Psychophysiology, 52 (2015), 225–237. Wiley Periodicals, Inc. Printed in the USA.

DOI: 10.1111/psyp.12300

Ambulatory measurement of the ECG T-wave amplitude

RENÉ VAN LIEN, MELANIE NEIJTS, GONNEKE WILLEMSEN, AND ECO J. C. DE GEUS

Department of Biological Psychology, VU University Amsterdam, Amsterdam, The Netherlands, and
EMGO$^+$ Institute, VU University Medical Center, Amsterdam, The Netherlands

Abstract

Ambulatory recording of the preejection period (PEP) can be used to measure changes in cardiac sympathetic nervous system (SNS) activity under naturalistic conditions. Here, we test the ECG T-wave amplitude (TWA) as an alternative measure, using 24-h ambulatory monitoring of PEP and TWA in a sample of 564 healthy adults. The TWA showed a decrease in response to mental stress and a monotonic decrease from nighttime sleep to daytime sitting and more physically active behaviors. Within-participant changes in TWA were correlated with changes in the PEP across the standardized stressors ($r = .42$) and the unstandardized naturalistic conditions (mean $r = .35$). Partialling out changes in heart rate and vagal effects attenuated these correlations, but they remained significant. Ambulatory TWA cannot replace PEP, but simultaneous recording of TWA and PEP provides a more comprehensive picture of changes in cardiac SNS activity in real-life settings.

Descriptors: Behavioral medicine, Individual differences, Genetics, Heart rate, PEP, RSA

Functional disturbances of the autonomic nervous system have been frequently linked to several diseases, and hyperactivity of the sympathetic nervous system (SNS) may be an important cause for the detrimental effects of stress on cardiovascular health (Lambert, Schlaich, Lambert, Dawood, & Esler, 2010; Parati & Esler, 2012). At the moment, the preejection period (PEP) is the measure of choice to monitor changes in cardiac SNS activity noninvasively in psychophysiological stress research (Berntson, Lozano, Chen, & Cacioppo, 2004; Kelsey & Guethlein, 1990; Kelsey, Ornduff, & Alpert, 2007; Sherwood et al., 1990; Vrijkotte, van Doornen, & de Geus, 2004). PEP can be obtained by simultaneous recording of the thoracic impedance cardiogram (ICG) and electrocardiogram (ECG) and is defined as the interval from the onset of left ventricular depolarization, reflected by the Q-wave onset (Qonset) in the ECG to the opening of the aortic valve, reflected by the B-point in the ICG signal (Labidi, Ehmke, Durnin, Leaverton, & Lauer, 1970; Lozano et al., 2007; Sherwood et al., 1990; van Lien, Schutte, Meijer, & de Geus, 2013; Willemsen, de Geus, Klaver, van Doornen, & Carroll, 1996).

The literature supports changes in PEP as a valid measure of SNS-induced changes in contractility of the left ventricle (Berntson et al., 1994; Goedhart, Kupper, Willemsen, Boomsma, & de Geus, 2006; Harris, Schoenfeld, & Weissler, 1967; Houtveen, Groot, & Geus, 2005; Krzeminski et al., 2000; Kupper, Willemsen, Boomsma, & de Geus, 2006; Mezzacappa, Kelsey, & Katkin, 1999; Miyamoto et al., 1983; Nelesen, Shaw, Ziegler, & Dimsdale, 1999; Newlin & Levenson, 1979; Richter & Gendolla, 2009; Schachinger, Weinbacher, Kiss, Ritz, & Langewitz, 2001; Sherwood, Allen, Obrist, & Langer, 1986; Smith et al., 1989; Svedenhag, Martinsson, Ekblom, & Hjemdahl, 1986; Vrijkotte et al., 2004; Williams, Puddey, Beilin, & Vandongen, 1993; Winzer et al., 1999). A large advantage of the PEP is that it can be measured outside the confines of a hospital or laboratory setting by using ambulatory monitoring devices (Nakonezny et al., 2001; Sherwood, McFetridge, & Hutcheson, 1998; Wilhelm, Roth, & Sackner, 2003; Willemsen et al., 1996). This allows examination of individual differences in cardiac SNS activity in a natural setting, for instance, during sleep or during job-related activities with a substantial mental load. These naturalistic conditions may have the largest clinical relevance (Kubiak & Stone, 2012; Trull & Ebner-Priemer, 2013).

However, when ambulatory research moves to an epidemiological scale, collecting data in thousands of participants across extended periods of time, the practical feasibility of PEP measurement becomes an issue. Automated scoring of the ambulatory PEP has been made more efficient over the years by implementing large-scale ensemble averaging (Riese et al., 2003). Unfortunately, the time saved in the total amount of visual inspection needed for large-scale ensemble averages comes with the disadvantage of

We thank all twins and their family members for their participation in the Netherlands Twin Register Biobank project, and we also thank Lena Zuskova, Rianne Bosch, Eline Bangert, Hester Verheijen, Naomi Vorstermans, and Kirsten Planting for their assistance with the data collection. Funding was obtained from the VU-AMS fund, the Netherlands Organization for Scientific Research (NWO), and Biobanking and Biomolecular Resources Research Infrastructure (BBMRI–NL, 184.021.007).

Address correspondence to: Eco J. C. de Geus, EMGO$^+$ Institute for Health and Care Research, VU Medical Center & VU University Amsterdam, van der Boechorststraat 7, Room D-452, 1081 BT Amsterdam, The Netherlands. E-mail: j.degeus@vumc.nl

PSYCHOPHYSIOLOGY

increased latency jitter that makes it more difficult to score the inflection points of interest on the dZ/dt waveform. Ambulatory PEP scoring, therefore, remains a time-consuming process requiring convergence in the visual inspection of multiple raters to ensure sufficient reliability of identification of the relevant landmarks (Berntson et al., 2004; Lozano et al., 2007; van Lien et al., 2013). Considering the laborious visual scoring required, it would be extremely valuable to have alternative noninvasive measures of cardiac SNS activity that could be assessed through ambulatory monitoring with more ease.

Two such alternative measures are currently in use: the ratio of power in the low to high frequency bands of the heart rate power, (LF/HF ratio; Pagani et al., 1986, 1991, 1997) and salivary alpha-amylase (sAA; Nater & Rohleder, 2009). Unfortunately, the validity of these measures as indices of cardiac or salivary SNS activity has been strongly questioned. Although its use has become widespread, the LF/HF ratio is theoretically a poor measure of cardiac SNS activity (Eckberg, 1997; Reyes Del Paso, Langewitz, Mulder, van Roon, & Duschek, 2013) that does not correlate with other indicators of this activity (Goedhart, Willemsen, Houtveen, Boomsma, & de Geus, 2008; Grassi & Esler, 1999). sAA, particularly when collected by cotton rolls (as is typical), has severe methodological drawbacks and seems to capture parasympathetic activity in addition to sympathetic activity (Bosch, Veerman, de Geus, & Proctor, 2011). Also, it cannot be sampled with a high temporal resolution during recordings in naturalistic settings or during the nighttime.

In this study, we set out to test a third, and seemingly somewhat forgotten, alternative to measure cardiac SNS activity, namely, 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 ms. It reflects ventricular repolarization (Abildskov, Burgess, Urie, Lux, & Wyatt, 1977; Burgess, 1979; Haarmark et al., 2010; Randall & Hasson, 1977) in which the sympathetic nerves play an important role (Abildskov, 1985). TWA is often defined as the difference between the peak of the T wave and the isoelectric level during the same heart cycle (Furedy & Heslegrave, 1984; Furedy, Heslegrave, & Scher, 1984; Kline, Ginsburg, & Johnston, 1998). Alternative isoelectric levels are in use, such as the midpoint of the PQ interval (Matyas, 1976) and the period between the T-wave offset and the P-wave onset (Contrada et al., 1989). These isoelectric periods represent moments where only a negligible number of fibers in the cardiac conduction system are depolarizing.

Decreases in TWA and even TWA inversion were seen to occur after local stimulation of the stellate sympathetic ganglia (Anitchkov & Vedeneyeva, 1961; Yanowitz, Preston, & Abildskov, 1966), intracoronary infusion of epinephrine (Barger, Herd, & Liebowitz, 1961), norepinephrine (Russell & Dart, 1986), or the nonselective beta-adrenergic agonist isoproteronol (Autenrieth, Surawicz, Kuo, & Arita, 1975) in dogs. In humans, TWA decrease was seen after subcutaneous or intramuscular administration of epinephrine (Hartwell, Burrett, Graybiel, & White, 1942; Katz, Hamburger, & Lev, 1932; Levine, Ernstene, & Jacobson, 1930), and after administration of a nonselective β-agonist (isoproteronol; Contrada et al., 1989; Contrada, Dimsdale, Levy, & Weiss, 1991).

Importantly, such functional TWA decreases could be reversed by β-blockade with propanolol (Contrada et al., 1989; Furberg, 1967, 1968; Guazzi et al., 1975; Noskowicz & Chrzanowski, 1968). Additionally, TWA has been shown to be a useful indicator of cardiac SNS activity during laboratory testing of stress reactivity (Furedy & Heslegrave, 1984; Heslegrave & Furedy, 1979; Furedy & Shulhan, 1986; Furedy, Szabo, & Peronnet, 1996; Matyas, 1976; Matyas & King, 1976; Scher, Furedy, & Heslegrave, 1984) where the stress-induced TWA decreases could also be blocked by beta-adrenergic antagonists (Contrada et al., 1989; Rau, 1991).

The initial enthusiasm for the TWA was tempered when Bunnell (1980) showed only a modest correlation (mean $r = .41$) between TWA decreases and other, at the time, accepted cardiac sympathetic measures (pulse transit time, carotid dP/dt, and heart rate). Furedy and Heslegrave (1983) rightfully noted that the modest correlations of TWA decreases to criterion measures merely showed that it is not a *perfect* measure of cardiac SNS activity, and that the other cardiac measures also did not correlate more than modestly among themselves. A more serious concern raised about the TWA was how "purely" it reflects SNS activity. Increases in cardiac SNS activity are often accompanied by increases in heart rate and decreases in vagal activity. Increased heart rate leads to a shortening of the interbeat interval (IBI), which could decrease TWA simply by reducing the rise time of the T wave. Decreases in vagal activity could also directly contribute to a decrease in TWA (Schwartz & Weiss, 1983; Weiss, Del, Reichek, & Engelman, 1980).

Dauchot and Gravenstein (1971) and Annila, Yli-Hankala, and Lindgren (1993) indeed found that an acetylcholinergic antagonist (atropine) led to a decrease in the TWA. Contrada et al. (1989, 1991) further reported a sudden paradoxical TWA increase during very high doses of isoproterenol infusion and reasoned that (baroreflex-induced) increases in vagal activity might have caused the increase in the TWA. These findings suggest that changes in cardiac vagal activity also affect the TWA, which would invalidate it as a pure SNS measure. Furedy et al. (1996) have argued that the effects of cardiac vagal activity may partly act by modulating cardiac SNS activity through the mechanism of accentuated antagonism (Levy, 1977; Levy & Zieske, 1969). Various studies indeed reported an enhanced decrease of TWA by isoproterenol after atropine infusion compared to isoproterenol alone (Fukudo et al., 1992; Stratton, Pfeifer, & Halter, 1987). Accentuated antagonism requires sympathetic and parasympathetic nerves to converge on the same neuromuscular synapses in the ventricular myocardium. There is increasing evidence for vagal innervation of the human ventricle, although the functional role of this innervation remains to be elucidated (Coote, 2013).

One way to resolve the role of PNS activity in the TWA is to coregister TWA with heart rate variability in the respiratory frequency range (RSA), which has been proposed as a proxy for cardiac parasympathetic nervous system (PNS) activity (Berntson et al., 1997; Task Force of the European Society of Cardiology and the North American Society of Pacing, 1996). A number of studies recording both TWA and RSA have reported a decrease in TWA to co-occur with vagal withdrawal (Kreibig, Wilhelm, Roth, & Gross, 2007; Palomba, Sarlo, Angrilli, Mini, & Stegagno, 2000; Pan & Li, 2007; Roth et al., 1992). However, these studies did not correlate TWA to other measures of cardiac SNS activity, and only one study related changes in TWA directly to changes in RSA. After controlling for between-person variance, changes in RSA across various stress tasks (Valsalva's maneuver, serial subtraction, and cold pressor) in 20 participants did not correlate to changes in TWA (Kline et al., 1998).

Taken together, the past studies on the TWA are overall suggestive of a role of the SNS in this measure, whereas the additional role of the PNS remains unclear. It is possible that the relative popularity of the PEP, rather than poor performance of the TWA, has led to its demise in the past two decades. Although TWA is a measure of

ventricular repolarization and PEP is a measure of ventricular contractility, both are influenced by the beta-adrenergic effects of the ventricular sympathetic innervation, so much of the information contained in PEP reactivity might be recaptured by studying TWA reactivity. As stated before, the automated scoring of the PEP from the ECG and ICG has a number of practical setbacks and requires visual inspection that can become prohibitive when PEP is used in long-term recordings in epidemiology-sized samples. This breathes new life into the attractiveness of the TWA that might not suffer from this setback as it only requires detection of clear landmarks in the ECG. In addition to being a potential alternative to the PEP, the TWA may prove useful in its own right to help understand the basic mechanisms of autonomic control, justifying a detailed examination of the properties of the TWA in an ambulatory setting.

Here, we report on 24-h ambulatory monitoring of the TWA in a sample of 564 healthy adults. First, the feasibility was assessed of automated scoring of ECG landmarks required for the TWA using large-scale ensemble averaging of the ECG. Large-scale ensemble averaging has been successfully applied to the ambulatory ICG but not yet to the ambulatory ECG. Second, to compare to previous literature, the TWA was scored in an ensemble-averaged ECG across a 4-min resting baseline and 4-min stress condition. These standardized conditions were embedded within the larger 24-h recording. Third, in the ambulatory recording it was tested whether the TWA varies in a predictable way within a wake/sleep cycle and across different levels of physical activity. Finally, it was tested whether the within-participant changes in TWA across the 24-h recording are more robustly correlated with the changes in PEP (convergent validity) than with the changes in RSA (discriminant validity). TWA was expected to vary in a predictable way between rest and stress conditions and across arousal and physical activity level. It was further expected that within-participant changes in TWA would show significant correspondence with changes in PEP but not with changes in RSA, and that the TWA-PEP correspondence survives correction for parallel changes in RSA and IBI. In short, we expected to provide evidence that the TWA can provide meaningful information on cardiac SNS activity in ambulatory recordings.

Method

Participants

Participants were all registered in the Netherlands Twin Register (NTR) and had previously participated in a larger biobank project (Willemsen et al., 2010). A priori reasons for exclusion were participation in an earlier ambulatory recording study (Kupper et al., 2005, 2006), heart transplantation, presence of a pacemaker and known ischemic heart disease, congestive heart failure, or diabetic neuropathy and pregnancy. Ambulatory cardiovascular recordings of 582 participants were available, of which 11 recordings had either a missing or noisy ECG or thorax impedance signal due to equipment failure, and were therefore excluded from the analysis. Seven participants using beta-blockers were excluded from the analysis. The final sample consisted of 277 identical twins (97 men), 234 fraternal twins (96 men), and 53 singleton siblings (21 men) from 297 families. Mean age was 36.9 years (*SD* 5.4). Zygosity of the twins was determined by DNA typing for 98.6% of the same-sex twin pairs. For the other same-sex pairs, zygosity was based on survey questions on physical similarity and the frequency of confusion of the twins by parents, other family members, and strangers. Agreement between zygosity based on these items and zygosity based on DNA was 96.1% (Willemsen et al., 2013). The Medical Ethical Committee of the VU University Medical Centre Amsterdam approved of the study protocol, and all participants gave written consent before entering the study. Participants received a payment of 10€ and an annotated review of their ambulatory ECG recording.

Procedure

A detailed description of the general ambulatory monitoring procedure has been provided elsewhere (Kupper et al., 2005, 2006). Briefly, participants were asked not to engage in vigorous exercise on the day before the ambulatory recording and to drink no more than one or two glasses of alcohol on the night before. Participants who had fallen ill were always rescheduled. On the recording day, participants were visited between 6 and 12 in the morning at home or at the work location, when this was deemed more convenient. They were fitted with the 5fs version of the VU-AMS device (VU University Amsterdam, www.vu-ams.nl), which records the ECG and the ICG continuously during a 24-h period (daytime and sleep) through seven disposable, pregelled Ag/AgCl electrodes (de Geus, Willemsen, Klaver, & van Doornen, 1995; van Dijk et al., 2013; Willemsen et al., 1996). After visually establishing proper signal quality, the recording was started, and participants were first interviewed on health, medication, lifestyle, and socioeconomic and demographic information, after which they filled out a questionnaire on psychological well-being. The questionnaire lasted on average 10 min and was completed while quietly sitting in a secluded part of the house/work area. The last 4 min of this quiet sitting period functioned as a baseline. Next, participants were instructed to execute a 2-min serial subtraction task directly followed by a 2-min Stroop Color Word conflict task. After a final equipment check, the experimenter departed, and participants were left to their daily routine until the next morning, when they were asked to remove the VU-AMS device and cables. These were mailed back to the experimenter in a prepared return envelope with a special protective layer. During the daytime and the evening before bedtime, participants were asked to give a chronological account of posture, physical activity, physical load, location, and social situation every 60-min period.

Physiological Recording

The VU-AMS device is worn on the hip and contains a triaxial accelerometer to assess body movement. ECG electrodes were carefully placed according to a standard protocol to obtain a lead II derivation, which yields the most prominent R-wave peak as well as a clear T-wave amplitude. Cleaning of the skin with alcohol before electrode application ensured that electrode resistance was kept low. The first ECG electrode (V-) is placed slightly below the right collar bone 4 cm to the right of the sternum. The second ECG electrode (V+) is placed at the apex of the heart over the ninth rib on the left lateral margin of the chest approximately at the level of the processus xiphoideus. The third ECG electrode is a ground electrode and is placed on the right side, between the lower two ribs at the right abdomen. The first ICG-measuring electrode (V1) is placed at the top end of the sternum, between the tips of the collar bones. The second ICG-measuring electrode (V2) is placed at the xiphoid complex of the sternum. The two current electrodes are placed on the back: I- on the spine over the cervical vertebra C4, at least 3 cm (1″) above the ICG-measuring electrode V-, and

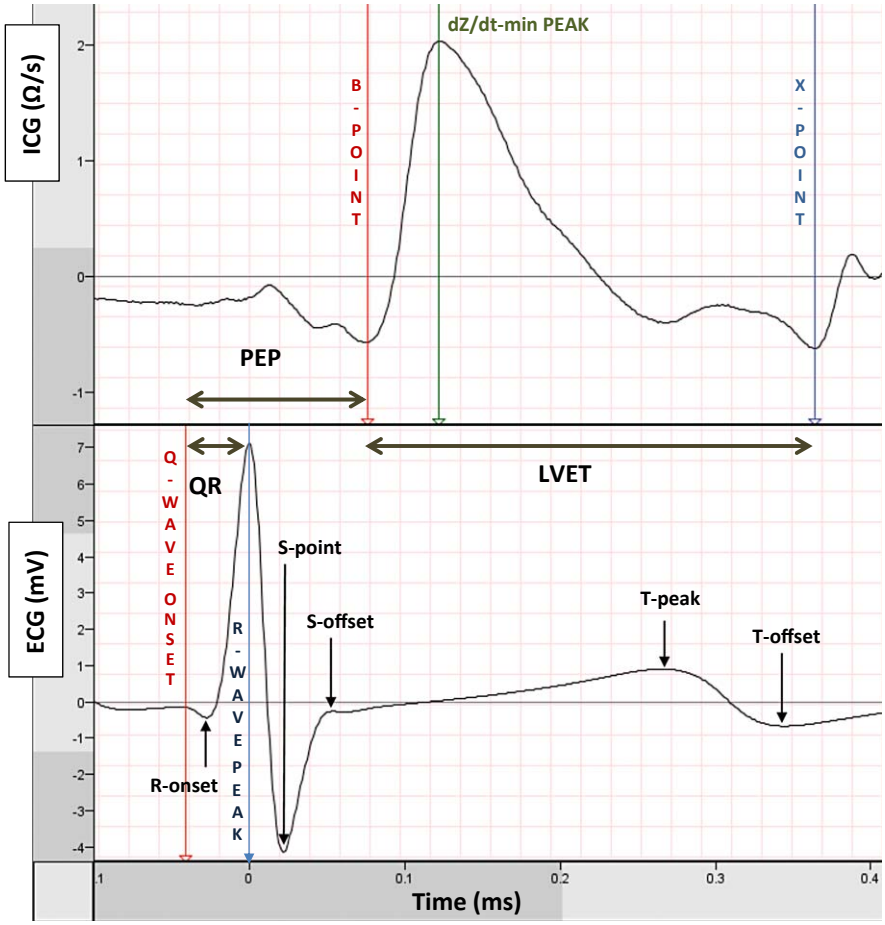

Figure 1. The impedance cardiogram (top) and the electrocardiogram (bottom) with the four landmarks defining the preejection period (PEP, Q-wave onset to B-point) and TWA (T-peak and T-offset). LVET = left ventricular ejection time. QR = Q-wave onset to R-wave peak interval.

I+ between thoracic vertebrates T8 and T9 on the spine, at least 3 cm (1″) below the ICG-measuring electrode V2.

Data reduction. The ECG and ICG signals were imported into the VU-DAMS software (version 3.2, VU University Amsterdam, www.vu-ams.nl). After automated detection of bad ECG signal fragments (artifacts), R-wave peak detection was done using a modified version of the algorithm by Christov (2004). From the R-wave peaks, an IBI (ms) time series is constructed that was visually displayed for interactive correction of missed or incorrect R-wave peaks. This correction takes on average 2 min per 24-h recording.

Large-scale ensemble averaging. Using the activity diary entries in combination with a visual display of the in-built triaxial accelerometer signal, the entire 24-h recording was divided into fixed periods coded for posture (e.g., lying, sitting, standing), ongoing activity (e.g., desk work, eating/drinking, meetings, watching TV), physical activity (none, light, medium, and heavy), location (e.g., work, home, outside), and social situation (e.g., alone, with colleagues, with friends). The ECG and ICG signals were ensemble averaged across these periods, time locking both signals to the R-wave peaks. This reduced the data set to, on average, 49 (range: 17–88) ensemble-averaged ECG and ICG traces per participant with an average length of 27 min (*SD* = 19). These had less than 0.5% ECG artifacts. An example of a large-scale ensemble-averaged ICG and ECG is presented in Figure 1. In these ensemble averages, the mean Q-wave onset (Qonset), R-wave onset (Ronset), S-wave peak (Spoint), S-wave offset (Soffset), T-wave peak (Ttop), T-wave offset (Toffset) in the ECG and the B-point, dZ/dt min (C-point), and incisura (X-point) in the ICG were automatically detected and presented for interactive visual scoring.

During interactive scoring, undetectable points were deleted, which effectively sets them to missing per complex. Interactive ECG and ICG scoring took, on average, 15 min per 24-h recording, the bulk of which was spent on the ICG. In some participants, Qonset and/or Ronset tended to be systematically missing, which has been reported before (Lozano et al., 2007). In these participants, Qonset and/or Ronset were set to missing. The ICG points, which caused the largest ambiguity, were scored by two raters as recommended (van Lien et al., 2013), and a third rater arbitrated when no consensus could be reached. To remove outliers, *Z* scores were calculated for the timing of Qonset, Ronset, Spoint, Soffset, Ttop, Toffset, B-point, C-point, and X-point across all participants and conditions and, per point, the top and bottom 2 percent of *Z* values of each of these parameters were set to missing.

Cardiac time intervals. From the ECG landmarks indicated in Figure 1, we computed the Qonset to R-wave onset (QRonset) interval, the Q-wave onset to R-wave peak (QR), the Ronset to

R-wave peak (RonsetR) interval, and the QRS, RS, QT, and ST intervals in milliseconds. From the ICG landmarks, we computed the left ventricular ejection time (LVET) as the interval between the B-point and the X-point, and by combining with the ECG, the R-wave peak to B-point (RB) interval and the PEP as the interval from the Q-wave onset in the ECG to the B-point in the ICG signal. As indicated before, a number of participants had no detectable Q-wave onset. For these participants, we used the procedure typically used to estimate PEP from the R-wave peak only (Lozano et al., 2007; van Lien et al., 2013) but modified it to exploit the large dataset available. If only Qonset was missing but Ronset was present, PEP was estimated by adding the grand-average QRonset interval across 531 participants with valid QRonset (12.6 ms) to the sum of the individual participants' RonsetR + RB intervals. If Ronset was additionally missing we defaulted to the usual estimation of PEP by adding the grand-average QR interval across 455 participants with valid QR (43.7 ms) to the individual participants' RB interval.

T-wave amplitude. We computed the TWA (in μV) using different baseline values in the ECG. First, we calculated TWA_zero as the difference between the ECG value at the T-wave peak and the isoelectric level represented by the zero μV line in the ECG. Because ECG signals are prone to drifting due to movement, especially during ambulatory recordings, the TWA might be under- or overestimated in some participants by using this static baseline. We therefore secondly calculated TWA_Toffset as the difference between the ECG value at the T-wave peak and the ECG value at the T-offset. We deviated from the usual approach that averages the entire T-offset to P-onset interval with the intention to obtain a more stable baseline. We found that at T-offset there is indeed negligible electrical activity, whereas activity is not always low in the entire T-offset to P-onset interval. In addition, we note that the T-offset is more reliably detected than P-onset.

RSA. Combining the ECG with the respiration signal extracted from the thorax impedance signal (dZ), the "peak–valley" RSA method was used to assess vagal chronotropic effects (de Geus et al., 1995; Grossman, van Beek, & Wientjes, 1990; Grossman & Wientjes, 1986). In this method, RSA is scored from the combined respiration and IBI time series by detecting the shortest IBI during inspiration and the longest IBI during expiration on a breath-to-breath basis according to the procedures detailed elsewhere (de Geus et al., 1995; Houtveen et al., 2005; van Lien et al., 2011). Breathing cycles that showed irregularities like gasps, breath holding, coughing, etc., were considered invalid and were removed from further processing. If no shortest or longest IBI could be detected in inspiration and expiration, respectively, the breath was either set to missing or to zero when computing the condition average for RSA. Similar results were found for RSA computed either way, and we employed only one (breaths set to missing) in further statistical analyses.

Statistical Analyses

For statistical analyses, the total recording, consisting of the standardized and the ambulatory part, was first converted into six experimental conditions. The first two experimental conditions were the baseline of quietly sitting and the mean of the two stress tasks. The next four conditions came from the ambulatory part of the recording. Because ambulatory activities were not standardized and could differ per participant, all coded periods were aggregated into four comparable conditions that were determined by the posture and the level of physical activity of the participant: sleep, sitting activities during the day, light physical activity in awake time, moderate physical activity in awake time.

To test the effect of mental stress on TWA, a mixed model analysis of variance (ANOVA) with age, sex, and body mass index (BMI) as covariates and family as a random factor, and baseline versus stress during the experimental part of the recording as the repeated measures fixed factor was first applied. We expected TWA to be systematically decreased in response to mental stress. Next, a similar mixed model ANOVA was applied to the four ambulatory conditions, where we expect the TWA to significantly decrease with increases in SNS activity from sleep to sitting awake to physical activity. Significant main effects of the repeated measures fixed factor were followed up by testing post hoc contrasts between sleep versus sitting, sitting versus light physical activity, and light versus moderate physical activity. We expressed the effect sizes for these contrasts in pooled within-condition standard deviation units, conditional on the covariates sex, BMI, and age.

To provide a measure of the internal consistency reliability of TWA, we selected the first 7 h of sleep and pools of 10 consecutive periods from the three daytime conditions to compute Cronbach's alphas across these repeated "items." Convergent validity of TWA in the experimental setting was then tested by computing the correlations between the participants' TWA and PEP reactivity to the stress tasks. Convergent validity of TWA in the naturalistic setting was assessed by computing the within-participant correlations between TWA and PEP across the day on all coded ambulatory periods available for a particular participant. The mean number of coded ambulatory periods used in within-participant correlations was 49. We selected only participants that had complete TWA, PEP, RSA, and IBI data in at least 15 coded ambulatory periods with a minimum of two periods in at least three of the four conditions (sleep, sitting, light and moderate activity). We next plotted the distribution of these within-participant correlations and tested whether the mean deviated from zero (the expected value if TWA and PEP are not systematically correlated during the recording day) after a Fisher *Z* transformation on the coefficients (Preacher, 2002).

To provide a test of discriminant validity of the TWA, we also computed the correlations between TWA and RSA, both during the standardized stressors and throughout the ambulatory recording day. Finally, to take into account the often-occurring parallel changes in PEP, RSA, and IBI, we recomputed the within-participant TWA-PEP correlations as a partial correlation using the IBI, RSA, or IBI and RSA values during the condition as a covariate. We compared the distributions of the uncorrected and partial TWA-PEP correlations using a test of the difference in correlation coefficients after Fisher *Z* transformation (Preacher, 2002).

Because the TWA-PEP comparison could suffer from poor quality of scoring of the criterion variable (PEP) in some participants, the analyses were repeated in the 101 participants for whom the raters expressed the highest confidence in ICG B-point scoring quality.

Results

Cardiac Time Intervals from the Large-Scale Ensemble-Averaged ECG

The means and standard deviations of the intervals derived from the large-scale ensemble-averaged ECG are shown per condition in

Table 1. *Means (Standard Deviations) for the Intervals Derived from the Ensemble- Averaged ECG During 24-h Ambulatory Recording*

	N range	Sleep	Sitting activities	Light physical activity	Moderate physical activity
QR (ms)	427–443	44.5 (4.1)	43.7 (4.0)[a]	43.5 (4.0)	43.4 (4.1)
RonsetR (ms)	504–521	31.6 (2.9)	31.2 (3.0)[a]	31 (3.0)	30.6 (3.1)
QRS (ms)	508–529	93.2 (9.5)	91.2 (9.3)[a]	90.9 (9.3)	91.4 (9.5)
Qronset (ms)	416–433	13.2 (4.1)	13.0 (4.0)	13.0 (3.9)	13.3 (3.9)
QT (ms)	483–519	411.0 (21.2)	376.9 (21.6)[a]	367.1 (21.0)[b]	356.0 (18.3)[c]
ST (ms)	469–504	317.8 (23.1)	285.4 (23.0)[a]	275.7 (22.2)[b]	263.9 (19.4)[c]
RS (ms)	523–552	24.6 (2.7)	24.0 (2.5)[a]	23.7 (2.5)	23.5 (2.4)
RB (ms)	539–559	61.7 (13.6)	57.7 (15.9)[a]	54.8 (16.6)[b]	48.6 (15.9)[c]

Note. Sample sizes (*N*) vary depending on the number of participants in which the particular ECG landmark could be scored or in which an entire condition was missing. Maximal sample size was $N = 564$ during the daytime and $N = 549$ during sleep.
[a]Significantly different compared to sleep, $p < .001$. Effect sizes were 0.22 *SD* for QR, 0.15 for RonsetR, 0.24 for QRS, 1.72 for QT, 1.57 for ST, 0.31 for RS, and 0.27 for RB.
[b]Significantly different compared to sitting activities, $p < .001$. Effect sizes were 0.48 *SD* for QT, 0.45 for ST, and 0.19 for RB.
[c]Significantly different compared to light physical activity, $p < .001$. Effect sizes were 0.54 *SD* for QT, 0.56 for ST, and 0.42 for RB.

Table 1. In general, the relevant landmarks could be clearly detected in the ensemble-averaged ECGs but, as noted before, Qonset and Ronset are not always detectable. Overall, the Qonset was missing and estimated in 19.3% of the participants (109 of 564), and Ronset was additionally missing and estimated in 5.8% (33 of 564). These percentages were not significantly different across the various ambulatory conditions.

Mixed ANOVA analysis with correction for family relatedness, sex, age, and BMI showed a significant main effect of ambulatory condition on most intervals derived from the ensemble-averaged ECG; QR, $F(3,1289) = 258.0$, $p < .001$; RonsetR, $F(3,1523) = 286.9$, $p < .001$; QRS, $F(3,1537) = 161.0$, $p < .001$; RS, $F(3,1253) = 13.0$, $p < .001$; QT, $F(3,1491) = 4092.1$, $p < .001$; ST, $F(3,1450) = 3535.4$, $p < .001$; RB, $F(3,1631) = 243.7$, $p < .001$. Post hoc analyses of the condition effects revealed that the QT, ST, RS, and RB intervals showed the expected significant monotonic decrease with increased arousal and physical activity. The effect of condition on QR, RonsetR, QRS, and Qronset was negligible and largely driven by the discrepancy between sleep and physically active periods.

From the covariates considered, sex had the strongest effect on these intervals. Female participants had shorter Qonset, Ronset, RS, and QRS durations and longer ST and QT intervals than males (*p*s $< .001$; effect sizes ranging from 0.21 to 0.40 *SD*). The Qonset, Ronset, QRonset, and QRS duration became longer with a higher age of the participants ($.05 < r_{age} < .13$, *p*s $< .001$). A greater BMI was significantly associated with shorter Qonset ($r = -.111$, $p < .001$), Ronset ($r = -.148$, $p < .001$), and ST ($r = -.074$, $p < .001$).

Response of the TWA to a Standardized Stressor

The mean values and standard deviation for IBI, LVET, PEP, RSA, TWA_Toffset, and TWA_Zero during baseline and stress task are presented in Table 2. Mixed model analyses with correction for family relatedness, age, sex, and BMI showed a significant main effect of the stress task on IBI, $F(1,525) = 588.1$, $p < .001$; LVET, $F(1,481) = 73.1$ $p < .001$; PEP, $F(1,456) = 109.5$, $p < .001$; RSA, $F(1,489) = 10.1$, $p < .01$; TWA_Toffset, $F(1,442) = 211.4$, $p < .001$; and TWA_Zero, $F(1,456) = 307.4$, $p < .001$. Post hoc analyses on the effect of the mental stress tasks generally showed the expected effect of our manipulation on the ANS measures. The IBI, LVET, PEP, TWA_Toffset, and TWA_Zero decreased significantly during the stress task, although RSA did not.

Response of the TWA to Increased Levels of Arousal and Physical Activity in a Naturalistic Setting

The mean values and standard deviation for IBI, LVET, PEP, TWA_Toffset, and TWA_Zero during the ambulatory recording are presented in Table 3. Automated TWA_Zero detection was confirmed as correct by visual inspection in 92% of the ensemble ECGs during sleep and in 93% of the ensembles based on daytime recording. TWA_Toffset was confirmed in slightly lower percentages (89% and 91%) due to additional uncertainty about the Toffset point. Mixed ANOVA analysis with correction for family relatedness, sex, age, and BMI showed a significant main effect of physical activity on IBI, $F(3,1664) = 3958.2$, $p < .001$; LVET, $F(3,1647) = 1237.0$, $p < .001$; PEP, $F(3,1630) = 281.5$, $p < .001$; RSA, $F(3,1630) = 303.3$, $p < .001$; TWA_Toffset, $F(3,1491) = 487.1$, $p < .001$; and TWA_zero $F(3,1528) = 291.7$, $p < .001$. Post hoc analyses of the ambulatory condition effects revealed the expected significant monotonic decrease of IBI, LVET, PEP, and RSA with increased arousal and physical activity. The correlation between TWA_Toffset and TWA_Zero was very high in all conditions (range .96–.98) but, as expected, the TWA_Toffset, which uses a dynamic baseline, performed slightly better than the TWA_Zero measure, which uses a static baseline. Post hoc analyses revealed the expected significant decrease of TWA_Toffset with increased arousal and physical activity. TWA_Zero also showed a significant decrease across sitting to increased levels of physical activity but, in contrast to TWA_Toffset, failed to

Table 2. *Means (Standard Deviations) for the Variables Derived from the Ensemble-Averaged ECG and ICG During the Standardizd Baseline and Stress Conditions*

	N baseline–*N* stress task	Baseline	Stress task	Reactivity (Δ)
IBI (ms)	524–542	833.7 (123.5)	769.0 (112.6)	−64.7*
LVET (ms)	491–510	283.7 (34.2)	276.6 (35.7)	−7.1*
PEP (ms)	472–488	106.6 (18.7)	102.6 (19.1)	−4.0*
RSA (ms)	498–530	58.3 (23.9)	60.3 (22.3)	2*
TWA_Toffset (mV)	452–474	1.48 (0.55)	1.37 (0.54)	−.11*
TWA_Zero (mV)	460–485	.97 (.32)	.88 (.31)	−.09*

*Significantly different during stress compared to baseline, $p < .05$. Effect sizes were 0.55 *SD* for IBI, 0.26 for LVET, 0.22 for PEP, 0.27 for TWA_Toffset, and 0.32 for TWA_Zero.

Table 3. *Means (Standard Deviations) for the Variables Derived from the ECG and ICG During the Ambulatory Conditions*

	N range	Sleep	Sitting activities	Light physical activity	Moderate physical activity
IBI (ms)	549–564	982.7 (132.5)	815.0 (101.5)[a]	743.0 (92.9)[b]	669.2 (77.3)[c]
LVET (ms)	545–561	314.9 (28.1)	280.8 (29.1)[a]	270.4 (30.9)[b]	257.5 (29.6)[c]
PEP (ms)	539–559	106.5 (13.9)	101.7 (16.1)[a]	98.5 (16.5)[b]	92. (16.1)[c]
RSA (ms)	539–555	62.9 (23.7)	57.9 (19.7)[a]	53.6 (18.1)[b]	43.6 (14.2)[c]
TWA_Toffset (mV)	492–516	1.46 (0.55)	1.38 (0.52)[a]	1.19 (0.51)[b]	1.07 (0.49)[c]
TWA_Zero (mV)	504–525	0.92 (0.32)	0.90 (0.30)	0.80 (0.29)[b]	0.75 (0.29)[c]

Note. Sample sizes (*N*) vary depending on the number of participants in which the particular ECG landmark could be scored or in which an entire condition was missing.
[a]Significantly different compared to sleep, $p < .05$. Effect sizes were 1.68 *SD* for IBI, 1.17 for LVET, 0.32 for PEP, 0.28 for RSA, 0.16 for TWA_Toffset.
[b]Significantly different compared to sitting activities, $p < .05$. Effect sizes were 0.72 *SD* for IBI, 0.35 for LVET, 0.21 for PEP, 0.23 for RSA, 0.39 for TWA_Toffset, 0.35 for TWA_Zero.
[c]Significantly different compared to light physical activity, $p < .05$. Effect sizes were 0.75 *SD* for IBI, 0.45 for LVET, 0.42 for PEP, 0.54 for RSA, 0.26 for TWA_Toffset, 0.17 for TWA_Zero.

differentiate between sleep and sitting activities. TWA_Toffset was therefore selected as the TWA measure for further analyses.

Female participants had lower IBI (−41 ms), TWA_Zero (−.20 mV), and TWA_Toffset (−.36 mV) values but a longer PEP (6.2 ms) than males (*p*s < .001, effect sizes ranging between .28 and .65 *SD*). RSA was significantly lower in older participants ($r_{age} = -.25$, $p < .001$), and a larger BMI was associated with shorter IBI ($r = -.08$, $p < .001$) and PEP ($r = -.15$, $p < .001$), lower RSA ($r = -.11$, $p < .001$) and TWA_Toffset ($r = -.11$, $p < .001$), but a longer LVET ($r = .07$, $p < .001$).

Internal Consistency Reliability of TWA in a Naturalistic Setting

To obtain a measure of the internal consistency reliability, we averaged IBI, LVET, PEP, RSA, and TWA across half-hour periods during sleep for a minimum of 7 h. This left us with 414 participants across which Cronbach's alpha was computed as a measure of internal consistency validity. As shown in the first column of Table 4, very high internal consistency was found during sleep for all measures including the TWA_Toffset and TWA_Zero. The other columns present Cronbach's alpha across pools of the first 10 periods of sitting activities, light physical activity, and moderate physical activity. Whereas a slight decrease in consistency of IBI and RSA was seen, all variables remain highly consistent even during moderate physical activity, which is the most heterogenous condition.

Validity of the TWA During the Standardized Stressors

Significant correlations of the TWA_Toffset reactivity scores were found, with PEP reactivity ($r = .41$, $p < .001$) and IBI reactivity ($r = .69$, $p < .001$). TWA_Toffset decreases were also significantly correlated with RSA reactivity, albeit more modestly ($r = .17$, $p < .001$). As expected, IBI reactivity was correlated with both RSA ($r = .23$, $p < .001$) and PEP ($r = .44$, $p < .001$) reactivity. The correlation between PEP and TWA_Toffset reactivity did not significantly decrease after controlling for RSA reactivity, as shown in the partial correlation coefficient ($r_{part} = .42$, $p < .001$). A significant decrease did occur after controlling for IBI reactivity, although the partial correlations between TWA and PEP reactivity remained significant after adding IBI reactivity ($r_{part} = .17$, $p < .001$) or both IBI and RSA reactivity ($r_{part} = .17$, $p < .001$). In contrast, the correlation between RSA and TWA_Toffset reactivity disappeared after controlling for IBI reactivity ($r_{part} = .02$, $p = .41$) or both IBI and PEP reactivity ($r_{part} = .04$, $p = .16$).

Validity of the TWA in a Naturalistic Setting

TWA_Toffset, PEP, RSA, and IBI could be obtained in more than 15 ambulatory conditions in 480 participants. The distribution of the within-participant correlations between TWA_Toffset and PEP for these participants is plotted in the upper panel in Figure 2. This panel also gives the correlations between TWA_Toffset and RSA and TWA_Toffset and IBI. The TWA_Toffset showed a mean within-participant correlation of .35 with PEP. The number of participants that had a positive within-participant correlation between TWA and PEP meeting a nominal $p = .05$ significance threshold of .11 was 278. In 10 participants, a significant negative correlation was found.

The mean within-participant correlation between TWA_offset and RSA was .38 and between TWA_Toffset and IBI it was .61. The lower panel in Figure 2 shows the partial correlation between PEP and TWA_Toffset after partialling out IBI, RSA, and joint

Table 4. *Cronbach's Alpha for the ECG- and ICG-Derived Variables from Repeated Periods of Sleep and Daytime Conditions Within Each Participant*

	First 7 hours of sleep ($N = 414$)	First 10 periods of sitting activity ($N = 511$)	First 10 periods of light physical activity ($N = 272$)	First 10 periods of moderate physical activity ($N = 413$)
IBI (ms)	.98	.96	.95	.91
LVET (ms)	.96	.95	.94	.94
PEP (ms)	.96	.97	.94	.94
RSA (ms)	.97	.95	.95	.90
TWA_Toffset (mV)	.98	.97	.96	.97
TWA_Zero (mV)	.98	.97	.96	.97

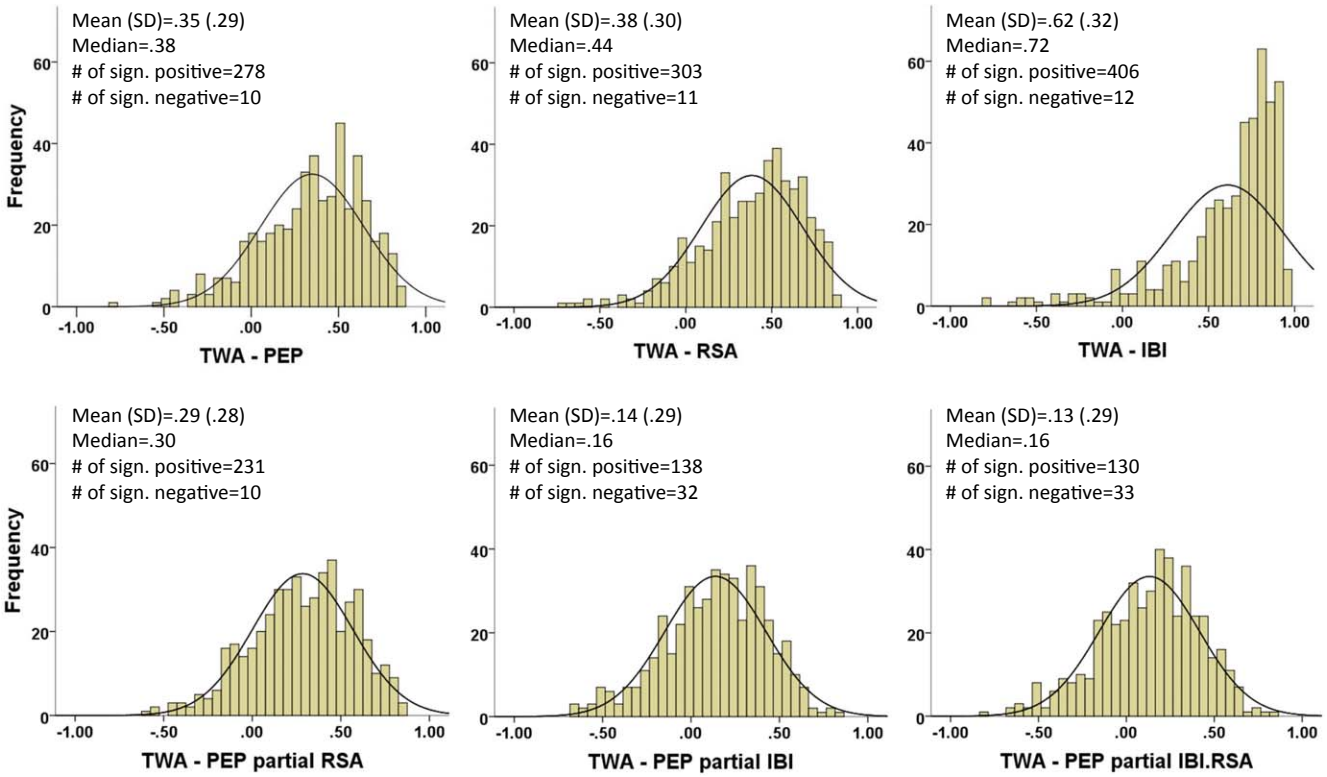

Figure 2. Distribution of the within-participant correlations of TWA-PEP, TWA-RSA, and TWA-IBI in the full sample ($N = 480$). TWA-PEP correlations are replotted with IBI, RSA, and joint IBI/RSA partialled out.

IBI/RSA. The drop in the mean correlation between TWA and PEP before and after taking changes in RSA into account was not significant ($p = .31$), but taking changes in IBI into account or changes in both IBI and vagal activity significantly reduced the correlation between PEP and TWA_Toffset ($ps < .001$). In spite of this attenuation, the mean within-participant correlation between PEP and TWA_Toffset remained significantly higher than zero after controlling for within-participant changes in RSA ($r_{part} = .29$, $p < .001$), IBI ($r_{part} = .14$, $p < .05$), or joint changes in IBI and RSA ($r_{part} = .13$, $p < .05$). In contrast, the mean within-participant correlation between RSA and TWA_Toffset was no longer significant after controlling for IBI ($r_{part} = .09$, $p = .10$) or IBI and PEP ($r_{part} = .08$, $p = .11$).

Optimal ICG Signal Recording and PEP Scoring Quality

Poor ICG signal quality could have led to low-quality PEP scoring and thus an underestimation of the PEP TWA_Toffset correlation. Repeating the analyses in the 101 participants with the highest quality of the ICG B-point scoring yielded higher within-participant correlations between TWA_Toffset and PEP, RSA, and IBI, although a similar distribution was seen when including all participants (upper panel in Figure 3). The TWA_Toffset showed a mean within-participant correlation of .43 with PEP. The number of participants that had a positive within-participant correlation between TWA_Toffset and PEP meeting a nominal $p = .05$ significance threshold of .24 was 69. In 32 participants, no significant correlation between PEP and TWA_Toffset was found in the expected direction. The mean within-participant correlation of TWA_Toffset with RSA was .43 and with IBI it was .71.

The lower panel in Figure 3 shows the partial correlation between PEP and TWA_Toffset after partialling out IBI, RSA, and joint IBI/RSA. The drop in the mean correlation between TWA_Toffset and PEP before and after taking RSA into account was not significant ($p = .47$) but taking either IBI or IBI and RSA jointly into account again significantly reduced the correlation between PEP and TWA_Toffset ($ps < 0.01$). Nonetheless, the mean within-participant correlation between PEP and TWA_Toffset remained significantly above zero after controlling for within-participant changes in RSA ($r_{part} = .34$, $p < .01$), IBI ($r_{part} = .25$, $p < .05$), or joint IBI and RSA ($r_{part} = .23$, $p < .05$). In contrast, the RSA-TWA correlation did not survive correction for IBI. The mean within-participant correlation between RSA and TWA_Toffset was no longer significant after controlling for IBI ($r_{part} = .10$, $p = .24$) or IBI and PEP jointly ($r_{part} = .07$, $p = .31$).

Discussion

Ambulatory assessment of fluctuations in cardiac SNS activity could greatly help epidemiologists understand the link between psychosocial stress and unfavorable health outcomes. The PEP is currently the measure of choice to assess cardiac SNS activity in ambulatory studies. The aim of the present study was to test the ECG T-wave amplitude as an alternative measure of cardiac SNS activity. Although TWA is a measure of ventricular repolarization and PEP is a measure of ventricular contractility, both are influenced by the beta-adrenergic effects of the ventricular sympathetic innervation, so that much of the information contained in PEP reactivity might be recaptured by studying TWA reactivity. The advantage of ambulatory TWA over PEP is that it can be derived

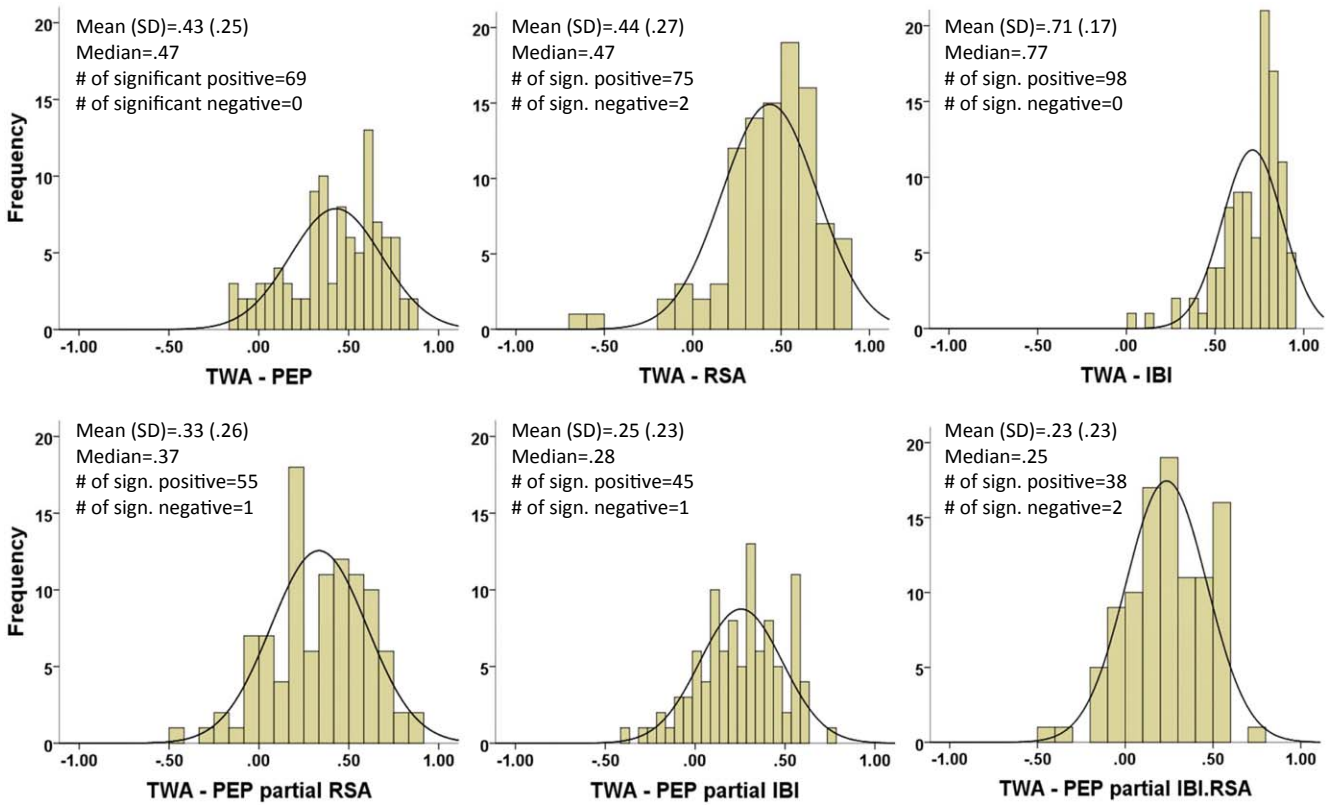

Figure 3. Distribution of the within-participant correlations of TWA-PEP, TWA-RSA, and TWA-IBI in the sample including only participants with unambiguous ICG B-point scoring ($N = 101$). TWA-PEP correlations are replotted with IBI, RSA and joint IBI/RSA partialled out.

from three spot electrodes that only present a minimal burden to the participant. If obtained from the ensemble-averaged ECG, the TWA requires less laborious data scoring and may prove more amenable to automation than PEP scoring. PEP scoring requires a detectable Q-onset, which is missing in about 19% of participants, as well as visual inspection of the B-point scoring in the ensemble-averaged ICG waveform, which requires multiple raters because it can be ambiguous, particularly in ambulatory recordings.

In our large sample of healthy adult participants, the TWA could be reliably extracted from the ensemble-averaged ECG in over 89% of the participants. In those participants, Cronbach's alpha for TWA across multiple periods varied between .96 and .98, suggesting high internal consistency. TWA also showed a small but significant decrease in response to a mental stress task in line with earlier research on the TWA (Contrada et al., 1989; Furedy & Heslegrave, 1984; Furedy et al., 1996; Heslegrave & Furedy, 1979; Scher, Hartman, Furedy, & Heslegrave, 1986). TWA further showed a monotonic decrease from nighttime sleep to daytime sitting to more physically active behaviors during an ambulatory 24-h recording, echoing the expected pattern of SNS activity across these various conditions. In addition, within-participant changes in TWA across the standardized as well as the unstandardized naturalistic conditions were correlated in the expected direction with parallel changes in the PEP, which we used as a criterion variable to establish convergent validity. The decreases in TWA in response to mental stress, and when going from sitting to active, as well as the correlation between decreases in TWA and decreases in PEP are compatible with the idea that TWA decreases can be used to measure increased cardiac SNS activity. It is important to note, however, that these findings are a necessary but not a sufficient condition, because heart rate and cardiac vagal activity also respond to increased arousal and the PEP is not a perfect measure of cardiac SNS activity. Also, the within-participant TWA-PEP correlations were significant in 69% of the participants only, even after selecting the subset of participants with the best quality ICG data. A first conclusion of our data, therefore, is that the ambulatory TWA can be a valuable addition to the epidemiologist's psychophysiology toolbox, but that caution is needed to interpret changes in TWA as purely reflecting cardiac SNS activity, particularly when doing so at the level of a single individual.

The literature examining the TWA as a measure of cardiac SNS activity in standardized laboratory conditions precedes the first application of regional cardiac norepinephrine (NE) spillover (Eisenhofer, Lambie, & Johnson, 1985; Esler et al., 1988), which is probably the only true gold standard of cardiac SNS activity. This is nontrivial, because low or absent correlation to cardiac NE spillover has been the major reason to distrust the LF/HF ratio as an indicator of cardiac SNS activity. It could be reasonably argued that true validity of the TWA likewise can only be assessed by a comparison of TWA to NE spillover or direct SNS nerve recording (Goedhart et al., 2008; Grassi & Esler, 1999). A large amount of pharmacological studies nonetheless bodes well for TWA. There is a clear pattern of decreased TWA with beta-adrenergic agonists (Barger et al., 1961; Contrada et al., 1989; Hartwell et al., 1942; Katz et al., 1932; Levine et al., 1930; Russell & Dart, 1986), and the effect is attenuated or disappears with beta-adrenergic antagonists (Contrada et al., 1989; Furberg, 1967, 1968; Guazzi et al., 1975; Noskowicz & Chrzanowski, 1968; Rau, 1991), although not all studies have been able to reproduce this pattern (Contrada et al.,

1991; Russell & Dart, 1986; Schwartz, Stone, & Brown, 1976; Taggart et al., 1979).

Admittedly, pharmacological studies have disadvantages in that they engage cardiac and vascular reflex regulation, which may prominently include cardiac vagal activity. There has been some debate on whether vagal activity itself might cause a decrease in TWA, which would invalidate it as an exclusive cardiac SNS measure. We therefore also computed the correlation between changes in RSA, a measure of cardiac vagal activity, and changes in TWA to provide a measure of discriminant validity. Changes in TWA were significantly correlated to changes in RSA, which at first sight suggests that TWA is as much a measure of decreased PNS as increased SNS activity to the heart. However, the dual correlation of TWA to PNS decreases and SNS increases could simply be explained by their profound reduction of the IBI, which may itself be the primary driver of TWA decreases. The correlation between changes in TWA and IBI was strong. Furthermore, it remained significant after partialling out PEP ($r_{part} = .55$ in full set; $r_{part} = .62$ in best quality subset) or partialling out both PEP and RSA ($r_{part} = .47$ in full set; $r_{part} = .55$ in best quality subset). This suggests that the TWA is sensitive to the shortening of the cardiac cycle, independent of whether it is caused by changes in cardiac SNS or PNS activity. This reflects as yet unknown effects that may involve a reduction in latency jitter or an inherent inverse relation of the amplitude of waveforms to their duration due to the shortening of the rise time.

Nonetheless, even after correcting for parallel changes in IBI, a significant relationship between PEP and TWA reactivity was found ($r_{part} = .17$ in full set; $r_{part} = .25$ in best quality subset), whereas the relationship between RSA and TWA reactivity disappeared. We note that survival of the PEP-TWA correlation is particularly appealing because correcting for changes in IBI is essentially overcorrecting: the same postganglionic fibers (N. accelerantes) that innervate the ventricle also innervate the sinoatrial node, and its activity can influence IBI and PEP simultaneously. Correcting one for the other is therefore a conservative correction. The fact that the PEP-TWA correlation is robust to this correction supports the idea that TWA shows sensitivity to cardiac SNS activity. Furthermore, correction for RSA changes did not lead to a significant reduction in the within-participant correlation between PEP and TWA reactivity. This suggests that cardiac vagal activity as such is not a confounder of the relation between changes in TWA and cardiac SNS activity.

A necessary limitation of this study is that it focused on the use of TWA as an alternative for the PEP as an ambulatory measure of cardiac SNS activity. Although the PEP is the only available measure of cardiac SNS activity in ambulatory recording, it must also be recognized that the PEP is sensitive to postural or physical activity-driven changes in preload that influence contractility independent of the SNS through the Frank-Starling mechanism (Houtveen et al., 2005). In addition, the PEP is sensitive to changes in mean aortic pressure (afterload) that can prolong the PEP even under conditions of increased cardiac SNS activity, as is seen during exposure to cold or static muscle work (de Geus, van Doornen, & Orlebeke, 1993; Krzeminski et al., 2000). Changes in temperature, posture, and static or dynamic exercise activities are a necessary element of naturalistic ambulatory monitoring. The PEP itself, therefore, will imperfectly correlate with cardiac SNS activity. In ambulatory settings, it is the best possible measure, but certainly not a gold standard. A further limitation is that we have implicitly assumed that SNS effects on the heart are unitary, whereas the SNS effects on ventricular contractility and ventricular repolarization might be partially independent. Such independence might result from differential effects of neural norepinephrine versus hormonal epinephrine on contractility and depolarization, differential effects of preload and afterload on TWA and PEP, and differential distribution of the two subtypes of cardiac beta-adrenergic receptors to these sympathetic effects.

These limitations are balanced by a number of strengths of this study. First, we had a large sample size allowing us to provide a normative dataset for the main ECG intervals including the QonsetR and RonsetR intervals, which we used to improve estimation of the PEP in participants where these landmarks were difficult to score. Secondly, by grace of the large dataset, we could repeat our analyses on a high-quality ICG data set to avoid ambiguity in the PEP scoring as a potential source of poor cross-measure correlation. Thirdly, although our major aim was to examine the properties of the TWA in a naturalistic ambulatory setting, the addition of the standardized stress testing allowed us to also investigate TWA in the same participants independent of confounding posture and activity effects and diurnal variations.

In conclusion, we find support for the usefulness of ensemble-averaged ECG-derived TWA in epidemiological research to estimate changes in 24-h cardiac SNS activity. The data are not sufficiently convincing to recommend replacement of the PEP by the TWA; instead, recording and analysis of both measures seems prudent and feasible. Prospective follow-up of the physical and mental health of our participants, who are part of a nationwide longitudinal study, must resolve the clinical value of these ambulatory measures, separately and in combination.

References

Abildskov, J. A. (1985). Neural mechanisms involved in the regulation of ventricular repolarization [Supplement D]. *European Heart Journal*, *6*, 31–39. doi: 10.1093/eurheartj/6.suppl_D.31

Abildskov, J. A., Burgess, M. J., Urie, P. M., Lux, R. L., & Wyatt, R. F. (1977). The unidentified information content of the electrocardiogram. *Circulation Research*, *40*, 3–7. doi: 10.1161/01.RES.40.1.3

Anitchkov, S. V., & Vedeneyeva, Z. I. (1961). Sympathetic stimulation as cause of myocardial damage. *Acta Physiologica Academy of Sciences Hungary*, *19*, 17–18.

Annila, P., Yli-Hankala, A., & Lindgren, L. (1993). Effect of atropine on the QT interval and T-wave amplitude in healthy volunteers. *British Journal of Anaesthesia*, *71*, 736–737. doi: 10.1093/bja/71.5.736

Autenrieth, G., Surawicz, B., Kuo, C. S., & Arita, M. (1975). Primary T wave abnormalities caused by uniform and regional shortening of ventricular monophasic action potential in dog. *Circulation*, *51*, 668–676. doi: 10.1161/01.CIR.51.4.668

Barger, A. C., Herd, J. A., & Liebowitz, M. R. (1961). Chronic catheterization of coronary artery: Induction of ECG pattern of myocardial ischemia by intracoronary epinephrine. *Proceedings of the Society for Experimental Biology and Medicine*, *107*, 474–477. doi: 10.3181/00379727-107-26661

Berntson, G. G., Bigger, J. T., Jr., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., ... van der Molen, N. W. (1997). Heart rate variability: Origins, methods, and interpretive caveats. *Psychophysiology*, *34*, 623–648. doi: 10.1111/j.1469-8986.1997.tb02140.x

Berntson, G. G., Cacioppo, J. T., Binkley, P. F., Uchino, B. N., Quigley, K. S., & Fieldstone, A. (1994). Autonomic cardiac control. III. Psychological stress and cardiac response in autonomic space as revealed by pharmacological blockades. *Psychophysiology*, *31*, 599–608. doi: 10.1111/j.1469-8986.1997.tb02140.x

Berntson, G. G., Lozano, D. L., Chen, Y. J., & Cacioppo, J. T. (2004). Where to Q in PEP. *Psychophysiology*, *41*, 333–337. doi: 10.1111/j.1469-8986.2004.00156.x

Bosch, J. A., Veerman, E. C., de Geus, E. J., & Proctor, G. B. (2011). Alpha-amylase as a reliable and convenient measure of sympathetic activity: Don't start salivating just yet! *Psychoneuroendocrinology*, *36*, 449–453. doi: 10.1016/j.psyneuen.2010.12.019

Bunnell, D. E. (1980). T-wave amplitude and the P-Q interval: Relationships to noninvasive indices of myocardial performance. *Psychophysiology*, *17*, 592–597. doi: 10.1111/j.1469-8986.1980.tb02304.x

Burgess, M. J. (1979). Relation of ventricular repolarization to electrocardiographic T wave-form and arrhythmia vulnerability. *American Journal of Physiology*, *236*, H391–H402.

Christov, I. I. (2004). Real time electrocardiogram QRS detection using combined adaptive threshold. *Biomedical Engineering Online*, *3*, 28.

Contrada, R. J., Dimsdale, J., Levy, L., & Weiss, T. (1991). Effects of isoproterenol on T-wave amplitude and heart-rate—A dose-response study. *Psychophysiology*, *28*, 458–462. doi: 10.1111/j.1469-8986.1991.tb00731.x

Contrada, R. J., Krantz, D. S., Durel, L. A., Levy, L., LaRiccia, P. J., Anderson, J. R., & Weiss, T. (1989). Effects of beta-adrenergic activity on T-wave amplitude. *Psychophysiology*, *26*, 488–492. doi: 10.1111/j.1469-8986.1989.tb01957.x

Coote, J. H. (2013). Myths and realities of the cardiac vagus. *Journal of Physiology*, *591*, 4073–4085. doi: 10.1113/jphysiol.2013.257758

Dauchot, P., & Gravenstein, J. S. (1971). Effects of atropine on the electrocardiogram in different age groups. *Clinical Pharmacology and Therapeutics*, *12*, 274–280. doi: 10.1097/00132586-197202000-00033

de Geus, E. J., Willemsen, G. H., Klaver, C. H., & van Doornen, L. J. (1995). Ambulatory measurement of respiratory sinus arrhythmia and respiration rate. *Biological Psychology*, *41*, 205–227. doi: 10.1016/0301-0511(95)05137-6

de Geus, E. J. C., van Doornen, L. J., & Orlebeke, J. F. (1993). Regular exercise and aerobic fitness in relation to psychological make-up and physiological stress reactivity. *Psychosomatic Medicine*, *55*, 347–363. doi: 10.1097/00006842-199307000-00003

Eckberg, D. L. (1997). Sympathovagal balance: A critical appraisal. *Circulation*, *96*, 3224–3232. doi: 10.1161/01.CIR.96.9.3224

Eisenhofer, G., Lambie, D. G., & Johnson, R. H. (1985). Beta-adrenoceptor responsiveness and plasma catecholamines as determinants of cardiovascular reactivity to mental stress. *Clinical Science*, *69*, 483–492.

Esler, M., Jennings, G., Korner, P., Willett, I., Dudley, F., Hasking, G., … Lambert, G. (1988). Assessment of human sympathetic nervous system activity from measurements of norepinephrine turnover. *Hypertension*, *11*, 3–20. doi: 10.1161/01.HYP.11.1.3

Fukudo, S., Lane, J. D., Anderson, N. B., Kuhn, C. M., Schanberg, S. M., McCown, N., … Williams, R. B., Jr. (1992). Accentuated vagal antagonism of beta-adrenergic effects on ventricular repolarization. Evidence of weaker antagonism in hostile type A men. *Circulation*, *85*, 2045–2053. doi: 10.1161/01.CIR.85.6.2045

Furberg, C. (1967). Adrenergic beta-blockade and electrocardiographical ST-T changes. *Acta Medica Scandinavica*, *181*, 21–32. doi: 10.1111/j.0954-6820.1967.tb07221.x

Furberg, C. (1968). Effects of repeated work tests and adrenergic beta-blockade on electrocardiographic ST and T changes. *Acta Medica Scandinavica*, *183*, 153–161. doi: 10.1111/j.0954-6820.1968.tb10457.x

Furedy, J. J., & Heslegrave, R. J. (1983). A consideration of recent criticisms of the T-wave amplitude index of myocardial sympathetic activity. *Psychophysiology*, *20*, 204–211. doi: 10.1111/j.1469-8986.1983.tb03289.x

Furedy, J. J., & Heslegrave, R. J. (1984). The relative sensitivities of heart rate and T-wave amplitude to stress: Comments on, and some alternative interpretations of, Penzien et al.'s results. *Biological Psychology*, *19*, 55–61. doi: 10.1016/0301-0511(84)90010-3

Furedy, J. J., Heslegrave, R. J., & Scher, H. (1984). Psychophysiological and physiological aspects of T-wave amplitude in the objective study of behavior. *Pavlov Journal of Biological Science*, *19*, 182–194.

Furedy, J. J., & Shulhan, D. L. (1986). Effects of respiratory depth and rate on HR and TWA: An indirect assessment of the respiratory confound in cognitive task difficulty manipulations. *Physiology & Behavior*, *37*, 515–517. doi: 10.1016/0031-9384(86)90217-9

Furedy, J. J., Szabo, A., & Peronnet, F. (1996). Effects of psychological and physiological challenges on heart rate, T-wave amplitude, and pulse-transit time. *International Journal of Psychophysiology*, *22*, 173–183. doi: 10.1016/0167-8760(96)00025-6

Goedhart, A. D., Kupper, N., Willemsen, G., Boomsma, D. I., & de Geus, E. J. (2006). Temporal stability of ambulatory stroke volume and cardiac output measured by impedance cardiography. *Biological Psychology*, *72*, 110–117. doi: 10.1016/j.biopsycho.2005.08.004

Goedhart, A. D., Willemsen, G., Houtveen, J. H., Boomsma, D. I., & de Geus, E. J. (2008). Comparing low frequency heart rate variability and preejection period: Two sides of a different coin. *Psychophysiology*, *45*, 1086–1090. doi: 10.1111/j.1469-8986.2008.00710.x

Grassi, G., & Esler, M. (1999). How to assess sympathetic activity in humans. *Journal of Hypertension*, *17*, 719–734. doi: 10.1097/00004872-199917060-00001

Grossman, P., van Beek, J., & Wientjes, C. (1990). A comparison of three quantification methods for estimation of respiratory sinus arrhythmia. *Psychophysiology*, *27*, 702–714. doi: 10.1111/j.1469-8986.1990.tb03198.x

Grossman, P., & Wientjes, C. J. E. (1986). Respiratory sinus arrhythmia and parasympathetic cardiac control: Some basic issues concerning quantification, applications and implications. In P. Grossman, K. H. Janssen, & D. Vaitl (Eds.), *Cardiorespiratory and somatic psychophysiology* (pp. 284–300). New York, NY: Plenum Press. doi: 10.1007/978-1-4757-0360-3_8

Guazzi, M., Polese, A., Fiorentini, C., Magrini, F., Olivari, M. T., & Bartorelli, C. (1975). Left and right heart haemodynamics during spontaneous angina pectoris. Comparison between angina with ST segment depression and angina with ST segment elevation. *British Heart Journal*, *37*, 401–413. doi: 10.1136/hrt.37.4.401

Haarmark, C., Graff, C., Andersen, M. P., Hardahl, T., Struijk, J. J., Toft, E., … Kanters, J. K. (2010). Reference values of electrocardiogram repolarization variables in a healthy population. *Journal of Electrocardiology*, *43*, 31–39. doi: 10.1016/j.jelectrocard.2009.08.001

Harris, W. S., Schoenfeld, C. D., & Weissler, A. M. (1967). Effects of adrenergic receptor activation and blockade on the systolic preejection period, heart rate, and arterial pressure in man. *Journal of Clinical Investigation*, *46*, 1704–1714. doi: 10.1172/JCI105661

Hartwell, A. S., Burrett, J. B., Graybiel, A., & White, P. D. (1942). The effect of exercise and of four commonly used drugs on the normal human electrocardiogram, with particular reference to T wave changes. *Journal of Clinical Investigation*, *21*, 409–417. doi: 10.1172/JCI101317

Heslegrave, R. J., & Furedy, J. J. (1979). Sensitivities of HR and T-wave amplitude for detecting cognitive and anticipatory stress. *Physiology & Behavior*, *22*, 17–23. doi: 10.1016/0031-9384(79)90397-4

Houtveen, J. H., Groot, P. F., & Geus, E. J. (2005). Effects of variation in posture and respiration on RSA and pre-ejection period. *Psychophysiology*, *42*, 713–719. doi: 10.1111/j.1469-8986.2005.00363.x

Katz, L. N., Hamburger, W. W., & Lev, M. (1932). The diagnostic value of epinephrine in angina pectoris. *American Heart Journal*, *7*, 371–379. doi: 10.1016/S0002-8703(32)90350-0

Kelsey, R. M., & Guethlein, W. (1990). An evaluation of the ensemble averaged impedance cardiogram. *Psychophysiology*, *27*, 24–33. doi: 10.1111/j.1469-8986.1990.tb02173.x

Kelsey, R. M., Ornduff, S. R., & Alpert, B. S. (2007). Reliability of cardiovascular reactivity to stress: Internal consistency. *Psychophysiology*, *44*, 216–225. doi: 10.1111/j.1469-8986.2007.00499.x

Kline, K. P., Ginsburg, G. P., & Johnston, J. R. (1998). T-wave amplitude: Relationships to phasic RSA and heart period changes. *International Journal of Psychophysiology*, *29*, 291–301. doi: 10.1016/S0167-8760(98)00021-X

Kreibig, S. D., Wilhelm, F. H., Roth, W. T., & Gross, J. J. (2007). Cardiovascular, electrodermal, and respiratory response patterns to fear- and sadness-inducing films. *Psychophysiology*, *44*, 787–806. doi: 10.1111/j.1469-8986.2007.00550.x

Krzeminski, K., Kruk, B., Nazar, K., Ziemba, A. W., Cybulski, G., & Niewiadomski, W. (2000). Cardiovascular, metabolic and plasma catecholamine responses to passive and active exercises. *Journal of Physiological Pharmacology*, *51*, 267–278.

Kubiak, T., & Stone, A. A. (2012). Ambulatory monitoring of biobehavioral processes in health and disease. *Psychosomatic Medicine*, *74*, 325–326. doi: 10.1097/PSY.0b013e31825878da

Kupper, N., Willemsen, G., Boomsma, D. I., & de Geus, E. J. (2006). Heritability of indices for cardiac contractility in ambulatory recordings. *Journal of Cardiovascular Electrophysiology*, *17*, 877–883. doi: 10.1111/j.1540-8167.2006.00535.x

Kupper, N., Willemsen, G., Posthuma, D., De Boer, P., Boomsma, D. I., & de Geus, E. J. C. (2005). A genetic analysis of ambulatory

cardiorespiratory coupling. *Psychophysiology*, *42*, 202–212. doi: 10.1111/j.1469-8986.2005.00276.x

Labidi, Z., Ehmke, D. A., Durnin, R. E., Leaverton, P. E., & Lauer, R. M. (1970). The first derivative thoracic impedance cardiogram. *Circulation*, *41*, 651–658.

Lambert, G., Schlaich, M., Lambert, E., Dawood, T., & Esler, M. (2010). Stress reactivity and its association with increased cardiovascular risk: A role for the sympathetic nervous system? *Hypertension*, *55*, e20. doi: 10.1161/HYPERTENSIONAHA.110.153841

Levine, S. A., Ernstene, A. C., & Jacobson, B. M. (1930). The use of epinephrine as a diagnostic test for angina pectoris: With observations on the electrocardiographic changes following injections of epinephrine into normal subjects and into patients with angina pectoris. *Archives of Internal Medicine*, *45*, 191–200. doi: 10.1001/archinte.1930.00140080033002

Levy, M. N. (1977). Parasympathetic control of the heart. In W. C.Randall (Ed.), *Neural regulation of the heart* (pp. 97–129). New York, NY: Oxford University Press.

Levy, M. N., & Zieske, H. (1969). Autonomic control of cardiac pacemaker activity and atrioventricular transmission. *Journal of Applied Physiology*, *27*, 465–470.

Lozano, D. L., Norman, G., Knox, D., Wood, B. L., Miller, B. D., Emery, C. F. & Berntson, G. G. (2007). Where to B in dZ/dt. *Psychophysiology*, *44*, 113–119. doi: 10.1111/j.1469-8986.2006.00468.x

Matyas, T. A. (1976). T wave amplitude stability during sinus arrhythmia in the resting man. *Physiology & Behavior*, *16*, 115–117. doi: 10.1016/0031-9384(76)90204-3

Matyas, T. A., & King, M. G. (1976). Stable T wave effects during improvement of heart rate control with biofeedback. *Physiology & Behavior*, *16*, 15–20. doi: 10.1016/0031-9384(76)90186-4

Mezzacappa, E. S., Kelsey, R. M., & Katkin, E. S. (1999). The effects of epinephrine administration on impedance cardiographic measures of cardiovascular function. *International Journal of Psychophysiology*, *31*, 189–196. doi: 10.1016/S0167-8760(98)00058-0

Miyamoto, Y., Higuchi, J., Abe, Y., Hiura, T., Nakazono, Y., & Mikami, T. (1983). Dynamics of cardiac output and systolic time intervals in supine and upright exercise. *Journal of Applied Physiology*, *55*, 1674–1681.

Nakonezny, P. A., Kowalewski, R. B., Ernst, J. M., Hawkley, L. C., Lozano, D. L., Litvack, D. A., … Lovallo, W. R. (2001). New ambulatory impedance cardiograph validated against the Minnesota Impedance Cardiograph. *Psychophysiology*, *38*, 465–473. doi: 10.1111/1469-8986.3830465

Nater, U. M., & Rohleder, N. (2009). Salivary alpha-amylase as a noninvasive biomarker for the sympathetic nervous system: Current state of research. *Psychoneuroendocrinology*, *34*, 486–496. doi: 10.1016/j.psyneuen.2009.01.014

Nelesen, R. A., Shaw, R., Ziegler, M. G., & Dimsdale, J. E. (1999). Impedance cardiography-derived hemodynamic responses during baroreceptor testing with amyl nitrite and phenylephrine: A validity and reliability study. *Psychophysiology*, *36*, 105–108. doi: 10.1017/S0048577299971500

Newlin, D. B., & Levenson, R. W. (1979). Pre-ejection period: Measuring beta-adrenergic influences upon the heart. *Psychophysiology*, *16*, 546–553. doi: 10.1111/j.1469-8986.1979.tb01519.x

Noskowicz, T., & Chrzanowski, W. (1968). The influence of propranolol on functional alterations of the electrocardiogram. *Cardiologia*, *52*, 324–329. doi: 10.1159/000166134

Pagani, M., Lombardi, F., Guzzetti, S., Rimoldi, O., Furlan, R., Pizzinelli, P., … Piccaluga, E. (1986). Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympathovagal interaction in man and conscious dog. *Circulation Research*, *59*, 178–193. doi: 10.1161/01.RES.59.2.178

Pagani, M., Mazzuero, G., Ferrari, A., Liberati, D., Cerutti, S., Vaitl, D., … Malliani, A. (1991). Sympathovagal interaction during mental stress—A study using spectral-analysis of heart-rate-variability in healthy control subjects and patients with a prior myocardial-infarction. *Circulation*, *83*, 43–51.

Pagani, M., Montano, N., Porta, A., Malliani, A., Abboud, F. M., Birkett, C., & Somers, V. K. (1997). Relationship between spectral components of cardiovascular variabilities and direct measures of muscle sympa thetic nerve activity in humans. *Circulation*, *95*, 1441–1448. doi: 10.1161/01.CIR.95.6.1441

Palomba, D., Sarlo, M., Angrilli, A., Mini, A., & Stegagno, L. (2000). Cardiac responses associated with affective processing of unpleasant film stimuli. *International Journal of Psychophysiology*, *36*, 45–57. doi: 10.1016/S0167-8760(99)00099-9

Pan, R. L.-C., & Li, J. K. J. (2007). A noninvasive parametric evaluation of stress effects on global cardiovascular function. *Cardiovascular Engineering*, *7*, 74–80. doi: 10.1007/s10558-007-9028-6

Parati, G., & Esler, M. (2012). The human sympathetic nervous system: Its relevance in hypertension and heart failure. *European Heart Journal*, *33*, 1058–1066. doi: 10.1093/eurheartj/ehs041

Preacher, K. J. (2002). Calculation for the test of the difference between two independent correlation coefficients. Retrieved from http://www.quantpsy.org/corrtest/corrtest.htm

Randall, D. C., & Hasson, D. M. (1977). A note on ECG changes observed during Pavlovian conditioning in a rhesus monkey following coronary arterial occlusion. *Pavlov Journal of Biological Science*, *12*, 229–231.

Rau, H. (1991). Responses of the T-wave amplitude as a function of active and passive tasks and beta-adrenergic blockade. *Psychophysiology*, *28*, 231–239. doi: 10.1111/j.1469-8986.1991.tb00415.x

Reyes Del Paso, G. A., Langewitz, W., Mulder, L. J., van Roon, A., & Duschek, S. (2013). The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: A review with emphasis on a reanalysis of previous studies. *Psychophysiology*, *50*, 477–487. doi: 10.1111/psyp.12027

Richter, M., & Gendolla, G. H. (2009). The heart contracts to reward: Monetary incentives and preejection period. *Psychophysiology*, *46*, 451–457. doi: 10.1111/j.1469-8986.2009.00795.x

Riese, H., Groot, P. F. C., van den Berg, M., Kupper, N. H. M., Magnee, E. H. B., Rohaan, E. J., … de Geus, E. J. (2003). Large-scale ensemble averaging of ambulatory impedance cardiograms. *Behavior Research Methods Instruments & Computers*, *35*, 467–477. doi: 10.3758/BF03195525

Roth, W. T., Margraf, J., Ehlers, A., Haddad, J. M., Maddock, R. J., Agras, W. S., & Taylor, C. B. (1992). Imipramine and alprazolam effects on stress test reactivity in panic disorder. *Biological Psychiatry*, *31*, 35–51. doi: 10.1016/0006-3223(92)90005-K

Russell, D. C., & Dart, A. (1986). T wave amplitude as a quantitative index of regional myocardial sympathetic responsiveness. *Journal of Cardiovascular Pharmacology*, *8*, 629–635. doi: 10.1097/00005344-198605000-00028

Schachinger, H., Weinbacher, M., Kiss, A., Ritz, R., & Langewitz, W. (2001). Cardiovascular indices of peripheral and central sympathetic activation. *Psychosomatic Medicine*, *63*, 788–796. doi: 10.1097/00006842-200109000-00012

Scher, H., Furedy, J. J., & Heslegrave, R. J. (1984). Phasic T-wave amplitude and heart rate changes as indices of mental effort and task incentive. *Psychophysiology*, *21*, 326–333. doi: 10.1111/j.1469-8986.1984.tb02942.x

Scher, H., Hartman, L. M., Furedy, J. J., & Heslegrave, R. J. (1986). Electrocardiographic T-wave changes are more pronounced in type A than in type B men during mental work. *Psychosomatic Medicine*, *48*, 159–166. doi: 10.1097/00006842-198603000-00001

Schwartz, P. J., Stone, H. L., & Brown, A. M. (1976). Effects of unilateral stellate ganglion blockade on the arrhythmias associated with coronary occlusion. *American Heart Journal*, *92*, 589–599. doi: 10.1016/S0002-8703(76)80078-6

Schwartz, P. J., & Weiss, T. (1983). T-wave amplitude as an index of cardiac sympathetic activity: A misleading concept. *Psychophysiology*, *20*, 696–701. doi: 10.1111/j.1469-8986.1983.tb00941.x

Sherwood, A., Allen, M. T., Fahrenberg, J., Kelsey, R. M., Lovallo, W. R., & van Doornen, L. J. (1990). Methodological guidelines for impedance cardiography. *Psychophysiology*, *27*, 1–23.

Sherwood, A., Allen, M. T., Obrist, P. A., & Langer, A. W. (1986). Evaluation of beta-adrenergic influences on cardiovascular and metabolic adjustments to physical and psychological stress. *Psychophysiology*, *23*, 89–105. doi: 10.1111/j.1469-8986.1986.tb00602.x

Sherwood, A., McFetridge, J., & Hutcheson, J. S. (1998). Ambulatory impedance cardiography: A feasibility study. *Journal of Applied Physiology*, *85*, 2365–2369.

Smith, J. J., Muzi, M., Barney, J. A., Ceschi, J., Hayes, J., & Ebert, T. J. (1989). Impedance-derived cardiac indices in supine and upright exercise. *Annals of Biomedical Engineering*, *17*, 507–515. doi: 10.1007/BF02368070

Stratton, J. R., Pfeifer, M. A., & Halter, J. B. (1987). The hemodynamic effects of sympathetic stimulation combined with parasympathetic blockade in man. *Circulation*, *75*, 922–929.

Svedenhag, J., Martinsson, A., Ekblom, B., & Hjemdahl, P. (1986). Altered cardiovascular responsiveness to adrenaline in endurance-trained subjects. *Acta Physiologica Scandinavica*, *126*, 539–550. doi: 10.1111/j.1748-1716.1986.tb07853.x

Taggart, P., Carruthers, M., Joseph, S., Kelly, H. B., Marcomichelakis, J., Noble, D., … Somerville, W. (1979). Electrocardiographic changes resembling myocardial ischaemia in asymptomatic men with normal coronary arteriograms. *British Heart Journal*, *41*, 214–225. doi: 10.1136/hrt.41.2.214

Task Force of the European Society of Cardiology and the North American Society of Pacing (1996). Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. *Circulation*, *93*, 1043–1065.

Trull, T. J., & Ebner-Priemer, U. (2013). Ambulatory assessment. *Annual Review of Clinical Psychology*, *9*, 151–176. doi: 10.1146/annurev-clinpsy-050212-185510

van Dijk, A. E., van Lien R., van Eijsden M., Gemke, R. J., Vrijkotte, T. G., & de Geus, E. J. (2013). Measuring cardiac autonomic nervous system (ANS) activity in children. *Journal of Visualized Experiments*, *74*, e50073. doi: 10.3791/50073

van Lien, R., Goedhart, A., Kupper, N., Boomsma, D., Willemsen, G., & de Geus, E. J. (2011). Underestimation of cardiac vagal control in regular exercisers by 24-hour heart rate variability recordings. *International Journal of Psychophysiology*, *81*, 169–176. doi: 10.1016/j.ijpsycho.2011.06.007

van Lien, R., Schutte, N. M., Meijer, J. H., & de Geus, E. J. (2013). Estimated preejection period (PEP) based on the detection of the R-wave and dZ/dt-min peaks does not adequately reflect the actual PEP across a wide range of laboratory and ambulatory conditions. *International Journal of Psychophysiology*, *87*, 60–69. doi: 10.1016/j.ijpsycho.2012.11.001

Vrijkotte, T. G., van Doornen, L. J., & de Geus, E. J. (2004). Overcommitment to work is associated with changes in cardiac sympathetic regulation. *Psychosomatic Medicine*, *66*, 656–663. doi: 10.1097/01.psy.0000138283.65547.78

Weiss, T., Del, B. A., Reichek, N., & Engelman, K. (1980). Pulse transit time in the analysis of autonomic nervous system effects on the cardiovascular system. *Psychophysiology*, *17*, 202–207. doi: 10.1111/j.1469-8986.1980.tb00136.x

Wilhelm, F. H., Roth, W. T., & Sackner, M. A. (2003). The lifeShirt. An advanced system for ambulatory measurement of respiratory and cardiac function. *Behavior Modification*, *27*, 671–691. doi: 10.1177/0145445503256321

Willemsen, G., de Geus, E. J., Bartels, M., Van Beijsterveldt, C. E., Brooks, A. I., Estourgie-van Burk, G. F., … Boomsma, D. I. (2010). The Netherlands twin register biobank: A resource for genetic epidemiological studies. *Twin Research and Human Genetics*, *13*, 231–245. doi: 10.1375/twin.13.3.231

Willemsen, G., Vink, J. M., Abdellaoui, A., den, B. A., van Beek, J. H., Draisma, H. H., … Boomsma, D. I. (2013). The adult Netherlands twin register: Twenty-five years of survey and biological data collection. *Twin Research and Human Genetics*, *16*, 271–281. doi: 10.1017/thg.2012.140

Willemsen, G. H., de Geus, E. J., Klaver, C. H., van Doornen, L. J., & Carroll, D. (1996). Ambulatory monitoring of the impedance cardiogram. *Psychophysiology*, *33*, 184–193. doi: 10.1111/j.1469-8986.1996.tb02122.x

Williams, P. D., Puddey, I. B., Beilin, L. J., & Vandongen, R. (1993). Genetic influences on plasma catecholamines in human twins. *Journal of Clinical Endocrinology and Metabolism*, *77*, 794–799. doi: 10.1210/jcem.77.3.8370701

Winzer, A., Ring, C., Carroll, D., Willemsen, G., Drayson, M., & Kendall, M. (1999). Secretory immunoglobulin A and cardiovascular reactions to mental arithmetic, cold pressor, and exercise: Effects of beta-adrenergic blockade. *Psychophysiology*, *36*, 591–601. doi: 10.1111/1469-8986.3650591

Yanowitz, F., Preston, J. B., & Abildskov, J. A. (1966). Functional distribution of right and left stellate innervation to the ventricles. Production of neurogenic electrocardiographic changes by unilateral alteration of sympathetic tone. *Circulation Research*, *18*, 416–428. doi: 10.1161/01.RES.18.4.416

(Received December 24, 2013; Accepted July 3, 2014)