CHAPTER 8

Summary & conclusions

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

The aim of my PhD project was three-fold. First, to critically re-examine the validity of the current strategies for ambulatory assessment of parasympathetic nervous system (PNS) and sympathetic nervous system (SNS) activity. The second aim was to test the feasibility and validity of new ambulatory measures of SNS activity. The third aim was to actively disseminate the knowledge gathered throughout the development of the VU-AMS device, and in particular to ensure a correct use of VU-AMS hardware and software by the growing community of VU-AMS users. In this final chapter, I briefly summarize the outcomes of the studies I conducted on current and new indices of ANS activity for use in ambulatory monitoring. These studies used data from four new experiments conducted at the VU University and two existing data sets from the Netherlands Twin Register. I conclude with some concrete implications of my research, and possible next steps to increase the use of ambulatory monitoring of autonomic nervous system function in the behavioral sciences.

Current available indices of SNS and PNS activity

Chapter one critically reviewed the currently available indices for the measurement of PNS and SNS activity and the feasibility of measurement of these parameters in daily life. The last is important, as individual differences in autonomic nervous system (ANS) responses to laboratory challenges do not generalize very well to real life situations, and this may be reflected in the low predictive value of laboratory ANS reactivity to future cardiovascular disease. Invasive measures, even though they have good content and criterion validity for measuring SNS and PNS activity (microneurography, microdialysis, pharmacological blockade, regional spillover and plasma catecholamines), thus need to be discarded as they cannot be measured in real-life without severely hindering normal life function. Looking at the non-invasive indices available, it was concluded that when conducting ambulatory monitoring the best index of PNS activity is Respiratory Sinus Arrhytmia (RSA). Both HF and RMSSD measures of RSA can be easily obtained, while pvRSA becomes available when respiration is also measured. For SNS activity in real life, the preejection period (PEP) was seen as the measure of choice. However, due to the labor-intensive scoring procedures and the sensitivity to preload and afterload effect, an alternative measure of SNS activity would be welcome.

In chapters two and three, the focus was on the limitations of these two ambulatory measures of choice, RSA and PEP, with the aim of providing the means to detect or even overcome these limitations. Chapter two focused on the possible underestimation of RSA in participants with low heart rates, due to ceiling effects in the acetylcholinergic neurotransmission. Such effects would be most pronounced during sleep. The 24-hour ECG and respiration recordings were examined in 26 regularly exercising participants who enrolled in a 6-week supervised training program and in 26 age- and sex matched non-exercisers. The IBI-RSA relationship was examined by visual inspection of the IBI-RSA plot for each individual. A subgroup of exercisers showed an IBI-RSA relationship that was characterized by a quadratic profile, compared to the expected linear profile, indicating an underestimation of vagal control in this group. Indeed, only when accounting for the ceiling effect a beneficial effect of exercise on cardiac vagal control was apparent. Inspection of the IBI-RSA relationship is thus mandatory when using HRV measures to index vagal control.

Chapter three focused on the detection of the specific points in the impedance signal which are used to estimate PEP. PEP is defined as the interval from the onset of the left ventricular depolarization, reflected by the Q-wave onset in the ECG, to the opening of the aortic valve, reflected

by the B-point in the ICG signal. However, the position of the Q-wave onset and the B-point are not always easily detected and automatically scored points need to be visually detected. This is not only labor-intensive but also makes the PEP estimate more error-prone. Using data obtained during several different postures and physical activity conditions from two studies conducted in different settings (laboratory versus ambulatory), I evaluated two alternatives to the detection of the Q-wave onset and B-point: computing PEP from a fixed value for the Q-wave onset to R-wave peak (QR) interval and from an R-wave peak to B-point interval that is estimated from the R-peak to dZ/dt-min peak (ISTI) interval. The evaluation of the QR interval and ISTI provided meaningful estimates of the expected location of Q-wave and B-point. Also, ISTI by itself may provide an additional measure of cardiac contractility, reflecting the time it takes to reach peak ventricular ejection. However, as discrepancies between estimated PEP and actual PEP were large, the detection of the Q-wave onset and the B-point remains highly advisable.

Evaluation of two alternative measures of SNS activity

In the chapters four to six, two alternative indices of SNS activity were studied: salivary alpha-amylase (sAA) and the T-wave amplitude (Twave). Chapters four and five focused on the enzyme sAA, which can be easily obtained from saliva and has gained interest as a potential non-invasive biomarker for SNS activity. In a first study, presented in chapter four, ECG and ICG signals were registered during 24 hours to obtain PEP and RSA, and participants provided 7 saliva samples throughout the day by gently chewing on Salivette cotton rolls. In contrast to what is known about diurnal patterns in SNS activity, sAA increased throughout the day and there was no significant association between PEP and sAA, not even when correcting for RSA. To exclude the possibility that the economic but suboptimal Salivette sampling method for sAA had caused the negative findings, a second study was conducted in which saliva was more carefully collected using the passive drooling method before and after cycling, a task certain to elicit SNS activation. In addition to sAA activity, which is the most commonly used sAA measure, actual sAA protein concentration and the ratio of sAA protein to salivary protein were also determined. The sAA responses to exercise were compared to changes in PEP and RSA in response to exercise. As expected, cycling increased SNS activity and decreased PNS activity, as indicated by a decreasing PEP and decreasing RSA. SAA activity and concentration also increased in response to the task. Participants who showed a combination of SNS activation and a small loss of PNS activation in response to the exercise task did show the strongest increase in SAA activity, but PEP and RSA changes were not related to sAA output. Based on the results of these two studies presented in chapters four and five, the use of sAA output or sAA activity as proxy of SNS activation is not recommended.

In chapter six the use of the ECG T-wave amplitude (TWA) as an indicator of SNS activity was determined. Using a large sample of 24 hour data it was shown that large scale ensemble averaging of the ECG is feasible and allows meaningful scoring of the major ECG landmarks. The TWA, for instance, could be reliably determined in over 90% of the participants and showed a clear increase in response to a mental task. Also, during 24 hour monitoring TWA decreased stepwise from nighttime sleep to daytime sitting to physical activity. As such TWA does seem to covary with expected changes in SNS activation. Within subjects, TWA also correlated with PEP, with an average correlation of .35 after partialling out RSA and IBI. This suggests that TWA cannot replace PEP as indicator of SNS activity but that the inclusion of both TWA and PEP may provide a more comprehensive picture of SNS activation.

The VU-AMS; development and dissemination

The studies on ambulatory monitoring of ANS activity made use of the VU University Ambulatory Monitoring System (VU-AMS) that was specifically developed for this purpose at the VU University, through collaboration between ICT/electronics specialists and psychology researchers. Chapter seven describes the history of the development of the VU-AMS, and shows how technological innovation linked to user input improved signal recording, signal quality, data processing and general usability of the VU-AMS. Improvements of the VU-AMS in turn lead to new applications and study designs that generated again more requests for further development, creating a positive feedback loop. It is in the triangle of the VU-AMS development team, technological progress and user community that ambulatory monitoring with the VU-AMS will continue to progress and this model seems very suitable for the development of ambulatory monitoring in general. Chapter 7, together with the appendices, also review the various tools created to support the dissemination of the correct application of the VU-AMS in behavioral research.

Main conclusions

- At the moment RSA and PEP are the indices of choice for measuring PNS and SNS activity in daily life.
- In individuals with lower heart rates, such as regular exercisers, vagal control may be underestimated by RSA, particularly during sleep. Inspection of IBI-RSA plots is mandatory in these subjects to detect underestimation of RSA due to ceiling effects
- Although the QR interval and ISTI provide meaningful estimates of the expected location of Q-wave and B-point, the detection of the Q-wave onset and the B-point remains highly advisable to obtain the actual PEP.
- ISTI by itself may provide an additional measure of cardiac contractility, reflecting the time it takes to reach peak ventricular ejection.
- The current evidence does not support the use of sAA activity or SAA output as an index of sympathetic nervous system activity.
- The TWA seems sensitive to SNS activity, but should not be seen as a replacement for the measurement of PEP. The joint measurement of T wave amplitude and PEP may provide a more complete picture of SNS activity.
- To further progress of ambulatory monitoring, a continuous and close interaction between behavioral researchers and the VU-AMS development team is essential.

Suggestions for further research

Based on the results presented in this study TWA and ISTI emerged as possible indicators of SNS activity to be used in addition to PEP. To provide more insight in the validity of TWA or ISTI as SNS indices, studies are now needed that compare changes in TWA, ISTI (but also PEP itself) to the other measurements of cardiac contractility for instance the ejection fraction obtained by echocardiography. In fact it is nigh time to do a large study that compares the various other indices extracted from thorax impedance recording that were not yet discussed in this thesis, including heather index and stroke volume, to similar measures extracted from echocardiographic recordings.

At the moment we have the tools to measure ANS activity in daily life, and a fair number of studies have studied ANS activity in relation to current health (depressed versus non-depressed) or

current situation (high versus low work stress). However, the move from the laboratory to real life monitoring was given in by the low predictive value of laboratory assessments for future disease development, for which low lab-real life generalizability was seen as one of the reasons. It is essential that longitudinal studies are now conducted to determine the importance of ANS activity in daily life for future disease development.

The rapid development within the communication technology opens up new possibilities for ambulatory studies. For instance, the precise determination of time and location has become possible, as well as repeated prompting for mood indications via mobile phones. The integration of this information with the continuously recording of ANS activity is expected in the near future and will be another step forward in understanding the influence of everyday life on our biology.