This thesis addresses autonomic nervous system function in a (pre)adolescent population cohort. There has been wide interest in the identification of biological correlates of behavioral characteristics over the past decades. Behavioral features are known to be influenced by a complex interplay between both environmental and genetic factors, which may be reflected in autonomic nervous system functioning. Studying the relationship between autonomic function and behavioral characteristics may therefore further our understanding of the etiology of behavioral functioning.

Measures such as heart rate (HR), heart rate variability (HRV), and baroreflex sensitivity [BRS; a measure of the quality of short-term blood pressure (BP) control] are important indicators of autonomic nervous system function that have been found to be associated with a variety of psychosocial variables. However, findings are still inconsistent, which may be partly explained by the use of small, non-representative samples. So far, studies on the relationship between autonomic function and behavioral characteristics have rarely been conducted in large population samples.

In the present set of studies, we investigated autonomic nervous system function in relationship to three important areas of functioning in a large (pre)adolescent population cohort. The first study regards the demographic determinants age and gender in addition to the general health indices body mass index and physical activity in relation to autonomic function. This is followed by two studies concerning the relationship between autonomic nervous system function and temperament and psychopathology, respectively. Finally, we describe the short-term reproducibility of various important autonomic function measures in a smaller sample of (pre)adolescents.

Before turning to the different studies of this thesis, this chapter provides a short introduction of the study objectives and methods, followed by an outline of this thesis. More detailed information about the function and structure of the autonomic nervous system, as well as the autonomic measures relevant to our study is described in chapter 2.

**STUDY OBJECTIVES**

**Baroreflex sensitivity in relation to demographic and general health determinants**

This first study focused exclusively on BRS, a relatively novel and sophisticated integrated measure of both sympathetic and parasympathetic autonomic function. While a considerable number of studies on normal HRV in healthy children is available (e.g., Finley et al. 1987, Massin & von Bernuth 1997, Urbina et al. 1998) large studies on normal BRS values and determinants of spontaneous BRS in children and adolescents from the general population are lacking. To better understand pathophysiological processes, knowledge of normal functions in apparently healthy individuals is a prerequisite. Therefore, the possible association of
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gender, age, pubertal stage, body mass index, and physical activity with baroreflex function in children is described in chapter 2, as studied in a large, population-based cohort of 10-to-13-year-old (pre)adolescents. In addition to measurements in the supine and standing positions, difference scores between both were calculated.

Based on the literature, we expected a negative association of BRS with age and pubertal stage, although the age range studied was quite small. Also based on studies in adults, we hypothesized BRS to be negatively related to female gender (Beske et al. 2001, Laitinen et al. 1998) and obesity (Emdin et al. 2001). Given the increasing number of children with obesity, this factor might be of particular interest as a potential risk factor for autonomic dysfunction.

Autonomic nervous system in relation to temperament and psychopathology

In general, temperament refers to individual differences in overt behavior, emotion, and motivational styles. In the literature, little agreement exists as to what exactly is temperament, thus many definitions of temperament are available. For example, Rothbart & Derryberry (1981) think of temperament as reflecting individual differences in behavioral regulation, reactivity, affectivity, and motivation. Also well-known is the personality theory of Eysenck, who described three main personality factors, i.e., neuroticism, extraversion, and psychoticism (Eysenck 1967).

In the present thesis, we use two common dimensions underlying temperament that may be distinguished: activation (approach) and inhibition (avoidance) (Elliot & Thrash 2002). Temperamental activation refers to an approaching and uninhibited behavioral style, motivated by positive feelings and sensitivities to reward and pleasure (Gray 1991). Examples of related personality constructs are stimulation-seeking, extraversion, and positive affectivity (Carver & White 1994, Elliot & Thrash 2002, Ravaja 2004). Temperamental inhibition, on the other hand, comprises avoidant behaviors and withdrawal responses guided by feelings of anxiety (Gray 1991). Personality constructs related to temperamental inhibition include shyness, introversion, and negative affectivity (Elliot & Thrash 2002, Kagan et al. 1988, Ravaja 2004).

The distinction between the concepts temperament and personality is considerably vague and many authors use both terms interchangeably. In an attempt to distinguish both concepts, it has been argued that temperament refers to stable patterns beginning early in life, whereas personality is shaped in later periods of development and is influenced by the social environment (Strelau 1994). A relationship between temperament and autonomic function is conceivable, given that temperament is considered to be an inherited behavioral disposition with an underlying neurobiological basis: individual differences in temperament are thought to parallel individual differences in neurobiology (Strelau 1994).

Whereas temperament concerns potentially normal, adaptive psychosocial functioning, psychopathology refers to abnormality. Psychopathology may be
manifested in different kinds of behavioral and emotional problems, such as aggression, rule-breaking behavior, anxiety, depression, attention and social problems, or somatic complaints.

In this thesis, we focused on two primary broad-band dimensions of internalizing and externalizing behavior which are thought to underlie psychopathology in children and adolescents (Achenbach 1978, Krueger et al. 1998). Children with internalizing behaviors are characterized by withdrawal from the external world (e.g., anxious and depressive symptoms, somatic complaints), while children with externalizing behaviors can be portrayed as moving to the outside world (e.g., aggression, delinquency) (Krueger et al. 1998).

An extensive literature has suggested an association not only between temperament and autonomic nervous system functioning (e.g., Garcia Coll et al. 1984, Kagan et al. 1988, Raine et al. 1997, Richards & Cameron 1989, Scarpa et al. 1997), but also between psychopathology and autonomic nervous system measures (e.g., Allen et al. 2000, Mezzacappa et al. 1997, Monk et al. 2001, Ortiz & Raine 2004, Rubin et al. 1997, Virtanen et al. 2003, Watkins et al. 1999), in adults as well as in children and adolescents. The available findings with regard to temperament and psychopathology have been reviewed in the introductory sections of chapter 4 and 5, respectively.

A theoretical framework to interpret the findings may be found in arousal theory. According to this, individuals with lower physiological arousal would be likely to engage in risk-taking or rule-breaking behavior in order to increase their arousal level to a physiologically more pleasant one (Eysenck 1997, Raine 2002, Zuckerman 1990). In contrast, persons with high physiological arousal would tend to avoid such behaviors and would be inclined to behavioral withdrawal and inhibition. Although the concept of arousal does not specifically refer to underlying brain structures or processes, underarousal and overarousal may be reflected in a decreased and an increased HR, respectively.

Another influential theory is Porges’ vagal brake hypothesis (Porges 1995, 1996) which states, in short, that increased vagal activity is associated with ‘healthy psychological functioning’. He pointed to the regulatory function of vagal activity serving as a “brake” to promote physiological flexibility and adaptability to meet environmental demands, which would also be reflected in behavioral parameters, such as increased openness to new experiences, emotional expressivity, and social skills (Porges et al. 1994). A low level of vagal activity may, in contrast, be associated with abnormal psychological functioning (Beauchaine 2001, Thayer & Brosschot 2005).

Even when some of the literature on the relationship between autonomic and behavioral parameters is in line with the arousal theory and Porges’ vagal brake hypothesis, this field of research is characterized by many inconsistencies, perhaps partially explained by small and non-representative samples. In addition, literature is sparse regarding certain relationships, for instance, between temperamental
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activation and HR, and between both externalizing and internalizing problems and vagal activity. Also, to our knowledge, only one other pediatric study has reported on the association between behavior and BRS (Allen et al. 2000). Furthermore, the role of behavioral and emotional problems that were reported to be already present early in life has rarely been studied in association with autonomic function. Finally, a few previous studies have suggested an association between orthostatic stress-induced autonomic reactivity and psychopathology (Mezzacappa et al. 1997, Stein et al. 1992, Yeragani et al. 1991). However, the relevance of this physical stressor to temperament and psychopathology is not yet clear. We aimed to address this by conducting the studies described in chapter 4 and 5, concerning the relationship of various autonomic measures with temperament and broadband psychopathology dimensions, respectively.

Overall, we expected different autonomic patterns to be associated with the temperamental dimensions ‘activation versus inhibition’, and with the psychopathological dimensions ‘externalizing versus internalizing problems’. We were interested in whether we could find a decreased HR in both temperamental activation and externalizing problems as an indication of autonomic underarousal, and an increased HR in both temperamental inhibition and internalizing problems as an indication of overarousal. We also wanted to explore whether autonomic function would be differentially related to temperament versus psychopathology.

Short-term reproducibility of autonomic measures
Test-retest data of the autonomic measures enables a proper interpretation of the strength of the relationships of autonomic function with the various parameters as described in the first three studies, as well as work from other authors. So far, a myriad of studies has examined autonomic measures in relation to psychosocial variables (Beauchaine 2001, Eisenberg et al. 1996, Ortiz & Raine 2004). These measures have also been widely applied in medical research and clinical practice, for instance in individuals with syncope (Suhra et al. 1999), diabetes mellitus (Rollins et al. 1992), and cardiac (Massin & von Bernuth 1998) or other diseases (Hrstkova et al. 2001).

However, much to our surprise, the short-term reproducibility of autonomic indices has rarely been investigated, especially in children. The few studies in children and adolescents show inconsistent results, which we have reviewed in detail in chapter 3. Whereas some studies reported sufficient, mostly moderate, reproducibility, other studies indicated poor reproducibility. Other gaps in the pediatric literature are the lack of studies on indices of autonomic function other than HR and HRV, as well as studies on the reproducibility of measurements in non-resting conditions, such as in response to orthostatic stress. Therefore, as described in chapter 3, we studied the short-term reproducibility of different autonomic measures related to HR and BP, to get more insight into the reliability of autonomic measurements in our (pre)adolescent study population and to facilitate the interpretation of findings of the other studies of this thesis. Also, we provide the
test-retest reproducibility of autonomic measures in response to orthostatic stress, which is used to gain more insight into autonomic regulation compared to static comparisons (Pagani & Malliani 2000). In addition, we used a more complementary statistical approach, other than only relying on correlational analyses, with methods that are commonly used in reliability research (such as the coefficient of variation and Bland & Altman plots). The results of the study described in chapter 6 may be of use to other researchers who use a similar methodology as in our study.

METHODS OF THE CURRENT STUDY

This thesis is embedded in the prospective cohort study “TRacking Adolescents’ Individual Lives Survey” (TRAILS) of Dutch (pre)adolescents (N=2,230), with the aim to chart and explain the development of mental health from preadolescence into adulthood, both at the level of psychopathology and the levels of underlying vulnerability and environmental risk. This thesis include data from the first (T1) assessment wave of TRAILS, which ran from March 2001 to July 2002. A subsample of (pre)adolescents (N=1,868) participated in autonomic measurements, which took place between July 2001 and December 2002 (the main start of these measurements was in October 2001). In addition to that, a small sample of (pre)adolescents (N=17) was recruited from a school that participated in TRAILS, but the children themselves did not. These data were collected in May 2004 to investigate the reproducibility of autonomic measures. In the remainder of this section, sample selection and data collection of TRAILS in general are described followed by that of the TRAILS subsample of which autonomic measurements are available.

TRAILS
Sample selection
Sample selection involved two steps. First, five municipalities in the North of the Netherlands, including both urban and rural areas, were requested to give names and addresses of all inhabitants born between 10-01-1989 and 09-30-1990 (first two municipalities) or 10-01-1990 and 09-30-1991 (last three municipalities), yielding 3483 names. Simultaneously, primary schools (including schools for special education) within these municipalities were approached with the request to participate in TRAILS; that is, pass on students’ lists, provide information about TRAILS participants’ behavior and performance at school, and allow class administration of questionnaires and individual testing (neurocognitive, intelligence, and physical) at school. School participation was a prerequisite for eligible children and their parents to be approached by the TRAILS staff, with the exception of those already attending secondary schools (<1%), who were contacted without involving their schools. Of the 135 primary schools within the municipalities, 122 (90.4%) of
the schools accommodating 90.3% of the children) agreed to participate in the study.

If schools agreed to participate, parents (or guardians) received two brochures, one for themselves and one for their children, with information about the study; and a TRAILS staff member visited the school to inform eligible children about the study. Shortly thereafter a TRAILS interviewer contacted parents by telephone to give additional information, answer questions, and ask whether they and their son or daughter were willing to participate in the study. Respondents with an unlisted telephone number were requested by mail to pass on their number. If they reacted neither to that letter, nor to a reminder letter sent a few weeks later, staff members paid personal visits to their house. Parents who refused to participate were asked for permission to call back in about two months to minimize the number of refusals due to temporary reasons. If both parents and children agreed to participate, parental written informed consent was obtained after the procedures had been fully explained. Children were excluded from the study if they were incapable of participating due to mental retardation or a serious physical illness or handicap, or if no Dutch-speaking parent or parent surrogate was available and it was not feasible to administer any of the measurements in the parent’s language. Of all children approached for enrollment in the study (i.e., selected by the municipalities and attending a school that was willing to participate, \( N = 3145 \)), 6.7% were excluded because of mental or physical incapability or language problems. Of the remaining 2935 children, 76.0% (\( N = 2230 \), mean age = 11.1, \( SD = 0.6 \), 50.8% girls) were enrolled in the study (i.e., both child and parent agreed to participate). Responders and non-responders did not differ with respect to the prevalence of teacher-rated problem behavior, nor regarding associations between sociodemographic variables and mental health outcomes (De Winter et al. 2005).

Data collection
At T1, well-trained interviewers visited one of the parents or guardians (preferably the mother, 95.6%) at their homes to administer an interview covering a wide range of topics, including developmental history and somatic health, parental psychopathology and care utilization. Besides the interview, the parent was asked to fill out a self-report questionnaire. Children filled out questionnaires at school, in the classroom, under the supervision of one or more TRAILS assistants. In addition to that, intelligence and a number of biological and neurocognitive parameters were assessed individually (at school, except for saliva samples, which were collected at home). Teachers were asked to fill out a brief questionnaire for all TRAILS-children in their class.

Autonomic Measurements
Sample
A subgroup of 1,868 (84%) from the 2,230 TRAILS participants (T1, first assessment wave) took part in autonomic measurements in the supine and standing
positions. Almost all TRAILS children from schools for special education participated in the autonomic measurements. Not all children have been included, mainly because autonomic measurements started a few months after the TRAILS data collection had begun and many children had changed from primary to secondary school (N=196). Additional attrition occurred because children were not present at school (e.g., holiday, school trip) and could not be rescheduled (N=62), were involved in the pilot measurements (N=56), had moved to another town (N=35), refused to perform measurements (N=12), or had a physical handicap (N=1). Children who were ill on the scheduled measurement day, were all rescheduled, either at school, in a community center, or at home.

Autonomic measurements as described in the present thesis all had to pass strict quality criteria. The quality examination started with visual inspection of BP and HR signals. Measurements were regarded unsuitable when adequate signal recording had failed. Also, children demonstrating arrhythmia (or extra-systoles) were excluded from the analyses. In addition, data of 15 children were lost. Thus, 1823 supine and 1792 standing recordings were suitable for further analysis. Autonomic variables were then calculated and checked for quality criteria, resulting in 1,472 (81%) supine and 1,129 (63%) standing measurements as described in detail in chapter 2. Finally, to avoid bias due to differences in group size, only those children were included in the studies of whom both reliable BRS measurements in the supine and standing positions were available (N=1,027).

The final subsample of 1,027 children did not differ from the general TRAILS sample (N=2,230) regarding gender, body mass index, pubertal stage, and all behavioral measures included in this thesis, except for age. The later start of the autonomic measurements compared to TRAILS in general explains why the present subsample was slightly older (mean age=11.6, SD=0.5, range 10-13 years) than the general TRAILS sample (mean age=11.1, SD=0.6, range 10-12 years; t=38.6, p<.001). Further sample characteristics, such as pubertal stage, body mass index, level of physical activity, substance use, and medical health are reported in the respective chapters. All studies described in this thesis are cross-sectional.

Autonomic measures
This thesis includes the cardiac measures HR and HRV (in the low and high frequency band), as well as the vascular measures systolic BP and blood pressure variability (BPV). Also, we investigated BRS as a measure of short-term BP regulation. These cardiovascular indices were derived from short-term non-invasive measurements in the supine and standing positions. The latter were performed to determine autonomic reactivity to orthostatic stress. Calculations of BPV, HRV, and BRS were based on spectral analysis. In chapter 3, the focus lies on BRS. In chapter 4 and 5, HR, HRV in the high frequency band [referred to as respiratory sinus arrhythmia (RSA)], and BRS have been related to behavioral variables. In chapter 6, the reproducibility of all of the above autonomic measures has been investigated. Methodological aspects of autonomic measurements are described in more detail in
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chapters 2 and 3.

Behavioral measures
All behavioral measures of the present study were based on parent questionnaires (generally provided by the mother), and not on child or teacher reports. We used parent reports, given that data from the other informants was unavailable to answer the specific research questions we were interested in. For example, to gain insight in children’s behavior at age four to five in relation to autonomic function, we had to rely on parent’s retrospective reports. In addition, parent questionnaires of temperament proved to be psychometrically superior to child reports.

Furthermore, we adopted a dimensional approach when investigating behavioral variables by viewing these as continuous variables and studying their underlying basic dimensions. Thus, we studied the dimensions of temperamental activation and inhibition, and externalizing and internalizing problems as their psychopathological counterparts.

OUTLINE OF THIS THESIS

Chapter 2 provides detailed information about the autonomic nervous system and the autonomic measures included in this thesis.

Chapter 3 describes a study on spontaneous BRS measurements in the supine and standing positions in a large population cohort of Dutch (pre)adolescents (N=1,027). Normal values of BRS are presented in relation to posture, gender, age, pubertal stage, body mass index, and physical activity level.

Chapter 4 investigates the relationship between autonomic function and temperament in (pre)adolescents from the general population. We were specifically interested in whether we could find different autonomic profiles to be associated with temperamental activation (i.e., high-intensity pleasure) versus temperamental inhibition (i.e., shyness). In this study, we examined HR, HRV in the high frequency band (or RSA), and BRS during supine rest and in reaction to standing (i.e., autonomic reactivity).

Chapter 5 examines the relationship between autonomic function and externalizing and internalizing problems in a population cohort of (pre)adolescents. We expected autonomic function to be differentially related to both types of problems. We also investigated whether these associations might be specifically found in children with problems retrospectively reported to have been present also at preschool age. As in chapter 4, we investigated HR, HRV in the high frequency band (or RSA), and BRS during supine rest and standing-induced autonomic reactivity.

Chapter 6 reports the short-term reproducibility of important autonomic measures obtained in the supine and standing positions and of autonomic reactivity
scores. Measures included HR, HRV in the low and high frequency band, systolic BP, BPV, and BRS. This study was conducted in a small group (N=17) of (pre)adolescents, applying a comparable methodology as in our other studies included in this thesis.

Finally, chapter 7 summarizes the results of these studies and provides general conclusions.
REFERENCES


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American Psychological Association, Washington DC.


