute pre-testing baseline. Glycopyrrolate is a non-specific, peripheral, muscarinic cholinergic receptor antagonist. The drug does not cross the blood-brain barrier. Since all peripheral postganglionic receptors of both the myelinated and unmyelinated vagal efferents to cardiac tissue are muscarinic, this competitive muscarinic antagonist was an appropriate choice to significantly inhibit all vagal input to the heart. In addition, it was expected that inhibitory presynaptic muscarinic receptors of sympathetic neurons would also be affected. Blockade of these receptors would disinhibit sympathetic activity
at the heart. The bolus concentration used here is calculated to have produced an eighty percent blockade of muscarinic receptors in the periphery.

Equipment:

ECG, respiration, and activity of each participant were monitored using the LifeShirt® System by Vivometrics®, a continuous ambulatory monitoring system. The system consists of the LifeShirt® vest, a small recorder, and a cable connecting vest to the recorder. The LifeShirt® vest is form-fitting with incorporated elastic bands across the chest and abdomen to measure expansion and contraction with respiration. An accelerometer is embedded in the vest to measure generalized torso movement. The LifeShirt® recorder is a small digital device with a data card that was placed in a small pouch secured around the participant’s waist. Following data collection, data are exported to a computer for analysis and permanent storage. Data are sampled at three discrete sampling rates: ECG voltage to the nearest millisecond, respiration band resistance at 50Hz, and generalized activity at 100Hz. Vivometrics® breathing calibration tubes were used to calibrate the LifeShirt® system to each participant’s tidal respiratory volume during each session.

Quantification of High and Low Frequency Heart Rate Variability:

Preprocessing of ECG and beat-to-beat heart rate data

Data collected by the LifeShirt® System were imported to computers using VivoLogic® software (Vivometrics; Ventura, CA), which automatically detects R-waves in the ECG recording and transforms continuous ECG signal into a time series of sequential heart periods defined by millisecond interval between sequential R-waves in the ECG. The heart period time series were edited for artifacts with CardioEdit (Brain-Body Center, University of Illinois at Chicago). Each heart period file was edited independently by two researchers, who had successfully completed
strict reliability training in the Brain-Body Center. A reliability ≥ 95% was achieved between the two researchers’ edited files prior to further processing and analysis.

**Porges-Bohrer Method (PB-RSA and PB-THM)**

For each edited heart period data file, mean heart period, mean heart rate, natural log variance of the heart period, and natural log variance of amplitude of respiratory sinus arrhythmia (RSA) were calculated using CardioBatch software (Brain-Body Center, University of Illinois at Chicago). To calculate the amplitude of RSA, CardioBatch applies the signal processing procedures developed by Porges (1985). These procedures enable the quantification of heart period oscillations in the frequencies of spontaneous breathing and include the following steps: (1) sequential heart period values are resampled at 500 ms intervals, (2) the equal interval time series is detrended with a moving 21-point cubic polynomial (Porges & Bohrer, 1990), (3) the detrended time series is processed by a bandpass filter to extract the variance in the heart period time series associated with the frequencies of spontaneous breathing in adults (passband = .12 - .40 Hz), and (4) the natural logarithm of the variance of the bandpassed time series is calculated as the measure of the amplitude of RSA (Riniolo & Porges, 1997). This last step is necessary to normalize the RSA distribution, so parametric statistics can be applied to answer study questions. These procedures are statistically equivalent to frequency domain methods (i.e., spectral analyses), which define RSA as the sum of the spectral densities in the frequency band associated with spontaneous breathing, when the heart period time series does not seriously violate the assumption of stationarity (Denver, Reed, & Porges, 2007; S. W. Porges & Byrne, 1992). Unlike spectral methods, however, the Porges-Bohrer procedures (the second step, in particular) accommodate the non-stationary nature of the inter-beat-interval pattern and correct it. The above method was modified to quantify the amplitude of a lower frequency component of HRV (THM) associated with vasomotor and blood
pressure oscillations (0.06-0.10 Hz). This was accomplished by applying a moving 51-point cubic polynomial filter to detrend and shifting the passband to 0.06 – 0.1 Hz.

*Spectral Method (HF-spec and LF-spec)*

Spectral analyses were applied to extract the measures of heart rate variability (HRV) described above (i.e., RSA, LF). Custom software (Brain-Body Center, University of Illinois at Chicago) in MATLAB was used to process the resampled heart period time series. The spectral methods included the following steps: (1) the linear trend was removed, (2) the detrended data were decomposed in constituent frequency components with a 1024-point fast Fourier transform (FFT), (3) magnitude spectral estimates derived from the FFT were stabilized with a 15-point Parzen smoothing window, 4) the spectral densities were accumulated within the two frequency bands used to define RSA and LF above.

*Peak to Trough Method (Pk-tr)*

Consistent with the literature (Grossman, van Beek, & Wientjes, 1990), the peak to trough measurement was defined as the average difference between the maximum and minimum heart period in each breath cycle. Custom software designed in MATLAB (Brain-Body Center, University of Illinois at Chicago) was used to quantify RSA with the peak-to-trough method. Each edited heart period time series, was upsampled to 50Hz to synchronize with continuous sampled chest wall movements from the tidal volume measure. Each breath cycle was identified by slope, and the Mean Respiration Period (MRP) was calculated. The potential lag between respiration pattern and RSA was calculated as the lag at which the maximal cross-correlation between the heart period and respiration trends occurred. The heart period series was shifted back by this lag to synchronize the respiration trend with the changes in heart period associated with respiration. Breath cycles in which either the inhalation or exhalation component was shorter than 20% of the MRP were
excluded from analysis. For all other cycles a peak to trough difference in heart period, measured in milliseconds was quantified, and for each recording, the mean difference, total number of breaths (mean = 43.1, s.d. = 12.9), and number of excluded breath cycles was reported (mean = 3.5, s.d. = 5.0).

2.4 Results

2.4.1 Effect of Glycopyrrolate (vagal blockade) on Physiological Measures

Both myelinated and unmyelinated vagal efferents to cardiac tissue terminate on muscarinic cholinergic receptors. Vagal efferents have also been shown to regulate (inhibit) sympathetic activity through activation of presynaptic muscarinic receptors on sympathetic neurons in pacemaker tissue (Manabe et al., 1991; Schmid, Whiteis, Oda, & Lund, 1986). Physiologic measures that are sensitive to glycopyrrolate must represent neurophysiologic mechanisms that rely on muscarinic, cholinergic transmission, i.e., inhibition of vagal activity and disinhibition of sympathetic neurotransmission in the heart.

In this study, 25 of the 52 participants were randomly assigned to receive glycopyrrolate infusion during the CRC research session. The other 27 participants received saline vehicle only. Repeated measures ANOVA was used to determine the effects of glycopyrrolate versus saline infusion on HRV measures recorded during a 5-minute pre-testing baseline and a 3-minute post-infusion baseline. Three common methods of HRV quantification were used. Significant interactions between glycopyrrolate/saline infusion and pre/post-infusion baseline were observed for all measures (see line graphs in Figure 2.1.). All measures of low-frequency and high-frequency HRV decreased significantly in response to muscarinic blockade. Measures of low-frequency HRV, then, must, in large part, reflect vagal (or parasympathetic) activity.
Figure 2.1. Effects of Glycopyrrolate on LF and HF HRV. Line graphs illustrate significant decreases in heart period (HP) and all measures of HRV in response to peripheral cholinergic blockade. The F tests represent significant interactions between drug infusion and pre/post infusion baselines.

2.4.2 Relative Sensitivity of Different HRV Measures to Glycopyrrolate—Effect Size

We compared relative sensitivities of popular quantification methods to glycopyrrolate, which directly manipulates vagal cholinergic transmission at the heart. Since the purpose of measuring HRV is to gain insight into the underlying functioning of different components of the autonomic nervous system, it is necessary to use a tool of quantification that is sensitive to direct changes in the physiologic mechanisms of interest.
All high-frequency and low-frequency HRV measures decreased significantly in response to peripheral muscarinic blockade, as expected (see Figure 2.1.). The Cohen’s $d$ statistic was used to determine standardized effect sizes of each of these variables, contrasting pre-testing and post-infusion physiology of those study participants, who received the glycopyrrolate infusion ($n = 25$) at the CRC (see Figure 2.2.). Since the effects of muscarinic antagonism by glycopyrrolate are strong, the effect sizes of all the HRV measures are considered large. However, the relative size of effect for each of the measures, when compared to each other, provides further insight into the relative sensitivity of each measure to changes in efferent vagal activity (specifically manipulated in this study) and not to secondary changes in heart rate or rhythm. The PB-RSA measure of high-frequency HRV and PB-THM measure of low-frequency HRV had the largest Cohen’s $d$ effect sizes, indicating that these measures are most sensitive to changes in efferent cholinergic transmission. These effect sizes were each even larger than HP. This was expected, because HP is affected by more than just autonomic activity alone (e.g., changes in intrathoracic pressure during breath cycles and changes in arterial blood pressure). Only the 95% confidence interval for heart period overlapped with the lower bounds of the PB-RSA and PB-THM confidence intervals. All other HRV measures, including HF-spec, Pk-tr, and LF-spec, fell significantly below the PB measures.
2.4.3 Specificity of HRV Quantification Strategy to Cholinergic Antagonism

In addition to being sensitive to changes in cholinergic (vagal) activity, good quantification methods must also change specifically in response to changes in autonomic activity. While all variables changed significantly in response to glycopyrrolate, only the peak-to-trough measure also changed significantly in response to saline vehicle infusion [F(1,26) = 10.204, p = .004]. This
suggests that changes in the Pk-tr measure, while sensitive, are not specific to changes in vagal tone (see Table I.).

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<th>Variable</th>
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<td></td>
<td>F, sig.</td>
<td>F, sig.</td>
</tr>
<tr>
<td>HP</td>
<td>F(1,24) = 198.09, .000</td>
<td>F(1,26) = .199, .659</td>
</tr>
<tr>
<td></td>
<td>.892, 1.00</td>
<td>.008, .071</td>
</tr>
<tr>
<td>PB-RSA</td>
<td>F(1,24) = 161.02, .000</td>
<td>F(1,26) = .647, .429</td>
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<tr>
<td></td>
<td>.870, 1.00</td>
<td>.024, .121</td>
</tr>
<tr>
<td>HF-spec</td>
<td>F(1,24) = 16.407, .000</td>
<td>F(1,26) = .139, .712</td>
</tr>
<tr>
<td></td>
<td>.406, .973</td>
<td>.005, .065</td>
</tr>
<tr>
<td>Pk-tr</td>
<td>F(1,24) = 42.18, .000</td>
<td>F(1,26) = 10.204, .004</td>
</tr>
<tr>
<td></td>
<td>.637, 1.00</td>
<td>.282, .867</td>
</tr>
<tr>
<td>PB-THM</td>
<td>F(1,24) = 87.60, .000</td>
<td>F(1,26) = .032, .860</td>
</tr>
<tr>
<td></td>
<td>.785, 1.00</td>
<td>.001, .053</td>
</tr>
<tr>
<td>LF-spec</td>
<td>F(1,24) = 39.04, .000</td>
<td>F(1,26) = .080, .779</td>
</tr>
<tr>
<td></td>
<td>.619, 1.00</td>
<td>.003, .059</td>
</tr>
</tbody>
</table>

Table I. Main Effects of Glycopyrrolate and Vehicle on Pre/Post-Infusion Baselines with different measures of Physiological activity. In the CRC, 25 participants received glycopyrrolate infusion; 27 participants received infusion of saline vehicle. Physiological variables that are sensitive to glycopyrrolate and not sensitive to vehicle can be considered measures of vagal tone, specifically. Repeated measures analysis of variance (ANOVA) revealed that while all measures of heart rate variability were sensitive to glycopyrrolate, only the Pk-tr measure also changed significantly in response to saline vehicle, suggesting that changes this measure are not specific to changes in vagal tone.

2.5 Discussion

2.5.1 Construct Validity and Neurophysiologic Mechanisms of HRV
Understanding heart rate variability and the neurophysiologic mechanisms it represents is important to many disciplines in translational research and medicine. Complex, functional and anatomic interactions between components of the autonomic nervous system (ANS) and the heart vary widely across mammalian species. Thus, even though we have gained vital insights into human autonomic physiology from animal studies, it is imperative that we continue to explore and refine methods to study humans directly. As with all scientific inquiry, the most important question to ask when designing an experiment to study human ANS function is whether the study procedures will yield results that will answer the study questions as directly as possible. When interpreting the results, this question must be repeated: Can these results be used to understand underlying ANS activity with reasonable specificity and confidence?

Construct validity is paramount. That is, the value of the variables of interest must, in fact, represent the theoretically-determined processes those variables were designed to measure. To start, in the case of heart rate variability (HRV), it is accepted that high and low-frequency patterns result from extracardiac autonomic neurons and not from the intrinsic cardiac nervous system. Strongest support for this in humans comes from the observation that HRV is essentially absent following cardiac transplantation in humans until extrinsic re-innervation develops (Murphy et al., 2000). It follows, then, that appropriate questions regarding underlying physiology of HRV will center on understanding the extent to which different components of the ANS are responsible for different frequency HRV rhythms. In this study, we were interested in two prominent HRV frequencies: high (HF) and low (LF). And there are three main sources of extracardiac autonomic innervation of the heart—(1) the vagal fibers from the nucleus ambiguus (NA), (2) the spinal sympathetic neurons, and (3) the vagal neurons from the dorsal motor nucleus of X (DMNX). Like previous studies, we used knowledge of each anatomical system to manipulate neurophysiologic function and looked for corresponding changes in HF and LF HRV measures.
Interpretation of high-frequency HRV, or respiratory sinus arrhythmia (RSA), is much better established than that of low-frequency HRV. RSA refers to the naturally occurring phenomenon of increased heart rate during inspiration followed by decreased heart rate during post-inspiration periods. Clinically, RSA is important, because it facilitates pulmonary gas exchange by optimizing the ventilation-perfusion ratio during respiration cycles. RSA has long been accepted as an index of efferent brainstem, and, therefore, parasympathetic, activity. Historically, Traube (1865) is credited with presenting the concept that “respiratory cardiac arrhythmias” were mediated by the central nervous system. The theory was later proved experimentally by Frédéricq (1882), and central regulation became dogma, despite Hering’s 1871 conclusions that respiratory arrhythmia was more likely the result of a peripheral reflex mechanism (Anrep, Pascual, & Rossler, 1936a, 1936b). By 1936, when Anrep et al reported their results from extensive experiments in dogs, the mechanisms mediating the effects of vagus tone on the heart were known (remember Loewi and Feldberg above), and the group concluded that vagal and pulmonary reflex mechanisms were interrelated (Anrep et al., 1936a, 1936b). While central neuronal function could have a more robust influence in many conditions, both contributed to RSA. More recent experiments have shown that RSA remains synchronized with respiratory rhythms of NA vagal neurons, even during mechanical ventilation occurring at different, non-synchronous intervals (Daly, 1991), suggesting that central regulation can override peripheral reflex. RSA also persists in human tetraplegic patients with spinal cord injuries that interrupt normal sympathetic efferent function (Koh, Brown, Beightol, Ha, & Eckberg, 1994). In the current study, vagal regulation of RSA was re-confirmed and, more importantly, isolated in humans by demonstrating that RSA was abolished by peripheral muscarinic blockade, which specifically interrupted vagal-cardiac neurotransmission. While less parasympathetic activity leads to lower RSA, the relationship between vagal activity and HF-HRV might not be linear across the entire range of levels of cholinergic transmission at the SA node, as is often implied in the literature. Some studies and communications have challenged the strength of
the vagal activity-HF HRV relationship on the whole by demonstrating that overstimulation of parasympathetic activity (often using indirect phenylephrine activation of parasympathetic regulation in the baroreflex) actually causes a decrease in HF-HRV (Goldberger, Ahmed, Parker, & Kadish, 1994; Goldberger, Challapalli, Tung, Parker, & Kadish, 2001; Goldberger, Kim, Ahmed, & Kadish, 1996; Malik & Camm, 1993). Further study is warranted into this proposed parabolic association and into defining physiologic ranges of vagal activity, however, these findings do not negate a significant link between parasympathetic activity and RSA, which remains clear.

Interpretation of low-frequency HRV is more complex than RSA for two main reasons: (1) there are strong relationships between LF HRV, blood pressure waves, and the baroreceptor reflex, which has both sympathetic and parasympathetic components; (2) the stubborn conception of the ANS as consisting of two systems in paired opposition persists. The latter notion allows LF to be continually interpreted as sympathetic, mostly because it is different from RSA, which is parasympathetic. In some fields, the “LF/HF ratio” is still thought of as an “index” of “sympathovagal balance,” even after lengthy, considered, published discussions (Berntson et al., 1997) and critical indictments (Eckberg, 1997) refuting the “balance” concept on the basis that it has little to no construct validity. One compelling argument that sympathetic drive cannot be primarily responsible for LF HRV is Koh et al.'s (1994) report that tetraplegic patients with no sympathetic innervation of cardiac tissue have prominent LF HRV that is significantly diminished by atropine (Koh et al., 1994). However, the goal of this paper is not to overcorrect current misconceptions by claiming that LF HRV is purely a parasympathetic phenomenon. There is important evidence that links sympathetic activity to LF HRV—increased sympathetic muscle activity and circulating catecholamines following nitroprusside infusion has been significantly correlated with increases in LF HRV (Saul et al., 1990); beta-adrenergic blockade has been associated with decreases in LF HRV (Akselrod et al., 1981), although results and interpretation of this association are not consistent (Cacioppo et al., 1994; Taylor et al., 1998). More clues about the
neurophysiology underlying LF HRV have come from studying the baroreflex response, which minimizes changes in cerebral blood perfusion with changes in posture—a necessary evolutionary adaptation to accommodate upright posture in humans. Pagani et al (1986) reported that LF HRV increased with rising blood pressure in response to upright tilt. Additionally, that increase could be attenuated by beta-adrenergic receptor blockade. The group also demonstrated increased LF HRV with intravenous infusion of nitroglycerin, which likely causes reflex sympathetic activation and decreased vagal activity (Pagani et al., 1986). Other studies have cited the increase in LF HRV during the baroreflex as evidence that LF HRV primarily reflects sympathetic influences at the heart. However, increased arterial pressure, such as that induced by sympathetic activation with the baroreflex, causes initial reflex-induced decreases in heart rate mediated predominately (some say exclusively) by vagal efferent activity (Stornetta, Guyenet, & McCarty, 1987; Sullebarger, Liang, Woolf, Willick, & Richeson, 1990). When using HRV to determine relative contributions of ANS components to phases of a complicated process, such as the baroreflex, temporal resolution and accuracy of monitoring equipment and methods of quantification become major concerns. There is still considerable heterogeneity in the literature regarding these particular aspects of baroreflex study.

The complexities of the interacting mechanisms that likely contribute to the LF HRV pattern also make interpretation difficult. The interaction between vagal and sympathetic effects on heart rate were explored by Levy and Zieske (1969), who demonstrated that parasympathetic-induced bradycardia was actually more pronounced under conditions when sympathetic efferent activity was high. Further, they demonstrated that the effects of sympathetic activity on changes in heart rate were significantly diminished in the presence of increased parasympathetic neuronal firing (Levy & Zieske, 1969). This interaction might be due to presynaptic muscarinic cholinergic receptors on sympathetic nerve terminals, which, when bound by acetylcholine from active nearby parasympathetic neurons, inhibit release of norepinephrine (Rardon & Bailey, 1983; Schmid et al.,
Hypotheses like this are difficult to test in awake, behaving humans, where there are limited options to experimentally link neurobiochemical processes with outcome measures like HRV. For this reason, pharmacological studies are often attractive, because they offer opportunity for direct intervention in awake, behaving human participants.

Designing biochemical based studies to study the ANS, however, is still difficult. First, many cholinergic and adrenergic agonists and antagonists cross the blood-brain barrier, which hinders the ability to distinguish between the effects of autonomic efferent activity directly at the heart and more complex interrelationships between autonomic brainstem and spinal cord structures. Effects of cholinergic agents that cross the blood-brain barrier are especially difficult to interpret, because the preganglionic receptors of all three autonomic cardiac systems are either muscarinic (DMNX vagal fibers) or nicotinic (NA vagal fibers and spinal sympathetic neurons). In addition, cholinergic interneurons, which modulate autonomic nucleus activity in the brainstem and spinal cord, can be affected. Several groups have demonstrated that LF HRV decreases in response to atropine (Cacioppo et al., 1994; Taylor et al., 1998), which has both peripheral and central effects. The findings in this study are unique, however, because for the first time, it is clear that LF HRV is sensitive to changes in peripheral, muscarinic, cholinergic, vagal neurotransmission.

2.5.2 Construct Validity and Quantification of HRV

A discussion of construct validity would certainly be incomplete here without evaluation of the methods used to quantify HRV variables. In this study, we employed the three most widely used methods to evaluate the effects of peripheral muscarinic blockade on heart rate variability. It is important to use a quantification strategy that can distinguish between RSA and LF HRV in order to distinguish the relative influences of underlying neurophysiologic mechanisms. The strategy must demonstrate robust statistical stability; it must not depend on control of other physiological parameters, such as respiration rate or tidal volume; it must not violate statistical assumptions.
required to use subsequent parametric statistics to answer study questions. Most important, it
must be both sensitive and specific to changes in autonomic efferent activity on heart rate and
rhythm. (For a detailed analysis and comparison of statistical properties of these methods, please
read Lewis et al, 2012.) A significant advantage of this study design was the use of glycopyrrolate
to directly manipulate muscarinic cholinergic receptors limited to the peripheral nervous system.
The measures with greatest sensitivity and specificity in response to change in vagus nerve
transmission have greatest construct validity and can, therefore, be considered superior measures
of cholinergic activity at the SA node.

An increasing number of studies in various scientific and clinical fields measures autonomic,
specifically parasympathetic, influence on cardiac rate and rhythms in humans. It is, therefore,
increasingly important that the methods used to obtain so many important results demonstrate
construct validity. Otherwise, the translational utility of too many studies will remain limited. It
has been suggested that the three methods employed in this study are essentially equivalent
(Grossman et al., 1990). However, we found that the Porges-Bohrer method of quantifying both
low-frequency and high-frequency HRV was, by far, most sensitive and specific to direct
manipulation of vagus nerve influence on cardiac rhythm. The superior sensitivity and specificity
of this method are due to two important strategies that were designed specifically to address the
statistical challenges presented by the non-stationarity of raw heart rate data. First, the inter-beat-
interval data are initially preconditioned using a moving polynomial filter, which removes complex
slow trends and reduces the influences of non-stationarity. This step is essential, because spectral
analysis can only appropriately be applied to a stationary sequence—an important assumption that
would be violated without polynomial detrending. Second, and equally important, the Porges-
Bohrer method is the only method of quantification, which applies a natural log transform to yield a
normally distributed value that can be used appropriately in parametric statistical analysis of study
findings. The distributions of the outputs from both spectral and peak-to-trough methods are not
normal and, therefore, violate the assumptions required for parametric analyses (e.g., skewness, kurtosis, sphericity, and heteroscedacity). Applying the appropriate moving polynomial filter to the data and logarithmically transforming the output of either the spectral or peak-to-trough methods would result in similar, improved sensitivity and specificity of these methods to changes in vagal activity (see Table II.).

<table>
<thead>
<tr>
<th>Variable</th>
<th>GLYCOPPYRROLATE</th>
<th>Effect Size, Obs. Power</th>
<th>VEHICLE</th>
<th>Effect Size, Obs. Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP</td>
<td>$F(1,24) = 198.09, .000$</td>
<td>.892, 1.00</td>
<td>$F(1,26) = .199, .659$</td>
<td>.008, .071</td>
</tr>
<tr>
<td>PB-RSA</td>
<td>$F(1,24) = 161.02, .000$</td>
<td>.870, 1.00</td>
<td>$F(1,26) = .647, .429$</td>
<td>.024, .121</td>
</tr>
<tr>
<td>HF-spec</td>
<td>$F(1,24) = 16.407, .000$</td>
<td>.406, .973</td>
<td>$F(1,26) = .139, .712$</td>
<td>.005, .065</td>
</tr>
<tr>
<td>Ln HF-21mpf</td>
<td>$F(1,24) = 172.97, .000$</td>
<td>.878, 1.00</td>
<td>$F(1,26) = 1.97, .172$</td>
<td>.070, .272</td>
</tr>
<tr>
<td>Pk-tr</td>
<td>$F(1,24) = 42.18, .000$</td>
<td>.637, 1.00</td>
<td>$F(1,26) = 10.20, .004$</td>
<td>.282, .867</td>
</tr>
<tr>
<td>Ln Pk-tr-21mpf</td>
<td>$F(1,24) = 149.06, .000$</td>
<td>.820, 1.00</td>
<td>$F(1,26) = 1.138, .296$</td>
<td>.042, .177</td>
</tr>
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<td>PB-THM</td>
<td>$F(1,24) = 87.60, .000$</td>
<td>.785, 1.00</td>
<td>$F(1,26) = .032, .860$</td>
<td>.001, .053</td>
</tr>
<tr>
<td>LF-spec</td>
<td>$F(1,24) = 39.04, .000$</td>
<td>.619, 1.00</td>
<td>$F(1,26) = .080, .779$</td>
<td>.003, .059</td>
</tr>
<tr>
<td>Ln LF-51mpf</td>
<td>$F(1,24) = 78.49, .000$</td>
<td>.766, 1.00</td>
<td>$F(1,26) = 1.15, .293$</td>
<td>.042, .178</td>
</tr>
</tbody>
</table>

Table II. Main Effects of Glycopyrrolate and Vehicle on Pre/Post-Infusion Baselines when the moving polynomial filter and natural log transform are applied to spectral and Pk-tr quantification strategies. Original F values from repeated measures ANOVA contrasting pre-post infusion baselines measured by spectral and Pk-tr methods are printed in bold italics. Addition of the moving polynomial detrending during preprocessing of the edited heart period series and natural log transform of the detrended series yields much higher F values with both Spectral and
Pk-tr strategies (bold). These values are now comparable to those achieved using the Porges-Bohrer method, and the measures are, thus, more sensitive to changes specifically in vagal activity.

2.5.3 Limitations and Future Directions

The findings presented in this paper were obtained as part of larger behavioral protocol designed to test the effects of peripheral vagal blockade on centrally-mediated social engagement behaviors. As such, there is no dose-response analysis to detect incremental changes, which may correspond between drug and HRV response. In addition, this study does not include vagus nerve or sympathetic stimulation to explore the effects these manipulations can have on HRV variables. Although our focus was on measuring heart rate variability, it might have also been helpful to measure beat-to-beat blood pressure and finger pulse activity, since the baroreceptors and peripheral vasomotor activity are both involved in the feedback circuit mediating LF HRV.

Future studies should investigate all of these scenarios. Additionally, new studies should be designed to examine the physiologic ranges of both low-frequency and high-frequency HRV in humans of different ages and healthy populations under varying conditions in order to establish a normative dataset that can truly be useful when detecting abnormalities in the clinic.

References


3. EMOTIONAL AND BEHAVIORAL SELF-REGULATION IN INFANTS—AN OVERVIEW

The ability to self-regulate emotions and behavior is essential to successful, adaptive psychosocial functioning and, thus, has received significant attention in developmental psychology and behavioral neuroscience literature for decades. Historically, the literature and research concerning self-regulation in children stem from observational studies of personality and emotion development. Mary Jones and Barbara Burks (1936) described individual differences in emotional and social behavior, as substrates for subsequent development of personality, from the neonatal period through school age. In their summary of infancy, they wrote that the infant is “an organism which can respond positively or negatively toward a limited number and kind of factors in his environment. . . . In a short time . . . the approach and avoidance responses become clearly elaborated and adapted so that the observer does not hesitate to ascribe a variety of emotional expressiveness to the child.” They concluded that “[t]he picture of infancy . . . is predominantly one of change: change from diffuse activity to adaptive behavior, from meager stimulus-response functioning to integrated responses in the light of . . . situation . . .” (Jones, Burks, & Jones, 1936).

This early thesis reflected both of the major themes of the time that would continue to dominate research in infant emotion and behavior for the next fifty years. One theme centered on the study of individual differences and, eventually, temperament. Chaney and McGraw (1932) studied individual differences in reflex and other motor reactions in newborn infants in the first ten days of life and concluded that the more complex the type of reaction, the larger the individual variation. They left the problem of understanding the “extent to which individual differences in the reflex activities of newborn infants may be suggestive of variability in development” for “future investigation.” However, they did suggest that such future research should be conducted with
“correlated studies of the structural development of the nervous system” (Chaney & McGraw, 1932). Developmental psychologists reasoned that if variations in development of the nervous system could lead to variations in motor reflex responses, then such variation could also underlie differences in more complex behaviors that constitute the core components of personality. Studying biological variability in the 1920s and 1930s proved a significant challenge, however, because the technology of the time was not yet sophisticated enough to explore the enormous complexity of brain and body coordination required to achieve the constellations of behaviors, which make up an emotional expression, for example. Measurements of blood pressure, pulse rate, and respiration as indices of autonomic activity had been used to understand mechanisms of sleep state in adults (Richter, 1926), but obtaining autonomic measures in awake, behaving infants was nearly impossible. One exception was the galvanic skin response (GSR), which was (and still is) used as an index of “thoracico-lumbar,” or sympathetic, peripheral nervous activity. Jones (1930) disproved earlier reports that GSR was absent or underdeveloped in the first year of life by successfully quantifying such responses or “reflexes” in eight infants aged three to eleven months during conditions of “startle” and “frustration” (Jones, 1930). Jones found that “[c]hildren who are the most ready to cry are frequently the least reactive on the galvanometer . . .” and used this observation to promote the concept of an inverse relationship between “overt and visceral expression,” which could be used to distinguish different “types” of children. Over time, the collections of characteristics that defined differences in infant behavior and emotional reactivity that were both consistent across behavioral tasks, such as maternal separation and startling sensory stimuli, and persistent over time, as in throughout infancy and toddlerhood, were characterized in detail. Individual differences appeared to be constitutional and were, thus, assumed to be rooted in genetics and nervous system biology. Eventually, categories of child “temperament” were defined, and these categories have become central to contemporary study of individual differences in infants and children. The definitions and approaches to studying
temperament are still debated (Goldsmith et al., 1987; Zentner & Bates, 2008), however, and will be discussed later.

The other major theme of infant behavioral research focused on detailed characterization of cognition and emotional development in infancy and early childhood. Arnold Gesell was one particularly influential developmental psychologist, pediatrician, and above all “maturationist”, who used a biological, naturalistic approach to explain development of behavior in infancy and childhood (Knobloch, 1961). Gesell wrote that “[p]atterns of behavior in all species tend to follow an orderly genetic sequence in their emergence” (Gesell, 1933). About the nervous system, Gesell asserted that “[g]rowth is a patterning process. It produces patterned changes in the nerve cells; it produces corresponding changes in patterns of behavior” (Gesell & Ilg, 1943). Anatomical and functional studies of nervous system development in humans was (and still is) difficult to conduct, so while it was generally accepted that there must be neurobiological correlates of behavior, there were few empirical studies to demonstrate any specific relationships. In the 1950s and 1960s, developmental psychologists, most notably Jean Piaget, began to describe stages of cognitive and sensorimotor development, which was thought to dominantly mediate the developing person’s relationship with environment. Like Gesell, Piaget downplayed individual differences in favor of prescribed developmental stages (or “schedules,” as Gesell called them) that were conserved across infants and children of congruent age. He wrote that “[t]he development of ... knowledge is a spontaneous process, tied to the whole process of embryogenesis. Embryogenesis concerns the development of the body, but it concerns as well the development of the nervous system, and the development of mental functions. In the case of the development of knowledge in children, embryogenesis ends only in adulthood” (Piaget, 1964). In terms of cognitive development, which he distinguished sharply from learning, Piaget described four main stages: (1) the sensorimotor, preverbal stage during which the practical knowledge that serves as the “substructure of later representational knowledge” develops; (2) the stage of pre-operational representation, which
includes the beginnings of language and symbolic function; (3) the concrete operational stage during which all of the “fundamental operations of elementary logic of classes and relations” appear; and (4) the formal or “hypothetic-deductive” operational stage when the child “attains new structures which are on the one hand combinatorial . . . [and] on the other hand, more complicated group structures” (Piaget, 1964). Descriptions of the stages of emotional development by Erikson (1950), Spock (1946), and others compliment theories of cognitive maturation: (1) the trust stage, when infants are physically helpless and emotionally agreeable; (2) the stage of autonomy, when toddlers develop a sense of their own individuality; (3) the initiative stage, when the pre-schooler begins to imitate through admiration and learns about friends; (4) the stage of industry, when school-age children begin to gain independence from parents and develop a sense of conscience and morality; and (5) the identity stage during adolescence, when adult identity begins to form (Erikson, 1950; Spock, 1946; Sroufe, 1982). The scope of the present discussion is limited to the period of infancy, specifically the first year of life. Therefore, the cognitive and emotional stages of preverbal, sensorimotor exploration and trust or emotional vulnerability to others are of primary concern.

While Piaget did describe a process of “equilibration” as a fundamental factor underlying his developmental stages (Piaget, 1964), it was not until the mid- to late 1980s, when development of cognitive and emotional states were considered well-understood. This marks the period when investigators began to rigorously pursue a subtle, but important conceptual shift toward studying regulation of emotions. “In the past, emotions were considered to be feeling states indexed by behavioral expressions; now, emotions are considered to be processes of establishing, maintaining, or disrupting the relation between the organism and the environment on matters of significance to the person” (Campos, Campos, & Barrett, 1989). Kopp (1989) defined self-regulation as “the term used to characterize the processes and the characteristics involved in coping with heightened levels of positive and negative emotions . . .” (Kopp, 1989). However, just as the defining parameters of
the temperament construct continue to change, operational definitions of emotion and behavior regulation are continually refined and debated in attempts to standardize terms investigators use to communicate between labs and across fields and specific interests. In 2004, *Child Development* devoted an entire issue to exploring methodological and definitional issues when studying emotion regulation as a scientific construct in infants and children. Cole et al asked whether studying emotion-regulation offered anything unique to the study of emotion development. Their answer was yes, and they promoted emotion-regulation as a valid scientific construct distinct from emotional “stances.” They proposed a working definition in which “[t]he term emotion regulation can denote two types of regulatory phenomena: emotion as regulating and emotion as regulated. In each case, the regulatory aspects must be conceptualized independently of which emotion is activated initially” (Cole, Martin, & Dennis, 2004). According to this definition, methods used to quantify or describe emotion regulation must be able to detect change (either physiological or behavioral), unlike many emotion development research methodologies, which focus on identification of emotion valence. In a brief survey of methodological trends in regulation literature, Bridges et al identified five common, discrepant decisions often made during emotion-regulation research that lead to divergent conclusions for potentially unexplored reasons: “(a) focusing on types versus total amount of emotion regulation, (b) determining distinctiveness of measures of emotion versus emotion regulation strategies, (c) deciding whether and how to examine temporal sequencing of strategy use and emotion, (d) using discrete versus global emotion measures, and (e) determining when emotion is being regulated” (Bridges, Denham, & Ganiban, 2004). In response to Cole et al’s question earlier in the issue, they concluded that research in emotion-regulation does promise to generate knowledge not otherwise achieved by studying other aspects of emotional development in children. However, they called for greater conceptual clarity and corresponding attention to research design. Last, Campos et al presented a unitary approach to studying emotion and its regulation. They argued that “in the real world, the processes underlying
emotion and emotion regulation appear to be largely one and the same, rendering the value of the distinction largely for the benefit of analysis” (Campos, Frankel, & Camras, 2004). Efforts to create guidelines for empirical study of emotion regulation continue, as does research in individual differences and development of emotional behavior in early life. As recently as 2009, Ruth Feldman acknowledged that even though “[r]egulation . . . has emerged in recent years as a central construct in the conceptualization of developmental progress. . . . , [the] term [is] still awaiting a comprehensive definition” (Feldman, 2009).

Aside from clarifying the construct, recent literature has also focused on elucidating the cognitive and social or environmental substrates on which behavioral and emotional self-regulation strategies are developed and maintained from infancy through early childhood. Sheese et al (2008) studied early behavioral markers of executive attention, which has been considered a crucial component in developing self-regulation, or “effortful control,” of behavior and, presumably, of underlying thoughts and feelings (Rothbart, Sheese, & Posner, 2007). The authors measured anticipatory looking, a probable index of executive attention, in six and seven-month-old infants and asked whether the behavior was reliably linked to emotion regulation and self-soothing behaviors (e.g., self-touching, hand-clasping, sucking, orienting away from a distressing stimulus). The larger question, as with most studies in this field, was whether the behavior could eventually be used to predict self-regulation ability later in life (Sheese, Rothbart, Posner, White, & Fraundorf, 2008). The authors found that infants with greater skill in anticipatory looking also showed greater regulation or effortful control over their physical movements (interpreted from increased reticence to approach a new toy). The relationship between executive attention and emotion regulation was “not as clear-cut.” As hypothesized, infants with better executive attention abilities showed increased duration of distress (measured by facial expressivity and interpreted as fear) in response to distressing stimuli. However, the duration of distress was not related to frequency or intensity of physical self-soothing behaviors used by the infants during these stimuli. Duration of distress was
related to completion of fewer distressing trials, though. So it is possible, that instead of using physical self-soothing techniques, like sucking on fingers or self-touching, infants with better executive attention skills simply regulate their emotions (or distress) by avoiding the distressing stimulus altogether. Overall, the authors conclude that their findings “suggest that there is cognitive control over emotion during infancy” (Sheese et al., 2008). However, it is possible, for example, that increased caution when reaching for a novel object is less based in cognition and primitive reasoning and more based on underlying fearful emotion. The correlational nature of this study did not allow directional statements of causation regarding the relationship between cognition and emotion within an individual—a question that could be argued is moot from a research perspective, since the likelihood of bi-directional influence is high and cannot be disproved. Results from follow-up experiments in this longitudinal study to answer the question of predictive power of anticipatory looking as an indicator of self-regulation development have not yet been published. Like Sheese et al, Bell and Wolfe (2004) have also asserted that “research integrating cognition and emotion is essential in any attempt to comprehend emotion and to endorse it as a scientific construct, because cognition and emotion represent an intricately bound developmental process.” They cite patterns of electrical activity obtained by electroencephalograms (EEG) and the proposed dual cognitive and emotional regulatory functions of brain structures in the anterior attention system, specifically the anterior cingulate gyrus and “frontal brain systems” as physiological evidence to support the link between the two processes in emotion regulation even in early life (Bell & Wolfe, 2004).

While early cognitive processes do likely contribute to emotion regulation in infants, many argue that one must consider the social environment in order to achieve meaningful understanding of emotion or self-regulation. This is especially true when studying the developing infant, whose survival depends almost entirely on his or her relationship with the primary adult caregiver. Moreover, the most significant reason researchers are interested in self-regulation at all is that
dysfunctional regulation leads to poor social functioning, which in human children and adults, can be devastating and even life-threatening. Calkins and Fox (2002) underscore this point by demonstrating that “deficits in . . . levels of [attention and in emotional and physiological] self-regulation may underlie childhood social withdrawal and aggression” more than individual differences in temperament. They called for specific improvements in the ways future longitudinal studies, in particular, might continue to study the role that self-regulation plays in “pathways to disordered behavior” (Susan D. Calkins & Fox, 2002). In a 2006 review of emotion regulation in children and adolescents, Zeman et al wrote that in addition to developmental status and organization of “emotion systems,” a child’s ability to regulate emotion at any point in time “depends on previous interactions with the social environment” (Zeman, Cassano, Perry-Parrish, & Stegall, 2006). Fogel et al (1992) examined infant emotional development specifically in social contexts and proposed a Social Process Theory of Emotion. Consistent with the emerging interest in regulation of emotion at the time, they proposed that “emotions are neither states nor programs, but self-organizing dynamic processes that are created with respect to the flow of the individual’s activity in a context” (Fogel et al., 1992).

The infant’s social environment, especially during the first year of life, is significantly defined by the primary caregiver, often the mother. Psychologists often conceptualize the mother-child interaction according to the perspectives made popular in Bowlby’s 1969 book, *Attachment and Loss* (Bowlby, 1969). The fundamental concepts of Attachment Theory, however, were well-delineated a decade earlier in Bowlby’s proposed “hypothesis of Component Instinctual Responses” (Bowlby, 1958), which describes five primary behaviors, or “responses,” in infants that make up “attachment behavior”—sucking, clinging, following, crying, and smiling. While Bowlby did not specifically discuss biology, he, like Gesell, supported the idea that an infant, especially an early infant, is essentially hard-wired to perform certain “instinctual” behaviors. He wrote that “. . . even though there is good evidence that the human face and voice hold some special interest for the
infant even in his earliest weeks, it is probably mistaken to suppose that at this age he entertains anything which remotely resembles the concept of ‘human being’. . . . [S]miling [can be] . . . sensitive to patterns much simpler than the whole human face: it is in fact activated at first by a sign stimulus comprising no more than a pair of dots.” (Bowlby, 1958).

Like other mammals, the human infant is designed to behave in ways that allow him or her to get what is needed for survival—nourishment, warmth, safety, and at least one caring social relationship—from the environment. It follows, then, that since the human infant is unable to acquire many of these needs independently, he or she must at least be able to encourage the primary caregiver to provide them—to essentially co-opt caregiver cooperation as part of a strategy to “self”-regulate. An infant’s cry, for example, leads to caring attention from the mother, who will touch and hold and rock or feed her baby until the crying or fussing stops. A smiling infant receives more attention from the mother, whose own sense of success as a caregiver is reinforced and encouraged by the infant’s smile and melodic vocalizations. In turn, this mother is likely to continue to care for the child and might even be more responsive to her child’s cries than she would be if the infant did not smile as often. From a researcher’s perspective, the difficulty and importance of studying infant regulation in the context of his or her social environment becomes particularly apparent when trying to understand pathological behaviors. Persistent crying or fussing in infancy that is difficult to console, for example, has been repeatedly correlated with increased maternal depression and anxiety (M. Papoušek & von Hofacker, 1998; Parkin, Schwartz, & Manuel, 1993; Vik et al., 2009). However, it is difficult to determine causal relationships between infant crying and parental emotions and behaviors. For example, a depressed mother might engage in behaviors that comprise overall poor caretaking skills or neglect, which cause an infant to cry more than he or she would with a non-depressed or non-anxious mother. On the other hand, a mother could develop depression and anxiety secondary to extended periods of sleep deprivation caused by her infant’s persistent crying. Papoušek and von Hofacker (1995) referred to
investigating directional relationships between persistent crying and parenting as “search[ing] for a butterfly in a dynamic process.” They identified several interrelated effects that infant crying could have on parenting, including “nervous exhaustion,” “ambivalence,” “learned helplessness,” and “exacerbation of neurotic conflicts,” each and all of which could contribute to “inhibition of intuitive parenting.” The resulting avoidance of playful interactions; ignoring of infant signals; and delayed, unpredictable, inadequate, or inefficient responses to infant cries could then lead to “lack of regulatory support” and increased risk of neglect or abuse, which would reinforce infant crying behavior, especially in early infancy (Papoušek & von Hofacker, 1995).

When persistent criers reach school age, they are at greater risk for diagnosable psychiatric disorders, which actively limit family activities, and their mothers have a greater number of depressive symptoms and are more likely to worry about their children’s health than mothers in a typical community sample (Brown, Heine, & Jordan, 2009). In a study of very low birthweight infants, longer duration of crying and fussing behaviors in the first few months of infancy was associated with “less optimal development at 24 months of age” (Munck et al., 2008). Stifter and Spinrad (2002) found that male infants with excessive crying behaviors displayed poor emotion regulation as measured by behavioral microanalysis of infants’ responses to arm restraint stress and parental completion of infant temperament questionnaires and cry diaries (Stifter & Spinrad, 2002). Findings like these reinforce the importance of understanding disorders of emotional and behavioral regulation in infants, because even though colicky babies tend to “grow out of it” in terms of crying behavior, the potential for developmental compromise remains. While formal study of parents’ emotional responses to children with flattened affect or primary disorders of social engagement, such as autism spectrum disorders, is scarce, anecdotal evidence suggests that a child’s lack of smiling (or often eye contact) can lead parents to feel that their child might not love them. This is not a scenario that typically applies to infants, however.
In light of these examples, it seems almost imperative to consider infant behaviors of regulation within social contexts, especially when evaluating behavior as appropriate or pathological and when designing potential interventions. For example, crying can be adaptive when it leads to a consoling behavior in the mother, which in turn causes the crying to stop. Crying becomes maladaptive, when it is persistent and inconsolable, despite appropriate and best efforts from a caregiver to console. Smiling can also be considered adaptive, when used, for example, during social play. Smiling or indifference in response to a stressful or painful stimulus, however, suggests underlying pathology. When interpreting results, it is equally important to remember the dynamic and bi-directional nature of the relationship and behaviors under examination. For example, crying can be a simultaneously adaptive and ineffective regulation strategy for an infant, if the caregiver fails to recognize or provide appropriate responses to the infant’s needs. Over time, if the pattern continues, the crying becomes maladaptive, futile, or even harmful, and the child may adopt non-traditional strategies in order to fulfill basic requirements. Alternative strategies, however, can be costly and might be developed at the expense of other important skills, and social-emotional and even physiological development may become compromised.

Regulation of emotional or physiological state in early infancy should neither be conceived as a process of independent self-regulation, nor as one completely dependent on another person. Instead, regulation in early life (especially the first few months) should be viewed as a fairly complex, dynamic process of social interaction initiated by the infant, responded to by the caregiver, and completed only when the back and forth interplay results in a satisfactory conclusion as determined (mostly) by the infant. An infant’s ability to regulate emotions and behaviors, then, depends not only on his or her own ability to elicit favorable, useful responses from the caregiver, but also on the caregiver’s ability to recognize the infant’s cues and to respond appropriately. In the end, the infant’s emotions and behaviors must be settled to satisfaction according to both parties, otherwise the interaction (or regulation) cannot be considered successful.
Social referencing—the ways infants use others’ emotional signals to regulate their own behaviors—is a phenomenon that has been studied for decades and is primarily concerned with the caregiver’s ability to influence the infant’s physical behaviors as he or she interacts with novel stimuli in the environment. The classic social referencing study is the investigation of visual cliff behavior in 108 12-month-olds tested by Sorce et al in 1985. The infants were placed to crawl toward the mother across what appeared to be a 12-inch cliff drop. As the infants approached the visual cliff, they hesitated and looked to the mother for more information to guide their behavior. In a series of experiments, mothers were asked to express joy or interest or fear or anger. Almost all of the infants of mothers who expressed joy or interest crossed the cliff. Very few infants whose mothers expressed fear or anger crossed. Interestingly, when there was no visual depth discrepancy in the crawling path, very few infants referenced the mother, and those who did tended to continue to crawl toward the mother, regardless of her expression (Sorce, Emde, Campos, & Klinnert, 1985). This suggests that, even in infants as young as one year, social referencing is only very important when confronted with an ambiguous or uncomfortable situation. Additionally, the study demonstrated two distinct phases in social referencing behaviors of infants: (1) seeking information from a trusted adult about an ambiguous situation and (2) using that information to guide subsequent behavior. Even at 12 months of age, infants in the Sorce et al study demonstrated relatively independent decision-making skills when the majority of them failed to heed the fearful or anger maternal expressions as warnings when there was no apparent visual depth problem.

Hornik and Gunnar (1988) studied 32 mother-infant dyads, half at 12 months and half at 18 months of age in a more naturalistic setting. Infants were presented with a rabbit in a large cage, while the mother sat in a chair in the corner of the room. The mother was instructed to first nod and use encouraging gestures without speaking whenever the child looked at her. Next, the mother was allowed to use vocal instructions and encouragement. Third, the mother approached the cage with the child and demonstrated how the child might interact with the rabbit. Last, the mother
returned to the chair and continued verbal instructions and encouragement. The results did not reveal any differences in amount or quality of social referencing between the 12-month and 18-month groups. There were also no significant differences in amount or quality of social referencing between infants who displayed more wary or more bold interacting behaviors when first encountering the rabbit. Interestingly, there was more social referencing during the segments when the mother was vocally interacting with the child than when she only nodded and made physical gestures (Hornik & Gunnar, 1988). The authors used this behavior to refute the widely held concept that novel situations must be ambiguous to evoke social referencing from a child. This might be true, however, in this study, there was also no significant relation between amount or quality of social referencing and subsequent behaviors, suggesting that while infants might have paid a lot of attention to the mother while exploring the rabbit, this interaction did not dictate any changes in behavior, which is what most experiments in social referencing seek to determine.

Mumme et al (1996) developed a novel toy social referencing paradigm, which allowed evaluation of the independent effects of facial and vocal emotional signals and of positive and negative signals on infant behavior. They found that in response to fearful vocalizations from the mother (facing away from the infant), infants looked longer at their mothers, approached the new toy less, and expressed more negative affect than they did in the neutral-voice or happy-voice conditions, which did not significantly differ from each other. In response to maternal facial expressions only (no vocalizations were allowed from the mother), the infants’ behaviors did not differ overall in response to happy, neutral, or fearful faces. The one significant difference that did appear in the face-only conditions was a main effect of sex of infant. Only girls showed a differential response to fearful faces by looking at the mother more often than in the neutral face and, surprisingly, by approaching the toy more. Boys’ behaviors did not differ between fearful and neutral maternal expressions (Mumme, Fernald, & Herrera, 1996). In a similar experiment, also using novel toy presentation, Stenberg (2003) attempted to separate the processes of social
referencing and attachment in 12-month-olds. She studied the effects of maternal inattentiveness on infants’ behaviors when presented with ambiguous and unambiguous toys. As expected, the infants displayed referencing during presentation of the ambiguous toy (a buzzing, mechanical dinosaur) than with the unambiguous toy (a buzzing, mechanical pig). Referencing also increased and toy exploration decreased with inattentive mothers. Once the mothers resumed attentiveness, however, exploration of the toy and environment increased, and referencing decreased (Stenberg, 2003). Taken together, these studies demonstrate that infants use social cues from the mother or available adult to help them gauge how to interact with novel objects in their environments. These cues are especially helpful when the infant experiences uncertainty, and adult cues signaling fear or anger or other information suggesting the infant should avoid a new stimulus or behavior tend to lead to greater contingency in infant behavior.

As illustrated in the examples above, most of the social referencing literature focuses on an infant’s use of others to guide regulation with respect to changes in the external environment. In light of the importance of the caregiver as a partner in the preverbal infant’s ability to interact appropriately with the environment, it is reasonable to extend this regulating role of the parent or caregiver to its influence on an infant’s ability to regulate his or her own internal environment, or emotions. Indeed, security of attachment (or quality of relationship between the infant and the caregiver) has recently been evaluated in terms of a parent’s ability to aid in regulation of a child’s emotional state during development, especially during the first year (Nichols, Gergely, & Fonagy, 2001). The Social Biofeedback Theory of Parental Affect-Mirroring (Gergely & Watson, 1996), for example, proposes that development of an infant’s ability to self-regulate depends heavily on “affect-regulative interactions” in which the parent “mirrors” the child’s affective states and, in essence, provides the child with a biofeedback mechanism with which he or she can learn to categorize different emotional states and subsequently learn to self-regulate. According to Gergely and Watson, deviant parental affect-mirroring styles may compromise developmental trajectory
and lead to adverse developmental outcomes for the child. From a research perspective, the degree of successful co-regulation or security of attachment should, then, be related to caregiver responsiveness and synchrony during the dyadic parent-child interaction. The degree of synchrony, in turn, could be measured using paradigms designed to examine social referencing. For example, when a toddler falls and hurts her knee, her first behavior is to look to the supervising adult for a reaction. If the adult says, “Oh, it’s okay. Come here. You’re fine,” the child might seem a little confused because of the disconnect between the pain she feels and the adult’s reaction, but she will, ultimately, not let the pain bother her too much and continue with her play. If, on the other hand, the adult appears to panic and runs toward the child asking if she is okay, that same child will now panic and cry and play will not likely resume.

Coincident with ongoing behavioral studies through the decades, researchers have maintained interest in developing technologies to directly study activity of the presumed neurophysiologic mechanisms that control behavior and regulation in infants. As technology has advanced, techniques for monitoring, measuring, and imaging dynamic changes in human neurophysiologic processes have become more refined. As a result, some of the early questions of which neural structures are important to individual variation and development of behavior are now beginning to be answered. In temperament research many significant studies have been designed to correlate hormonal and physiological activity to classic observed behavioral and questionnaire data evaluating individual differences. Biological systems of interest have included hypothalamus-pituitary-adrenal (HPA) axis “stress” hormones, including salivary cortisol (Gunnar, Porter, Wolf, Rigatuso, & Larson, 1995), and EEG recordings of frontal cortical activity (Calkins, Fox, & Marshall, 1996; Wolfe & Bell, 2007). Gunnar et al (1995) studied neurobiological indices of stress responses in infants before, during, and after a painful heel stick procedure. Increased salivary cortisol as well as increased heart rate, increased crying, and decreased vagal tone in response to the heel stick were all associated with lower scores on the “Distress to Limitations” measure of temperament.
measured by the Infant Behavior Questionnaire (IBQ). More recently, Wolfe and Bell (2007) associated frontal lobe EEG activity with individual differences in cognitive processing during infancy and early childhood, “especially during cognitive processing that involves the skills of working memory and inhibitory control,” or regulation. They hypothesized that temperament factors (as measured by the IBQ) related to regulation in infancy would be related to EEG assessment of inhibitory control in early childhood. They found that infant performance during an inhibitory control task was predicted from concurrent frontal EEG and from maternal report of temperament.

In studying neural mechanisms of self-regulation, several laboratories have offered innovative, evidence-based models to characterize brain structures and networks likely to contribute to regulatory processes. Many of them concentrate on structures that link cognitive processes and emotion, reflecting the increasingly popular view that cognitive suppression of emotion is central, or even equivalent to self-regulation (Bell & Wolfe, 2004). Also newer emerging questions of biological control mechanisms of regulation and their development can be explored. Posner et al (2007) have argued that the anterior cingulate gyrus plays an important functional role. They define self-regulation as a “natural function of brain networks, designed to control the influx of information from the environment through orienting, in order to avoid conflicting responses in behavior.” Based on EEG recordings in infants and fMRI data from adults, the authors propose that activity in the midfrontal cortex, particularly the anterior cingulate area, aids self-regulation by “processing . . . information from other networks, serving as a part of an executive attention network involved in the control of both cognition and emotion” (Posner, Rothbart, Sheese, & Tang, 2007). Beauregard et al (2001) used fMRI in adult males to study self-regulation and concluded that the right anterior cingulate gyrus was important during cognitive suppression of emotional responses in adults (Beauregard, Lévesque, & Bourgouin, 2001). Geva and Feldman (2008) created a developmental model to study early risk, which included consideration of brainstem physiology,
limbic system function, and socio-emotional-cognitive factors. They emphasize the importance of “assessing dysfunctions related to . . . brainstem . . , already at the NICU” and propose that “disruptions to the development of these lower level systems can serve as important indicators of risk for the emergence of self-regulatory capacities.” (Geva & Feldman, 2008). There are also several theories detailing the role of the autonomic nervous system in regulating states of vigilance, controlled by catecholaminergic systems, and states of calm, social interaction, controlled by increased activity in some (not all) parasympathetic pathways (Boulenger & Uhde, 1982; Porges, 1995; Redmond & Huang, 1979; Taylor, 1994). These theories are discussed in more detail throughout the experiments described in Chapter 4.

Overall, much of the research regarding biological mechanisms controlling self-regulation in human infants has focused on theoretical modeling based on findings in animal studies or adults. Direct neurophysiologic measurement can be difficult to obtain and interpret depending on the neurologic system of interest. Measuring changes in the autonomic nervous system through ECG recording offers an attractive solution to the problem of direct monitoring in awake, behaving human infants. This technique is used to study infant regulation during social challenge in the next chapter of this discussion.
References


4. NEUROPHYSIOLOGIC REGULATION IN INFANTS DURING SOCIAL CHALLENGE—
AN INTEGRATED APPROACH IN THE “MOTHER-CHILD PLAY PROJECT”

4.1 The Integrated Approach

The ability to appropriately self-regulate emotions and behavior is paramount to successful psychosocial functioning in humans. Dysfunctional self-regulation in children has been linked to poor impulse control, reduced academic achievement, slower mental processing, compromised social competence, and increased parental stress—all of which have been identified in the literature as early contributors to psychiatric disease in adolescents and young adults. Self-regulatory and social engagement skills develop early in life, and several laboratories have conducted extensive behavioral research in infants to explore the developmental process. To fully appreciate the complexities of development of regulation in human infants, however, it is necessary to consider, at least, the three major areas of research, which have contributed most to our current understanding of regulation in early life—development, individual differences, and self-regulation. The Mother-Child (MC) Play Project was designed to treat these three areas as well-integrated and interdependent within a single experimental design.

Examining each of these concepts separately would certainly be simpler, but interpreting the results would necessarily lead to incomplete and even mis-understanding. Individual differences in baseline physiology or temperament, for example, could influence patterns of reactivity, which are modulated by regulatory strategies that are, in part, determined by developmental stage and ability of the nervous system to react to changes in environment. Each of these relationships, in turn, could also be moderated by social context. In order to test whether these relationships, in fact, exist, it is important to incorporate elements of each in the experimental design. It is, of course, important to achieve a certain level of parsimony in research. However, it is also important, especially from a translational perspective, to create a meaningful, relevant picture of how a
particular process functions under both physiologic and pathological conditions. In the case of emotional and behavioral regulation in infants, accurate understanding cannot be achieved without considering changes that occur with age, as the nervous system develops; differences, which exist between infants of the same age group; and the effects of social context on infant regulation, i.e. the relationship between the infant and the adult who is available to help the infant obtain his or her needs. An objective, reliable method of quantifying the inherently elusive processes of regulation is also necessary. The approach behind the MC Play Project, then, has been to integrate certain existing perspectives in behavioral and developmental literature with each other and with neuroscience and psychophysiology principles and methods. The goal has been to begin to describe normal variations in development and their effects on physiologic regulation during social interaction in order to begin to build a frame of reference, or organizing principle, within which infant regulation can continue to be studied from integrated perspective.

In this project, measurement of parasympathetic nervous system activity provided an accessible, reproducible means of gauging how autonomic regulation of heart rhythms changed in response to particular changes in environment. Studying the ANS as a window to understanding how the nervous system facilitates self-regulation has several advantages, particularly when working with human infants. First, when contrasted with hormone and electroencephalogram (EEG) or evoked response potential (ERP) studies, data collection and quantification of electrocardiogram (ECG) data is relatively straightforward and non-invasive (i.e., small sticker electrodes attached to the chest). When collecting physiologic data in awake, behaving infants during behavioral tasks, it is important that the equipment be unobtrusive and that the data obtained be stable and reliable. Second, there is sound theoretical framework linking autonomic physiology and social engagement behaviors, which serves as an organizing principle to guide interpretation of results. Infant autonomic physiology has been proposed by many to be an especially sensitive index of temperament, which is also rooted in biology (Snidman, Kagan,
Riordan, & Shannon, 1995). Third, the reliability and stability of measurement of vagal activity in infancy have been rigorously studied. Fracasso et al (1994) demonstrated that individual differences in autonomic measures in infancy were reliable and stable across stressful or demanding conditions and over time (Fracasso, Porges, Lamb, & Rosenberg, 1994). This last point is particularly important and specific to the methods of vagus activity quantification used in the MC Play Project.

As detailed below, this project only studied typically developing infants, because it is always important to understand what is normal before it is possible to appropriately characterize and distinguish what is not. Dysfunctional states in infants who have been diagnosed with regulatory disorders or infants with persistent crying behaviors have been studied, mostly outside of the social context, and compared with infants who do not have regulatory disorders. However, few studies have systematically examined normal age-related changes in regulation, and there is no normative dataset addressing the neurophysiologic, specifically autonomic, mechanisms that underlie and ultimately enable self-regulation and social engagement. By gaining a better understanding of the underlying autonomic mechanisms regulating behavioral state and social engagement during the first year of life, it may become possible to identify early indicators of potential dysfunction and to develop more discrete targets for early intervention.

Last, since the primary caretaker, often the mother, plays an integral role in defining the physical, social, and, presumably, physiologic environment of the developing infant, this study was also designed to examine maternal neurophysiology and emotional state and the likely reciprocal relations between these variables in mother and child. Self-regulation in the first year of life is largely achieved by proxy (see Chapter 3), therefore the social context in which changes in emotions and behavior occur is important to consider when studying regulation in infants.
4.2 Theoretical Background

The hypotheses in this study with regard to autonomic measurements were largely informed by the Polyvagal Theory, proposed by Porges (Porges, 1995), which describes the evolution of hierarchical organization of the autonomic nervous system and its role in supporting social behaviors. According to the Polyvagal Theory, when the nervous system detects a safe, supportive environment, such as that created in the caring, nurturing relationship of a successful mother-infant dyad, there is a change in physiologic state mediated through the myelinated vagus nerve (Xth cranial nerve) that facilitates both physiologic homeostasis and behaviors of social engagement. The source nucleus of the myelinated vagus, the nucleus ambiguus, interacts in the brainstem with other cranial nerve nuclei that control the muscles of facial expression (allowing eye contact and smiling); muscles of the middle ear that allow preferential detection of the higher frequencies of human voice; and other muscles of the face, head, and neck, which allow vocalization and appropriate vocal prosody (cooing and mother-speak). The myelinated vagus, an integral part of the proposed “social engagement system,” is the most highly evolved component of the autonomic nervous system and is dynamically responsive to both internal and external environmental cues. Myelinated vagal tone to the heart and lungs can be decreased to achieve increases in heart rate to adapt to increased metabolic demand, such as during stressful or attention tasks. It can then be rapidly restored during recovery to re-engage positive social behaviors, as in during social play. Activity in this nerve and its upstream interactions in the brainstem, subcortical regions, and cortex serve as biological substrates that specifically support social engagement or defense and withdrawal, and within a given physiologic state, certain behavioral responses to environmental stimuli naturally emerge.

In a healthy nervous system, during environmental challenge in which expected social reciprocity is violated, as with the Face-to-Face Still Face (FFSF) procedure used in this study, or in which there is some other potential threat, vagal tone is characteristically withdrawn to support
gaze aversion; sensitivity to lower frequency, environmental sounds; and mobilization behaviors, often referred to as the fight-flight response. In a dysregulated system, the vagal response to environment is less dynamic. Several laboratories report that children with behavioral regulation problems have lower baseline RSA and dampened RSA responses during testing sessions (Blair & Peters, 2003; Calkins, 1997). Suppression of RSA in 24-month-olds is specifically associated with increasing distress across negative emotion tasks (Buss, Goldsmith, & Davidson, 2005). In 1996, Porges and others demonstrated that infants with difficulties in decreasing vagal tone appropriately during a social/attention task at 9 months of age had significantly more behavioral problems at 3 years of age, when compared to children with more dynamic vagal regulation (Porges, Doussard-Roosevelt, Portales, & Greenspan, 1996).

The ability of the nervous system to socially engage and disengage appropriately in response to environmental cues is important to the development of emotional and behavioral self-regulatory skills in an infant and is dependent, at least in part, on structural maturity of the myelinated vagal system. In humans, in a normally developing fetus, the number of myelinated vagal fibers from the nucleus ambiguus to the heart increases linearly from 24 to 28 weeks gestation until full-term parturition, when this number is comparable to those observed in adolescents (Sachis, Armstrong, Becker, & Bryan, 1982). Interestingly, there is also evidence of postnatal maturation of the vagal system in humans. Axonal dimensions (i.e. axon diameter) continue a slow increase during the first year of life, and active myelination of the nerve occurs at a fast rate during the first nine months. There is also a slow transition from unmyelinated to myelinated fibers during the first year, particularly in the first three months post-partum (Pereyra, Zhang, Schmidt, & Becker, 1992). Behaviorally, as infants age from three to six months, expressions of positive emotion and responsiveness during mother-infant interactions begin to increase (Cohn & Tronick, 1987; Kaye & Fogel, 1980). Presumably, observed increases in positive social engagement are due to increases in
self-regulatory capacity of infants, most likely supported by increasing structural development of the myelinated vagal system.

Critical development of self-regulatory strategies occurs primarily in the first years of life, and much of this development, especially in the first year, occurs within the context of the parent-child, often the mother-child, relationship. Maternal depression, for example, has been shown to adversely affect child development and ability to self-regulate emotions (Field, 1984; Field, Diego, & Hernandez-Reif, 2009; Pelaez-Nogueras, Field, Hossain, & Pickens, 1996). Several laboratories have explored behavior in mother-child interactions and its impact on developmental outcome. While social behaviors between mother and child have been observed, described, and qualified in numerous studies, few investigators have examined the important underlying neural mechanisms that specifically enable and maintain these behaviors by mediating physiologic and behavioral state. Even fewer studies have measured autonomic reactivity in both the mother and child to understand the neurophysiologic and biobehavioral aspects of the bi-directional interaction between the two.

In this project, in addition to infant physiology, maternal autonomic reactivity was also a important. In a 1956 conference paper on parent development, David Friedman, a pediatrician, defined the role of the pediatrician: “Pediatricians have three primary tasks: To treat children when they are ill, to keep children physically and mentally healthy, and to promote healthy parent-child relationships.” He added that “[t]he pediatrician who is accepting, sensitive and a good listener and who keeps in mind that parents as well as children have capacities for growth and development will be a potent factor in promoting good parent-child relationships and many times more effect in dealing with the child in health and disease.” In the MC Play Project, both maternal autonomic reactivity and subjective anxiety while mothers watched their infants undergo social challenge with a researcher (stranger) were evaluated. Higher RSA in adults has been shown to predict greater self-reported regulatory control and decreased negative emotional arousal in
response to stressors (Fabes & Eisenberg, 1997). More recently, also in adults, greater RSA suppression to sad video clips predicted recovery from depression (Rottenberg, Salomon, Gross, & Gotlib, 2005). Again, however, few studies have examined the relationship between physiologic characteristics in mothers and the impact on parenting behavior, child behavior, or child outcome.

It is important to note the bi-directional nature of the mother-child relationship. As early as 1928, Gesell often described the maturational processes of early human life within the context of the mother-infant relationship. When describing methods for experiments to study maturational changes in infant behavior, he not only described ways to test the infants, but also ways the experimenter could gain the trust of their mothers to help develop a good experimenter-infant relationship (Gesell, 1928). RSA baselines and reactivity in children have been shown to moderate parenting styles, and presumably affect maternal physiology. Children, from ages two to four years, with higher RSA have been reported to be able to moderate maternal restrictive-parenting practices, while children with lower RSA were more likely to have problems as the consequence of restrictive-parenting practices (Kennedy, Rubin, Hastings, & Maisel, 2004). Similarly, high RSA has been shown to contribute to child resiliency and might buffer the effects of maternal hostility on a child (Katz & Gottman, 1995). Unlike the current study, however, these investigations did not monitor maternal physiology, either during baseline or during the mother-child interactions.

Dysfunctional self-regulation in children when responding to environmental challenges has been linked to compromised social competence, parental stress, academic achievement, and mental processing (Doussard-Roosevelt, McClenny, & Porges, 2001). It is, therefore, important to investigate early indicators of self-regulation that may identify potential difficulties and that can become targets for early intervention. This study is unique in that it facilitates study of dynamic regulation of vagal tone in children and their mothers employing an approach, which incorporates development, individual differences, and social context—specifically the context of the mother-child relationship. Information about the impact of a parent’s or caregiver’s behavior on an infant’s
physiology may develop into future assessment tools for both parents and children and
development of aids designed to improve parenting behavior when needed. The MC Play Project
was developed in hopes to contribute to this overall effort.

4.3 **Aims and Hypotheses**

4.3.1 **Aim 1—Development**

The first aim of the MC Play Project was to study age-related trends in autonomic reactivity
in infants. As the autonomic nervous system matures, the amplitude of respiratory sinus
arrhythmia (RSA) increases and heart period (HP, the time between successive heart beats)
becomes longer. Developmental shifts in each of these physiologic variables are expected not only
as the child grows, but also as the vagal efferent fibers originating in the nucleus ambiguus continue
to myelinate. To date, however, very little is known about changes in autonomic *reactivity* to and
*recovery* from social challenge that may occur with development, as the infant improves skills in
self-regulation. A cross-sectional design (ages 3 – 12 months) will allow detailed examination of
age-related shifts in autonomic baselines, reactivity, and recovery.

The hypothesis behind this aim was that secondary to development of the myelinated vagal
system with age, older infants would have increased baseline RSA and better dynamic control of
RSA recovery following challenge, which should improve. Many significant neuronal changes occur
in the myelinated vagal system postpartum during the first year of life. Axon diameters increase,
active myelination continues at fast rate, and there is slow transition from unmyelinated to
myelinated fibers (Pereyra, Zhang, Schmidt, & Becker, 1992). As these structural changes occur,
functional changes in reactivity and recovery in response to social challenge should also emerge.
Due to increased myelination with age, recovery following challenge should be more robust in older
infants when compared to younger infants.
4.3.2 **Aim 2—Individual Differences**

The second aim was to evaluate individual differences in age-related shifts in the dynamic measures of physiologic activity (i.e., RSA and Heart Period). Individual differences in an infant’s ability to self-regulate are not only age-related. There is extensive literature documenting individual differences in temperament, attachment styles, and positive and negative reactivity to environmental stimuli in age-matched infants during the first year of life. The autonomic nervous system is integrally related to these behaviors, and, as a result, individual differences in behavior regulation strategies should be reflected in differences in autonomic reactivity.

The hypothesis here was that individual differences in infant physiology at baseline would be systematically related to patterns of physiologic reactivity and recovery from social challenge. As with younger infants when compared to older infants, within each age group, infants with lower baseline RSA (i.e., less well developed NA vagal systems) should display dampened reactivity to social challenge when compared to infants with higher baseline RSA (i.e., more developed NA vagal systems).

4.3.3 **Aim 3—Social Context**

The third central aim of this project was to compare infant reactivity to a mother-administered and researcher-administered social challenge. The Face-to-Face Still Face (FFSF) Challenge is typically administered by the infant’s mother. Increasing numbers of studies, however, are using a researcher to administer the social challenge. Separate administrations of the social challenge to the infant with the mother and a researcher (female stranger) enabled the evaluation of potential differential reactivity within and across ages.

The hypothesis was that since expected social reciprocity is well-established in a mother-infant dyad (especially with older infants), infant reactivity to a mother-administered FFSF procedure might be more pronounced than reactivity to a researcher-administered FFSF, where such
expectation might not exist. Differential behavioral responses to mother and stranger in infancy have been studied for decades. Bowlby's Attachment Theory (1969) examined further by Ainsworth’s Strange Situation experiment (Ainsworth & Bell, 1970) both highlight the unique relationship that develops between an infant and his or her mother in the first years of life. Attachment Theory emphasizes mother-infant relationships starting at six months, however, an infant’s ability to distinguish between mother and stranger has been demonstrated much earlier. In a longitudinal study of infants from three to nine months old, Roe (1978) observed that individual differences in vocalizations and motor activity responses to mothers versus strangers were related to cognitive development. More recently, in another longitudinal study beginning at four months, Lin and Green (2009) demonstrated differences in vocalization and smiling responses to three-minute social interactions between a mother and female experimenter. In the MC Play Project, potential differences in autonomic reactivity and recovery responses to the FFSF administered both by the mother and by a female researcher were examined in a cross-sectional design.

4.3.4 Additional Aims

Three other aims, in addition to the three main investigations were also considered during design of the MC Play Project and will be considered for future study.

4.3.4.1 Aim 4—Infant Physiology and Temperament

One was to evaluate the relationship between infant autonomic reactivity and behavioral self-regulation. Several standardized psychometric instruments and behavioral assessments are routinely used in clinical and laboratory settings to evaluate infant behavior and predict developmental outcome with regard to emotional, cognitive, and behavioral self-regulation. This study employed several of these traditional measures, and scores can be compared with neurophysiologic responses to social challenge in the laboratory setting. Social challenge is already
known to induce predictable behavioral responses in infants, which have been used in combination with questionnaire instruments to assess risk for compromised developmental outcome. Since the social challenge was expected to produce corresponding changes in infant physiology, the magnitude and direction of the physiologic changes that occur in response to social challenge should be related to behavioral and psychological indices of self-regulation.

Relations between individual physiologic differences and well-established measures of temperament are important to elucidate in order to more fully understand the dynamic functioning of neurophysiologic mechanisms that support and facilitate social behaviors and self-regulation early in life. Investment in this kind of study is crucial when developing strategies to identify early autonomic patterns that could be indicative of future self-regulation and behavior problems (Bazhenova, Plonskaia, & Porges, 2001; Greenspan, 1992; Porges et al., 1996).

Interest in individual differences and temperament is certainly not new. Numerous investigations in child psychology and development have centered on temperament because it is considered to be a constitutional, stable characteristic that can not only be measured in infancy, but can also be used to predict a child’s subsequent social functioning. Over the years, definitions and measurement of temperament in infancy have been revised and refined. Thomas et al (1968) used nine different characteristics to described three main categories of children: the child who is “slow to warm up”, the child with an “easy” temperament, and the child with a “difficult” temperament (Thomas, Chess, & Birch, 1968). Each of these was predicted to interact with certain features of environments and lead to different, more or less adaptive styles of social engagement throughout development (Chess, Thomas, Rutter, & Birch, 1963). Since Thomas and Chess, other theories and measures of temperament have been developed. Although differing on particular characteristics that are included (or not) in the operational definition of the temperament construct, all major perspectives of temperament agree that temperament remains relatively stable over time, serves as an important substrate for subsequent development of personality and social skills, and is
biologically driven (Goldsmith et al., 1987; Zentner & Bates, 2008).

The MC Play Project used the Infant Behavioral Questionnaire—Revised (Gartstein & Rothbart, 2003), which was designed to assess both continuity and change in patterns of reactivity and self-regulation in infants during the first year of life, to assess temperament. The IBQ-R is widely used in both clinical and research settings and identifies fourteen scales representing characteristics essential to “Surgency/Extraversion,” “Negative Affectivity,” and “Effortful Control” in infants. The IBQ-R has demonstrated high internal and inter-rater reliability; however, as a parent-report measure, it is subject to scrutiny regarding accuracy and validity of the information gathered and the relationship between parent perception and actual child behavior. Several studies have suggested that the consistencies in parent-report measures of temperament might not reflect stability of temperament as much as they do stability of parental perception of infant behavior (Bates, 1989; Huffman et al., 1998; Stifter & Jain, 1996; Tirosch, Harel, Abadi, Berger, & Cohen, 1992). Stifter and Jain (1996) noted a “lack of concurrent relations between maternal ratings and observed behavior” in their study of psychophysiology and temperament. DeGangi et al (1993) reported that higher parent ratings of “difficultness” were either positively or negatively correlated with developmental outcome depending on whether or not the child met criteria for regulatory disorder (Degangi, Porges, Sickel, & Greenspan, 1993). Since this study, like others, concluded that infants with untreated regulatory disorders might not outgrow their behavioral difficulties, it is important to find other, perhaps complimentary, ways to measure characteristics that might predict potential developmental compromise.

Previous research has demonstrated that neonatal vagal tone is related to motor and mental development during the first year of life (Fox & Porges, 1985). Increased vagal reactivity to cognitive challenges has also been related to better mental development in healthy infants (Degangi, Dipietro, Greenspan, & Porges, 1991). Consistent with the findings in this study, infants who decreased cardiac vagal tone most during laboratory attention tasks to assess temperament
have been rated as being more easily soothed in maternal reports of temperament (Huffman et al., 1998). In studies of infants with regulatory disorders, baseline vagal tone has been found to be less highly and inconsistently correlated with vagal reactivity to challenge (Degangi et al., 1991). Regulatory disordered infants can even increase RSA during environmental challenges unlike their healthy counterparts (Dale, O’Hara, Keen, & Porges, 2010). While autonomic measures have great potential to aid detection of regulatory difficulties early in life, clear relations between autonomic regulation and traditional measures of behavioral regulation have still not been found. Findings from the MC Play Project should contribute to developing more comprehensive understanding in this area of research.

4.3.4.2 Aim 5—Maternal Physiology and Anxiety

A second additional aim involves one of the most innovative aspects of the MC Play Project design, which was to evaluate the relationship between maternal autonomic reactivity and subjective anxiety while the mothers watched their infants being tested. Measurement of autonomic state in mothers is seldom done within the context of testing infants, and this study allows examination of the “stress” and sensitivity of the mother while monitoring her child. To date, no other project of this scale has recorded autonomic reactivity and recovery in both a mother and her child during social interaction and challenge. And no other study has measured maternal autonomic reactivity while the mother observes her child undergoing social challenge remotely or, by extension, been able to compare a mother’s response when administering a social challenge to her response when watching a stranger administer the challenge. In the MC Play Project protocol, the mother continuously rated her level of subjective anxiety using an “anxiety dial” that could be turned from 1 (not anxious at all) to 5 (extremely anxious) while she watched a researcher administer the social challenge to her child. This continuous rating was time-synched with her physiologic recordings, so the two can be compared. This expressed anxiety can also be compared
to questionnaire instruments designed to assess parental stress and maternal depression and anxiety symptoms.

It was expected that mothers with greater expressed anxiety while watching their children undergo social challenge with the researcher would show greater ANS reactivity (i.e., greater decreases in RSA amplitude) and less recovery immediately following the challenge. For many mothers, separation from their infants while watching them undergo social challenge with a stranger will cause increased concern and anxiety when compared to baseline and even to administering an experimental social challenge themselves. This increased concern for the child during the researcher-administered challenge should also be supported by the underlying neurophysiology, and these changes could be reflected in our measurements when monitoring physiologic activity. Individual differences in these maternal responses can also be studied in relation to their responses to questionnaires assessing parental stress and potential psychiatric symptoms.

4.3.4.3 Aim 6—Reciprocal Autonomic Regulation

The final aim of the MC Play Project was to examine the reciprocal relationship between autonomic reactivity in both mother and child. Mirroring behaviors that define healthy and efficient social interactions may be paralleled by reciprocal modulation of autonomic state, and, thus, can be monitored by examining changes in vagal activity during parent-child interactions. The hypothesis was that the violation of expected social reciprocity between mother and child would reveal individual differences in the patterns of mother-child neurophysiology that underlie adaptive and maladaptive regulatory behaviors. The Face-to-Face Still Face (FFSF) paradigm used in this study artificially disrupted playful social interaction between the mother and child. Concurrent monitoring of autonomic activity in both mother and child provided a unique
opportunity to examine a reciprocal, physiologic relationship that may exist between the dyad partners during social interaction and challenge.

4.4  Design and Methods

4.4.1  Participants and Eligibility

The MC Play Project employed a cross-sectional approach to study infants aged 3 to 12 months, in order to allow close examination of distributional characteristics across the entire 10-month range during normal development. To ensure inclusion of typically-developing children and healthy mothers in this study, certain criteria for eligibility were met before mother-child pairs were invited to participate. All children were between the ages of 3 and 12 months of age at the time of the research session. Children born prematurely or with respiratory distress, genetic, neurological, or cardiovascular abnormalities, or who had ever needed surgery or ophthalmologic procedures were excluded. Children taking medications, including antihistamines or any CNS stimulants or depressants at the time of the research were excluded. Also children with continuing medical conditions, including asthma, cardiovascular disease, or recurrent ear infections were excluded. Children were not fed within an hour of participating in either social challenge, due to possible interference with subdiaphragmatic vagus activity influencing heart rate changes of interest. Mothers were healthy and without respiratory, neurological, endocrine, or cardiovascular disease. They were not taking any illegal drugs or medications, including antihistamines or any CNS stimulants (such as ephedra or caffeine-containing products) or depressants (such as alcohol) at the time of research. Mothers were also asked not to consume any nicotine or caffeine-containing substances for at least 4 hours prior to the research session. Mother-child pairs who underwent significant pre- or perinatal complications, such as pre-eclampsia or gestational diabetes were also excluded. Pregnant mothers did not participate. Eligibility was determined via phone interview, and in addition to a “Demographic Information Form”, which included questions
about the primary and secondary caregivers of the child and the relations and ages of other people living in the child’s home, mothers were asked to complete a “BBC Checklist” and “Health Questionnaire” at the time of the research session in the laboratory to confirm that eligibility criteria were met prior to participation. Information about breast vs. bottle feeding and birth order was also collected for potential future analysis.

At the University of Illinois at Chicago, the location in the city allowed inclusion of research participants from a variety of communities. The MC Play Project was able to include significant numbers of families from traditionally medically underserved communities and affluent suburban areas, each including several different ethnic groups to participate.

### 4.4.2 Questionnaire Instruments

The research protocol was designed to address the aims of this study. To explore the relationship between autonomic reactivity and traditional psychometric measures of behavior (Aims 4 and 5), the mothers are asked to complete several questionnaires. Once eligibility was determined over the phone, questionnaires were mailed to the mother for her to complete in the few days prior to the laboratory session.

**Infant Behavioral Questionnaire-Revised (IBQ-R)** (Gartstein & Rothbart, 2003)

The IBQ-R instrument is a widely used, rationally derived, parent-report assessment of infant temperament that allows “investigation of relations among dimensions of temperament that may be obscured in the study of more global constructs” (Derryberry & Rothbart, 1988; Gartstein & Rothbart, 2003). The IBQ-R has fourteen scales that evaluate temperament characteristics manifested in infancy: Activity Level, Smiling and Laughter, Fear, Anger, Duration of Orienting, Soothability, Vocal Reactivity, High and Low Intensity Pleasure, Falling Reactivity/Arousal Regulation, Cuddliness/Affiliation, Perceptual Sensitivity, Sadness, and Approach. A three-factor
solution extracts the following dimensions: Positive Affectivity/Surgency (PAS), Negative Emotionality (NE), and Orienting/Regulatory Capacity (ORC).

Parenting Stress Index (PSI) (Abidin, 1995).
The PSI is a 120-item self-report instrument designed to measure the relative degree of stress in a parent-child system and to identify the sources of distress. Three major sources of stress—characteristics of the child, characteristics of the parent, and situational-demographic life stress—are assessed by the instrument.

Brief Symptom Inventory (BSI) (Derogatis, 1992).
The BSI measures nine psychiatric symptom patterns (somatic complaints, obsessive-compulsive behavior, interpersonal sensitivity, depression, anxiety, phobic anxiety, paranoid ideation, hostility, and psychoticism) that are clinically relevant (Derogatis & Clearly, 1977).

4.4.3 Autonomic Measurements
To assess physiologic activity, either the LifeShirt® System by Vivometrics™ or the ActiHeart® by MetriSense™ were used for each infant. The LifeShirt® garment allows continuous, wireless, ambulatory collection of electrocardiogram (ECG), thoracic and abdominal respiration, and gross motor activity data. For infants who were either too small or large to fit comfortably in the LifeShirt® garment, the smaller ActiHeart® device was used to collect ECG data and avoid any interference with the social challenge.

Heart rate data collected by the LifeShirt® System were imported to computers using VivoLogic® software (Vivometrics; Ventura, CA); data collected from the ActiHeart® were recorded in text format on computers using MetriSense™ software. Artifacts in R-R interval data were edited independently by two researchers who successfully completed strict reliability training.
using CardioEdit software developed at the Brain-Body Center. Each file was edited with ≥ .95
agreement between raters. For each ECG data file, the mean inter-beat interval, mean heart rate,
and natural log variance of amplitude of respiratory sinus arrhythmia (RSA) were calculated using
CardioBatch software (Brain-Body Center, University of Illinois at Chicago).

Quantification of RSA amplitude as an index of vagal tone creates a statistical challenge, because
the inter-beat interval data used to calculate RSA are not statistically stationary. RSA is a naturally
occurring rhythm within the heart rate pattern that occurs at about the frequency of spontaneous
breathing. Regulation of RSA via the myelinated vagus nerve is dynamic and sensitive to changes in
both internal and external environments. The sensitivity and dynamic response pattern of vagal
activity enable an individual to self-regulate and to interact appropriately with his or her
environment throughout the day. This dynamic, physiologic pattern is, of course, not stationary.

The CardioBatch software executes procedures that quantify the amplitude of RSA using
parameters that are sensitive to the frequency of spontaneous breathing (Porges, 1985). Sequential
inter-beat intervals are first resampled into 500 ms intervals to produce an equal interval time-
series data that is then detrended by a moving 21-point cubic polynomial (Porges & Bohrer, 1990).
This innovative moving polynomial function was designed to remove the non-stationary trends and
slow periodic processes in inter-beat interval data obtained via ECG, while having negligible impact
on the amplitude of RSA regardless of stationarity or time invariance. Next, a bandpass filter is
applied to the detrended time series to extract the variance in the inter-beat interval pattern
associated with the frequencies of spontaneous breathing (.3 to 1.3 Hz) for infants in this study.
For these infants, distributions of mean respiration frequency were determined to verify that
respiration frequencies of participants fall within these typical, preset parameters. Last, the natural
logarithm of the variance of the bandpassed time series is calculated and reported as the measure
of the amplitude of RSA (Riniolo & Porges, 1997).
These procedures are statistically equivalent to frequency domain methods (i.e., spectral analyses) that sum the spectral densities in the frequency band associated with spontaneous breathing in order to calculate the amplitude of RSA. However, spectral analyses assume that inter-beat intervals are stationary, and thus are less appropriate when applied to understanding dynamic, physiologic processes (Lewis, Furman, McCool, & Porges 2012; Denver, Reed, & Porges, 2007; Porges & Byrne, 1992). Also, the final natural log transformation performed by the CardioBatch program creates a normal distribution of values that can be appropriately used in parametric statistical analyses required to answer study questions (Lewis et al., 2012).

4.4.4 Social Challenge

In order to evaluate autonomic regulation during social challenge, the Face-to-Face Still Face (FFSF) paradigm was used. The Face-to-Face Still Face experiment was developed by Tronick et al in 1978 to study social reciprocity between mothers and infants. The original experiment consisted of two three-minute periods of interaction between infant and mother separated by 30 seconds of separation. During one three-minute period, the mother was instructed to play normally with her child. During the other period, she was instructed to look at the child with a neutral face and not interact in any way. The order of the periods was randomized to assess order effects. Overall, as expected, infants oriented toward the mother less and interacted less with the mothers during the still face period than during the normal interaction. During the still face, infants reacted with “intense wariness and eventual withdrawal” behaviors such as turning away from the mother and slumping down in their chairs (Tronick, Als, Adamson, Wise, & Brazelton, 1978). The behaviors originally described by Tronick et al are reminiscent of the patterns of behavior seen in learned helplessness models of depression in animals over long periods of time. In the case of the human infant, however, behavioral withdrawal occurs in just a few minutes. The original experiment included infants between ages 2 and 20 weeks and studied age differences in response to the still
face condition. The authors concluded that “the intentions and emotions of the older infant [were] similar to those of a younger infant. The richness and skill in reestablishing a reciprocal interaction, however, [were] greater.”

Since this first publication of the Face-to-Face Still Face experiment, the still face condition has become well-established as a mechanism to elicit a broad range of emotional and behavioral responses in infants. Several laboratories have used it to investigate the effects of the still face condition on infants of different ages, or infants with various regulatory disorders or temperaments (J. F. Cohn & Tronick, 1983; Moore & Calkins, 2004; Shapiro, Fagen, Prigot, Carroll, & Shalan, 1998; Weinberg & Tronick, 1994, 1996). To date, however, no published experiments have used the experimental paradigm to examine the effects of age and individual differences and identity of social partner on infant behavior or physiology. The Mother-Child Play Project was designed, in part, to fill that gap.

The FFSF procedure used in the MC Play Project consisted of three phases: The first phase, “Social Play,” involves two minutes of face-to-face social play with the child. During the second phase, “Still Face,” the mother is instructed to maintain a straight face while looking at her child for two minutes; she is instructed not to respond to the child in any way during this phase. In the third phase, “Reunion Play,” social play with the child is resumed. This protocol allowed evaluation of several aspects of infant regulation, including the ability of the infant to interact with others during play, the ability of the infant to self-regulate or to exert effortful control in order to respond appropriately to a social stressor, and the ability of the infant to use others to soothe during recovery from challenge. Since the social interaction requires two partners, however, the results also reflect the degree to which the mother was able to interact appropriately with the infant during the first and final stages of the six-minute FFSF procedure.

Because of the importance of social context in infant regulation, in addition to the traditional mother-administered FFSF challenge, the MC Play Project also included a separate,
researcher-administered challenge. This second administration made it possible to ask whether the identity of the available adult would impact the infant’s ability to self-regulate physiologic state (i.e., autonomic activity). Walden and Kim (2005) studied social looking toward mothers and female strangers in a social referencing experiment with children ages 18 and 24 months. When confronted with ambiguous situations requiring additional information from an available adult, children of both ages looked more often and with less latency toward the stranger when compared to looks to the mother (Walden & Kim, 2005). The authors offer several explanations for this. One is that a “novelty effect” led infants to look longer at the strangers, not only to gather information about the ambiguous tasks, but also to gather information about the stranger herself. Another explanation was the “familiarity effect”, which suggested that it simply took longer for the children to gather information from the new person, because they were less familiar with her than with the mother. This study did not evaluate contingency of children’s behaviors based on the positive or negative cues they received from either the stranger or the mother depending on the social referencing task presented. Therefore, this study could not be used to evaluate the functional value of the information the children may or may not have gathered from the mothers and strangers. The study did, however, demonstrate, that children do, not surprisingly, respond differently to strangers and mothers in social referencing paradigms.

The question addressed in the MC Play Project was whether or not this difference affected infant physiologic regulation. Further, with regard to methodology, it was also important to compare infant regulation in response to mothers and strangers, specifically during the FFSF challenge. Several laboratories use the FFSF paradigm to test various aspects of behavioral and emotional regulation in children, usually in the context of the mother-infant dynamic. However, some laboratories use researchers to evaluate infant regulation during the FFSF experiment, presumably due to some of the advantages a researcher-administered FFSF offers. For example, consistency-of-stimulus can me more or less assured if the same individual (the researcher)
administers the challenge to each infant in a particular study. In some ways, using the mother to administer the challenge might also introduce confounds of varying styles of attachment in the mother-infant relationships of study participants. These variables would require separate testing and introduce additional analytical considerations, which might not be of interest to the laboratory conducting the study. Also, since the FFSF is very difficult to administer (the children often become extremely distressed during the Still Face phase), mothers often have difficulty following directions. This potential problem, however, is relatively minor. The FFSF has not been studied extensively using a researcher administration, and it should not be assumed that this alternative strategy is equivalent to the traditional mother administration. Identity of the person administering the challenge does likely influence an infant’s regulation. Not only is the infant separated from the mother (a well-studied challenge itself), but the infant is also confronted with and asked to interact with a stranger. Both of these additional challenges might influence infant regulation patterns independently of the challenges presented by the Still Face phase alone.

Last, even though several studies have used the FFSF technique to evaluate qualitative aspects of the relationship between children and their mothers, no other studies have specifically examined the maternal physiology during the experiment or compared infant and maternal responses during the mother and researcher-administered conditions. The MC Play Project design is the first to allow such comparisons and contrasts to be made.

4.4.5 Protocol

The research protocol employs the Face-to-Face Still Face (FFSF) paradigm in two different administrations. The order of these administrations is randomized within child age groups (3 to 6 months, 7 to 9 months, and 10 to 12 months). This ensures that any differences observed between the mother-administered and researcher-administered FFSF procedures were not due to order of administration. Between each FFSF administration, the mother and child were allowed five
minutes of free play in the testing room with each other to allow enough time for both to return to “baseline” physiologic state.

Baseline measures of infants are difficult to define and obtain, because behavior at this age is largely environment and state-dependent and not yet well-regulated. Therefore, quiet, free play between the child and mother was used as a “baseline” condition.

The research protocol proceeded as follows for mother and child:

<table>
<thead>
<tr>
<th>MOTHER</th>
<th>CHILD</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-minute quiet play with Child</td>
<td>3-minute quiet play with Mother</td>
</tr>
<tr>
<td>Anxiety Rating while watching 6-minute Still Face Procedure between Child and Researcher</td>
<td>6-minute Still Face Procedure with Researcher</td>
</tr>
<tr>
<td>OR</td>
<td>OR</td>
</tr>
<tr>
<td>6-minute Still Face Procedure with Child</td>
<td>6-minute Still Face Procedure with Mother</td>
</tr>
<tr>
<td>5-minute quiet play with Child</td>
<td>5-minute quiet play with Mother</td>
</tr>
<tr>
<td>6-minute Still Face Procedure with Child</td>
<td>6-minute Still Face Procedure with Mother</td>
</tr>
<tr>
<td>OR</td>
<td>OR</td>
</tr>
<tr>
<td>Anxiety Rating while watching 6-minute Still Face Procedure between Child and Researcher</td>
<td>6-minute Still Face Procedure with Researcher</td>
</tr>
<tr>
<td>3-minute quiet play with Child</td>
<td>3-minute quiet play with Mother</td>
</tr>
</tbody>
</table>

Table III. Mother-Child Play Project research protocol.
4.5 Results

4.5.1 Participants and Demographics

Overall, 150 mother-infant dyads with infants ages 3 – 12 months participated in the MC Play Project. Of these, data from 122 infants participating in the mother-administered FFSF were used to examine effects of age and individual differences in baseline RSA on physiologic regulation (see section 4.5.2). Missing data were due to infants who were unable to complete social play due to fussiness, mothers who did not comply with instructions during FFSF administration, equipment failure, or data recordings with too many artifacts to ensure accurate editing. Statistical outliers of measurements in any of the three phases of the FFSF procedure were also excluded from analysis. The data reported here include 122 infants (64 female, 58 male), including 34 from 3 to 5 months, 45 from 6 to 8 months, and 43 from 9 to 12 months. 36.1% were reported as white/Caucasian; 34.4% were black/African-American; 1.6% were Asian; 9% were reported as more than one race. 17% of the infants were reported as Latino; 18% did not report race or ethnicity.

Due to natural developmental stages, including changes in responses to separation from mother and to interactions with strangers, not all infants, especially in the 9 – 12 month age group, were able to complete the researcher administered FFSF. As a result, fewer infants were included in the investigations evaluating the effects of social context on vagal regulations, since these analyses only included infants, who were able to complete all phases of both the mother- and researcher-administered FFSF procedures. Thus, analyses including 105 infants participating in both the mother and researcher-administered FFSF were used. This group of infants consisted of 56 females and 49 males, including 32 from 3 to 5 months, 43 from 6 to 8 months, and 30 from 9 to 12 months. 35.2% were reported as white/Caucasian; 38.1% were black/African-American; 2.9% were Asian; 8.6% were reported as more than one race. 14.3% of the infants were reported as Latino; 17.1% did not report race or ethnicity. These demographics are not different from those
used in the analyses of the mother-administered FFSF only, therefore any differences reported between the two samples of children cannot be due to differences in demographics.

4.5.2 Infant Regulation during the Mother-Administered Challenge

Structural maturation of the myelinated vagus system is ongoing throughout the first year of life in humans (Pereyra et al., 1992; Sachis et al., 1982). This development as well as allometric changes in heart rate with increasing body size should lead to increased RSA and longer heart period (length of time between successive heart beats, HP) as infants age. Consistent with this expectation, our data demonstrate a significant positive correlation between infant age and RSA and heart period (length of time between successive heart beats, HP). However, scatter plots of these relations also reveal a range of individual differences at baseline within age groups (Figure 4.1).

![Infant RSA at Baseline](image1)

![Infant HP at Baseline](image2)

**Figure 4.1.** Scatter plots of HP and RSA in infants at baseline during quiet play with mother illustrate significantly increasing HP and RSA with age. However, there is a range of individual differences in each variable within age groups.
In response to the FFSF challenge, it was hypothesized that due to differences in structural development of the myelinated vagal system, patterns of vagal regulation would differ between age groups. Specifically, infants in the youngest age group were expected to display decreased dynamic regulation during the challenge. During the mother-administered FFSF, this expectation proved true. Infants in the youngest age group, in addition to having overall lower HP and RSA, demonstrated significantly decreased regulation during recovery following the Still Face phase of the FFSF procedure (see Figure 4.2.).

**Figure 4.2.** Line graphs of infant RSA and HP during three successive time points during the phases of the FFSF procedure illustrate progressive increases in both variables during all phases of the FFSF in successively older age groups. The patterns of regulation in all three age groups do not significantly differ in HP. However, RSA patterns of regulation do differ. Specifically, infants in the
youngest age group differ significantly in recovery from the Still Face phase when contrasted with the two older groups.

When examining changes in autonomic regulation during social challenge in this project, it was important to ensure that any changes in HP or RSA were due to the experimental conditions (social challenge) and not to changes in the infant’s activity, which typically increases with distress, such as that caused during the Still Face phase of the FFSF procedure. Activity patterns during the FFSF did not differ between age groups, and there were no statistical interactions between activity level and any other physiologic variable (see Figure 4.3.). Differences between recovery in the youngest infants and that of older infants were, therefore, not due to differences in activity.

**Figure 4.3.** Patterns of changes in infant activity did not differ between age groups during the FFSF challenge. The difference observed in RSA regulation in youngest infants, then, is not due to differences in activity.
In addition to separating the infants based on age group to study age-related changes in autonomic regulation during the FFSF challenge, infants were also separated into groups based on individual differences in baseline vagal activity (RSA). This was accomplished using a median split within each age group. Individual differences in baseline RSA were found to moderate infant vagal regulation in response to FFSF. This moderation, however, was only present in older infants (see Figure 4.4).

Figure 4.4. Line graphs illustrate that individual differences in high and low baseline RSA moderate infant RSA regulation during different phases of the FFSF procedure. However, this moderation only occurs in infants older than 6 months of age, emphasizing the importance of integrating development and individual differences when creating a model to understand infant autonomic regulation.
In contrast to effects on RSA regulation during challenge, individual differences in baseline RSA did not moderate regulation of HP during the FFSF challenge, regardless of age (see Figure 4.5).

**Figure 4.5.** Line graphs of individual differences in infant HP regulation during the FFSF procedure separated by age illustrate that, unlike with RSA regulation, individual differences in baseline vagal activity does not moderate changes in HP during the procedure, regardless of age group.

One interesting observation here is the apparent difference in the strength of the correlation between RSA and HP in the groups with higher and lower baseline RSA. At rest, parasympathetic activity exerts a dominant influence on heart period (greater vagal activity leads to longer heart periods). Therefore, in a healthy, well-adapted system, vagal withdrawal during stress or challenge causes decreased heart period (i.e., increased heart rate). Indeed, this is the
observation in our infants with higher baseline RSA. Interestingly, the infants with lower baseline RSA were able to achieve shorter heart period in the absence of vagal reactivity or withdrawal. This means that these infants were able to increase cardiac output to meet increased metabolic demands presumably induced by the social stressor without relying on dynamic decreases in vagus nerve activity. In fact, the relation between RSA and HP could be investigated as its own measure of development and autonomic integrity. One possible mechanism for the decoupling trend between RSA and HP in lower RSA infants could be engagement of the sympathetic nervous system and release of circulating catecholamines, which comprises, essentially, a less well-controlled signal. Like hormonal signals, circulating catecholamines released in concert with sympathetic activation have slower onset and much slower reabsorption or turning off mechanism. This leads to poor (prolonged) physiologic recovery and, theoretically, less successful self-regulation.

4.5.3. Infant Regulation during the Mother and Researcher-Administered Challenges

Since human infants are physically unable to obtain their own environmental needs, such as nutrients, shelter, and comfort, they must co-opt the aid of a caregiver in order to survive. The term “self-regulation by proxy” was introduced in Chapter 3 to emphasize the active, though not necessarily intentional, role the infant plays in securing caregiver cooperation to maintain physiologic homeostasis and, thus, to achieve successful emotional and behavioral regulation during both internal and external challenges. Social context, then, becomes the third dimension of the integrated approach to understanding self-regulation in infants. In order to address the impact of one aspect of social context—identity of the caregiver—on infant regulation, the Mother-Child Play Project design included two separate administrations of the FFSF challenge. One FFSF challenge was administered by the mother; a second challenge was administered by a female researcher. The order of these two administrations was randomized, and infants were allowed
adequate quiet time with the mother alone to recover both behaviorally and physiologically from the first challenge, before proceeding with the second.

To analyze infant regulation during the FFSF and compare infants in both the mother-administered and researcher-administered contexts, it was important to only include infants who were able to participate in both FFSF administrations. This necessarily excluded infants (mostly in the 9 – 12 month age group), who were unable to complete the researcher-administered FFSF due to stranger or separation responses that were severe enough to preclude play with the female stranger. The results comparing mother and researcher, then, are necessarily dependent on a subset (albeit large) of infants who were amenable to both separation from mother and play with a stranger.

Before conducting analyses to compare and contrast autonomic regulation during the two FFSFs, a number of control analyses were conducted to ensure that (1) the correlation of increasing RSA amplitude and HP with increasing age remained significant in this new group of infants, (2) there was no order effect on infant responses during mother and researcher FFSFs, (3) there was a significant correlation between infants’ RSA amplitude during the first Social Play phase of each FFSF, and (4) there was no significant difference between infants’ RSA amplitude during the first Social Play phase of each FFSF. Indeed, as expected, RSA amplitude and HP both increased significantly with increasing age in these 105 infants (see Figure 4.6.).
Revised measures ANOVA demonstrated no significant main effect of testing order on the difference between RSA during the mother and researcher administered FFSF challenges \([F(1,93) = .452, p = .503]\). Testing order did not interact with mom-res \([F(1,93) = 1.014, p = .317]\), age \([F(2,93) = .969, p = .383]\), individual difference in baseline RSA \([F(1,93) = .723, p = .397]\), or FFSF phase \([F(2,186) = .631, p = .533]\). The 5-way interaction between all of these conditions was also non-significant \([F(2,186) = .083, p = .921]\). Within each age group, there was a significant correlation between infants’ RSA amplitude values during the first Social Play phase of each FFSF: In the 3 – 5 month group, \(r(32) = .645, p = .000\); in the 6 – 8 month group, \(r(43) = .405, p = .007\); and in the 9 – 12 month group, \(r(30) = .771, p = .000\). Last, paired t-tests between Social Play phase in each FFSF were performed to ensure there were no significant differences in starting values at the beginnings
of each FFSF administration. The paired t-tests revealed no significant differences in any of the age
groups: In the 3 – 5 month group, $t(32) = -1.45$, $p = .157$; in the 6 – 8 month group, $t(43) = 1.304$, $p$
$= .199$; and in the 9 – 12 month group, $t(30) = .131$.

Once these important control parameters were confirmed, contrasts between the effects of
age and the effects of individual baseline differences on physiologic regulation in the two FFSF
administrations could be determined with confidence. First, repeated measures ANOVA
demonstrated that the regulation patterns observed in the group of infants who completed both the
mother and researcher FFSFs challenges were similar to those obtained from among all infants who
completed the mother FFSF (see Figure 4.7.). Once again, the infants in the youngest group did
appear to have a different trajectory of RSA amplitude during recovery from the Still Face phase
during the mother-administered FFSF. However, unlike the full group of infants, the difference
between the change from Still Face to Reunion Play in these youngest infants was not significantly
different from the older infants, who no longer include 15 infants in the 9 – 12 month group. HP
patterns of regulation between the three age groups did not differ, as expected.
Figure 4.7. Line graphs of infant RSA and HP during three successive time points during the phases of the mother-administered FFSF procedure illustrate progressive increases in both variables during all phases of the FFSF as infant age increases. The patterns of regulation in all three age groups do not significantly differ in HP. However, RSA patterns of regulation do appear to differ. While not significant in the sample of infants, which is limited to those who completed both the FFSF administrations, infants in the youngest age group have a different trajectory during recovery from the Still Face phase when contrasted with the two older groups.
During the researcher-administered FFSF, the trajectory of the RSA amplitude from Still Face phase to Reunion Play in the youngest infants did not at all differ from the regulation pattern of the older infants (see Figure 4.8.). This suggests that the failure of youngest infants to return toward baseline during the mother-administered FFSF might not be due to structural difference alone and might also be related to social context.

**Figure 4.8.** Line graphs of infant RSA and HP during three successive time points during the phases of the researcher-administered FFSF procedure illustrate progressive increases in both variables during all phases of the FFSF. The patterns of regulation in all three age groups do not significantly differ in HP, as with the mother-administered FFSF. However, RSA patterns of regulation during the researcher administration are also not significantly different between age groups, suggesting that differences observed during the mother-administered challenge must not be due solely to structural, maturational differences between infants in the first and second half-years of life.
The next analyses were designed to examine potential effects of individual differences in baseline RSA on infant regulation during the mother and researcher FFSFs. With the complete sample of infants completing the mother-administered challenge, individual differences in baseline RSA amplitude were found to moderate infant regulation only in older infants. In this sample of 105 infants, repeated measures ANOVA revealed the same result (see Figure 4.9).

**Figure 4.9.** As expected, line graphs illustrate that individual differences in high and low baseline RSA moderate infant RSA regulation during different phases of the FFSF procedure. Consistent with previous analyses, this moderation only occurs in infants older than 6 months of age, which, again, emphasizes the importance of integrating development and individual differences studying infant autonomic regulation.

Individual differences in baseline autonomic activity did not moderate HP regulation during the mother-administered FFSF procedure in any of the age groups (see Figure 4.10.).
Figure 4.10. Line graphs of individual differences in infant HP regulation during the FFSF procedure separated by age illustrate that individual differences in baseline vagal activity does not moderate changes in HP during the procedure, regardless of age group. This emphasizes the importance of considering RSA specifically, when using heart rate data to gather information about activity in the nervous system.

To examine whether or not social context, specifically the identity of the available adult, influenced baseline RSA moderation of regulation patterns during the FFSF, the analyses completed above were repeated with the same infants during a researcher-administered challenge. Again, the two FFSF administrations were completed in randomized order, and there was no order effect on any variables or conditions of interest. Also, there was significant correlation and no significant difference between autonomic activity during the Social Play phases of the mother and researcher FFSF challenges within subjects.
There was no significant difference found in RSA regulation pattern between the age groups during the mother or researcher-administered FFSF challenges in this group of infants (see Figures 4.7 and 4.8). However, during the mother-administered procedure, RSA regulation in infants in the two older age groups was moderated by individual differences in baseline vagal activity. Specifically, infants during the second half-year who had higher baseline RSA levels displayed significant RSA regulation during the FFSF procedure when administered by the mother. These same infants, however, failed to demonstrate the same regulation patterns during the researcher-administered challenge, despite similar starting RSA during the Social Play phase (see Figure 4.11).

**Figure 4.11.** Line graphs illustrate that, unlike during the mother FFSF challenge, individual differences in high and low baseline RSA do not moderate infant RSA regulation during different phases of the researcher-administered FFSF procedure, regardless of age group. This result demonstrates the importance of considering social context when interpreting data designed to measure regulation in infants during the first year of life.
Again, as expected, there was no significant moderation of HP regulation by baseline vagal activity during the researcher-administered challenge when infants were separated by baseline RSA (see Figure 4.12.).

**Figure 4.12.** Line graphs of individual differences in infant HP regulation during the FFSF procedure separated by age illustrate that individual differences in baseline vagal activity do not moderate changes in HP during the researcher-administered procedure, regardless of age group.

Although the preceding analyses suggest that the influences of development and individual differences on RSA regulation are impacted by social context, direct contrasts of infants between the two FFSF administrations were necessary to determine whether these different patterns were statistically significant. To accomplish this, repeated measures ANOVA was performed to contrast RSA regulation during the mother and researcher-administered FFSF challenges. Since only the
infants with higher baseline RSA displayed significant regulation during the challenges separately, only these infants were included. Additionally, since the both of the older groups of infants displayed significant regulation during the FFSF phases, these groups were collapsed into one analysis to maintain statistical power. As expected, there was no effect of social context on RSA regulation in the 15 infants from 3 to 5 months who had higher baseline RSA. Social context, however, was found to moderate RSA regulation in the 37 infants between 6 and 12 months of age (see Figure 4.13).

Figure 4.13. Line graphs of infant RSA regulation during the mother and researcher-administered FFSF procedures illustrate that social context moderates changes in RSA in older infants with higher baseline RSA.
4.5.4 Maternal Regulation during the Mother and Researcher-Administered Challenges

Accomplishing Aims 5 and 6 of the MC Play Project require analysis of maternal physiology during both the researcher and mother-administered challenges. The additional aims will not be discussed in this dissertation. However, some preliminary findings of maternal RSA and HP regulation during both FFSF administrations are presented below. As with the infant data, potential effects of order and relations between Social Play RSA between administrations were explored before proceeding to compare regulation patterns. There was no main effect of order on maternal RSA \([F(1,100) = .174, p = .677]\) and RSA during the Social Play phases were significantly correlated \([r(102) = .729, p = .000]\). However, RSA levels during the Social Play phase did differ significantly \([t(102) = -2.845]\), in that RSA during the researcher FFSF was higher (mean = 6.19 ln(msec)², s.d. = 1.40) than that during the mother FFSF (mean = 5.92 ln(msec)², s.d. = 1.15).

Overall the pattern of physiologic regulation in mothers was a mirror image of regulation in infants. While administering the FFSF, both maternal RSA and HP increased during the Still Face phase. This could be explained, in part, by decreased activity, which would, over a two-minute period, cause a decrease in heart rate due to decreased metabolic demand. While watching the researcher administer the FFSF challenge, maternal HP did not increase during the Still Face phase, as expected, and there is a significant interaction between HP regulation and FFSF administration in the mothers. RSA regulation, however, did increase during the Still Face phase of the researcher administration revealing changes in nervous system regulation not exposed by measuring heart period alone (see Figure 4.14.). Despite the initial difference in RSA during Social Play, there was no interaction between FFSF phase and mom-res condition with RSA level.
**Figure 4.14.** Line graphs of maternal regulation during the mother and researcher-administered FFSF procedures illustrate an interaction between HP changes during the mother and researcher FFSF challenges. There is no interaction present in the RSA regulation patterns, revealing changes in vagal activity not exposed by the HP measure.

### 4.6 Discussion

#### 4.6.1 Infant Physiology

Development of appropriate behavioral and emotional self-regulation in infants and toddlers is vital to development of successful social engagement skills. Over the past few decades, it has become increasingly clear that behavioral self-regulation and regulation of neurophysiologic
state are closely linked. Gaining a more sophisticated understanding of the biological substrates of behavior, especially in early life, is an increasingly central goal of research in psychology, psychophysiology, behavioral pediatrics, and many other developmental disciplines. One biological system of great interest is the autonomic nervous system (ANS), which has evolved to specifically support both social engagement behaviors and withdrawal or mobilization behaviors, contingent on environment (Porges, 1995). The focus of this work was the parasympathetic division of the ANS. Specifically, the MC Play Project centered on activity in the myelinated vagus nerve, which not only supports behaviors of social engagement, but also innervates cardiac pacemaker tissue, where its influence can be noninvasively and reliably measured.

Sequential development of specific neural structures, including cranial nerves of the parasympathetic nervous system and cortical–brainstem pathways, provide the infant with the ability to obtain basic physiologic needs and ultimately to participate in reciprocal social interaction during the first year of life (Porges & Furman, 2011). Development of the myelinated vagal system, the central link between social engagement and visceral, physiologic state, begins in the third trimester of fetal life (see Chapter 1) and provides support for healthy cardiopulmonary function at birth. At term, ingestive-vagal reflexes, triggered by feeding behaviors (i.e., sucking), “exercise” a face-heart connection mediated by brainstem structures that coordinate the regulation of striated muscles of the face with myelinated vagal regulation of the heart. During the first year postpartum, cortical development is expansive, corticobulbar tracts begin to communicate with cardiac vagal neurons in the brainstem, and the face-heart connection is strengthened. As these pathways continue to mature, the infant is increasingly able to use social cues to elicit what he or she needs from a caregiver. Eventually, this very basic social interaction develops into an ability to engage in prosocial behaviors, and sophisticated, reciprocal social engagement becomes possible.

Activity in the vagus nerve is a powerful indicator of physiologic state and is specifically reflected in RSA (see Chapters 1 and 2). As the autonomic nervous system matures, the amplitude
RSA increases, and HP becomes longer. In resting adults, high vagal tone is associated with health and well-being. In infants, the relationship between high resting vagal tone and health or pathology is less clear. In this project, higher baseline vagal tone was associated with increased, adaptive regulation during social challenge. Higher baseline vagal tone has also been associated with positive developmental outcome over time (Fox & Porges, 1985). Counter to expectations, however, infants with regulatory disorders can also have high vagal tone when compared with typically behaving infants. In some studies, this higher level of baseline vagal tone has been associated with poorer developmental outcome and increased behavioral difficulty over time (Degangi et al., 1993).

Since interpretation of baseline measures seems to depend on the study and population, measures of dynamic autonomic functioning (i.e., regulation during challenge) might be more helpful when attempting to gain better insight into autonomic mechanisms underlying self-regulation in infants. When presented with environmental or emotional challenges, a well-regulated nervous system should withdraw vagal tone in order to decrease heart period (increase heart rate) and support increased metabolic demand, which allows the individual to cope with any given stressor. A dysregulated system might be less reactive and have less dynamic vagal withdrawal. This has been tested by several groups, and poor reactivity to stressful, environmental challenges in infants and toddlers has been linked to negative emotionality (Buss et al., 2005) and behavior problems in early childhood (Porges et al., 1996). Regulation during recovery from challenge, however, has not been well-studied. The social challenge used in the MC Play Project included a “Reunion Play” phase in order to examine recovery more closely. Discovery of age differences in recovery in healthy infants might reflect developmental, structural changes in humans, which will help us gain more insight into the biological mechanisms of autonomic and behavior regulation.

One goal of the MC Play Project was to study age-related differences in autonomic
regulation during social challenge (see Aim 1). Another goal was to investigate relations between individual differences in infant baseline autonomic activity and autonomic reactivity to social challenge (see Aim 2). A third primary goal was to consider the effects of social context on infant autonomic regulation (see Aim 3). As expected, the results of each of these investigations could not be interpreted independently of each other. Analysis of all infants during the mother-administered FFSF revealed that regulation during recovery from the Still Face phase was affected by age, in that the youngest infants, from 3 to 5 months of age, had a different trajectory of RSA change than the older infants. Following modest vagal withdrawal, the infants from 3 to 5 months in this study were unable to reinstate vagal control of heart rate variability in response to social re-engagement. While this pattern appeared to persist during the mother-administered challenge including only those infants who completed both FFSF administrations, the difference between the youngest infants and the older groups was not statistically significant. Further, there was no difference in regulation pattern observed in the youngest infants during the researcher-administered FFSF procedure.

Overall, when considering only the relations between FFSF regulation and age group, age did not consistently moderate RSA regulation during social challenge, as hypothesized. However, age group was an important factor when considering the effects of individual differences in baseline RSA on autonomic regulation. When a median split was applied to each age group, dividing the groups based on higher and lower baseline RSA, individual differences were found to moderate autonomic regulation. Infants with higher baseline RSA displayed significantly more regulation when responding to social challenge than infants with lower baseline RSA. This moderation only occurred in infants in the two older age groups. Individual difference in baseline RSA were apparent in the youngest age group, however, the differences did not affect patterns of RSA regulation during challenge.

Regarding age differences and individual differences in baseline vagal activity only,
individual difference in baseline RSA did moderate infant vagal regulation, as hypothesized. However, further investigation revealed that this moderation was not consistent, even in the older infants. Individual differences only moderated regulation in older infants during the mother-administered social challenge. No such moderation was observed during the researcher-administered FFSF procedure. Taken together, the results of the MC Play Project, concerning infant physiology, indicate that individual differences in baseline vagal activity moderate infant regulation only in older infants and only during the maternal interaction.

When searching for mechanisms to explain these results, one conspicuous factor of interest, especially regarding age-related differences in social behavior and physiology in infancy, is anatomical change. It was hypothesized that due to structural development of the myelinated vagal system, older infants would have higher baseline RSA and increased RSA regulation during challenge. Anatomical research in human autonomic development, especially in the first year of life, is understandably limited. Two major studies, however, have addressed postpartum maturation of the vagus nerve in humans, and findings in these studies contributed to age grouping decisions made in this study. Sachis et al. (1982) studied vagus nerves obtained from thirty-three term and premature infants who had died in the first year of life to compare to vagus nerves obtained from autopsies of five adolescents. They focused their investigations on determining age-related changes in the number of myelinated vagal fibers in the left cervical vagus and found that the number of myelinated fibers increased rapidly from 20 to 40 weeks post-conceptional age, at which point the number of myelinated fibers was comparable to adolescents ages 12 to 22 years (Sachis et al., 1982). Ten years later, Pereyra et al. (1992) pursued more qualitative study of the myelinated fibers of the vagus in 27 term infants who had died in the first year of life. They compared fiber number, axon diameter, and myelin thickness in vagus nerves from infants aged 0 – 3 months, 3 – 6 months, 6 – 9 months, and 9 – 12 months. Rapid myelination was most prominent through the ninth month and slowed toward the end of the first year. Also, larger axon diameters
did not develop until after 6 months, and the 3 – 6 month period was the only period that displayed a significant increase in density of myelinated fibers (Pereyra et al., 1992).

As these structural changes occur, functional changes in regulation during social challenge might also emerge. However, increased myelination does not necessarily lead directly to greater conduction velocity. In fact, the ratio between axon diameter and thickness of the myelin sheath varies throughout development of the nerve until finally settling into a range that is optimal for maximal conduction velocity and, thus, nerve function (Williams & Wendell-Smith, 1971). The relative proportions of different fibers within a nerve at various stages in this maturation process must also be considered when trying to extrapolate functionality from anatomical studies. According to the distributions published by Pereyra et al., infants in the 3 – 6 month range have a greater proportion of hypomyelinated axons and are engaged in rapid myelination—two factors, which should compromise conduction velocity. This age-dependent, decrease in functioning could account for the lack of significant vagal regulation in the youngest age group regardless of individual difference in baseline RSA or social context.

In addition to changes in anatomy, developmental milestones in infant behavior also contributed to the MC Play Project questions and design. Since the primary interest was in the relationship between autonomic regulation and social behavior, a social challenge, instead of a cognitive or attention task, was used to assess provoke regulatory activity. The use of a social challenge in this study, instead of a heel stick stressor or toy-based cognitive or visual attention task, lends great ecological validity to the findings. Social interactions and behavioral self-regulation in response to social situations is a primary indicator of social success especially in early life. Attention and cognitive ability certainly contribute to social functioning, however, using a social challenge in the context of a familiar relationship is a more direct test of the relations between autonomic physiology and behavioral or temperament characteristics. The FFSF procedure forced infants to rely completely on social cues to regulate emotion and behavioral
responses (i.e., infants were not allowed access to pacifiers; mothers were not allowed to hold or rock their infants during the procedure). The youngest age group was intentionally bound by the age of emergence of the social smile (3 months) and the age of the emergence of intentional social behaviors (about 26 weeks) (Kaye & Fogel, 1980). Interestingly, social referencing becomes apparent during the second half-year of life, which encompasses the infants whose autonomic regulation was moderated by social context (Campos & Sternberg, 1981; Zeman, Cassano, Perry-Parrish, & Stegall, 2006).

4.6.2 Maternal Physiology

At the time the MC Play Project began, no other published studies had measured maternal autonomic regulation during mother-child interaction. Since then one other study has monitored maternal RSA during the FFSF procedure. This study's findings that maternal RSA patterns are opposite to those of infant patterns were consistent with results from the MC Play Project (Moore et al., 2009).

The explanation for increased RSA during the Still Face phase is difficult to elucidate and will require further study. Increased RSA is typically associated with states of calm and safety in adults. And, as expected, baseline RSA in the mother in the MC Play Project was significantly, negatively correlated with indicators of distress in the questionnaire instruments: Total Life Stress \( r(102) = -0.195, p = 0.050 \) score from the Parent Stress Index; Depressive Symptoms \( r(102) = -0.269, p = 0.011 \), and Phobic Symptoms \( r(102) = -0.247, p = 0.020 \) scores from the Brief Symptom Inventory. This background leads to an expectation that RSA would decrease to reflect greater anxiety or fear during the Still Face phase of the protocol. Moore et al (2009) concluded that increased maternal RSA while performing the Still Face suggested “self-regulation of distress.” It is possible that adults, who are more practiced in effortful control of emotions, could change
psychological factors, which counteract feelings of anxiety and result in increased RSA. Increased concentration, however, is typically associated with decreases in RSA.

4.7 Limitations

Limitations to the findings and analyses possible with the current MC Play Project dataset are mostly related to data collection and sample size. While the sample sizes included in these results have sufficient statistical power, it is preferable to include many more participants, especially when dividing participants into smaller groups to adequately explore various individual differences. Also, difficulties with data collection and analysis of anxiety dial data and questionnaire data have precluded exploration of all of the stated aims of the study at this time.

4.8 Future Investigations

4.8.1 Additional Analyses with the Current Dataset

First, Aims 4 through 6 should be explored further. For example, preliminary analysis of IBQ-R data show that infant RSA recovery (calculated by subtracting RSA_{Still Face} from RSA_{Reunion Play}) during mother FFSF is significantly correlated with “Falling reactivity / Difficulty Recovering from Stress” $r(105) = -.314, p = .003$. Infant RSA recovery during researcher FFSF was correlated with “Smiling and laughter” [$r(105) = -.216, p = .048$], “Distress to limitations” [$r(105) = .275, p = .011$], and “Falling reactivity” [$r(105) = -.325, p = .002$]. Infant RSA reactivity (calculated by subtracting RSA_{Still Face} from RSA_{Social Play}) during researcher FFSF was significantly correlated with “Sadness” [$r(105) = .229, p = .035$] and “Distress to limitations” [$r(105) = .253, p = .020$].

Strategies to calculate continuous RSA need to be developed to relate maternal physiology with maternal subjective anxiety as recorded by the “anxiety dial.” (see Figure 4.15.)
Figure 4.15. Examples of maternal anxiety recorded during the researcher-administered FFSF challenge. 1 = not anxious at all, 2 = somewhat anxious, 3 = moderately anxious, 4 = highly anxious, 5 = extremely anxious.

Pattern recognition software or another method should be used to further characterize individual differences in dyadic mother-infant regulation patterns (see examples in Figure 4.16.) There is a range of differences in individual mother-infant patterns of regulation, which might relate to information obtained from questionnaire data or to other information collected from the mothers during the protocol, including breast-feeding and birth order. Additionally, these patterns might differ with age.
**Figure 4.16.** Line Graphs of three individual mother-infant dyad examples chosen to illustrate different possible patterns of reciprocal autonomic regulation.

### 4.8.2 Project Modifications for Further Study

The basic MC Play Project Protocol could be used to investigate special populations of infants, including premature infants born between 30 and 36 weeks gestation (remember maturation of the myelinated vagus nerve and the nucleus ambiguous, Chapter 1). Babies with gastroesophageal reflux disease (GERD) could be studied. Also, dyads who experienced certain complications during pregnancy that might have affected development of the ANS, including gestational diabetes, pre-eclampsia, and maternal smoking could be included.

In terms of protocol modification, a longitudinal component could be added. The current protocol calls for collection of follow-up questionnaires six and twelve months following the initial laboratory session. However, there are no additional laboratory visits, which would be helpful in
determining true developmental changes in autonomic regulation. Adding additional Still Face periods to the protocol might also be interesting to assess whether there is an extinction of effect over time. While watching the infants undergo the researcher-administered challenge, eye tracking could also be added to the mother’s physiology to see where on her infant’s face she looks to gather information that might contribute to her responses to the child’s remote experiences. Most important, many more participants would be extremely useful to explore several aspects of social context, individual differences, and age-related or developmental changes that might all contribute to autonomic regulation in various populations.

References


VITA

SENTA A. FURMAN

EDUCATION

<table>
<thead>
<tr>
<th>Institution and Location</th>
<th>Degree</th>
<th>Years</th>
<th>Field of Study</th>
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<tr>
<td>University of Illinois at Chicago—College of Medicine; Chicago, IL</td>
<td>Ph.D. expected May 2013</td>
<td>2005 – present</td>
<td>Medicine, Neuroscience</td>
</tr>
<tr>
<td></td>
<td>M.D. expected May 2013</td>
<td></td>
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<tr>
<td>Wayne State University; Detroit, MI</td>
<td>B.S.</td>
<td>1999 – 2001</td>
<td>Biological Sciences, Psychology</td>
</tr>
<tr>
<td>Duke University; Durham, NC</td>
<td></td>
<td>1996 – 1998</td>
<td>Neurobiology, French Literature</td>
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UNIVERSITY SERVICE

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<tr>
<td><strong>MSTP M3 Transition Course</strong></td>
<td>Student Coordinator. With two other MSTP students, I helped design and coordinate a pilot of this course designed to help MSTP students in graduate school transition to the M3 clinical curriculum following completion of the PhD. The past two years, since the other two students graduated, I have coordinated a new course incorporating student feedback from the pilot course. Tasks include soliciting participants and coordinating topics and schedules for participating attending physicians, PharmDs, and standardized patients. I have also designed and compiled evaluation tools to continue to develop the course, which will soon be added to the general curriculum under supervision of the UIC UGME office.</td>
<td>October 2009 to present</td>
</tr>
<tr>
<td><strong>MSTP Student Advisory Committee</strong></td>
<td>Executive Board Member. These few positions are determined by general MSTP student elections. My annual responsibilities included planning and coordinating activities for the annual MSTP retreat and running the annual MSTP student meeting.</td>
<td>August 2007 to present</td>
</tr>
<tr>
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<tr>
<td>MSTP Admissions Committee</td>
<td>Student member. I was invited to participate as the student member of the MSTP admissions committee.</td>
<td>August 2010 to May 2011</td>
</tr>
<tr>
<td>MSTP Programming Committee</td>
<td>Student member. I was invited to participate as the student member of this committee, which oversees program administration and prepares MSTP grant application at times of renewal.</td>
<td>August 2010 to June 2011</td>
</tr>
<tr>
<td>MSTP Student Advisory Committee</td>
<td>President. This position is elected by general MSTP students.</td>
<td>August 2010 to July 2011</td>
</tr>
<tr>
<td>Student Clinician Ceremony</td>
<td>Student Coordinator. I was asked to coordinate the white coat ceremony for M3 students entering clinical clerkships.</td>
<td>Summer 2007</td>
</tr>
<tr>
<td>M1 Orientation</td>
<td>Co-Chair. I was one of four students charged with design and implementation of M1 orientation week. In addition to several group tasks, I was also specifically in charge of coordinating the M1 white coat ceremony.</td>
<td>Summer 2006</td>
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**RESEARCH EXPERIENCE**

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<tr>
<td>Brain-Body Center; Department of Psychiatry, University of Illinois at Chicago, College of Medicine</td>
<td>This is the lab in which I will complete my dissertation in Neuroscience. My research focus here is on the autonomic nervous system and its regulation of cardiorespiratory systems. I also study how the autonomic nervous system supports (or fails to support) social behaviors in children with disorders such as social anxiety and autism.</td>
<td>July 2006 to present</td>
</tr>
<tr>
<td>Mental Illness Needs Discussion Sessions (MINDS), Inc.</td>
<td>As Research Coordinator for this non-profit organization, I was in charge of grant writing and development of survey instruments to evaluate the efficacy and effectiveness of the MINDS curriculum to positively impact students' knowledge of mental illnesses, knowledge of available resources for help with mental health problems, and most important, changes in help-seeking behaviors.</td>
<td>August 2001 to August 2004</td>
</tr>
<tr>
<td>Department of Psychiatry and Neurosciences, Wayne State School of Medicine</td>
<td>Worked in Dr. Michael Bannon's lab using <em>in situ</em> hybridization to study the localization and concentration of certain proteins in the brain that are activated during cocaine use.</td>
<td>March 2001 to August 2001</td>
</tr>
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<tr>
<td>Department of Biological Sciences, Wayne State University</td>
<td>Worked as an undergraduate research assistant in Mark VanBerkum's lab. My project examined various proteins involved in axon guidance during the development of the central and peripheral nervous systems.</td>
<td>January 1999 to March 2001</td>
</tr>
<tr>
<td>University of Michigan Cancer Center</td>
<td>Worked in Dr. Alfred E. Chang's immunology laboratory. Here I became proficient in basic cell culture techniques and immunological assays. I also gained experience in working with rodent models. The results of the analyses and figures I completed and created during my project were included in a publication at the end of the summer.</td>
<td>Summer 1997</td>
</tr>
</tbody>
</table>

**OTHER RESEARCH ACTIVITIES**

<table>
<thead>
<tr>
<th>Research Project</th>
<th>Position</th>
<th>Dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicago Health Atlas: Exchanging de-identified data between hospitals for city-wide health analysis in the Chicago area</td>
<td>Co-Investigator</td>
<td>November 2011 to present</td>
</tr>
<tr>
<td>Migratory Patterns of Admissions in Chicago Among Different Hospitals</td>
<td>Key Research Personnel</td>
<td>November 2011 to present</td>
</tr>
<tr>
<td>The Effects of Vagus Nerve Stimulation on Autonomic Physiology</td>
<td>Co-Investigator</td>
<td>April 2010 to present</td>
</tr>
<tr>
<td>Reciprocal Autonomic Regulation in Mother-Child Dyads During Social Interaction</td>
<td>Principal Investigator</td>
<td>November 2007 to present</td>
</tr>
<tr>
<td>Effect of Partial Vagal Blockade on Social Engagement Behaviors</td>
<td>Co-Investigator</td>
<td>August 2006 to present</td>
</tr>
<tr>
<td>The Growing Baby Study: Changes in autonomic and behavioral regulation in infants between ages 6 and 36 months</td>
<td>Co-Investigator</td>
<td>April 2008 to June 2011</td>
</tr>
<tr>
<td>Infant Crying and Developmental outcome: A Biobehavioral Approach</td>
<td>Key Research Personnel</td>
<td>March 2007 to June 2011</td>
</tr>
</tbody>
</table>
### Scholarships, Prizes, Honors or Other Recognition

<table>
<thead>
<tr>
<th>Award</th>
<th>Awarded By</th>
<th>Date</th>
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</thead>
<tbody>
<tr>
<td><strong>Honorable Mention—UIC College of Medicine Research Forum</strong></td>
<td>University of Illinois at Chicago, College of Medicine</td>
<td>November 2009</td>
</tr>
<tr>
<td><strong>The Neurobiology Course at the Marine Biological Laboratory, Woods Hole, MA</strong>—one of 12 international Students and Assistant Professors accepted</td>
<td>Neurobiology Course and MBL Directors</td>
<td>June 2009 to August 2009</td>
</tr>
<tr>
<td><strong>Diversity Program in Neuroscience American Psychological Association Individual Graduate Fellowship</strong></td>
<td>APA-DPN Grant Review Committee</td>
<td>September 2008 to September 2010</td>
</tr>
<tr>
<td><strong>Summer Program in Neuroscience, Ethics, and Survival (SPINES) Course at the Marine Biological Laboratory, Woods Hole, MA</strong></td>
<td>SPINES and MBL Directors</td>
<td>June 2008 to July 2008</td>
</tr>
<tr>
<td><strong>Training Grant in the Neuroscience of Mental Health (5 T32 MH067631-04)</strong></td>
<td>T32 Committee, Department of Psychiatry</td>
<td>August 2007 to August 2008</td>
</tr>
<tr>
<td><strong>MSTP Graduate Research Assistantship</strong></td>
<td>UIC-COM Medical Scientist Training Program (MSTP)</td>
<td>June 2006</td>
</tr>
<tr>
<td><strong>University of Illinois at Chicago, College of Medicine Alumni Scholarship</strong></td>
<td>UIC-COM Alumni Association</td>
<td>May 2006</td>
</tr>
<tr>
<td><strong>Nominee finalist for the David D. Henry Undergraduate Award</strong>—Nominated and selected finalist for this University award given to one male and one female annually for outstanding performance and service in the university and the community.</td>
<td>Dean of Students Office—Wayne State University</td>
<td>October 2001</td>
</tr>
<tr>
<td><strong>MBRS Scholarship</strong>—This is a program similar to the MARC program. It was while I was participating in MBRS that I worked with Dr. Bannon.</td>
<td>MBRS Program Office—Wayne State School of Medicine</td>
<td>March 2001</td>
</tr>
<tr>
<td><strong>First Place Minority Programs Research Award</strong>—Presented my research project from Dr. VanBerkum’s lab along with graduate students at an annual research symposium at Wayne State School of Medicine.</td>
<td>Minority Programs Research Symposium—Wayne State University</td>
<td>August 1999, 2000, 2001</td>
</tr>
</tbody>
</table>
### Awards

<table>
<thead>
<tr>
<th>Award</th>
<th>Awarded By</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preceptorship at Oakwood Hospital, Dearborn, MI</strong></td>
<td>Oakwood Hospital, Dearborn, MI</td>
<td>Summer 1998</td>
</tr>
<tr>
<td>Received a preceptorship position in the Department of Neurosurgery at Oakwood Hospital. I worked with Norman Rotter and Peter Zahos attending routine medical appointments in the office, patient rounds in the hospital, and numerous surgeries.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Biomedical Research Fellow</strong></td>
<td>University of Michigan Medical School</td>
<td>Summer 1997</td>
</tr>
<tr>
<td>Received this fellowship to do biomedical research at the University of Michigan-Ann Arbor. There, I worked in Dr. Alfred E. Chang's lab in the Cancer Center. My project studied various aspects of adoptive immunotherapy, a possible treatment technique for cancer. Since Dr. Chang was also the Division Chief of Surgical Oncology at the hospital, I attended patient rounds and surgeries regularly.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Angier B. Duke Memorial Scholarship</strong></td>
<td>Duke University</td>
<td>June 1996</td>
</tr>
<tr>
<td>This is a full academic scholarship appointed to fifteen incoming freshmen each year at Duke University.</td>
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</tbody>
</table>

### Presentations


Distribution of Axonal Mitochondrial Transport across Asymmetrical Branchpoints in the MitoKaede mouse model.
Imaging Section. Project facilitated by Thomas Misgeld. July 2009

Microarray characterization of Mad3 gene expression patterns in medulloblastoma cell lines versus control.


Research Talk: Social Engagement Behaviors and the Autonomic Nervous System, a Drug Study.


Irish, H., S Riolo, S Furman, CA King. Mental Illness Needs Discussion Sessions (MINDS): An Educational Intervention for High School Students.


**Publications**

**Furman, SA.** Neurophysiologic Regulation in Infants during Social Challenge—An Integrative Approach. In preparation for submission to *Child Development*

**Furman, SA, GF Lewis, MA McCool.** Neurophysiologic Mechanisms of Heart Rate Variability—The Importance of Appropriate Criterion Variables. Submitted to *Circulation*.


