

Separation anxiety disorder in children: disorder-specific responses to experimental separation from the mother

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Background: Separation anxiety disorder (SAD) is one of the most common anxiety disorders in childhood and is predictive of adult anxiety disorders, especially panic disorder. However, the disorder has seldom been studied and the attempt to distinguish SAD from other anxiety disorders with regard to psychophysiology has not been made. We expected exaggerated anxiety as well as sympathetic and respiratory reactivity in SAD during separation from the mother. **Method:** Participants were 49 children with a principal diagnosis of SAD, 21 clinical controls (CC) with a principal diagnosis of anxiety disorder other than SAD, and 39 healthy controls (HC) not meeting criteria for any current diagnosis. Analyses of covariance controlling for age were used to assess sympathetic and parasympathetic activation (preejection period and respiratory sinus arrhythmia) as well as cardiovascular (heart rate, mean arterial pressure, total peripheral resistance), respiratory (total breath time, minute ventilation, tidal volume, end-tidal CO₂, respiratory variability), electrodermal, and self-report (anxiety, cognitions, symptoms) variables during baseline, 4-min separation from, and reunion with the mother. **Results:** Children with a diagnosis of SAD were characterized by elevated self-reported anxiety responses to separation and increased sympathetic reactivity compared with CC and HC groups. The SAD group also displayed greater vagal withdrawal and higher reactivity in multiple cardiovascular, respiratory, and electrodermal measures compared with the HC group, while corresponding responses were less in the CC group and not significantly different from the other groups. **Conclusions:** Separation from the mother elicits greater autonomic, respiratory, and experiential responses in children with SAD. Our findings based on brief experimental separation demonstrate differential subjective and physiological manifestations of specific anxiety diagnoses, thus supporting the validity of the diagnostic category of SAD. **Key words:** Childhood anxiety, separation anxiety disorder, autonomic nervous system, respiration.

Introduction

Separation anxiety disorder (SAD) is conceptualized as developmentally inappropriate distress during separation from a significant other. The most frequently reported symptoms are separation-related distress, avoidance of being alone or without an adult, and sleeping away from caregivers or from home (Allen, Lavalley, Herren, Ruhe, & Schneider, 2010). In a comprehensive review, prevalence rates (point- to 1-year-prevalence) for SAD were between 0.5% and 20.2% with a median of almost 4% (Cartwright-Hatton, McNicol, & Doubleday, 2006). Comparable lifetime prevalence rates of childhood SAD in other recent studies were 4.1% (Shear, Jin, Ruscio, Walters, & Kessler, 2006) and 5.1% (Kessler et al., 2005). The research has identified SAD as a specific risk factor for adult panic disorder (PD; Battaglia et al., 2009). It has also identified SAD as a general risk factor for multiple adult anxiety disorders (Brückl et al., 2007) and has shown that SAD

may continue into adulthood (Manicavasagar, Silove, & Hadzi-Pavlovic, 1998).

Despite its high prevalence and unfavorable long-term prognosis, SAD remains neglected and underresearched with respect to etiology and treatment. One area in critical need of empirical investigation is its psychophysiology, which could provide information about the biological systems involved. Psychophysiological measures could be clinically useful in anxiety disorders if they led to (a) an understanding of basic processes responsible for their etiology and maintenance (Pine, Coplan, et al., 1998; Pine et al., 2000), (b) clarification of the boundaries and relationships between anxiety disorder subtypes previously classified solely on the basis of behavioral observation or self-report (e.g., Biber & Alkin, 1999), and (c) prediction of treatment outcomes (Mataix-Cols & Phillips, 2007).

Psychophysiological studies of childhood anxiety have focused mainly on two physiological systems responding to stress: the autonomic nervous system (ANS) and the respiratory system. Studies suggest an association between internalizing psychopathology

Conflict of interest statement: No conflicts declared.

and ANS functioning in both adults and children. Elevated heart rate (HR) during baseline has been linked to numerous internalizing disorders (e.g., Monk et al., 2001) including childhood separation anxiety (Rogeness, Cepeda, Macedo, Fischer, & Harris, 1990). However, HR, while easily obtainable, is influenced by interaction between the parasympathetic and sympathetic branches of the ANS (Berntson, Cacioppo, Quigley, & Fabro, 1994). Respiratory sinus arrhythmia (RSA), a measure of the magnitude of rhythmic fluctuations in HR caused by respiration, is the preferred indicator of vagal activity (Beauchaine, 2001; Berntson et al., 1994). Some studies have linked childhood anxiety disorders to decreased baseline RSA activity (Beauchaine, 2001; Pine, Wasserman, et al., 1998) and weak parasympathetic responses to stress (e.g., Friedman, 2007; Monk et al., 2001), while others have reported heightened parasympathetic responses to stress (Boyce et al., 2001). However, reactivity in SAD specifically is not known.

Preejection period (PEP) derived from systolic time intervals is a noninvasive marker of sympathetic control of the heart and is inversely related to sympathetic activation – the shorter the PEP, the greater the sympathetic arousal (Berntson et al., 1994). While studies have found heightened baseline sympathetic activation in a mixed anxiety group (Rogeness et al., 1990) and during stress (van Lang et al., 2007), the relationship between PEP and SAD during baseline and stress has yet to be examined. Furthermore, no studies have examined the autonomic reactivity of children with SAD during a separation situation.

Studies investigating respiratory abnormalities have found differences between children with anxiety disorders, including SAD, and nonanxious children: enhanced respiratory rate during CO₂ inhalation, as well as elevated minute ventilation, increased tidal volume, and lower end-tidal CO₂ during room-air breathing (Pine et al., 2000). However, these findings have been documented only in CO₂-induced stress situations and not in separation situations.

The current study is the first to investigate autonomic and respiratory responses in children diagnosed with SAD, children diagnosed with other anxiety disorders beside SAD (clinical controls, CC), and children without current or past mental disorders (healthy controls, HC) during a standardized disorder-specific stress situation, separation from the mother. Our measures were selected to register a wide variety of autonomic and respiratory indications of anxiety (Pine et al., 2000; Wilhelm & Roth, 1998). We expected disorder-specific reaction patterns in the three groups in line with the diagnostic category and DSM-IV criteria. First is that SAD children would report significantly more subjective anxiety, physical symptoms, and anxiety-related cognitions during separation than would the CC and HC children. Second is that the CC and SAD groups would exhibit lower RSA and higher HR at baseline (Monk et al., 2001) but that

the disorder-specific stimulus (i.e., separation from the mother) would induce higher sympathetic activation (as indicated by increased HR and blood pressure and reduced PEP) and more vagal withdrawal in the SAD compared with the other groups (Boyce et al., 2001; van Lang et al., 2007). Third is that compared with the HC and CC groups, children with SAD would exhibit abnormal respiratory control during baseline (Pine et al., 2000), as well as elevated ventilatory response during the separation task (as indicated by lower total breath time and expired CO₂ concentration, and increased tidal volume and minute ventilation). Finally, we investigated to what degree anxiety responses would be modulated by high or low anxiety provoking instructions.

Methods

Participants

The groups consisted of 49 children with a principal diagnosis of SAD, 21 CC with a principal diagnosis of anxiety disorder other than SAD, and 39 HC not meeting criteria for any current or past diagnosis. The ages of the sample can be found in Table 1. Seven participants in the SAD group and four participants in the CC group were recruited through local child and adolescent psychiatrists, psychologists, and pediatricians. The rest of the sample was recruited through newspaper advertisements and flyers. Inclusion criteria were knowledge of the German language, the children's and their parent's informed consent, and completion of psychological assessments. Children were excluded if they were taking psychotropic medication, and none of the anxious children were receiving treatment of any kind. Children with SAD were offered free diagnostic assessment and treatment for their participation in the study, while children in the control groups received a monetary reward. The local ethics committee for medical research approved the study.

The diagnoses were assessed by trained doctoral students in clinical child psychology, blinded to group status, using the Diagnostic Interview for Mental Disorders in Children and Adolescents (Kinder-DIPS; Schneider, Unnewehr, & Margraf, 2009). It is a well-validated structured interview for diagnosing DSM-IV disorders in children, which has alternate forms for children and parents. The structured interview assesses all anxiety disorders, depression, attention-deficit hyperactivity disorder (ADHD), oppositional defiant disorder, sleep disorders, eating disorders, and elimination disorders. The Kinder-DIPS has good validity and reliability for anxiety disorders (child version: kappa = .88; parent version: kappa = .85) and other axis I disorders (child version, kappa = .48–.88, parent version, kappa = .85–.94; Schneider et al., 2009). For children below the age of 8 years ($n = 13$), only the parent's version was used. Kappa values for the interrater reliability of SAD diagnosis in the present study were .88 (95% CI: .80–.97), and 76 (95% CI: .61–.92) for other anxiety diagnoses.

In the CC group the following principal diagnoses were present: 10 children (47.6%) with social phobia, 6 children (28.6%) with specific phobia, 3 children (14.4%) with generalized anxiety disorder (GAD), and

Table 1 Demographic and psychometric values of the study groups

	SAD group (mean ± SD)	CC group (mean ± SD)	HC group (mean ± SD)	Statistic	Post hoc significance
<i>n</i> in sample	49	21	39		
% female	51.0	66.7	48.7	$\chi^2(2) = 1.93$	
Age (years)	8.3 ± 2.5	9.5 ± 2.4	9.9 ± 2.3	$F(2,106) = 4.71^*$	SAD < CC = HC
Age range	5–13	5–14	6–14		
SAI–C	1.82 ± 0.91	1.44 ± 0.57	0.56 ± 0.61	$F(2,83) = 23.41^{***}$	SAD = CC > HC
SAI–P	2.35 ± 0.81	1.72 ± 0.84	0.56 ± 0.46	$F(2,89) = 52.93^{***}$	SAD > CC > HC
SASC–R	2.38 ± 0.58	2.84 ± 0.84	1.93 ± 0.63	$F(2,57) = 7.02^{**}$	SAD = CC > HC
RCMAS–C	0.43 ± 0.16	0.43 ± 0.13	0.27 ± 0.14	$F(2,96) = 12.72^{***}$	SAD = CC > HC
RCMAS–P	0.41 ± 0.17	0.39 ± 0.16	0.27 ± 0.14	$F(2,72) = 5.94^{**}$	SAD = CC > HC

SAD, separation anxiety disorder; CC, clinical controls; HC, healthy controls; SAI–C, Separation Anxiety Inventory for Children; SAI–P, Separation Anxiety Inventory for Parents; SASC–R, Social Anxiety Scale for Children–Revised; RCMAS–C, Revised Children’s Manifest Anxiety Scale–Child version; RCMAS–P, Revised Children’s Manifest Anxiety Scale–Parent version.

* $p < .05$; ** $p < .01$; *** $p < .001$.

one each with OCD (Obsessive-compulsive disorder) (4.7%) and PD with agoraphobia (4.7%). In the SAD group, 19 children (38.8%) had comorbid mental disorders, of which specific phobia was the most frequent (31.6%). GAD, social phobia, primary insomnia, ADHD, and oppositional defiant disorder were each diagnosed in two cases. Nightmare disorder, transient tic disorder, and agoraphobia without PD were each diagnosed once. In the CC group, the most common combinations of diagnoses were specific phobia and social phobia, social phobia and GAD, and specific phobia and GAD. While the groups differed significantly in age [$F(2,106) = 4.71$, $p = .011$], their ages overlapped to a high degree (age overlap: 6–13 years; see Table 1). The groups did not differ in sex distribution [$\chi^2(2) = 1.94$, *ns*].

Psychometric assessment of the study groups included the disorder-specific Child and Parent versions of the Separation Anxiety Inventory for Children (SAI–C/P; In-Albon & Schneider, in press), in which parents and children assessed the degree of avoidance of separations in a variety of settings (e.g., ‘I/my child avoid/s going to sleep alone’) using a 5-point scale ranging from 0 (never) to 4 (always). The psychometric properties of the SAI–C were good, with a test–retest reliability of .84. Internal consistency (Cronbach’s α) of the current sample was .88 (children) and .93 (parents). Furthermore, to measure trait anxiety, the Revised Children’s Manifest Anxiety Scale (RCMAS; Reynolds & Richmond, 1978) including ratings from both the children and the mothers was used. The Social Anxiety Scale for Children–Revised (SASC–R; La Greca & Stone, 1993) was included because we originally planned to recruit a CC group of children with a principal diagnosis of social phobia. Both questionnaires, the RCMAS and the SASC–R, demonstrated good internal consistency (.82–.92) in the current sample.

Procedure

Two experimenters conducted the individual testing sessions, which took approximately 90 min and consisted of three standardized tasks in succession, starting with the separation test, followed by video-supported social stress and hyperventilation, in a temperature- and sound-controlled room. Participants were seated in a comfortable armchair at a 90° angle from the mother, who was located in a corner of the

room approximately 3 m from the child, so that eye contact for both parties was possible. Electrodes and sensors were attached. The behaviors of the child and the mother were monitored in an adjoining control room through two discreetly placed cameras. Communication with the participant was via an intercom.

Prior to the experiment, the child was told that our aim of the study was to find out what happens in their body during different situations such as when they are sitting still or watching a video. The separation paradigm began with a baseline, for which the child was instructed to find a comfortable position and sit calmly for 5 min, neither to move nor speak, to keep their eyes open, and to breathe through their nose. Then a second, unfamiliar experimenter in a white coat entered and asked the mother to leave the room with her. The child received one of two randomly selected standardized instructions via intercom. In the low-anxiety expectation group, the child was told that their mother was called for a talk in the adjoining room and would be back soon. In the high-anxiety expectation group, the child was told that their mother had to go to another building and that it was not clear when she would return. The mother and experimenters watched the child for 4 min from a visual monitor in the adjoining room. Both the mother and the child were informed upon the return of the mother that it was important that they stay still and not talk to each other. The mother then returned to her seat in the testing room for 2 min. The child completed a questionnaire after each phase. If necessary, the experimenter helped the child in filling out the questionnaires.

Self-report measures

Subjective ratings by the children were recorded during the physiological testing. For this purpose a specially developed child-friendly, 11-point Likert Scale questionnaire (0 = not at all, 10 = extremely) was used (Wilhelm, Schneider, & Friedman, 2005). Individual items assessed anxiety (AX) and restlessness (RL), and two subscales asked about panic symptoms (PS; palpitations, dizziness, shortness of breath) and panic cognitions (PC; fear of going crazy, fear of dying). A separation anxiety cognition subscale was added to assess symptoms specifically related to SAD (SA; fear of mother being harmed, fear of one’s self being harmed).

The items in the three scales were selected from questionnaires on anxiety such as the Body Sensations Questionnaire (Chambless, Caputo, Gallagher, & Bright, 1984) and the Panic Rating Scales by Clark et al. (1994). As answering the questionnaires was sometimes difficult for children younger than 8 years old, a short version with five items was created. It assessed anxiety (AX), restlessness (RL), the two separation anxiety cognitions (SA), and whether the child felt he/she would have liked to end the task (ET). A total of 13 children completed the shortened version, of which 10 were in the SAD group, because this group had somewhat younger children who needed a shorter questionnaire. No difference was found in the pattern of results between the SAD children completing the short version and those completing the long version ($p > .2$, but note that power to detect differences was low). All questionnaires demonstrated good internal consistency (Cronbach's α : .83–.94) for this sample.

Physiological measures

Physiological channels were recorded at 1,000 Hz using BIOPAC hardware and AcqKnowledge software (BIOPAC Systems, Inc., Goleta, CA, USA). Data reduction and editing of artifacts were performed using ANSLAB software (Wilhelm & Peyk, 2005). Placement of electrodes/sensors, recording and data reduction were performed in accordance with published guidelines and conventions established for psychophysiological research (e.g., Fowles et al., 1981; Sherwood et al., 1990).

Cardiovascular measures. R-waves from a standard Lead-II electrocardiogram (ECG) were identified automatically. Cubic spline interpolation and resampling at 4 Hz were used to convert interbeat intervals (IBI) into an equidistant time series. Blood pressure was recorded once during each task using an inflatable arm cuff device (Dinamap 1846SX (Critikon, Tampa, FL, USA)). Eight spot electrodes were attached pairwise to the neck and thorax to obtain an impedance cardiogram (ICG). The raw ICG dz/dt -signal was ensemble averaged over 1-min experimental intervals to allow for reliable identification of the B-, Z- and X-points. PEP was calculated as the interval from ECG Q-point to ICG B-point. Stroke volume was calculated using the Kubicek formula and multiplied by HR to obtain cardiac output CO. Total peripheral resistance (TPR) was calculated by dividing mean arterial pressure (MAP) by CO and multiplying the quotient by 80. We examined the individual respiratory rates to see if they lay outside the 9–30 cycles/min range (0.15–0.5 Hz). As this was never the case, RSA was quantified by the natural logarithm of the summed Welch power spectral density of IBI in this range, corresponding to the high-frequency (HF) range of heart period variability. The choice of the 0.5-Hz cutoff as the higher bound for the HF band is also in line with prior studies in children (e.g., Pine, Wasserman, et al., 1998). PEP and RSA are the best available noninvasive measures of cardiac sympathetic and parasympathetic efferent activity, respectively.

Electrodermal measures. Two Ag/AgCl Beckman electrodes filled with isotonic electrode gel were

attached to the volar surfaces of the medial index and middle fingers of the child's nondominant hand. A constant voltage difference of 0.5 V between the electrodes afforded calculation of average skin conductance level (SCL). The signal was scanned for rises greater than 0.02 microSiemens from a zero-slope baseline, which were counted as the number of nonspecific skin conductance fluctuations (NSFn).

Respiratory measure. The following respiratory variables were calculated from thoracic and abdominal pneumographic channels (James Long, Inc., New York, NY, USA) calibrated for each individual using the mean of two fixed volume breathing calibration procedures: tidal volume (V_t), minute ventilation (V_{min}), total breath time (T_t , corresponding to 60 per respiratory rate; Wilhelm & Roth, 2001). The standard deviation (SD) was the metric of breath-by-breath respiratory variability for the following respiratory indices: variability of V_t (V_tSD), T_t (T_tSD), and V_{min} ($V_{min}SD$). Expiratory pCO_2 (end-tidal partial pressure of expired CO_2) was measured continuously using capnography (N-1000; Nellcor (Boulder, CO, USA)) drawing air from dual nostril prongs. Subjects were instructed to keep their mouth closed and breathe only through their nose. End-tidal pCO_2 was defined as the level at which pCO_2 stopped rising at the end of an expiration.

Data analyses

Data were visually inspected for normal distribution and outliers. For each subject, a spreadsheet was assembled containing physiological parameters for each test segment for review. Data points 2SDs above or below the mean for a subject during an epoch, prompted reanalysis for validation without knowledge of the clinical status. If data within conditions were missing, the interpolation method described by Stemmler (1989) was applied. Percentage of missing data for single physiological variables ranged between 0% and 6%. Three comparisons between parallel self-report and physiological data were made: baseline differences between the groups, differences in reactivity (separation phase minus baseline measurement), and differences in recovery (reunion phase minus baseline measurement, so that recovery is not confounded with reactivity differences and can show how completely children recover to their prestress levels).

To test our predictions, we assessed MAP, HR, PEP, RSA, TPR, and NSFn as the primary physiological measures of autonomic dysregulation in anxiety (Wilhelm & Roth, 1998); pCO_2 , T_t , V_t , and V_{min} , and their respective variabilities (T_tSD , V_tSD , $V_{min}SD$) were chosen as indices of the quality and quantity of respiratory changes (Wilhelm & Roth, 2001).

To avoid alpha inflation, the selected physiological variables were submitted to multivariate analyses of covariance (MANCOVA) controlling for age. Linearity was assessed from scatterplots. If the MANCOVA was significant, the individual ANCOVAs controlling for age were analyzed. If they were significant, group contrasts controlling for age are reported. This procedure was performed separately for baseline, reactivity, and recovery. For each set of measures at each assessment, 2 (Instruction) \times 3 (Group) MANCOVAs were originally

calculated but as instruction was not significant and did not interact significantly with the group, one-way MANCOVAs with group as the independent variable are reported here.

Nonparametric Kruskal–Wallis ANOVA and Mann–Whitney *U*-tests were used to analyze the self-report data as their distribution was strongly left-skewed and could not be normalized by transformation. Multivariate nonparametric tests are not available. Our nonparametric tests were performed once with the whole sample and once with an age-stratified sample (overlapping age range: 6–13). The patterns of results were equivalent, and only the analysis for the whole sample is reported. Significant Kruskal–Wallis ANOVA results were followed by Mann–Whitney *U*-tests. Finally, a Spearman correlation was calculated to assess the association between the physiologic and subjective (anxiety and restlessness) reactivities.

Significance levels were set at $p < .05$ for measures relevant to the reported hypotheses and $p < .01$ for other measures. All statistical tests were two-tailed. Effect sizes (Cohen's *d*) were calculated for the three pairwise group comparisons on primary measures. The numerous indices investigated are grouped together according to the models and hypotheses discussed before. Group comparisons used uncorrected *p*-values.

Results

Sample characteristics

Table 1 compares the clinical characteristics of the SAD, CC, and HC groups. While the groups differed significantly in age, these ages overlapped to a high degree (overlap in 6–13 years represents 89% of children). The groups did not differ in sex distribution. SAD and CC reported higher mean scores than HC on all clinical questionnaires administered during the diagnostic phase. As expected, SAD scored significantly higher on the disorder-specific SAI than CC but only on the parent form (SAI-P).

Self-report measures

Figure 1 presents the group means. Group differences during *baseline* were found for anxiety [$\chi^2(2, N = 107) = 10.24, p = .006$], restlessness [$\chi^2(2, N = 107) = 6.55, p = .038$], and desire to end task [$\chi^2(2, N = 107) = 6.30, p = .043$]. Compared with HC, CC reported higher anxiety [$U(39,21) = 250.5, p = .001, d = .91$], restlessness [$U(39,21) = 260.0, p = .013, d = .67$], and desire to end task [$U(39,21) = 260.0, p = .012, d = .69$]. However, no child chose to terminate the experiment.

Group differences in *reactivity* were found for anxiety [$\chi^2(2, N = 107) = 10.39, p = .006$] and restlessness [$\chi^2(2, N = 107) = 10.96, p = .004$]. As expected, reactivity was higher for anxiety [$U(47,39) = 629.5, p = .005, d = .62$] and restlessness [$U(47,39) = 602.5, p = .004, d = .66$], in SAD than HC, and for anxiety [$U(47,21) = 324.0, p = .014, d = .61$], in SAD than CC. No significant reactivity or *recovery* differences between CC and HC were found. Groups differed in separation anxiety cognitions during separation [$\chi^2(2, N = 82) = 11.46, p = .003$], and separation anxiety cognitions during the reunion phase [$\chi^2(2, N = 84) = 6.58, p = .037$]. Compared with HC, SAD showed more separation anxiety cognitions during separation [$U(31,33) = 295.0, p = .001, d = .88$], even after the mother returned [$U(34,32) = 420.5, p = .018, d = .60$]. No significant differences in separation anxiety cognitions were found between SAD and CC.

Physiological measures

Figure 2 presents the group means. The *baseline* MANCOVA was significant [Wilks $\lambda = .661; F(26,186) = 1.65, p = .031$]. Significant group differences were found in the following measures: MAP

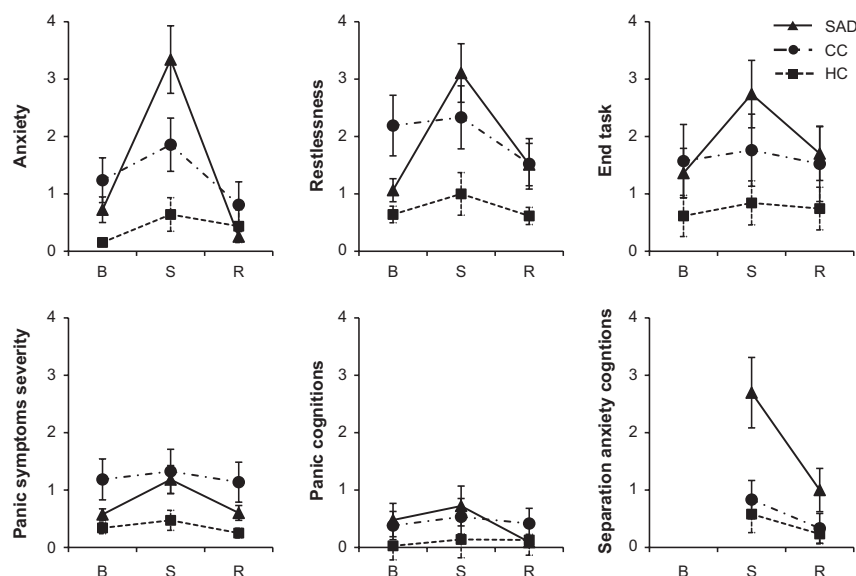


Figure 1 Self-report data of the significant primary measures of the separation anxiety group (SAD), clinical controls (CC), and healthy controls (HC). Vertical lines depict standard errors of the means. B, baseline; S, separation; R, reunion; total potential range of scores: 0–10

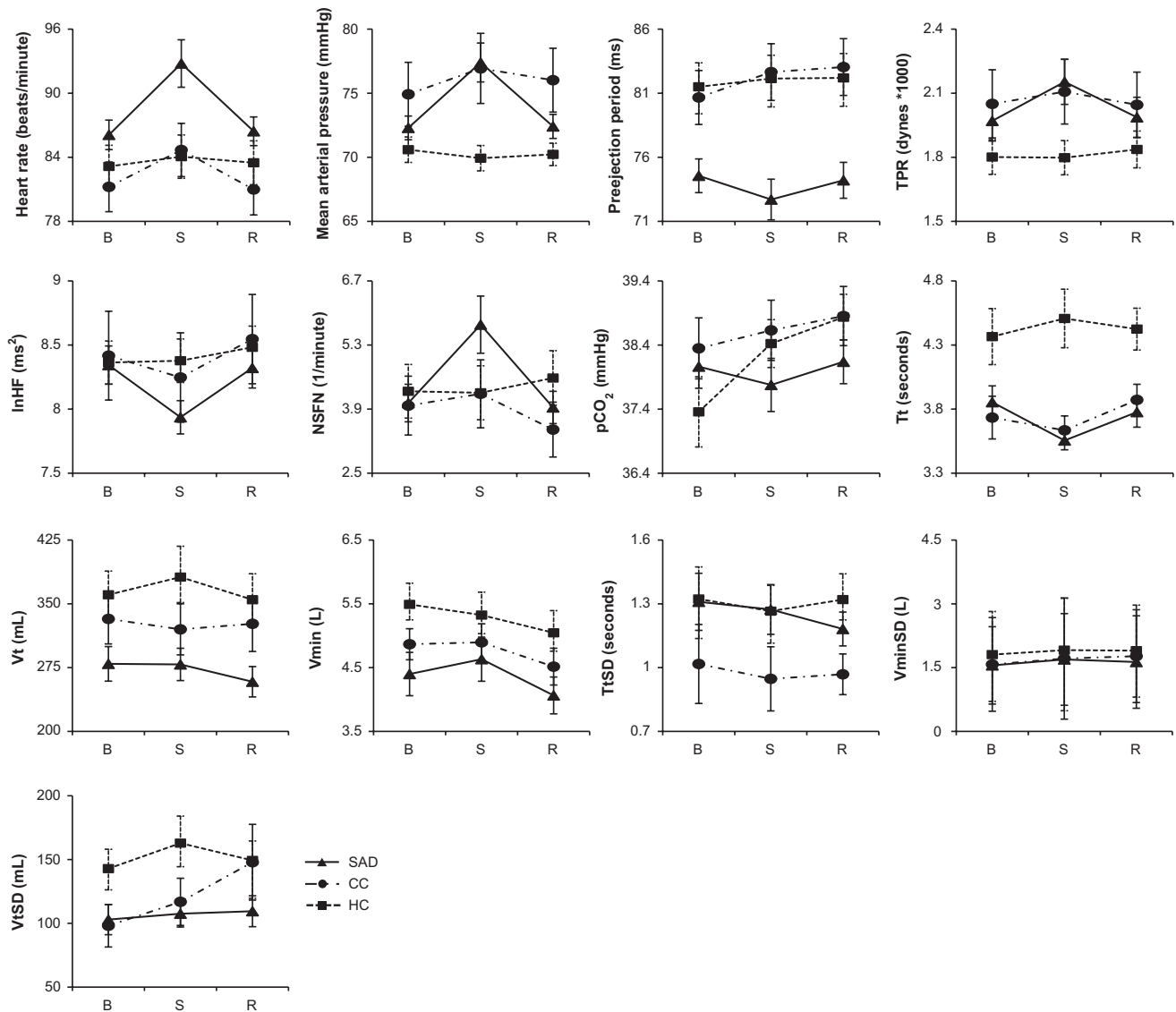


Figure 2 Principal physiological measures for the separation anxiety group (SAD), clinical controls (CC) and healthy controls (HC). Vertical lines depict standard errors of the means. B = baseline; S = separation; R = reunion; lnHF = natural logarithm of high frequency spectral power of heart period variability; NSFn = number of non-specific skin conductance fluctuations; pCO₂ = end-tidal partial pressure of expired CO₂; TPR = total peripheral resistance; T_t = total breath time; V_t = tidal volume; V_{min} = minute ventilation

[$F(2,105) = 3.07$, $p = .050$, T_t , $F(2,105) = 3.79$, $p = .041$] and PEP [$F(2,105) = 3.48$, $p = .035$]. Contrasts showed that compared with HC, CC had increased MAP ($p = .049$, $d = .39$) and decreased T_t ($p = .031$, $d = .42$). Compared with HC, SAD also had increased MAP ($p = .032$, $d = .42$) and decreased T_t ($p = .034$, $d = .42$) as well as a significantly lower PEP ($p = .011$, $d = .51$). No difference was found between SAD and CC.

The reactivity MANCOVA was significant [Wilks $\lambda = .577$; $F(26,182) = 2.26$, $p = .001$]. Significant group reactivity differences were found in the following cardiovascular measures: HR [$F(2,105) = 3.39$, $p = .037$], MAP [$F(2,105) = 7.65$, $p = .001$], TPR [$F(2,105) = 4.30$, $p = .016$], natural logarithm of high-frequency spectral power of heart period variability [lnHF; $F(2,105) = 3.64$, $p = .030$], and PEP [$F(2,105) = 4.74$, $p = .011$]. Contrasts showed significantly higher increases in SAD compared with HC in

HR ($p = .011$, $d = .50$), MAP ($p < .001$, $d = .72$), TPR ($p = .004$, $d = .57$), as well as significantly larger decreases in PEP ($p = .027$, $d = .41$) and lnHF ($p = .008$, $d = .52$). In addition, SAD displayed significantly more PEP decrease than CC ($p = .005$, $d = .55$). No difference was found between HC and CC.

Significant group differences in respiration were found for CO₂ [$F(2,105) = 5.40$, $p = .006$] and a barely significant difference in T_t [$F(2,105) = 3.67$, $p = .056$]. The differences were because of greater decrease in CO₂ ($p = .001$, $d = .63$) and T_t ($p = .017$, $d = .47$) in SAD than in HC. No differences were found between HC and CC. Among electrodermal measures, the index NSFn [$F(2,105) = 3.48$, $p = .034$], displayed significant group reactivity differences because of greater reactivity in SAD than HC ($p = .012$, $d = .50$). Again, no difference was found between HC and CC. Groups did not differ in any of the respiratory variability measures ($ps > .05$).

The recovery MANCOVA was significant [Wilks $\lambda = .558$; $F(26,182) = 2.42$, $p < .001$]. Significant group differences were found for $p\text{CO}_2$ [$F(2,105) = 6.24$, $p = .003$] and PEP [$F(2,105) = 6.69$, $p = .002$]. The differences were because of reduced increase in $p\text{CO}_2$ ($p = .001$, $d = .68$) in SAD compared with HC, and reduced increase in PEP ($p < .001$, $d = .71$) in SAD compared with CC.

Relationships between self-report and physiological measures

Correlations between anxiety and restlessness and the physiological variables that displayed significant reactivity differences between the groups are shown in Table 2. Self-report variables were associated with cardiovascular measures only in the SAD group, while the association with $p\text{CO}_2$ was only significant in the CC group. HC displayed no significant correlations between physiological and subjective measures.

Discussion

Using a novel standardized laboratory separation test, this study is the first to investigate autonomic and respiratory responses during separation from the mother in children diagnosed with SAD, CC, and HC. A wide range of cardiac, respiratory, electrodermal, and experiential responses were examined, extending prior psychophysiological findings of childhood SAD during a baseline and a respiratory challenge (Pine et al., 2000). Consistent with our expectations, we found that separation from the mother elicits exaggerated experiential and physiological responses in children with SAD.

During separation, SAD children reported significantly greater increases in anxiety and restlessness compared with HC children, and their separation anxiety cognitions were elevated compared with both the other groups. This supports the ecological validity of the separation test used in the study, which is an important prerequisite for interpretation

of the physiological responses. SAD children but not children with other anxiety disorders were characterized by hyperreactivity to separation in numerous autonomically regulated cardiovascular measures. Unlike a study that found blunted autonomic reactivity to CO_2 inhalation in pediatric anxiety (Monk et al., 2001), we found that the ANS was highly reactive in SAD children during our brief disorder-specific task. Both ANS branches were hyperreactive as measured by our noninvasive measures of sympathetic (PEP) and parasympathetic (lnHF) functioning. In terms of the autonomic space model (Berntson et al., 1994), our data indicate that separation was stressful for SAD children, leading to a reciprocal autonomic activation pattern of sympathetic activation and vagal withdrawal. The large changes in the dually controlled HR are consistent with this. Interestingly, the marked increases in MAP and TPR that have been specifically related to cognitive appraisal of stressful stimuli as threatening (vs. challenging; see Blascovich & Tomaka, 1996) indicate in our study that SAD children felt less able to cope with the separation situation than did the HC children. Sympathetic reactivity as indicated by PEP reduction from baseline to separation was specific to SAD children, while the other groups showed rise in PEP. This points to a disorder-specific sympathetic hyperreactivity in SAD but not in other anxiety disorders. During reunion with the mother, PEP did not return as much to baseline levels in SAD children as in HC children, indicating that sympathetic down-regulation was less in SAD children after separation. The lack of differences in respiratory reactivity to separation between SAD and CC children shows that this system is not specifically sensitive to separation challenge in SAD. However, SAD children showed a striking drop in $p\text{CO}_2$ with separation while $p\text{CO}_2$ increased in the other groups (the differential reactivity was significant for SAD vs. HC, but not for SAD vs. CC). $p\text{CO}_2$ normalization during reunion was incomplete in the SAD group.

Our finding of increased vagal reactivity in SAD contrasts with previous reports of little reactivity in

Table 2 Spearman correlations between selected physiological measures and self-reported anxiety and restlessness (correlations are based on reactivity scores, i.e., separation minus baseline)

	Separation anxiety ($n = 49$)		Clinical controls ($n = 21$)		Healthy controls ($n = 39$)	
	Anxiety	Restlessness	Anxiety	Restlessness	Anxiety	Restlessness
HR	.48**	.42**	.27	.04	.03	.02
MAP	.37**	.40**	.12	.29	.21	.01
TPR	.50**	.42**	.08	.21	.14	-.06
PEP	-.24	-.39**	.31	.11	.09	.03
lnHF	-.26	-.09	.05	-.16	-.17	-.03
NSF n	.09	.46**	.40	.06	.13	.15
$p\text{CO}_2$	-.28	-.12	-.50*	-.46*	-.01	.03

HR, heart rate; MAP, mean arterial pressure; TPR, total peripheral resistance; PEP, pre-ejection period; lnHF, natural logarithm of high-frequency spectral power of heart period variability; NSF n , number of nonspecific skin conductance fluctuations; $p\text{CO}_2$, end-tidal partial pressure of expired CO_2 .

* $p < .05$; ** $p < .01$.

mixed anxiety groups (Friedman, 2007), perhaps because of the specific fit of the stressor with the disorder of interest. Our pattern of findings supports Beauchaine's (2001) model in which excessive vagal withdrawal in response to challenge is a sign of greater emotional lability in negative emotional states, especially panic. Similar physiological reactions to stress have appeared in adult studies, where PD patients exhibited greater vagal withdrawal to stress than depressed patients, other anxiety-disordered patients, or controls (Beauchaine, 2001).

Our hypotheses regarding baseline differences were partially confirmed in that both clinical groups showed increased MAP, confirming the finding of Rogness et al. (1990). Lower PEP in the SAD than in the HC group extends this finding by documenting higher sympathetic cardiac control during baseline in SAD. Apart from the lower T_t (higher respiratory rate) in the clinical groups, the absence of respiratory abnormalities such as elevated respiratory variability during baseline is in contrast to previous reports (Pine, Coplan, et al., 1998; Pine et al., 2000), which may be related to differences between settings, apparatus, or instructions. For example, in the studies of Pine et al. children lay in a sealed plastic canopy and were warned that they would undergo a respiratory challenge and that this might lead to a panic attack. In any case, only ambulatory assessment can insure that baseline differences observed in the laboratory are sustained trait features of a disorder. Anxiety disordered patients in particular are prone to react anxiously to often-intimidating laboratory environments (Wilhelm & Grossman, 2010).

Self-report of psychological states is subject to bias and may be particularly inaccurate in children because of their not yet fully developed competence in emotion perception and verbalization (Wilhelm et al., 2005). Our study demonstrates that differences between SAD children in self-reported anxiety and restlessness in response to their mother leaving were related to reactivity in various autonomic measures. This indicates that not only at the between-group level (anxious vs. nonanxious groups of children), but also at the individual level, our physiological measures of anxiety such as HR and blood pressure were concordant with experienced anxiety. Although moderate, the level of concordance supports the use of psychophysiological measurement in a separation test to make better diagnostic decisions about SAD in children (Wilhelm & Roth, 2001).

In sum, our results help clarify the boundaries and relationships between SAD and other anxiety disorders, which currently are classified solely on the basis of behavioral observation or self-report (e.g., Biber & Alkin, 1999). Despite comorbidities and overlapping symptoms in our two anxious groups, the separation test elicited a pattern of physiological

hyperreactivity characteristic for the SAD children. This lends credence to SAD being a distinct diagnostic category distinguishable from a mixed anxiety group. Several results were unexpected. The SAD children significantly differed from the CC children on the parent version of the SAI but not on the child version. This result is in line with other studies finding discrepancies between parent and children reports of symptoms (Kraemer et al., 2003). The higher level of subjective distress during baseline in the CC group than in the HC and SAD groups may be explained by the fact that during baseline the mother was in the room with the child which made the SAD children feel safe. In contrast, for the 33.3% of the CC group with a principal diagnosis of social phobia, this situation may have been perceived as a test situation and thus as threatening.

The current study has distinct limitations. First, there was a significant age difference between groups, which we addressed by adding age as a covariate where possible. We performed the non-parametric analyses twice, once with the whole sample and once with an age-stratified sample. Only after determining that age had no significant effect, did we consider the results from the whole sample to be valid. Second, the two different instructions given (mother is near and will return soon vs. mother is far away) did not produce different effects. Unfortunately, we did not check if these instructions were perceived by children differently from what we had intended. Assuming that this cognitive intervention was valid, our negative finding may point to a direct elicitation of fear responses in SAD during separation from the mother with little cognitive reappraisal potential.

Based on the current findings it would be worthwhile to study if hyperreactivity to separation in SAD children can be normalized by treatment, and if the observed reactivity pattern is a precursor of SAD in a high-risk sample. Answering these questions should help us better understand the development and maintenance of SAD and thus lay the basis for successful prevention and treatment.

Acknowledgements

This study was supported by grant project PP001-68701; 105314-116517) awarded to Silvia Schneider by the Swiss National Science Foundation. The authors thank Peter Peyk for his research assistance. The authors report no biomedical financial interests or potential conflicts of interest.

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Key points

- Despite a high prevalence of SAD and the apparent relevance of separation situations for this disorder, responses to maternal separation have not been studied systematically in these patients.
- Children with SAD were characterized by autonomic and respiratory hyperreactivity to separation. Subjective responses and cardiac sympathetic control could successfully distinguish SAD children from a CC group with mixed anxiety diagnoses.
- Results support SAD as a distinct diagnostic category, the classification of which is not solely dependent on behavioral observation or self-report.
- Including a behavioral separation challenge and psychophysiological measures in future studies of SAD in children may enhance differential diagnosis and treatment evaluation.

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Accepted for publication: 22 July 2011

Published online: 16 September 2011