Improving the methodology for non-invasive autonomic nervous system recording and its implementation in behavioral research

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CHAPTER 1

Measuring autonomic nervous system activity

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Abstract

Cardiovascular disease (CVD) is one of the main causes of death in Westernized countries. The etiology of CVD is complex, with many different factors (demographical, lifestyle, psychological, and genetic) contributing to an increased risk of CVD development (Brotman, Golden, & Wittstein, 2007; Brotman, Walker, Lauer, & O'Brien, 2005). The physiological risk factors that form the final common pathway to CVD include the metabolic syndrome, with hypertension, hyperlipidemia, hyperglycemia, and android obesity as core features (Bayturan et al., 2010); inflammation (Danesh et al., 2008); coagulation/fibrinolysis imbalance (Libby & Theroux, 2005); reduced heart rate variability (Dekker et al., 2000; Dekker et al., 1997); and increased heart rate (Fox et al., 2007). Strikingly, activity of the autonomic nervous system (ANS) is associated with all of these physiological risk factors (Charkoudian & Rabbitts, 2009; Lambert & Lambert, 2011; Malpas, 2010; Straub, Wiest, Strauch, Harle, & Scholmerich, 2006; Task Force of the European Society of Cardiology the North American Society of Pacing, 1996; Tracey, 2009; von Kanel, Mills, Fainman, & Dimsdale, 2001). Because the ANS is very sensitive to psychosocial stress, it plays a key role in almost all models in biobehavioral medicine that try to account for the well-known role of social (Karasek et al., 1988; Rosengren et al., 2004; Siegrist, Peter, Junge, Cremer, & Seidel, 1990) and psychological (Nicholson et al., 2006) sources of chronic stress in hypertension, diabetes, and cardiac disease.

There are large individual differences in the activity of the ANS in the basal resting state (Berntson et al., 1994a; Berntson et al., 1994b; Grossman & Kollai, 1993; Light, Kothandapani, & Allen, 1998; Salomon, Matthews, & Allen, 2000). These differences in ANS activity are further amplified in response to brief laboratory stressors (de Geus et al., 2007; Houtveen et al., 2002; Lucini, Norbiato, Clerici, & Pagani, 2002; Wang et al., 2009) as well as prolonged psychosocial stress (Riese, van Doornen, Houtman, & de Geus, 2000; Vrijkotte, van Doornen, & de Geus, 2004). This chapter reviews the available measures to capture differences in ANS activity at rest and during stress. We first present a short overview of the ANS and a detailed review of the measurement strategies used to study its activity. We close by discussing the ANS measures that are both sufficiently valid and applicable for ambulatory assessment in population-based samples that are sufficiently large to allow epidemiological studies and genetic analyses.

The Autonomic Nervous System

The term “autonomic nervous system” was coined by Langley in 1898. Based on anatomical and functional criteria, he divided the ANS into three separate branches: the sympathetic nervous system (SNS) including the adrenal medulla, the parasympathetic nervous system (PNS), and the enteric nervous system, a collection of neurons embedded within the walls of the entire gastrointestinal tract that control gastrointestinal motility and secretions. In more recent use of the term, the enteric system is discarded and ANS is usually synonymous with the sympathetic and parasympathetic branches.

The sympathetic branch is best known for its key role in the “fight-or-flight” response. Activity of the SNS causes, among other things, an increase in heart rate, contractility, blood pressure, breathing rate, bronchodilation, sweat production, epinephrine secretion, and a redistribution of blood flow favoring the muscles. The PNS, on the other hand, promotes the maintenance of the body by acquiring energy from food and getting rid of wastes. The PNS is therefore often labeled as the “rest and digest” branch of the ANS. Its activity causes slowing of the

heart, constriction of the pupils, stimulation of the gut and salivary glands, and other responses that help restore energy. Many organs are innervated by both the sympathetic and the parasympathetic branches of the ANS, and an increase in the activity of these branches typically exerts opposing actions. However, some organs are not dually innervated (e.g. sweat glands) and, even for dually innervated organs, the autonomic branches may have synergistic rather than opposing effects (e.g. salivary glands).

The main function of the ANS is coordinating bodily functions to ensure homeostasis and performing adaptive responses when faced with changes in the external and internal environment, such as those due to physical activity, posture change, food consumption, or hemorrhage. In addition, the ANS is capable of substantial heterostatic action; it can prepare the body for anticipated threats to homeostasis even in the absence of actual changes in bodily activity. The best known example is the anticipatory response that prepares the body for physical activity in response to a vast range of stressors that can be purely symbolic in nature and are often not followed by actual physical activity (fight-or-flight) or changes in internal environment (e.g. through blood loss or infection). This response is called the physiological stress response.

In humans, subjective experience of stress can be sufficient to trigger the physiological stress response. Subjective experience of stress typically occurs when there is an imbalance between perceived threats/demands and perceived abilities/resources. In the brain, the perception of internal (thoughts) or external (environmental events) threats by neocortical areas leads to the activation of limbic areas, in particular the amygdala (Lovallo, 2005). The amygdala, in turn, projects to paraventricular and other hypothalamic nuclei as well as to a network of neurons in the rostral ventrolateral medulla (RVLM) and the nucleus of the solitary tract (NTS) that initiates changes in the activity of sympathetic neurons in the intermediolateral (IML) column and in activity of the parasympathetic neurons in the n. ambiguus.

Parasympathetic Nervous System Activity

The vagus nerve (CN X) carries preganglionic fibers of the PNS to the heart and lungs (as well as to other organs) and is the primary source of parasympathetic innervation of these organs. Many efferent fibers in the vagus originate in the n. ambiguus. Other preganglionic fibers of the PNS leave from the cell bodies of the motor nuclei of cranial nerves (CN) III, VII, IX, and X in the brainstem and from the second, third, and fourth sacral segments of the spinal cord. The preganglionic axons terminate in parasympathetic ganglia, which lie within or very close to the organs innervated by the short postganglionic neurons. The preganglionic neurons employ acetylcholine (ACh) as the primary neurotransmitter, which binds to a nicotinic receptor subtype on the postganglionic neurons in the ganglia. Postganglionic parasympathetic fibers also employ ACh as a primary neurotransmitter, but the receptor subtypes on the target organ are commonly muscarinic. For instance, the parasympathetic postganglionic receptors in the sinoatrial (SA) node of the heart are type 2 muscarinic (M2) and their activation reduces heart rate.

Sympathetic Nervous System Activity

The preganglionic fibers from neurons in the IML column leave the central nervous system from the thoracic and lumbar regions of the spinal cord. They synapse onto a chain of sympathetic ganglia that lie close to the spinal cord, known as the sympathetic trunk. The preganglionic neurons from the IML column to the sympathetic ganglia employ ACh as the primary neurotransmitter. The

postganglionic neurons from the sympathetic ganglia to the organs employ norepinephrine as the primary neurotransmitter, which can act on α1-adrenergic (e.g. in arterioles) or β1- and β2-adrenergic receptors (e.g. on the heart). Stimulation of the α1-adrenergic receptors causes vasoconstriction by acting on the smooth muscles in the medial layer of the blood vessels. Stimulation of the cardiac β-adrenergic receptors by norepinephrine released from the cardiac sympathetic nerves (nn. accelerantes) increases the pacemaker frequency of the SA node (i.e., heart rate), as well as contractility of the ventricles. Together, vasoconstriction and increased cardiac performance account for the increase in blood pressure seen during increased sympathetic activity.

A first exception to the use of norepinephrine as the final effector in the SNS is found in the sympathetic innervation of eccrine sweat glands, which is cholinergic rather than adrenergic. A second exception is a set of preganglionic neurons that end in a special ganglion, namely the adrenal medulla. On activation by preganglionic neurons, the adrenal medulla releases a small amount of norepinephrine into the bloodstream, but most of the released norepinephrine is converted to epinephrine, which is excreted in much larger amounts than norepinephrine (5:1). Circulating epinephrine preferentially binds to β2-receptors in the vessels and on the heart, causing vasodilatation (mostly in muscle tissue) and increases in heart rate and contractility.

Measurements of Autonomic Nervous System Activity

Many studies of the ANS have focused on the fight-or-flight response, which is often characterized by reciprocal increases in SNS activity and decreases in PNS activity. Such a pattern gives rise to increases in heart rate and blood pressure, and heart rate and blood pressure reactivity are still among the most used variables to indicate changes in ANS activity. Laboratory studies generally involve the measurement of heart rate and blood pressure during one or more rest periods and during mental and physical challenges, with each period often lasting no more than 5–15 minutes. Such studies provide valuable information on the mechanisms underlying ANS responses to stress and have been instrumental in establishing the existence of stable individual differences in the physiological stress response.

Notwithstanding that much has been learned from studies focusing on heart rate and blood pressure as indicators of ANS activity, a disadvantage of these variables is that they represent an unknown mix of sympathetic and parasympathetic effects. It has been shown that the classical reciprocal pattern of sympathetic activation with parasympathetic deactivation describes only a limited part of the total autonomic space (Berntson et al., 1991). Different patterns of co-activation, reciprocal activation and co-inhibition are found across individuals performing the same task or within individuals performing different tasks. For example, dental phobia patients engaged in a stressful mental arithmetic task showed an increase in their SNS activity with decreased PNS activity; but, when exposed to phobic stimuli, the same participants showed increased SNS activity with increased PNS activity (Bosch, de Geus, Veerman, & Amerongen, 2000). Most importantly, health outcomes of sympathetic hyperreactivity need not be the same as those of parasympathetic hyperreactivity. Hyperactivity of the SNS has been mostly associated with an increased risk for hypertension, the metabolic syndrome, and left ventricular failure (Brotman et al., 2007; Esler, 2010; Esler et al., 2008; Esler, Lambert, & Schlaich, 2010; Lambert, Schlaich, Lambert, Dawood, & Esler, 2010), whereas loss of PNS activity causes a reduction in the electrical stability of the heart (Schwartz et al., 2003; Vanoli et al., 1991) and may play a key role in the proinflammatory state (Rosas-Ballina & Tracey, 2009; Tracey, 2009).

Because heart rate and blood pressure do not reveal the underlying pattern of ANS activity, studies in the past two decades began indexing sympathetic and parasympathetic activity separately. Next, we review the various measures of SNS and PNS activity currently in use (see Table 1 for an overview).

Table 1. *Measurement strategies of autonomic nervous system activity in humans.*

Technique	Invasiveness	Principle	Measure	References
PARASYMPATHETIC				
Parasympathetic microneurography	Very High	Direct measurement of action potentials in the parasympathetic nerves converted to bursts with a fixed time constant integrator	Vagal (burst count/time)	(Cerati & Schwartz, 1991; Jewett , 1964; Kunze, 1972)
Microdialysis	Very High	Measurement of acetylcholine (ACh) concentrations in the dialysate samples, for instance in the sino-atrial (SA) node, using high-performance liquid chromatography	[Ach]	(Shimizu et al., 2009)
Pharmacological blockade	Moderate	When the heart rate measured in the unblocked state is subtracted from the heart rate measured during full muscarinic blockade, the change in heart rate (ΔHR) yields a measure of vagal activity.	ΔHR (bpm)	(Berntson et al., 1994a; Berntson et al., 1991; Cacioppo et al., 1994)
Respiratory Sinus Arrhythmia	Noninvasive	Heart rate variability due to respiratory gating of tonic vagal effects on the SA node scales with the height of that activity	RMSSD (ms) pvRSA (ms) HF (ms^2)	(Akselrod et al., 1981; Cerutti, Bianchi, & Mainardi, 2001; Katona & Jih, 1975; Sztajzel, 2004)
Heart Rate Variability (TP, ULF, VLF, LF)	Noninvasive	Same principle as RSA above, but lower	SDNN (ms)/TP (ms^2) SDANN/ULF	(Task Force of the European Society of

		frequencies in heart rate variability are additionally influenced by modulation of the sympathetic effects on the SA node.	(ms^2) VLF (ms^2) LF (ms^2)	Cardiology the North American Society of Pacing, 1996)
Baroreflex sensitivity (BRS)	High (if intra-arterial BP) Noninvasive (if Finapres)	The BRS is computed as the mean slope of the regression line relating beat-to-beat changes in SBP to changes in IBI. As the sympathetic contribution to fast changes in the IBI is minimal, this reflects mainly cardiac vagal activity.	BRS (ms/mmHg)	(Di Rienzo, Parati, Radaelli, & Castiglioni, 2009; La Rovere, Pinna, & Raczak, 2008)
SYMPATHETIC				
Sympathetic microneurography	High	Direct measurement of action potentials by a tungsten microelectrode inserted into the fascicle of a sympathetic nerve in the skin or muscle (m. peroneus). The voltage is rectified and integrated with a fixed time constant (100 ms).	MSNA (burst count/time) SSNA (burst count/time)	(Hagbarth & Vallbo, 1968; Wallin, 1984; Wallin, 2004)
Regional norepinephrine spillover	Very High	During constant-rate infusion of radiolabeled NE , and with regional catheterization, the organ-specific rate of spillover of NE to plasma can be determined based on the degree of dilution of infused radioactive-labeled NE by	Total NE spillover (ng/min) Organ NE spillover (ng/min)	(Eisenhofer, 2005; Esler et al., 1988; Esler & Kaye, 2000b; Grassi & Esler, 1999)

		endogenous-released NE.		
Pharmacological blockade	Moderate	When the response measured in the unblocked state is subtracted from the response measured during blockade, this yields a measure of sympathetic activity to the organ. For instance, (1) the heart rate decrease (ΔHR) that is induced by blockade of β-adrenergic receptors, (2) the blood pressure increase that is induced by blockade of α-adrenergic receptors.	ΔHR (bpm) ΔDBP (bpm)	(Berntson et al., 1994a; Cacioppo et al., 1994; Julius, Pascual, & London, 1971)
Plasma catecholamines	Low (if venous) High (if arterial)	Measurements of concentrations of NE or E in arterial or venous blood using high-performance liquid chromatography. Caveat: intraneuronal vesicular storage/leakage and synaptic reuptake contribute in unknown ways to plasma NE concentrations and (changes in) tissue clearance strongly co-determine the level of both catecholamines.	[NE] pg/mL [E] pg/mL	(Esler et al., 1990; Goldstein, Eisenhofer, & Kopin, 2003; Hjemdahl, 1990)
Urinary catecholamines	Noninvasive	Measurement of (24-h) urinary excretion of NE or E (relative to creatinine). Caveat: same as for plasma levels, and the kidneys	[NE] (pg/mg creatinine) [E] (pg/mg creatinine)	(Esler et al., 1990; Goldstein et al., 2003; Hjemdahl, 1990)

		themselves also produce NE.		
Skin conductance	Noninvasive	Sympathetic activity of skin nerves increases the activity of the sweat glands, which in turn yields a measurable change in the conductance of an applied current across the skin.	SCL (µS) nsSCRs (counts/time)	(Boucsein, 1992; Dawson, Schell, & Filion, 2000; Fowles, 1986)
Salivary α-amylase activity	Noninvasive	NE release from sympathetic nerve terminals near adrenoceptors in the acinar cells of the saliva glands cause an immediate increase in the protein-to-fluid ratio of many salivary proteins, including α-amylase. Caveat: PNS activity also increases α-amylase secretion.	sAA (U/mL)	(Nater & Rohleder, 2009)
LF/HF ratio	Noninvasive	The LF/HF ratio of spectral power of the heart rate in the lower frequencies centered around 0.1 Hz (LF) divided by the power in the higher frequencies centered around the respiratory frequency (HF). Also expressed as LF/(LF+HF) = LFnu. Caveat: validity is very controversial.	LF/HF (dimensionless) LFnu (dimensionless)	(Pagani & Malliani, 2000)
Ejection Fraction	High (if using contrast MRI), Moderate (if using MRI) Low (if using echocardiography)	The ejection fraction (EF) is the ratio between stroke volume and enddiastolic volume which can be derived	EF (%)	(Sherwood et al., 1990)

		from (contrast) MRI or echocardiography. Increased sympathetic activity leads to increased cardiac contractility that causes a higher EF.		
ECG T-Wave amplitude	Noninvasive	The amplitude difference between an isoelectric baseline (e.g. P-Q interval) of the ECG and the peak of the T-wave.	TWA (mV)	(Heslegrave & Furedy, 1979; Malm, Frigstad, Sagberg, Larsson, & Skjaerpe, 2004)
Systolic Time Intervals	Noninvasive	From the thorax impedance cardiogram, the preejection period is derived as the time interval between the onset of ventricular depolarization and the opening of the semilunar valves. This interval shortens with increased sympathetic drive to the left ventricle. Caveat: posture should be controlled.	PEP (ms)	(Sherwood et al., 1990)

Measuring Parasympathetic Activity

The ideal way to assess parasympathetic activity is the direct measurement of action potentials in the parasympathetic nerves (Cerati et al., 1991; Jewett, 1964; Kunze, 1972). For cardiac vagal activity, an alternative was developed that assesses the changes in (ACh) concentration in the SA node by microdialysis (Shimizu et al., 2009). Unfortunately, both these "golden standard" measures are too invasive to be used in research with humans. A theoretical alternative would be to measure the spillover of ACh to plasma by venipuncture, but this is not feasible because of the rapid and extensive clearance of the transmitter in the synaptic space by acetylcholinesterase.

Human studies of parasympathetic activity have therefore focused on the effects of parasympathetic activity on the innervated organs rather than on activity per se. For instance, to assess cardiac parasympathetic activity to the SA node, the heart rate change can be measured in response to pharmacological blockade of the muscarinic receptors (Berntson et al., 1994a; Cacioppo

et al., 1994; Martinmaki, Rusko, Kooistra, Kettunen, & Saalasti, 2006). High doses of M2-antagonists, like atropine, effectively remove all parasympathetic effects on the heart. When the heart rate measured in the unblocked state is subtracted from the heart rate measured during full blockade, participants with higher parasympathetic activity will show a larger increase in heart rate during blockade than will participants with lower activity. The increase in heart rate is not a perfect measure of parasympathetic activity, since the SNS and PNS are known to interact (Mizuno et al., 2008). High levels of SNS activity will act to reduce the release of ACh through the action of the α2-autoreceptors on the terminal buttons of vagal axons. However, these interactive effects can be dealt with using a dual blockade strategy (Berntson et al., 1994a; Berntson et al., 1994b; Cacioppo et al., 1994).

A disadvantage of pharmacological blockade studies is that they are confined to well-controlled (hospital) settings and are not readily amenable to be used in larger samples or in recordings in naturalistic settings. Fortunately, reliable noninvasive estimation of parasympathetic cardiac effects is possible by measuring time- or frequency-domain indices of heart rate variability in the respiratory frequency range, also called respiratory sinus arrhythmia (RSA). Respiratory sinus arrhythmia is the difference in heart rate during the inspiration and expiration phases of the respiratory cycle. Respiratory sinus arrhythmia is generated when tonic firing of motor neurons in the n. ambiguus and sympathetic nuclei is modulated by phasic inhibition and excitation coupled to the respiratory cycle. This modulation is caused by connections between the nuclei that control the respiratory generator in the pre-Bötzinger and Bötzinger complexes and the vagal and sympathetic motor neurons, which lie in close proximity in the brainstem (Rekling & Feldman, 1998). This respiration–ANS coupling yields an oscillatory pattern in the release of norepinephrine and ACh in the SA node, such that ACh levels increase during expiration and decrease during inspiration, whereas norepinephrine shows the reverse pattern of increases during inspiration and decreases during expiration. The effect of this respiratory "gating" (Eckberg, 2003) is that the heart rate increases during inspiration and decreases during expiration. This has an advantageous effect on the efficiency of oxygen exchange in the lungs, at least in the resting state (Yasuma & Hayano, 2004).

Fortuitously, the effect of the respiratory-related changes in vagal activity on heart rate variability is much more prominent than the effect of the respiratory-related changes in sympathetic activity. This is due to the differential filter characteristics of the muscarinergic and adrenergic receptors (Berntson, Cacioppo, & Quigley, 1993b). The muscarinergic receptor rapidly opens potassium channels after ACh release, and closure of calcium channels with parallel changes in the hyperpolarization of the SA node cells occur within hundreds of milliseconds. The β-receptors, that first need to activate protein kinases before channels for sodium and calcium are opened, act much slower and influence the speed of depolarization of the SA cells only on the scale of seconds. This causes the high-frequency changes due to respiration to be filtered from the sympathetic effects on the heart. In keeping, RSA shows relatively little sensitivity to sympathetic blockade but is affected in a dose–response way by muscarinergic blockers in humans (Berntson et al., 1994a; Cacioppo et al., 1994; Martinmaki et al., 2006) or vagal cooling in animals (Katona et al., 1975). This has led to the use of RSA as a proxy for vagal cardiac activity (Berntson et al., 1997), although it is acknowledged that large changes in sensitivity of chemoreceptor and baroreceptor reflexes (Berntson et al., 1997; Berntson et al., 1993b; Houtveen et al., 2002) or respiratory behavior (Grossman et al., 1993; Grossman, Wilhelm, & Spoerle, 2004; Ritz & Dahme, 2006) are important confounders.

Respiratory sinus arrhythmia can be derived from the interbeat interval (IBI) time series obtained from the R waves in the electrocardiogram (ECG) by taking the root mean square of differences (RMSSD) between successive IBIs (Sztajzel, 2004; Task Force of the European Society of

Cardiology the North American Society of Pacing, 1996). When the respiratory signal is co-registered with the ECG, RSA can also be derived by peak-valley estimation (pvRSA). Estimates of pvRSA are obtained by subtracting the shortest IBI during heart rate acceleration in the inspiration phase from the longest IBI during heart rate deceleration in the expiration phase (Katona et al., 1975). Respiratory sinus arrhythmia can also be derived in the frequency domain by Fourier (Akselrod et al., 1981) or wavelet analysis (Houtveen & Molenaar, 2001; Pichot et al., 1999; Wiklund, Akay, & Niklasson, 1997) or by autoregressive model estimation (Cerutti et al., 2001). These analyses describe the periodic oscillations of the heart rate signal decomposed at different frequencies and amplitudes and provide information on the amount of their relative contribution to the variance (also termed power) in the heart rate. Power in the respiratory frequency range of 0.15–0.40 Hz (HF power) can be used to index RSA.

In standardized laboratory recordings, as well as in ambulatory settings, the different time- and frequency-domain measures of RSA (e.g. RMSSD, HF power, pvRSA) were highly correlated, with all r's being greater than 0.80 (Grossman, van Beek, & Wientjes, 1990; Hayano et al., 1991b; Houtveen et al., 2001; Penttila et al., 2001), and this high intercorrelation of the various RSA measures proved stable across a wide range of values for respiration and heart rates (Goedhart, Kupper, Willemsen, Boomsma, & de Geus, 2006). An important feature of the RSA measures is that they can be reliably measured under naturalistic conditions with the use of ambulatory monitoring (de Geus et al., 1995; Wilhelm, Roth, & Sackner, 2003). For the average 24-hour levels of RMSSD and HF power, high test–retest correlations ($.63 < r < .90$) were found after 3 to 65 days in both healthy individuals and cardiac patients (Bigger, Jr. et al., 1992; Hohnloser, Klingenheben, Zabel, Schroder, & Just, 1992; Kleiger et al., 1991; Sinnreich, Kark, Friedlander, Sapoznikov, & Luria, 1998; Stein, Rich, Rottman, & Kleiger, 1995). Good long-term temporal stability ($.58 < r < .76$) for 24-hour levels of pvRSA, HF, and RMSSD has been shown over a period of 7 months (Pitzalis et al., 1996) to 3.4 years (Goedhart, van der Sluis, Houtveen, Willemsen, & de Geus, 2007)

Respiratory sinus arrhythmia is only one component of the total variability in the heart rate. Other heart rate variability measures include the power in the low-frequency (LF, 0.04–0.15 Hz), very-low-frequency (VLF, 0.003–0.04 Hz), and ultra-low-frequency (ULF, < 0.003 Hz) bands (Sztajzel, 2004; Task Force of the European Society of Cardiology the North American Society of Pacing, 1996). Together with RSA, these measures have received a lot of attention in medicine because lowered levels of heart rate variability predict adverse cardiovascular events, including atrial fibrillation, myocardial infarction, congestive heart failure, and coronary artery disease (Bigger et al., 1990; Bigger, Jr. et al., 1992; Bigger, Fleiss, Rolnitzky, & Steinman, 1993; Dekker et al., 1997; Hayano et al., 1991a; Kleiger, Miller, Bigger, & Moss, 1987; Lombardi et al., 1987; Nolan et al., 1998; Saul et al., 1988; Singer et al., 1988; Tsuji et al., 1996; Vikman et al., 2003). Heart rate variability in the LF band arises from so-called Mayer waves, which are periodic oscillations in arterial blood pressure around the 0.1 Hz frequency (Julien, 2006). Through the action of the baroreceptors, these periodic changes in blood pressure are met by parallel changes in vagal activity and sympathetic vascular tone to keep blood pressure constant. This gives rise to a "10-s rhythm" in the heart rate that can be detected by spectral analysis as the power in the LF band. Heart rate variability in the ULF and VLF bands has been hypothesized to reflect circadian rhythms in bradykinin, renin, or angiotensin release, or thermoregulatory effects on peripheral vasomotor tone. However, in ambulatory recordings, the ULF, which is highly correlated to the standard deviation of the average heart rate across all 5-minute segments of an entire recording (SDANN), seems to arise predominantly from changes in behavior from passive to physically active and vice versa, particularly around the transitions to and from sleep

(Roach, Wilson, Ritchie, & Sheldon, 2004). This may also be true of the standard deviation of the heart rate (SDNN), which reflects all of these same components. The SDNN can also be indexed by the total power (TP).

A final measure that has been used to index cardiac vagal activity, particularly in the context of increased risk for cardiac disease, is the sensitivity of the baroreflex (BRS) (La Rovere, Bigger, Marcus, Mortara, & Schwartz, 1998; La Rovere et al., 2003). The baroreflex loop counteracts deviations in blood pressure from an ongoing setpoint by changing sympathetic outflow to the blood vessels to affect peripheral resistance and by changing sympathetic and vagal activity to the SA node to affect cardiac output (Di Rienzo et al., 2009; La Rovere et al., 2008). Deviation of blood pressure from the setpoint is detected by stretch receptors (baroreceptors), mainly located on the wall of the aorta and the carotid arteries. These receptors interface with the sympathetic and parasympathetic motor neurons of the n. ambiguus and the n. tractus solitarius to generate the required ANS responses. To allow blood pressure to increase in emergency situations, the sensitivity of the baroreflex is kept variable; substantial within-participant variation in the BRS over time is found, as well as large between-participant differences (DiRienzo, Parati, Radaelli, & Castiglioni, 2009). Classical approaches to measure BRS have manipulated baroreceptor firing through pharmacological agents that affect blood pressure and by lower body negative pressure, forced expiration against resistance (the Valsalva maneuver), or a neck suction/pressure cuff over the carotid baroreceptors. Changes in mean arterial blood pressure are then regressed on the changes in heart rate to obtain the cardiac BRS, which is defined as the change in heart rate induced by a fixed rise in blood pressure. These methods assess the integrated effects of baroreflex-induced changes in sympathetic vascular activity and sympathetic and vagal cardiac activity.

To selectively index the cardiac vagal component of the baroreflex loop, two methods are available. Both need the continuous time series of IBIs as well as the beat-to-beat changes in blood pressure that can be obtained from intra-arterial pressure recordings or noninvasively from the "Finapres" vascular unloading technique to determine finger arterial pressure (Langewouters, Settels, Roelandt, & Wesseling, 1998). In the sequence method, all occurrences of three or more consecutive beats are identified with progressive increases/decreases in systolic blood pressure of at least 1 mm Hg that are followed by progressive increases/decreases in the IBI. The BRS is computed as the mean slope of the regression line relating changes in systolic blood pressure to changes in IBI (Steptoe & Sawada, 1989). In the spectral method, the BRS can be obtained by calculating the strength of the linear coupling (coherence) between the beat-to-beat fluctuations in the systolic blood pressure and the IBI time series in the low-frequency (0.04-0.15 Hz) band (Robbe et al., 1987). Because the effects of cardiac sympathetic activity are again too slow to follow the rapid beat-to-beat changes in blood pressure, changes in the cardiac BRS are mainly thought to reflect changes in cardiac vagal activity. A depressed BRS (<3 ms/mm Hg) was shown to increase the risk for cardiac mortality by 2.8 times, independent of left heart failure and mostly by increasing sudden death (La Rovere et al., 1998).

Measuring Sympathetic Activity

The golden standards to measure SNS activity in humans are the direct microneurographic recording of action potentials from superficial nerves innervating the skeletal muscle (MSNA) or the skin (SSNA) (Hagbarth, Hallin, Wallin, Torebjork, & Hongell, 1972; Hagbarth et al., 1968; Svedenhag, Wallin, Sundlof, & Henriksson, 1984; Wallin, 1984; Wallin, 2004) or the measurement of spillover of

the postganglionic neurotransmitter norepinephrine using radioactive tracers (Eisenhofer, 2005; Esler et al., 1988; Esler & Kaye, 2000a; Kingwell et al., 1994). The advantage of norepinephrine spillover is that it can be measured on an organ-to-organ basis, which allows separate measurement of, for instance, renal, lung, or cardiac sympathetic activity (Eisenhofer, 2005). This is important because the notion of a single emergency SNS response that affects all organs to the same extent has proven untenable. In some circumstances, like exercise, the SNS acts as a "unitary system," but in many other situations it is capable of differentiated regulation of its activity to separate organs to a substantial degree (Hjemdahl, Freyschuss, Juhlin-Dannfelt, & Linde, 1984).

Much less invasive measurements of norepinephrine and/or its metabolites in antecubital venous blood are possible by venipuncture or by assessing the excretion of norepinephrine and/or metabolites in urine. However, concerns have been raised about differences in intraneuronal vesicular storage and leakage, reuptake, extraneuronal clearance, and urinary filtration/secretion that may (severely) distort the relation between actual SNS activity and plasma and urine norepinephrine concentrations (Eisenhofer, Kopin, & Goldstein, 2004; Esler et al., 1990; Goldstein et al., 2003). Only a very small proportion of norepinephrine released from sympathetic nerves reaches the bloodstream, and the final plasma levels depend on an unknown mixture of changes in the rate of release, as well as on the rate of removal by tissues. Plasma epinephrine levels reflect neural outflow to the adrenal medulla rather well (Goldstein et al., 2003), but the substantial clearance of adrenaline by adrenoceptors in the tissues, which further increases during vasoconstriction, causes antecubital plasma levels to be much lower than the arterial levels (Hjemdahl, 1990). Nonetheless, venous epinephrine levels show systematic increases to psychosocial stress that seem to selectively enhance adrenomedullary activation over general sympathetic nerve activation.

As was true for the PNS, most human studies of parasympathetic activity have focused on the effects of sympathetic activity on the innervated organs rather than on activity per se. Sympathetic nervous system effects can be measured using pharmacological blockade of either α-receptors (e.g. phentolamine) or β-receptors (e.g. propranolol) or even specific β1- (e.g. metoprolol) or β2-adrenergic (ICI 118-551) receptors. This has been extensively done for the assessment of cardiac and vascular sympathetic activity. Cardiac sympathetic activity, for instance, is estimated as the difference between heart period in the unblocked state and during complete blockade of cardiac sympathetic effects (Berntson et al., 1994a; Berntson et al., 1994b; Cacioppo et al., 1994; Lewis, Nylander, Gad, & Areskog, 1980; Shi, Stevens, Foresman, Stern, & Raven, 1995).

Skin SNS effects can be noninvasively measured by the activity of the eccrine sweat glands. Acetylcholine release from the preganglionic sympathetic nerves increases the activity of the sweat glands, which in turn increases the conductance of an applied current across the skin (Foster & Weiner, 1970; Fowles, 1986). Because sweat glands are at the highest density in palmar and plantar regions, approximately 400/mm², most researchers measure skin conductance at these sites. Electrodermal activity incorporates both slow tonic shifts in basal skin conductance level (SCL) and more rapid phasic transient events, that is, skin conductance responses (SCRs), which are also referred to as galvanic skin responses (Boucsein, 1992; Dawson et al., 2000; Fowles, 1986). The frequency of the nonspecific SCRs (nsSCRs) is termed electrodermal lability (Mundy-Castle & McKiever, 1953). Both SCL and nsSCRs have been shown to be influenced by emotional stress (Boucsein, 1992; Critchley, 2002; Schell, Dawson, & Filion, 1988). The test–retest reliability coefficients over time periods encompassing 1 day to a year for SCL levels ranged from 0.40 to 0.85 and from 0.40 to 0.76 for nsSCRs (Freixa i Baque, 1982; Iacono et al., 1984; Schell et al., 1988; Schell, Dawson, Nuechterlein, Subotnik, & Ventura, 2002; Vossel & Zimmer, 1990).

The salivary glands are also innervated by the ANS, and salivary α-amylase secretion (sAA) has been suggested to be a noninvasive marker for SNS activity (Nater et al., 2009). Salivary amylase is a digestive enzyme that breaks down insoluble starch into soluble maltose and dextrin. It comprises approximately 30 percent of total protein secretion from the parotid glands, submandibular glands, and sublingual glands, as well as from minor glands in the submucosa underlying the lips, cheeks, and palate (Humphrey & Williamson, 2001). Various studies have revealed that conditions known to evoke sympathetic activation uniformly increase sAA secretion, including stressful academic examination (Bosch, de Geus, Ring, Nieuw Amerongen, & Stowell, 2004; Chatterton, Jr., Vogelsong, Lu, Ellman, & Hudgens, 1996), stressful computer games (Skosnik, Chatterton, Jr., Swisher, & Park, 2000; Takai et al., 2004), watching a stressful video (Bosch et al., 2003b), mental arithmetic test (Noto, Sato, Kudo, Kurata, & Hirota, 2005), cold pressor test (van Stegeren, Wolf, & Kindt, 2008), and the Trier Social Stress Test (Nater et al., 2006; Nater et al., 2005; Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004). Moreover, administration of the β-adrenergic antagonists reduces sAA concentration in unstimulated whole mouth saliva (Nederfors & Dahlof, 1992) and attenuates the stress-induced increases in sAA concentration (Van Stegeren, Rohleder, Everaerd, & Wolf, 2006).

Unfortunately, most studies used an inadequate methodology that was validated for cortisol research but is not suitable for sAA measurements (Bosch, Veerman, de Geus, & Proctor, 2011). Even more problematic is that the salivary glands are innervated by both branches of the ANS, not just the sympathetic branch (Proctor & Carpenter, 2001). Parasympathetic activity can influence sAA concentrations: (1) via α-amylase release from glands that are solely or mainly parasympathetically innervated (e.g. the palate and sublingual glands); (2) via synergistic sympathetic–parasympathetic interactions whereby parasympathetic activity amplifies sympathetic effects; and (3) via the effects of (parasympathetically-mediated) salivary flow rate (Bosch et al., 2011). In fact, Bosch et al. (Bosch et al., 2003b) showed that a passive-coping stressor that evoked parasympathetic activation (viewing a surgical video), as measured by increases in salivary flow and RSA, also evoked a strong sAA release, which was much larger than the release during a stressor that elicited a dominant cardiac sympathetic activation (a time-paced memory task). The important role of the PNS in sAA and protein secretion invalidates the use of sAA secretion as an exclusive read-out of sympathetic activity.

Pagani and coworkers have advanced the notion that a single ratio, the power of the heart period time series in the LF band divided by the power in the HF band, may capture changes in the ratio of sympathetic to vagus nerve traffic to the heart (Malliani et al., 1991; Montano et al., 1994; Pagani et al., 1997; Pagani et al., 1986; Pagani et al., 2000). The idea behind the LF/HF ratio is that, during sympathetic activation, LF and HF power both decrease as does the TP, but the decrease in LF power relative to the decrease in total power is less strong than that in HF power, whereas the reverse occurs during vagal activation, where the increase in LF power relative to the increase in total power is less strong than that in HF power. Expressing the spectral components in absolute units prevents the appreciation of this fractional redistribution of the power across the LF and HF bands. This information is regained when LF and HF are expressed as a ratio, or when LF and HF power are measured in normalized units (LFnu), which represent the relative value of each power component in proportion to the TP minus the VLF/ULF components (Burr, 2007).

The predictive power of LF power for CVD is beyond question (Bigger et al., 1993; Dekker et al., 2000; Tsuji et al., 1996). However, the use of the normalized LF power as a potential measure of cardiac sympathetic control is the subject of controversy (Eckberg, 1997; Goedhart, Willemsen,

Houtveen, Boomsma, & de Geus, 2008b). The strongest concern about the validity of the LF/HF ratio is provided by those studies that directly compare it against invasive measures of sympathetic activity, such as peroneal muscle nerve activity or cardiac norepinephrine spillover. Although some studies did report a correlation of these measures with the LF/HF ratio (Pagani et al., 1997), most studies did not find this correlation across a range of clinical contexts (Grassi et al., 1999; Kingwell et al., 1994).

Together with electrodermal activity, measurement of cardiac contractility is currently the preferred noninvasive method to measure SNS activity. Contractility is influenced only by the sympathetic branch of the ANS because there is an abundance of functional β-adrenergic receptors on the human ventricle but no ACh receptors. Activation of the β-receptors exerts inotropic effects on the cardiac muscle through the opening of calcium channels in the membrane, as well as of the T tubules of the muscle fibers. The calcium influx increases contractile force and contraction speed of the ventricle. This increased contractility is reflected in a larger ejection fraction of the left ventricle. The ejection fraction reflects the ratio between the stroke volume and the end diastolic volume. The ejection fraction can be obtained from recordings of end diastolic and end systolic volumes (the difference equals the stroke volume) by echocardiography or (contrast) magnetic resonance imaging (Malm et al., 2004).

Contractility is also reflected in a more rapid start of the ejection phase after the onset of ventricular depolarization, a time interval referred to as the preejection period (PEP). The PEP can be measured by using impedance cardiography, in which a HF alternating current is introduced across the thorax by electrodes at the level of the neck and belly (Sherwood et al., 1990). Electrodes at the level of the top and bottom of the sternum measure the changes in the impedance of the enclosed thorax column (dZ). The first derivative of the pulsatile changes in transthoracic impedance (dZ/dt) is called the impedance cardiogram (ICG), and it reflects the momentary changes in aortic blood flow during the systolic phase. From the combined ECG and ICG, the PEP can be derived as the time interval between the onset of ventricular depolarization (QRST-onset) and the opening of the semilunar valves (sharp upstroke in the dZ/dt). Within-participant changes in PEP reliably index changes in β-adrenergic drive to the left ventricle, as was shown in laboratory studies that employed manipulations known to increase cardiac sympathetic activity such as epinephrine infusion, amyl nitrite inhalation, mental stress, and exercise. These manipulations systematically decrease PEP (de Geus et al., 2007; Houtveen et al., 2005; Krzeminski et al., 2000; Mezzacappa et al., 1999; Miyamoto et al., 1983a; Nelesen et al., 1999; Newlin et al., 1979; Richter et al., 2009; Schachinger et al., 2001; Sherwood et al., 1986; Smith et al., 1989a). In addition, pharmacological blockade of cardiac sympathetic effects results in the expected prolongation of the PEP (Berntson et al., 1994a; Cacioppo et al., 1994; Harris, Schoenfeld, & Weissler, 1967; Schachinger et al., 2001; Winzer et al., 1999), whereas PEP is hardly affected by blockade of cardiac vagal effects (Berntson et al., 1994a; Cacioppo et al., 1994; Martinsson, Larsson, & Hjemdahl, 1987).

Since the PEP can be reliably obtained noninvasively from only 5–7 spot electrodes, a number of ambulatory devices are available that allow recording of the PEP in naturalistic settings (Cybulski, 2000; Martinsson et al., 1987; Nakonezny et al., 2001; Sherwood, McFetridge, & Hutcheson, 1998; Willemsen et al., 1996). The only caveat in using PEP as an index of changes in cardiac sympathetic activity is its sensitivity to preload and afterload effects. Cardiac contractility can increase independently from sympathetic effects when the stretch of the myocardial muscle fibers increases through the Frank-Starling mechanism. Thus, increases in end diastolic volume (preload) can shorten the PEP in the absence of increased sympathetic activity, leading to the erroneous suggestion of

increased cardiac sympathetic effects. The reverse problem occurs when the pressure in the aorta is increased (afterload) in the presence of unchanged sympathetic activity. Because it takes longer for the aortic valves to open, the PEP becomes longer, erroneously suggesting decreased cardiac sympathetic effects. Postural changes have a major effect on preload and afterload and indeed lead to paradoxical responses of the PEP. Head-up tilting from supine to upright systematically prolongs the PEP (Frey & Kenney, 1979; Lewis, Rittgers, Forester, & Boudoulas, 1977; Ovadia, Gear, Thoele, & Marcus, 1995), and longer PEPs have also been demonstrated when participants go from supine to sitting to standing (Houtveen et al., 2005; Sherwood & Turner, 1993; Waldstein, Neumann, & Merrill, 1998). Clearly, posture needs to be taken into account when using PEP as a measure of sympathetic activity.

When posture is controlled, the PEP has been shown to be a stable individual characteristic. In the laboratory, test–retest correlations between 0.45 and 0.88 have been found for baseline and stress-task levels of PEP across retest intervals ranging from 28 days to 3 years (Burleson et al., 2003; Matthews, Salomon, Kenyon, & Allen, 2002; Willemsen et al., 1998). For ambulatory 24-hour PEP, high stability ($.67 < r < .93$) has been found across a few days (Vrijkotte et al., 2004), as well as across a much longer period of 2–5 years (Goedhart et al., 2006). It is important to note that the between-participant differences in PEP reflect the extent to which participants differ in the degree of sympathetic effects on their cardiac contractility. These effects are likely to be highly correlated with differences in sympathetic activity, but the correlation is not perfect. Inotropic responses to norepinephrine and circulating epinephrine will be modulated by individual differences in the effectiveness of the cardiac β1- and β2-adrenergic receptors. Density, affinity, and distribution of these receptors may show large individual differences (Liggett, 1995; Liggett et al., 2006). These individual differences in receptor status may, for instance, lead to a paradoxically long PEP in patients with high levels of cardiac sympathetic nerve activity who have very low ventricular β-receptor densities. In healthy participants, however, a high interindividual correlation (0.82) was found between PEP levels and cardiac sympathetic effects as assessed through dual blockade (Cacioppo et al., 1994).

Although the PEP provides a reliable non-invasive index of SNS activity, algorithm detection and even visual detection of its crucial landmarks can be very difficult or even impossible due to noise in the ICG signal caused by movement (Berntson, Lozano, Chen, & Cacioppo, 2004; Lozano et al., 2007; Willemsen et al., 1996). The initial systolic time interval (ISTI) has recently been proposed as an easier to measure alternative to the PEP. Changes in cardiac contractility may not only be reflected in the time it takes the left ventricle to build up sufficient force to open the aortic valve (B-point) but also in the time it takes to reach peak ventricular ejection or maximal aortic diameter (dZ/dt-min peak or C-point), thus extending the PEP (ventricular depolarization plus isovolumetric contraction) with the rapid part of the ejection phase. The ISTI is therefore defined as the interval between the R-wave peak and the clear dZ/dt-min peak. The latter can be detected with much more fidelity than the B-point, which is often a (subtle) inflection defined by a zero-order crossing in the second derivative of the ICG rather than a true minimum. Two groups have independently confirmed that the ISTI is a significant predictor of both the R-wave peak to B-point (RB) interval as well as the PEP (Berntson et al., 2004; Lozano et al., 2007; Meijer, Boesveldt, Elbertse, & Berendse, 2007; Meijer, Boesveldt, Elbertse, & Berendse, 2008). Thus adequate estimation of sympathetic changes in contractility could be achieved by the detection of the two most salient features in the ECG and ICG, the R-wave peak and the dZ/dt-min peak respectively. Additional research, measuring PEP and ISTI

simultaneously and preferably in a blockade design, is needed to further support the validity of the ISTI as an alternative non-invasive SNS measure.

Another, seemingly somewhat forgotten, alternative to measure cardiac SNS activity is the amplitude of the T-wave in the ECG (TWA). The T-wave is the asymmetrical wave in the ECG that comes after the QRS-complex and typically lasts approximately 150 msec. It reflects ventricular repolarization (Abildskov, Burgess, Urie, Lux, & Wyatt, 1977; Burgess, 1979; Haarmark et al., 2010; Lozano et al., 2007; Randall & Hasson, 1977) in which the sympathetic nerves play an important role (Abildskov, 1985; Lozano et al., 2007). Decreases in TWA and even TWA inversion was seen to occur after local stimulation of the stellate sympathetic ganglia in dogs (Anitchkov & Vedeneyeva, 1961; Yanowitz, Preston, & Abildskov, 1966), intracoronary infusion of adrenaline in dogs (Barger , Herd, & Liebowitz, 1961), subcutaneous or intramuscular administration of adrenaline in man (Hartwell et al., 1942; Katz, Hamburger & Lev, 1931; Levine, Ernstene & Jacobson, 1930) and in reaction to pharmacological manipulation (isoproterenol) in man (Contrada et al., 1989; Contrada, Dimsdale, Levy, & Weiss, 1991; Guazzi et al., 1975). Importantly, these functional TWA decreases could be reversed by beta-blockade with propanolol (Contrada et al., 1989; Fukudo et al., 1992; Furberg, 1967; Furberg, 1968; Guazzi et al., 1975; Noskowicz & Chrzanowski, 1968; Rau, 1991). Additionally, TWA has been shown to be a useful indicator of cardiac SNS activity during laboratory testing of sympathetic stress reactivity (Furedy, Heslegrave, & Scher, 1984; Furedy & Shulhan, 1986; Furedy, Szabo, & Peronnet, 1996; Guazzi et al., 1975; Heslegrave et al., 1979; Matyas & King, 1976; Scher, Furedy, & Heslegrave, 1984).

In spite of these findings, TWA has not often been used, in part due to the "competition" from the more popular PEP that entered the literature at about the same time. However, a unresolved threat to the validity of TWA as a "pure" measure of SNS activity is the apparent contribution of vagal activity to decreases in TWA (Annila, Yli-Hankala, & Lindgren, 1993; Contrada, 1989; Contrada et al., 1989; Contrada et al., 1991; Dauchot & Gravenstein, 1971; Schwartz & Weiss, 1983; Weiss, Del, Reichek, & Engelman, 1980), although Gauzi and colleagues (Guazzi et al., 1975) found no changes in TWA in response to decreases in PNS activity by administration of atropine and Kline and colleagues (Kline, Ginsburg, & Johnston, 1998) did not find a significant correlation between TWA and RSA. Additional research, measuring TWA, RSA and PEP simultaneously, and preferably in a blockade design, is needed to further support the validity of the TWA as a non-invasive SNS measure.

ANS measurement in ambulatory monitoring studies

The above review showed that there are a number of invasive measures that have good content and criterion validity for measuring SNS and PNS activity. However, these invasive measures are suitable only for the investigation of individual differences in ANS responses to very standardized and controlled laboratory conditions, many requiring the equipment (or nearby safety precautions) of medical hospital facilities. Unfortunately, individual differences in ANS responses to standardized laboratory/hospital conditions, particularly conditions that should induce psychosocial stress, do not seem to transfer to individual differences in ANS responses to actual real-life stressors because the association between laboratory and ambulatory measurements is moderate at best (Gerin, Rosofsky, Pieper, & Pickering, 1994; Kamarck & Lovallo, 2003; van Doornen, Knol, Willemsen, & de Geus, 1994). It is likely that the psychological and physiological processes induced by laboratory stress are only a poor reflection of the actual processes in everyday real-life stress situations. Perhaps as a

consequence, the predictive value of ANS responses to laboratory challenges for future CVD is low, with the response to a challenge hardly contributing to the prediction of disease when basal levels have been taken into account (Barnett, Spence, Manuck, & Jennings, 1997; Carroll et al., 1998; Coresh, Klag, Mead, Liang, & Whelton, 1992).

As an alternative to bringing "everyday situations to the laboratory," researchers have increasingly tried to bring the "laboratory to everyday situations." This is done by using miniaturized versions of the recording equipment for non-invasive ANS measures that allow prolonged ambulatory monitoring in naturalistic settings (Fahrenberg, Myrtek, Pawlik, & Perrez, 2007; Houtveen & de Geus, 2009; Wilhelm & Grossman, 2010). The expectation is that ambulatory measurement of physiological levels and reactivity in the natural environment will lead to better prediction of morbidity and mortality. For blood pressure, the added value of the ambulatory approach has already been demonstrated (Mallion, Baguet, Siche, Tremel, & de Gaudemaris, 1999; Palatini & Julius, 2004; Verdecchia, 2000; Verdecchia, Schillaci, Reboldi, Franklin, & Porcellati, 2001).

An important question then becomes which of the non-invasive measures for PNS and SNS activity can be optimally employed for ambulatory monitoring. A major demand is that the measure is non-invasive. Secondly, we want the measure to be a relatively "pure" indicator of a single ANS branch only, so changes in the measure either reflecting predominantly PNS activity or predominantly SNS activity. Three additional considerations are (1) established validity, (2) feasibility, both in terms of technical implementation and participant-tolerance, and (3) cost, both in terms of equipment/consumables and labor for data cleaning. The latter becomes increasingly important if we want to study PNS and SNS activity in samples that are sufficiently large to allow epidemiological research and genetic analyses. Using the non-invasive measures listed in table 1 we select an optimal choice for ambulatory monitoring of PNS and SNS activity in terms of the balance between established validity and feasibility. To a minor extent we will also consider cost.

For PNS activity, we can choose from RSA, other HRV measures, or BRS. As HRV measures other than RSA reflect both SNS and PNS activity, our options rapidly reduce to the choice between RSA and BRS. Although changes in BRS are theoretically and empirically solidly linked to vagal activity, the non-invasive registration of BRS requires a beat to beat registration of blood pressure through a finger cuff measuring either pressure or photoplethysmographic changes in the fingertips. This (pulsating) cuff attached to the finger strongly hampers the daily routine of participants, and data collection and reduction is both expensive and very costly. Ambulatory recording of PNS activity should therefore preferably turn to RSA measures.

Two RSA measures, HF and RMSSD are highly feasible for prolonged recording across real life settings and are well-tolerated by participants as they only require a type II lead ECG recording, which can be obtained from chest bands or a minimum of three surface electrodes. The power in the high frequency band has a more clear-cut theoretical link to vagal activity, but empirically RMSSD proved highly correlated to HF across a wide range of conditions and its scoring is less labor-intensive and does not require a correct interpolation of missing IBIs (Goedhart et al., 2007). In principle, this makes RMSSD preferable to the third RSA measure, pvRSA, which requires the additional recording of the respiration signal, which increases both participant and experimenter burden. Yet, it has the clear advantage of allowing correction of pvRSA for both between-participant differences and within-participant changes in respiratory behavior which has been advocated as necessary for its validity as a proxy for (changes in) vagal tone by a variety of authors (Houtveen, Groot, & de Geus, 2006; Ritz et al., 2006). As ambulatory respiratory behavior can be easily captured from recordings of the ICG, a

signal paramount to what we will recommend for SNS activity below, we conclude that RMSSD and pvRSA are the best options for ambulatory monitoring of PNS activity.

For SNS activity, we can choose from urinary catecholamines, sAA, LF/HF ratio, SCL, TWA, and PEP. Urinary catecholamines is still a good measure of sympathetic activity, even with the caveats in validity mentioned before, but they only provide an aggregate measure over prolonged periods of time (e.g. an entire 24-hour period). They further lack precision in that the SNS activity cannot be linked to specific psychological or physiological stressor, although comparisons of workday versus resting day are still meaningful. Urine collection is tolerated rather well but logistics and costs of biochemical analyses make it challenging to use this method on larger scale samples.

Taken the current state of knowledge, perhaps the least attractive of these measures is sAA, in spite of its rapidly gaining popularity. Strong concerns have been voiced regarding its validity (Bosch et al., 2011) particularly when using the Salivette collection method. The alternative method of collection by passive 'drooling' has not been tested in an ambulatory setting, but a priori user-friendliness and tolerance does not seem very high. Additionally, although the literature is still inconclusive at present, it may also be strongly dependent on vagal influences, which refutes sAA as a relatively pure index of SNS activity.

Although highly feasible and cost-effective –it only requires a three lead ECG recording- the LF/HF ratio was discarded as a pure SNS measure due to an unsound theoretical underpinning and a complete lack of empirical criterion validity. In contrast, electrodermal measures like SCL and the nsSCL have a very good theoretical basis and criterion validity, but here it is feasibility in ambulatory monitoring that proves challenging. The typical electrode placement at the palm of the hand restricts individuals in their normal daily routine during 24-hour recordings. Technical challenges compound this problem as movement of the hand affect signal quality and changes in temperature make it hard to interpret SCL outside the laboratory. Alternative electrode placements at the soles of the feet are even less practical and electrodes at the sternum were shown to be relativity unresponsive to psychological stimuli (Freedman, 1989).

Currently, the PEP is the measure of choice for ambulatory recording of SNS activity as its validity is relatively undisputed and ICG measurements using four spot electrodes have been shown to be well tolerated (Kupper, Willemsen, Boomsma, & de Geus, 2006; Vrijkotte et al., 2004). In spite of validity and practical feasibility, two clear downsides of the PEP are that it requires laborious manual inspection despite data reduction strategies such as large scale ensemble averaging (Riese et al., 2003) and that the PEP is sensitive to confounding by preload and afterload effects that require careful co-registration of posture and physical activity as well as the appropriate activity/posture stratified analyses. An alternative ambulatory measure of SNS activity would be most welcome.

The TWA remains untested as such an alternative. TWA relies only on the ECG signal. If valid as an SNS index, a simple ECG recording, yielding TWA and RMSSD, could provide a new way to index both ANS branches with minimal burden and costs. Pending that, combined ECG and ICG recordings from 5 to 7 spot electrodes, yielding PEP and pvRSA, remains the preferred way to assess SNS and PNS activity in ambulatory recordings.