Ambulatory recording of within- and between-subject variation in autonomic nervous system activity

The central theme of this thesis was the non-invasive measurement of within- and between-subject variation in autonomic nervous system activity. Reliable and valid non-invasive measures are essential to ambulatory monitoring, which in turn is crucial for the ecologically valid study of the link between biology and behavior. In this final chapter, I first summarize the main results from the laboratory and two ambulatory studies presented in this thesis. Next, I describe the implications of these results for future ambulatory monitoring of ANS function.

Comparability of different measures of cardiac vagal control

Heart rate variability measures within the respiratory frequency range, also called respiratory sinus arrhythmia (RSA), provide us with a window on the modulation of heart rate (HR) by the parasympathetic branch of the autonomic nervous system. (Berntson *et al*, 1997; Task Force of the European Society of Cardiology the North American Society of Pacing, 1996). Cardiac parasympathetic control is paramount to the electrical stability of the heart (Ando *et al.*, 2005; Hull, Jr. *et al.*, 1990; Levy & Schwartz, 1994; Vanoli *et al.*, 1991) and lower levels of RSA have been shown to independently predict cardiac disease and cardiac mortality (Bigger, Jr. *et al.*, 1993; Dekker *et al.*, 1997; Dekker *et al.*, 2000; Hayano *et al.*, 1991; Lombardi *et al.*, 1987; Nolan *et al.*, 1998; Saul *et al.*, 1988; Singer *et al.*, 1988; Singh *et al.*, 1998; Tsuji *et al.*, 1996). The fact that RSA may be readily obtained from noninvasive measurement of the heart period, either with or without simultaneously measuring respiration, makes it a promising measure for large-scale ambulatory studies.

The second chapter of this thesis examined whether the assessment of individual differences in ambulatory assessed RSA is sensitive to the method used or, otherwise formulated, whether "high tech" pvRSA or HF power (labor-intensive) is superior to "low tech" RMSSD (readily obtained). The answer is a resounding no. The correlations between the three measures of RSA were high (r > .80) and these correlations remained stable over time and within different ambulatory conditions (sleep, daytime sitting and daytime standing/walking). This demonstrates that these measures are indications of the same underlying construct. None of the three RSA measures stood out in terms of short-term reliability or temporal stability over a period of more than 3 years. Excellent temporal stability was found for sleep (.72 - .81) and sitting (.68 -. 80) levels over an average period of 3 years and 4 months. However, temporal stability for periods of standing/walking was rather moderate, ranging from .44 to .57. These results underscore the need to take physical activity into account when interpreting ambulatory RSA measures (Grossman *et al*, 2004).

Independent of ambulatory condition, large differences in mean respiratory frequency and HR were found. We were concerned that such differences might distort the correlations between the three RSA measures, because respiratory frequency is known to affect RSA independent of cardiac vagal control and may do so differently for time- and frequency-based measures. For instance, breathing at a low respiration rate (RR) will tend to make the HF

power underestimate the RSA (visible mostly in LF power), whereas the pvRSA and RMSSD are not susceptible to this problem. Recently, Berntson and colleagues have also expressed mathematical concerns about the RMSSD, which they argued to be too sensitive to HR for a reliable measurement of RSA (Berntson *et al.*, 2005). Both theoretical concerns were greatly mitigated by empirical observation. Differences in mean resting HR or mean RR did not affect the correlations between the various RSA measures; the RSA measures were as highly correlated in a group with a mean HR of 83 bpm as in a group with a mean HR of 65 bpm, and as highly correlated in groups with a mean RR of 15 (range 14-16) versus 18 (range 17-20) breaths per minute. This contradicts the idea that one of these RSA measures is relatively more sensitive than the others to confounders such as individual differences in RR or in HR.

Taken together, our results suggest that each of the measures studied – pvRSA, HF power or RMSSD – can be used as a reliable and stable measure of ambulatory RSA. Since the different RSA measurement strategies have varying specific advantages, for instance, providing additional information on RR or on (very) low frequency power, the choice for a specific measure should be based on the exact research questions.

Temporal stability of ambulatory stroke volume and cardiac output

Chapter 3 focused on two cardiovascular measures, stroke volume (SV) and cardiac output (CO), that are routinely derived from impedance cardiography in laboratory settings, but have been used with great reluctance in ambulatory studies. That is unfortunate because these indices offer great potential to further our understanding of the way chronic cardiovascular activation in response to naturalistic events may contribute to cardiovascular disease (Carrasco & Van de Kar, 2003; Davis, 1992; Federenko *et al.*, 2004; Fritz & Levine, 1951; Imaki *et al.*, 1995; Nijsen *et al.*, 2001; Sapolsky, 2003; Sherwood *et al.*, 1986; Van de Kar & Blair, 1999). The reluctance to use ambulatory SV and CO is based on a number of considerations. Ever since the landmark publication of the guidelines committee (Sherwood *et al.*, 1990) there has been a general concern about the validity of impedance-derived SV and CO. A major reason is that the ICG waveform shows large individual variation which may depend on a multitude of factors including variation in electrode placement, anatomical differences in the size and shape of the thorax and the heart and other unknown factors. Amongst others, this can hamper reliable detection of the B-point in the first derivative of thoracic impedance signal which is used to compute the dZ/dt(min), a crucial parameter in SV computation. Doubts about the validity of impedance-derived SV and CO were reinforced by a meta-analysis of all studies comparing them to SV and CO obtained from different invasive methods (Raaijmakers *et al.,* 1999). A total of 154 studies comparing the ICG to a reference method (dye dilution, indirect Fick method, echocardiography) were analyzed. An overall correlation of .53 (95% CI: .43-.62) was found for CO values, which was increased to .67 if repeated measures were averaged. This points to reasonable validity for research on large groups, but for diagnostic interpretation, a correlation of .53 may not be sufficiently accurate.

Additional concerns arise when SV and CO are measured in an ambulatory setting. Shifts in posture and physical activity strongly affect cardiac sympathetic drive as well as cardiac afterload and preload which all have an impact on SV (Cacioppo *et al.*, 1994a; Fallo *et al.*, 1994). In addition, postural changes are expected to alter the relative position of measuring

and current electrodes, the exact shape of the enclosed thorax column, and the resulting basal thorax impedance (Z_0) (Grossman & Kollai, 1993; Mohapatra, 1981; Toska & Walloe, 2002) compromising valid use of the Kubicek equation (Raison & Miller, 2003) which assumes these to be stable.

In spite of the concerns above, a number of studies have demonstrated that, at a group level, ambulatory SV and CO are well-behaved across a 24-hr period (Riese *et al.*, 2003; Vrijkotte *et al.*, 2004; Willemsen *et al.*, 1996). SV and CO both increase with physical activity, whereas SV increases during the night to compensate for the lowered HR, leaving CO at comparable or only slightly lower levels to sitting at rest. The crucial question, however, is whether absolute levels or within-day changes in ambulatory SV and CO tap into stable individual differences in cardiac regulation. To address this question long-term temporal stability of these measures was assessed in chapter 3. We selected data only from periods with fixed posture and low physical activity and we used both the zero-crossing of the ICG and the B-point to compute SV and CO. Even so, only moderate stability was found for SV and CO, in the range of .29 to .46, which is in range with results of previous laboratory and ambulatory studies (Barnes *et al*, 2002; Barnes *et al*, 2004; Matthews *et al*, 2002).

Although the ICG waveforms can differ greatly between subjects they are reasonably stable within a subject. A common feeling, therefore, is that within-subject changes in impedance derived SV and cardiac output are probably reliable, even if absolute values are not. To address this 'feeling', we also computed percentual change scores for each individual on test and retest days, using the awake periods as the "active" state and sleep levels as the resting state. Again, only modest stability of SV and CO reactivity was found (.12 - .45), which, if anything, was lower than that for the absolute SV and CO levels.

A major cause for the low stability of both absolute SV values and change scores was the L_0^2/Z_0^2 ratio. In principle this should be a stable trait, at least within individuals, since the amount of impedance per area body surface is not expected to change that much. In contrast, the product of dZ/dt and LVET can be reasonably expected to change over time, due to true changes in cardiac control. In the actual observations, however, the L_0^2/Z_0^2 ratio proved to be less stable than the product of dZ/dt and LVET, particularly at night.

I conclude that ambulatory SV and CO may capture group differences, for instance when subjects with chronic stress are compared to non-stressed subjects, but are not sufficiently reliable to index individual differences in correlational designs, for instance in genetic studies. Having said this, it must be kept in mind that tracking coefficients for a major CVD risk factor like blood pressure itself are also not much higher than .5 (Hottenga *et al.*, 2005; Palti *et al.*, 1988; Woelk, 1994).

Ambulatory indices of sympathetic nervous activity: heart and skin

In addition to SV and CO, chapter 3 also addressed the temporal stability of ambulatory PEP. PEP is an index of contractility of the left ventricle. Contractility is influenced only by the sympathetic part of the ANS: there is an abundance of functional adrenergic receptors on the ventricle but no acetylcholine receptors. In psychophysiology, the PEP has become the first measure of choice to index cardiac sympathetic control. Its validity in within-subject designs has been shown in many studies that employed manipulations known to increase cardiac

sympathetic activity like epinephrine infusion, amyl nitrite inhalation, mental stress and exercise. These manipulations systematically decrease PEP (Berntson *et al.*, 1994; de Geus *et al.*, 2007; Krzeminski *et al.*, 2000; Kupper *et al.*, 2006; Mezzacappa *et al.*, 1999; Miyamoto *et al.*, 1983; Nelesen *et al.*, 1999; Newlin & Levenson, 1979; Houtveen *et al.*, 2002; Houtveen *et al.*, 2005; Schachinger *et al.*, 2001; Sherwood *et al.*, 1986; Smith *et al.*, 1989; Svedenhag *et al.*, 1986). In addition, pharmacological blockade of cardiac sympathetic effects results in the expected elongation of the PEP (Cacioppo *et al.*, 1994a; Harris *et al.*, 1967; Schachinger *et al.*, 2001; Winzer *et al.*, 1999), whereas PEP is hardly affected by blockade of cardiac vagal effects (Cacioppo *et al.*, 1994a; Martinsson *et al.*, 1991). Between-subject differences in absolute PEP have been shown to closely reflect individual differences in ß-adrenergic inotropic drive (Cacioppo *et al.*, 1994a). In a study of 10 female undergraduate students, a high correlation was found between absolute PEP and heart period increases in response to sympathetic blockade. In further support, a significant inverse correlation between a subjects' absolute PEP and their plasma adrenaline level was found (Levi *et al.*, 1982).

The only caveat in using PEP as an index of cardiac sympathetic control 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. Thus, when increased preload occurs in the absence of increased sympathetic activity, it decreases the PEP leading to the erroneous suggestion of increased cardiac sympathetic control. The reverse problem occurs when the pressure in the aorta is increased (afterload) in the presence of increased sympathetic activity; because it takes longer for the aortic valves to open, the PEP becomes longer erroneously suggesting *decreased* cardiac control. Preload and afterload effects explain why PEP did not decrease or even increase during the cold pressor test or a hand grip test (chapter 4), both of which should lead to increased cardiac sympathetic control, and why PEP paradoxically increases with head up tilting and in going from supine to standing (Houtveen *et al.*, 2005).

In spite of this caveat PEP can still reliably track decreases in cardiac sympathetic control under conditions of increased preload and reduced afterload. During sleep preload and afterload effects would tend to shorten PEP, but instead we find it to be systematically longer during sleep compared to daytime activities (see chapters 3 and 4), which confirms similar findings in other studies (Kupper *et al.*, 2006; Vrijkotte *et al.*, 2004). This suggests that the decrease in sympathetic activity during sleep is strong enough to overcome confounding of the PEP by pre- and afterload effects. During daytime recordings, preload and afterload effects on PEP need not be a problem if they are anticipated in the design and analysis strategy of the study. When this is done, very high test-retest correlations (r = .90) across a few days are observed for ambulatory recording of both daytime and sleep PEP (Vrijkotte, van Doornen, & de Geus, 2004). In addition, as demonstrated in chapter 3, very good long-term temporal stability of the 24-hr measurements across the four daily periods is seen (.66-.81), which is, in fact, as good as the stability of PEP obtained under standardized laboratory conditions (Burleson *et al*, 2003; Matthews *et al*, 2002; Willemsen *et al*, 1998). This leads me to conclude that, provided a study-design and a data-analysis strategy that appropriately take into account the independent effects of preload and afterload, ambulatory PEP is a reliable and stable index of cardiac sympathetic control.

Using PEP as the comparison measure, chapter 4 and 5 of this thesis explored two other often used indices of SNS activity; the ratio of LF and HF power in the IBI time series and skin conductance level. Both are non-invasive measures and can, in principle, be assessed in ambulatory designs. Chapter 4 reports on the comparison of PEP with the LF/HF ratio in an ambulatory design. The idea behind the LF/HF ratio is that the LF power is sensitive to both vagal and cardiac sympathetic activity, whereas the HF power is sensitive to vagal activity only. Increases in cardiac sympathetic activity should increase the absolute LF power, but because these are often paired to reciprocal decreases in vagal activity, an actual decrease in LF power may be observed. The LF/HF ratio aims to correct this by taking parallel decreases in vagal activity into account. Although it is accepted that the ratio is sensitive to both vagal and cardiac sympathetic activity, it is considered to be relatively more sensitive to the latter. Hence it may be used as an index of cardiac sympathetic control, even if imperfect.

In spite of its widespread use, the validity of the LF/HF ratio has been the subject of continued controversy (Eckberg, 1997; Malik & Eckberg, 1998; Malliani *et al.*, 1998; Sleight & Bernardi, 1998). The results obtained in chapter 4 have forced me into the camp of the LF/HF skeptics. This is regrettable because a second non-invasive measure of cardiac sympathetic control would have been most welcome. However, the findings were very clear cut. Most subjects failed to show the expected negative correlation between PEP and the LF/HF ratio across an average of 56 separate periods during the ambulatory recording. The average within-subject correlation was only -.11. Between-subject correlations, computed across the two test days but separately for sleep, sitting and physical activity, were .07, -.13 and -.20 respectively, all non-significant. The use of normalized LF power instead of the LF/HF ratio to index cardiac sympathetic control yielded similarly disappointing results. Furthermore, only PEP, but not the LF/HF ratio showed the expected reciprocal behavior to HF power indicative of the reciprocal vagal and sympathetic cardiac control, which one expects in the majority of subjects and across the majority of ambulatory conditions. I conclude, therefore, that the evidence to support the LF/HF ratio or (normalized) LF power as potential measures of cardiac sympathetic control in epidemiology-scaled research is currently insufficient.

Chapter 5 compared PEP with two skin conductance variables, number of nonspecific skin conductance responses (ns.SCRs) and skin conductance level (SCL), indices of sympathetic activity in the skin (Boucsein, 1992; Dawson *et al.*, 2000; Fowles, 1986; Schell *et al.*, 2002; Venables & Christie, 1980). The underlying idea was that in response to many stressors the SNS acts as a unitary system, such that increased sympathetic activity is seen in multiple effector systems, including the skin and the left ventricle. As expected, during mental and physical stressors known to engage the SNS there was a decrease in PEP and an increase in the two skin conductance indices. For the skin conductance indices, overall significant between-subject correlations ($.34 < r < .55$) and within-subject correlations (mean $r = .72$) were found and about 43% of the variance in these skin conductance indices overlapped. This suggests that these skin conductance parameters both reflect SNS activity but, since the overlap is imperfect, the two skin conductance indices contain partly unique information about ongoing skin SNS activity. Since they can be extracted from the same signal, it seems prudent to assess them in parallel in future studies.

Surprisingly, no solid association was found between either of the two skin conductance indices and PEP. Between-subjects correlation between PEP and ns.SCRs and SCL was non-significant at rest and during each of the stressors. This may partly reflect individual differences at the level of the effectors that act to hide a correlation of SNS activity to the heart and the skin. The $ß_1$- and $ß_2$-receptor density on the left ventricle is known to vary strongly between individuals (Brodde *et al.*, 2006) as is the number of sweat ducts per area of skin (Sato & Sato, 1983). Yet, these two traits are important determinants of absolute PEP and SCL levels. It is thus possible that a subject with a low ß-receptor sensitivity and a high number of sweat glands may have long PEP and high SCL, whereas another subject with identical SNS activity but high ß-receptor sensitivity and a low number of sweat glands may have a shorter PEP but lower SCL.

More alarming was the ambiguous pattern of within-subject correlations where such individual differences in the heart and skin effector systems cannot play a role. A strong and significant correlation was found between stressor elicited changes in PEP and the two measures of skin conductance *only* when short periods of moderately intense exercise were included in the analyses. These led to a strong decrease in PEP and a strong increase in ns.SCRs and SCL in most subjects. Across all other stressors, the changes in PEP and ns.SCRs and in PEP and SCL, were not significantly correlated. It is unclear why SNS activity was neatly reflected in both heart and skin responses, yet failed to show coherence across these effector systems. A first explanation is that the ß1- and ß2-receptors do not respond solely to noradrenaline released from the cardiac sympathetic nerve. They are also highly sensitive to circulating catecholamines, whereas sweat gland activity is controlled by sympathetic cholinergic fibers acting on muscarinergic receptors that are insensitive to circulating catecholamines. A second explanation is that the changes in PEP across the stressors were strongly influenced by posture and blood pressure changes. Our design was intended to create substantial changes in SNS activity. For this reason we added three conditions that are known to cause increased noradrenergic tone, the cold pressor test, the hand grip test and orthostasis. Clearly, this came at the price of confounding the manipulation of SNS activity with that of changes in preload and afterload effects. In an attempt to deal with this, we recomputed the correlations post-hoc using only across the sitting conditions that did not evoke strong afterload effects (i.e. rest1, stroop, tone avoidance, recovery1-4). Still no significant correlation was found, but now a restriction of range may be at work. Future studies could readdress this issue by using emotional stressors of different intensity (e.g. by varying the financial bonus or the level of ego-threat and deliberate harassment).

Apart from the methodological explanations above, the absence of a relation between PEP and skin conductance may also reflect true differences in the activation of the various branches of the SNS during stressful tasks. That is, the underlying idea that the SNS acts as a unitary system affecting multiple effector systems simultaneously to the same extent may be too naïve. Previous studies have shown that some differentiation is known to occur such that SNS activity does not uniformly increase to all effector organs (Grassi & Esler, 1999; Wallin, 1981). Baroreflex engagement, for instance, may be a powerful source of differences in vascular/cardiac versus skin SNS activity. Unlike cardiac SNS activity, skin SNS activity is not influenced by the baroreflexes (Bini *et al.*, 1981; Vissing *et al.*, 1994; Wallin *et al.*, 1975;

Wilson *et al.*, 2001). Task induced changes in baroreflex activity, therefore, will affect PEP but not SCL and ns.SCR. The well-known individual differences in baroreflex sensitivity (Riese *et al.*, 2006; Tank *et al.*, 2001) may also partly account for the low between-subject correlations.

Adding it all up, the results for SCL and ns.SCRs at first sight seem comparable to those obtained for the LF/HF ratio. Neither LF/HF ratio nor SCL measures showed solid correlation to the PEP, either within or between subjects. The interpretation of these two sets of 'zero' correlations, however, is quite different. In contrast to the LF/HF ratio, the SCL measures did systematically change in the expected direction with changes in SNS activity. Adding the exercise condition even induced a strong significant correlation. Electrodermal activity unequivocally shows good correlation to the number of sympathetic action potentials in skin sympathetic nerves (Wallin, 1981), whereas the LF/HF ratio does not correlate to the "golden standard" of cardiac sympathetic activity, cardiac noradrenaline spillover (Alvarenga *et al.*, 2006; Kingwell *et al.*, 1994). Finally, there are very plausible physiological pathways that link sympathetic nerve activity to variation in SCL and ns.SCRs whereas the exact source of LF fluctuations in HR remains to be established. Thus, the non-overlap of skin SNS activity with that of cardiac SNS activity is not a weakness but a strength that can be exploited in future research. I suggest that the *simultaneous measurement of PEP, SCL, ns.SCR* yields a multivariate measure of SNS activity that has more explanatory power than each measure alone.

Permissive effects of cortisol on sympathetic stress reactivity

Glucocorticoids (GCs), including cortisol and corticosterone, have powerful actions on the cardiovascular system. As reviewed by Sapolsky *et al*. (2000) these effects can be permissive, stimulating, suppressive, and preparative. The stimulating, suppressive, and preparative actions all refer to the action of *stress-induced* increases in GC levels. The permissive actions of GCs are unique in that they occur before and therefore independent of, the stress-induced increase in GC levels. Specifically, tonic increases in GC levels that precede the acute exposure to stressors by hours, are hypothesized to modulate sympathetic nervous system effects on the heart and blood vessels during the stressor, such that sympathetic effects on blood pressure, cardiac output, and vascular resistance reactivity are enhanced. Such time-delayed permissive effects on cardiovascular responsivity make good evolutionary sense in light of the clear diurnal rhythm in cortisol (Burleson *et al.*, 2003). Cortisol levels begin to rise sharply a few hours before awakening suggesting that – taken the genomic delays- its augmentation of sympathetic effects is optimal during the active phase (day in primates, night in nocturnal rodents) when fight-flight responses can be essential for survival.

In spite of the theoretical attractiveness, direct evidence for permissive actions of early morning cortisol levels on human sympathetic and cardiovascular stress-reactivity in the course of the day is currently lacking. In chapter 6 we tested the permissive effects of cortisol in two different ways. First, we tested whether the natural occurring variation in the early morning levels in cortisol could predict sympathetic and cardiovascular reactivity to a series of standardized mental and physical stressors to which participants were exposed exactly three hours after awakening. Next, we used a double–blind randomized trial to compare sympathetic

and cardiovascular reactivity during a placebo condition with reactivity during a condition in which the early morning cortisol peak was blocked by administration of the synthetic glucocorticoid DEX during the previous evening.

In line with the glucocorticoid-sympathetic interaction predicted by permissive effects we expected basal morning cortisol levels to modulate individual differences in sympathetic stress-reactivity at the level of the central sympathetic drive generated in the amygdale and associated limbic structures. Such effects would have been entirely blocked by DEX, which nearly completely depletes the brain of glucocorticoid action (de Kloet *et al.*, 1975; Meijer *et al.*, 1998), thereby eliminating the hypothesized permissive effects of cortisol on central sympathetic neurotransmission. However, successful blockade of the normal early morning peak in cortisol by administration of DEX had no effect on sympathetic and cardiovascular reactivity to any of the mental and physical stressors. Thus, no evidence for permissive effects of the early morning cortisol rise on daytime sympathetic and cardiovascular responses to stress were found at the level of *central* generators of sympathetic activity. This still leaves the possibility of permissive effects of cortisol on *peripheral* sympathetic neurotransmission which may have been taken over by circulating DEX. Such peripheral permissive actions of GCs can be brought about by enhancing sinoatrial, cardiomyocyte and vascular responsiveness to adrenaline and noradrenaline (Fritz & Levine, 1951; Grunfeld & Eloy, 1987; Ramey *et al.*, 1951; Schomig *et al.*, 1976; Tanz, 1960). We note, however, that peripheral permissive effects on reactivity should have created larger stress reactivity in subjects with larger cortisol responses in the placebo condition. We found only very circumstantial evidence for this. Of all 105 correlations tested, only 6 scores showed a significant correlation with various measures of the early morning cortisol rise, of which only 1 was in the predicted direction, i.e. larger sympathetic or cardiovascular reactivity in subjects with high early morning cortisol levels. This does not exceed the number of false positives that is to be expected due to multiple testing.

I conclude, therefore, that the permissive effects of cortisol on daytime sympathetic and cardiovascular responses to stress remain to be established

Effect training state on ambulatory cardiac autonomic control

An exercise-induced bradycardia with a shift to less sympathetic and more parasympathetic control over the heart rhythm is one of the mechanisms put forward to explain this reduced CVD risk in exercisers, and evidence in favor of this mechanism has accrued in animal studies and studies in cardiac patients (Billman, 2002; Goldsmith *et al.*, 2000; Gutin *et al.*, 2005; Mueller, 2007; Rosenwinkel *et al.*, 2001). As reviewed in chapter 7 the evidence for an exercise-induced shift in cardiac autonomic control is less strong in healthy humans, either in studies using invasive assessments of sympathetic and vagal effects (Alvarez *et al.*, 2005; Katona *et al.*, 1982; Kingwell *et al.*, 1992; Lewis *et al.*, 1980 Meredith *et al.*, 1991; Ray & Hume, 1998; Svedenhag *et al.*, 1984) or in studies using RSA and PEP (Boutcher & Stein, 1995; de Geus *et al.*, 1996; de Geus *et al.*, 1990; Loimaala *et al.*, 2000; Sherwood *et al.*, 1989; Svedenhag *et al.*, 1986; Svedenhag *et al.*, 1991; Uusitalo *et al.*, 2004). Of note, the bulk of these studies used laboratory resting conditions. Ambulatory recording of RSA was used in a few studies only (Goldsmith *et al.*, 1992; Goldsmith *et al.*, 1997; Loimaala *et al.*, 2000; Schuit *et al.*, 1999; Stahle *et al.*, 1999) and no study addressed the effects of exercise on ambulatory

PEP levels. Based on the idea that more consistent effects of exercise on autonomic cardiac control may emerge in ambulatory settings, chapter 7 addressed the link between training state and 24-hr recordings of RSA and PEP.

We first compared RSA and PEP levels from regular vigorous exercisers to those in age- and sex-matched sedentary controls. Next, the regular exercisers were subjected to a standardized training program of 6 weeks in which we attempted to make their training state as comparable as possible. After this run-in standardization phase they were randomized to 2 weeks of continued training or 2 weeks of de-training. We deliberately chose a detraining design to address a specific short coming in training studies. By necessity, training studies have to select subjects who were untrained at the start of the study, and preferably had a sedentary lifestyle in general. In view of the emerging evidence that there are strong genetic differences in the response to training of parameters like maximal oxygen uptake (VO_{2max}) (Bouchard & Rankinen, 2001) and HR (Rice *et al.*, 2002), sedentary subjects may potentially represent a selected group of autonomic low or non-responders. Low exercise responsiveness may even contribute to sedentary behavior if the same genetic factor that prevents large shifts in autonomic cardiac control also decreases the propensity to engage in regular exercise behavior (de Geus *et al.*, 1993). Detraining manipulations avoid this potential selection of 'autonomic non-responders' whilst still addressing causality.

Results showed that non-exercising controls had a significantly higher ambulatory HR compared to the regular exercisers but entirely comparable 24-hr levels of PEP and RSA. In the regular exercisers, 2 weeks of detraining did not significantly change the ambulatory levels of PEP or RSA. We conclude that the bradycardia in healthy regular exercisers paired to the paradoxical absence of clear cut effects of training and detraining on sympathovagal balance is best explained by an exercise-induced decrease in intrinsic HR. Dual blockade studies indeed point to a lower intrinsic HR as the most replicated source of resting bradycardia in exercisers (Katona *et al.*, 1982; Kingwell *et al.*, 1992; Lewis *et al.*, 1980; Smith *et al.*, 1989b; Uusitalo *et al.*, 1996) and this is supported by findings in animals (Lin & Horvath, 1972; Negrao *et al.*, 1992). Although the exact physiological mechanism causing a reduction in intrinsic HR remains elusive, it has been hypothesized that it may be caused by a mechanical effect on the pacemaker tissue imposed by cardiac hypertrophy or by an alteration in myocardial cell metabolism (Bhan & Scheuer, 1972; Katona *et al.*, 1982). The combined results from training and detraining studies reviewed in chapter 7 suggest that these adaptations apparently take time, but once in place are robust against short periods of detraining but ultimately reversible by longer periods of detraining (Mujika & Padilla, 2001).

Unintentionally, this study provided a compelling example of the need to do a full assessment of cardiac autonomic balance in ambulatory studies instead of just measuring HR, even though the latter can be done more cost-efficient and with commercial devices that minimize subject discomfort. Had we relied on HR recordings only as our proxy for sympathovagal balance, we would have probably concluded that exercise indeed shifts autonomic control towards vagal activity, and that the effects were robust to two weeks of detraining! As it stands, I must conclude differently. In healthy subject populations, training and detraining induced changes in ambulatory HR may have to be explained to a large extent

by changes in intrinsic heart rate. Changes in cardiac autonomic control seem to play a modest role at best.

Future ambulatory monitoring of ANS function

The results of the studies presented in this thesis have implications for large-scale ambulatory studies with the aim of exploring within- and between-subject variation in ANS function. In fact, part of my PhD thesis mission was to provide scientific guidance for the development of a new version of the Vrije Universiteit Ambulatory Monitoring System (VU-AMS) in close collaboration with the technical department (ITM) of the Faculty of Psychology and Education. This device, the VU-AMS.5fs, has now been thoroughly beta-tested and recently became available for researchers (see appendices I to III). Below, I describe the implications of the results in this thesis for data collection, study design, and data reduction in future ambulatory monitoring of ANS function, and some of the new features that were implemented in the VU-AMS.5fs to address the identified needs.

Data collection: which measures to choose?

Measuring parasympathetic nervous system activity. To measure cardiac vagal control either RMSSD, pvRSA, or HF power can be used. The results presented in this thesis do not lead me to favor one measure over the others. I do note that accurate assessment of all three measures depends crucially on the quality of the IBI time series from which they are computed. The old VU-AMS system (version 4.6) used a powerful dynamic online R-wave detector, but did not store the full ECG waveform. As such it did not outperform commercial wrist-watch type HR-recording devices, that need only a single elastic recording band around the chest to record the IBI time series. In an estimated 90% of the subjects, IBI time series will be of sufficient quality 90% of the time to extract reliable HR and RMSSD values with automated error checking of the IBI time series and only minimal visual inspection. However, that leaves out 19% of the potential data, and, if this missingness is non-random, may introduce bias. Using Compact Flash memory cards, storage capacity of the new VU-AMS.5fs is now >1 gigabyte which makes it possible to record the full ECG signal recording at a maximum sampling rate of 1000Hz with a 16-bit resolution. The resulting higher quality IBI time series allows for more sophisticated automated IBI detection and it makes visual inspection more informative. This will greatly increase the amount of data that can be salvaged for all three RSA measures, most strongly benefiting Wavelet- or Fourier-analysis which is the most sensitive to missing or incorrectly scored IBIs.

A remaining question is whether the additional recording of the respiration signal needed for pvRSA outweighs the added burden on the study participants that need to wear either additional electrodes or respiratory bands. However, this method has the huge advantage of providing extra information on respiration behavior (Houtveen *et al.*, 2006). Currently, the field is littered with concerns about the use of RSA as a measure of cardiac vagal control, without appropriate control for respiratory behavior (Eckberg, 2003; Grossman *et al.*, 2004; Grossman & Kollai, 1993; Houtveen *et al.*, 2002; Ritz & Dahme, 2006). Whether such concerns are valid in ambulatory recordings that take physical activity into account remains to be established. Analyses in chapter 2 and 5 that explicitly used between-subject variation in RR as

a potential modulator of RSA yielded essentially identical results with or without correction for RR. This may reflect the fact that RR did not show dramatic between-subject variation across sitting activities and sleep (range 14 to 20 breaths per minute). However, strong within-subject manipulation of respiratory behavior in a range of 5 to 25 breaths per minute clearly shows that interpretation of RSA can be compromised by respiratory behavior (Houtveen *et al.*, 2006). Ultimately, the choice for a specific measure must depend on the exact research question and ambulatory design. In very large-scale research that (1) focuses on prediction of disease risk, (2) aims for minimum discomfort to the participants, and (3) wants to avoid labor-intensive data reduction by the researcher, the RMSSD seems the most appropriate measure.

Measuring sympathetic nervous system activity. The present thesis examined four measures that have been used as indicators of sympathetic autonomic control; PEP, LF/HF ratio, SCL, and ns.SCRs. Among these measures, the LF/HF stood out as a very poor measure of SNS activity. In fact, based on these results I concluded that the LF/HF ratio should not currently be used as an index of sympathetic activation. The other three measures, PEP and two skin conductance indices proved valid indicators of sympathetic effects on the heart and the skin respectively, although for PEP strong effects on pre- or afterload need to be dealt with in design or analysis strategy. These three measures reflect non-overlapping information on changes in SNS activity and should, whenever possible, be measured simultaneously to provide the most complete picture of the sympathetic activation in the whole body. To enable future researchers to do so an important change was needed to the VU-AMS.5fs. Previous versions of the VU-AMS recorded either ECG and SCL or ECG and ICG, but simultaneous recording of the PEP and SCL was not feasible. The new device allows full parallel recording of the ECG, thorax impedance (dZ) and skin conductance.

Although technologically feasible, a serious problem with ambulatory skin conductance measurement is that the electrode placement may restrict individuals in their normal daily activities. The most common place to measure skin conductance is at palmar sites, because eccrine sweat glands are at the highest density in these regions. The next best alternative, the soles of the feet, is even less practical. An specific solution has been found by researchers using SC to detect hot flashes in menopausal women (Thurston *et al.*, 1994). Skin conductance electrodes placed on the sternum proved to be the most sensitive and specific physiological measure of hot flashes. However, in contrast to palmar and plantar skin conductance, sternal skin is relatively unresponsive to psychological stimuli (Freedman, 1989). Current studies of the feasibility of using alternative locations of the skin conductance electrodes, e.g. on the back or on the dorsal side of the hand, will hopefully remove this final restriction on ambulatory SCL recording and allow the measurement of PEP as well as SCL and ns.SCRs in ecologically valid settings.

Study design: factors to take into account

Appropriate technology to collect ANS data is only the first step in ambulatory recording. Interpreting the ANS data in terms of relevant within- and between-subject variation in psychological (e.g. work stress), lifestyle (exercise) or biological (e.g. genetics) factors requires the ANS data collection to be accompanied by the collection of information on

(confounding) variables that could influence the ANS independently of the relevant study variables.

Posture and physical load. As ambulatory recording is characterized by frequent changes in posture and activity, a solid strategy is needed to identify significant changes in posture and physical activity to be able to take these factors into account during data analysis. The classical way to collect information on activity and posture is through the participant reports. In the present study we used paper diaries in which participants entered their activities and posture for each half hour period of the day, a simple method which captures a large part of the variation in posture and activity. Still, diary information remains subjective and in particularly the exact timing of changes in behavior may be less accurately presented by the participants. We improved timing of the onset and offset of changes in posture and activity with the use of an inbuilt recorder for body movement. Specifically, a piezoelectric circuit in the VU-AMS device, which is attached by a belt to the hip, measures the vertical acceleration of the subject. Vertical acceleration is very sensitive to postural changes, but it does not discriminate well between bicycling and walking, for instance, and misses all more subtle 'movements in place'. In the new VU-AMS.5fs, we added a measurement of horizontal acceleration, in the hope to further increase the accuracy of the assessment of body movement.

Psychological factors. Before starting a large ambulatory study, one needs to be aware that ANS function may be influenced by both short-term mood changes (e.g. fluctuations in mood as results of a short lasting stressor) and more chronic mood state (e.g. depression). Many different questionnaires are available to measure general mood and stress status, with respect to the measurement day or to a prolonged period of time, e.g. the months preceding the ambulatory measurement. To control for, or study, the more acute mood effects throughout the day, repeated information needs to be obtained from participants throughout the day. This can be done by asking the subjects to add this information in the diary they are completing, stating at every entry how they felt or how stressed they were at the time of entry. An excellent alternative is provided by the use of electronic devices. For instance, participants may be provided with a small handheld device which prompts the subject to provide all the information needed (Houtveen & van Doornen, 2007). If the handheld device also assesses posture and ongoing activity it can even entirely replace paper diaries. The current omnipresence of mobile phones provides further options for the acquisition of information on daily mood or activities through short automated phone calls or Short Message Service (SMS) based queries. Electronic devices are more intrusive than diaries but they allow a more standardized and complete recording of mood and activity status, and can provide more accurate timing of specific ambulatory events.

Social factors. A neglected area so far is the effect of location (e.g. indoor, outdoor; home, in public, at work, etc.) and social situation (alone, with significant other, with colleagues, etc) on ANS activity. Data collection on these factors can be done through similar strategies as mood, posture and ongoing activity. Using diaries we have systematically collected data on a few social factors, but a careful analysis remains to be done. A first obvious problem that needs to be tackled is that there are very many social situations, which reduces the number of

subjects per cell. Appropriate clustering of social situations in meta-categories may require theoretical input from social psychology.

Data reduction: From prolonged continuous recordings to a few core numbers

Ambulatory monitoring creates a huge amount of physiological data. When storing one-minute ensemble averages of the impedance cardiogram, as was done in the present thesis, the measurement over 24-hr would result in 1440 complexes. The newly developed VU-AMS.5fs will even store the impedance complexes continuously on a beat-to-beat basis, which will result 100800 data points over a 24-hr period with an average HR of 70 beats per minute. Steps need to be taken to manage these data adequately. We use two major strategies for data reduction: (1) large scale averaging across periods with a fixed type of activity, and (2) nearly full automated data scoring that feeds directly into SPSS scripts to identify possible artifacts or outliers. This restricts (labor-intensive) visual inspection to suspect signal parts only rather than the entire recording.

For large-scale averaging, first the information from the activity diaries is used in combination with a visual display of the inbuilt vertical accelerometer signal to divide the entire 24-hr recording into fixed periods. These periods are coded for posture (e.g. lying, sitting, standing), type of ongoing activity (e.g. desk work, eating/drinking, meetings, watching TV), physical activity level (no, light, medium or heavy physical activity), location (e.g. work, home, outside) and social situation (e.g. alone, with colleagues, with friends). A full coding scheme is provided in the appendix of chapter 4. The coded periods are never shorter than 5 min or longer than 1 hr. If periods lasted more than 1 hr (as during sleep), they were divided into multiple periods of maximally 1 hr (e.g. sleep1, sleep2, etc). An average of about 30 (typical range 20 to 50) coded periods is thus created per subject with an average duration of 30 min. Scoring of the HR and RMSSD by the AMSGRA program or of the PEP, SV and CO by the AMSIMP program then proceeds on large-scale ensemble averages across these 20 to 50 coded periods. As described by Riese et al (2003) the average value of the PEP obtained on a small time scale (e.g. 60-s averages) is almost perfectly correlated to the PEP obtained from these large-scale ensembles, which substantially reduces interactive scoring of the impedance cardiogram (automated signal scoring of which is known to be very hazardous). Both AMSGRA and AMSIMP programs are freely available at www.psy.vu.nl/vu-ams.

All physiological measures are ultimately averaged across the same coded periods, i.e. per variable we end up with no more than 50 numbers. Depending on the research question we often aggregate this data set even further to arrive at mean values across all sleep periods, all sitting periods, all standing periods and all periods with light or moderate physical activity (heavy physical activity is usually avoided by asking the subjects not to engage in exercise on the measurement day). Alternatively we do this separately across morning, afternoon, and evening periods or, on a work day, across leisure time and work time.

Near-fully automated data scoring is done for the respiration signal by the AMSRES program (www.psy.vu.nl/vu-ams). Output is on a breath-to-breath basis, but a preprogrammed script can rapidly aggregate the data across the coded periods, whilst removing likely outliers or artifacts in the ECG and respiration signals. The overall strategy for VU-AMS data reduction

and the accompanying SPSS scripts can be found in appendices IV and V; the scripts are also available on request from vu-ams@psy.vu.nl.

In closing

This thesis shows that ambulatory recording of ECG, ICG and SC provides reliable and stable indices of within- and between-subject variation, which, even if imperfect, are valid indicators of vagal effects on the heart and sympathetic effects on the heart and the skin. This further opens the door for much needed large-scale ambulatory monitoring studies of individual differences in the delicate balance between the two branches of the ANS. A shift from parasympathetic to sympathetic activity is a main risk factor for CVD (Curtis & O'Keeffe, 2002; Dekker *et al.*, 2000; Verdecchia, 2000; Fox *et al.*, 2007). Detection of this shift is increasingly done by ambulatory monitoring under the expectation that this has higher predictive validity for long term health outcomes than laboratory measurements (Feldman & Weidenfeld, 2002; Goldstein *et al.*, 2006; Grossman, 2004). In view of the ongoing improvements in ambulatory technology, an appropriate closing conclusion is that this field is very much 'on the move'.