The startle reflex in children with neuropsychiatric disorders

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Increased whole-body auditory startle reflex and autonomic reactivity in children with anxiety disorders

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MAJ Tijssen
JN van der Meer
JHJM Koelman
F Boer
Abstract

Background: Young patients with anxiety disorders are thought to have a hypersensitive fear system including alterations of the early sensorimotor processing of threatening information. However, there is equivocal support in auditory blink studies for an enlarged auditory startle reflex (ASR) in such patients. We sought to investigate the ASR measured over multiple muscles (whole-body) in children and adolescents with anxiety disorders.

Methods: We assessed ASRs (elicited by 8 consecutive tones of 104 dB, interstimulus interval ±2 minutes) in 25 patients and 25 matched controls using a case-control design and in 9 non-affected siblings. We recorded the electromyographic activity of 6 muscles and the sympathetic skin response. We investigated response occurrence (probability %) and response magnitude (area-under-the-curve in μV.ms) of the combined response of six muscles and of the single blink response were investigated.

Results: in patients (17 girls, mean age 12.7 years; 13 social phobia, 9 generalized anxiety, 3 other) the combined response probability (p=0.027) of all muscles, the combined area-under-the-curve of all muscles (p=0.011) and the sympathetic skin response (p=0.006) were enlarged compared with matched controls. The response probability (p=0.478) and area-under-the-curve (p=0.069) of the blink response were normal in patients compared with matched controls. The ASR pattern was normal with normal latencies in patients compared with controls. In non-affected siblings, the sympathetic skin response (p=0.038), but not the combined response probability of all muscles (p=0.152), was enlarged compared with controls. Conclusions: The results point towards a hypersensitive central nervous system (fear system) including early sensorimotor processing alterations and autonomic hyperreactivity. The multiple muscle (whole-body) ASR is suggested to be a better tool to detect ASR abnormalities in anxiety disorder patients than the blink response alone. Abnormalities in ASR serve as a candidate endophenotype of anxiety disorders.
Introduction

A growing body of evidence supports the hypothesis that young patients with anxiety disorders have a highly sensitive fear system. Abnormalities of fear circuit activation, function of the hypothalamus-pituitary-adrenal axis and autonomic nervous system reactivity have been found. More specifically, anxiety disorder patients are thought to experience altered early sensorimotor processing of threatening information. One of the quickest reactions of the fear system to a suddenly imposed sensory stimulus is the auditory startle reflex (ASR). The ASR consists of the contraction of several muscles throughout the body and subsequent autonomic changes. Animal studies showed that this primitive defensive reflex is generated by the caudal brainstem and modulated by the amygdala. The amygdala is believed to play a key role in emotional processing and pathological anxiety.

There is evidence of an association between anxiety disorders and an enlarged ASR. However, the results are equivocal, both with regard to posttraumatic stress disorder as well as other anxiety disorders in adults. Findings in children with anxiety disorders range from attenuated to normal ASRs in posttraumatic stress disorder to normal ASRs in other anxiety disorders. Possibly methodological issues underlie some of the inconsistent findings of the ASR in anxiety disorder patients. In animals, the ASR is quantified by an electromyogram (EMG) measurement of the whole-body flinch or jump, involving multiple muscles. In contrast, in human research, the ASR is often electromyographically measured over a single muscle, the orbicularis oculi (blink response). However, there are indications that the blink response of the ASR is different from the ASR as it occurs in the whole body. First, the auditory blink response and the ASR measured over multiple muscles show different habituation patterns in both adults and children. Second, the auditory blink response and the ASR measured over multiple muscles show different abnormalities in several neurological disorders. Third, the auditory blink response consists of 2 different EMG responses that differ in size and latency. As the 2 responses extensively overlap, it is almost impossible to distinguish them. Together, these findings support the idea that the blink response does not fully represent the ASR. For these reasons, measuring the generalized ASR or the ‘whole-body ASR’ in the form of a multiple muscles EMG is considered to be more valid and appropriate than the single blink response in quantifying the ASR.

Our aim was to investigate an alternative ASR quantification to that used in blink response studies, the multiple muscle ASR, in young patients with anxiety disorders.
Furthermore, we compared the multiple muscles ASR, a method routinely used in the neurophysiological literature, with the blink response, a method routinely used in the psychophysiological literature. We investigated the following ASR parameters using a case control design: 1. The combined response probability (%) of six differentially located muscles and the combined EMG area-under-the-curve (μV·ms) of these responses. 2. Response probability (%) and EMG area-under-the-curve (μV·ms) of the blink response. 3. The sympathetic skin response (the maximal change in μV inside the hand following stimulation, standardized to the intra-individual maximum). We used the sympathetic skin response to assess the ‘orienting response’, which includes autonomic changes occurring with a latency of longer than 500 ms after an auditory stimulus. In addition, we studied basic ASR characteristics such as latencies and startle patterns and we assessed the multiple muscle ASR response probability and sympathetic skin response in a small group of siblings of patients with anxiety disorders. We hypothesized that the multiple muscle ASR and sympathetic skin response would be abnormal in patients compared with controls, and that the pattern of the blink response and multiple muscle ASR would be incongruent in patients compared with controls.

Methods

Participants

We approached all patients who were referred to the outpatient clinic of the Child and Adolescent Psychiatry Department of the Academical Medical Center between August and November 2006 and met our inclusion criteria for the study. Inclusion criteria for participation were at least one primary anxiety disorder diagnosis and age between 8 and 17 years. A clinician (MB) determined whether the patients had anxiety disorders. Further, the patient was screened for a comorbid major depression disorder; comorbid major depression was an exclusion criterion, as the ASR can be reduced in these patients. Subsequently, we invited the patients to participate in the study. Of the 28 patients we invited to participate in the study, 3 refused. Patients were excluded from the study if they took medication, had a hearing defect, or met criteria for the following disorders: major depression disorder, neurological disorder, mental retardation or schizophrenia or other psychotic disorders. None of the 25 patients had to be excluded. We also included a small group of siblings of the patients participating in the study. Nine siblings of the 25 patients enrolled in the study also agreed to participate (8 patients had no siblings aged 8 - 17 years, 4 siblings refused to participate, 2 patients refused the participation of their sibling and 2 siblings were known to suffer from autism and borderline personality disorder respectively). We invited 30 healthy controls matched for age and sex to participate in the study. We excluded controls if they met criteria
for psychopathology, had a hearing defect or a neurological disorder. We excluded 3 control participants owing to presence of an anxiety disorder (n=2) or evidence of a neurological disorder in the past (n=1). As 12 of the 27 controls were boys (44 %) and 8 of the 25 patients were boys (32 %), we excluded 2 control boys, selected on the basis of their age, to make the control and patient groups optimally similar in terms of number, sex and age. Thus we included 25 anxiety disorder patients (17 girls, mean age 12.7 years, mean height 154.9 cm) and 25 controls (15 girls, mean age 12.0 years, mean height 155.9 cm) in the study. Sex, age, height and handedness did not significantly differ between the groups (Student’s t-test, p=0.575, p=0.375, p=0.824, p=0.561). Comorbidity among the anxiety disorders was common, with most of the patients having more than one anxiety disorder. The primary type of anxiety disorders of the anxiety disorder patients are shown in Table 1. Comorbidity included dysthymia (n=8), attention-deficit disorder in remission (n=1) and suspicion of autistic spectrum disorders (n=1); we referred the patient in whom we suspected an autism spectrum disorder to another clinic for further testing. The 9 siblings (3 girls, mean age 10.5 years, mean height 147.3 cm) did not meet the criteria of a DSM-IV diagnosis according to the ADIS. All participants were non-smokers and we asked them to refrain from caffeinated beverages on the day of testing. The Institutional Board of the Academic Medical Center, Amsterdam, approved the study protocol and participants consent forms.

Table 1. Clinical characteristics patients.

<table>
<thead>
<tr>
<th>Primary anxiety disorder diagnosis</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social phobia</td>
<td>13</td>
</tr>
<tr>
<td>Generalized anxiety disorder</td>
<td>9</td>
</tr>
<tr>
<td>Phobia</td>
<td>2</td>
</tr>
<tr>
<td>Panic disorder</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL</td>
<td>25</td>
</tr>
</tbody>
</table>

Psychiatric assessment

We used the Anxiety Disorders Interview Schedule (ADIS)\textsuperscript{255} to formally establish and exclude anxiety disorders in all participants. The ADIS is a semi-structured interview based on DSM-IV classification of psychopathology\textsuperscript{36} and includes both a child and a parent interview (ADIS-C/P). It was translated to Dutch by Siebelink and Treffers and Dutch norms of the ADIS are available.\textsuperscript{256}

Stimulation

Experimental stimuli consisted of 8 consecutive 104 dB (A) (sound-pressure level), 50 ms, 2000 Hz pure tones with instantaneous rise and fall times.\textsuperscript{65} Following a digital trigger, we generated the tones using an audio stimulator (Audio ACB 1, Walter Messequeltronic, Kamenz, Germany) and presented them binaurally through stereo headphones (Optac,
Vario 4000 S, Dundee, United Kingdom). The triggers of auditory stimuli were stored in the computer synchronously with the EMG data. We presented the stimuli with varying time intervals (1.5 – 2.5 minutes)\textsuperscript{58,90} which were similar for all participants.

**Data collection**

We recorded physiological data, consisting of bipolar left orbicularis oculi, masseter, sternocleidomastoid, deltoid, abductor pollicis brevis, quadriceps EMG and sympathetic skin response measures, using Biosemi’s Active System (www.biosemi.nl). After skin preparation (Abrasive gel, NuPrep, D.O.Weaver and Co, Aurora, CO, USA), we filled the cutaneous silver-silver chloride flat (11mm width, 17mm length, 4.5mm height) active surface electrodes (equipped with pre-amplifiers to improve the signal/noise ratio and reduce the movement artifact ) with conductive paste (Ten20 Conductive, D.O.Weaver and Co, Aurora, CO, USA). We attached the electrodes 2 cm apart (except the electrodes for sympathetic skin response) using adhesive collars in accordance with recommendations by the Psychophysiology Committee\textsuperscript{65} Of the electrodes attached to the orbicularis oculi muscle, we placed one straight below the eye and the other 2 cm towards the ear and slightly higher. We checked the impedance of the electrodes (<10 kΩ). We recorded the sympathetic skin response from the palm of the hands with the reference electrode on the dorsum of the hands\textsuperscript{219,228} We placed the isolated ground electrode on the temple. The signal was analogue filtered high-pass (1\textsuperscript{st} order; -3dB at 0.16 Hz) and low-pass (5\textsuperscript{th} order anti-aliasing; -3dB at 3500 Hz). We continuously digitized filtered data with a sample frequency of 16384 Hz per channel using a 24-bit A/D converter.

**Procedure**

We tested most participants in the afternoon, usually between 4 PM and 6 PM; we tested three 3 participants and 1 anxiety disorder patient in the morning on the weekend. Participants sat on a bed (with backrest) in an upright position; we asked them to sit quietly and relaxed. We gave them the following instructions: 'Shortly you are going to hear a series of sounds. Please sit quietly and listen to the sounds as they come. Keep your eyes open throughout the entire procedure, which will last approximately 15 minutes'. Subsequently, we placed the headphones and began the stimulation procedure. The preparation took 30 minutes and the experiment lasted 15 minutes.

**Data processing**

We performed off-line data analysis with Brain Vision Analyzer 1.05 (Brain Products, GmbH, Munich, Germany). We digitally filtered orbicularis oculi signals at 100 Hz high-pass 12 dB/oct to minimize DC offsets and slow eye drifts\textsuperscript{229-231} The cut-off frequency of the high-pass filtering of the other muscles was 20 Hz.\textsuperscript{232} Owing to the high sample
rate employed (16384 Hz) the low-pass analogue filter could be chosen at 3500 Hz. We converted the 14 raw unipolar signals into 7 pairs of bipolar derivations. We segmented all 7 channels into parts of 1000 ms after the stimulus. We applied baseline correction using the mean of the 0-20 ms time interval. Subsequently, the signals were rectified. We placed markers manually using the Brain Vision Analyzer (see further) and we processed the EMG using Matlab 7.1 software (Mathworks).

We used several parameters to quantify the magnitude of the ASR: For the multiple muscle ASR, we used the combined response probability (%) of all muscles and the combined area-under-the-curve (μV·ms) of the responses of all muscles. For the blink response, we used the response probability (%) of the blink response in the single orbicularis oculi muscle and the area-under-the-curve (μV·ms) of the blink response (definitions of all ASR parameters are provided in the next paragraphs). We considered the first parameter, the multiple muscle ASR response probability, to be the primary motor ASR parameter; we obtained only this parameter and the sympathetic skin response in the siblings. We included the blink response in this study to compare this parameter to the multiple muscle ASR.

The response probability represents the chance (%) that a certain muscle responds following stimulation.58 We defined the combined response probability of all muscles as the average of the response probabilities (expressed in % chance of a muscle response following stimulation) of the six muscles.58 We defined the response probability of the orbicularis oculi response as the chance (%) of the occurrence of an orbicularis oculi response following stimulation. One of us (MB) visually inspected the EMG activity of all muscles after stimulation with Brain Vision Analyzer to determine which trials could be considered valid responses. We scored the response if an increase of EMG activity from baseline occurred in any of the 6 simultaneously recorded muscles at an appropriate latency.45, 47, 89 We performed scoring based on the following rules defined before scoring the responses: We defined a response as a clear increase (duration increase at least 30 ms, magnitude response at least 30 μV) from baseline. We marked the response onset (20-200 ms following stimulation) at the baseline (thus at the start of the μV increase). The same investigator scored all responses at the same screen sensitivity (200 μV on the screen, 100 μV below baseline and 100 μV above baseline).

We determined the response probability of the individual muscles by counting the total occurrence of these responses and dividing this value by the total amount of recorded traces (amount of participants per group times 8 stimuli), and multiplying this by 100. We defined latency as the period between stimulus onset and start of the response at the EMG baseline. One of us (MB) manually marked the response onset (latencies) and response offset. We marked trials considered artefacts (e.g. heart beat, loose electrodes) as such and we did not include them in the analysis.
The area-under-the-curve parameters represent the magnitude (expressed in μV·ms) of the scored responses, the quantification of the EMG signal between response onset and offset. Quantification of the EMG burst in area-under-the-curve (rectified integrated signal) is the most direct translation of the raw EMG into measurement units and a commonly used ASR quantification method.\textsuperscript{58, 65} We conformed to the psychophysiology guidelines for human startle eye blink electromyographic studies.\textsuperscript{65} In line with Blumenthal and colleagues, we did not (try to) remove the early auditory protective reflex component of the orbicularis oculi area-under-the-curve EMG response.\textsuperscript{74} Furthermore, we included only the EMG area-under-the-curve’s of the trials considered to be valid responses (see earlier) in the analysis. For the orbicularis oculi muscle, we included responses with onset latencies between 20 and 100 ms in the analysis.\textsuperscript{65} For the other muscles, onset latencies were between 20 and 200 ms. We defined the combined area-under-the-curve as the summated log transformed EMG area-under-the-curve of the 6 muscles.\textsuperscript{58} We performed a log transformation to normalize the area-under-the-curve values of the different muscles.

Although we scored responses conforming to the rules we defined, we could not totally rule out investigator’s bias as it was logistically impossible to keep the investigator completely blind for group membership. Therefore we also analyzed parameters using fixed time intervals (peak amplitude and area-under-the-curve between 20 – 200 ms following stimulation of all EMG trials). We identified the peak amplitude (μV) of the smoothed (with a 40 Hz filter low-pass filter\textsuperscript{65}) EMG trials between 20 and 200 ms following stimulation using software, and we averaged these amplitudes per muscle. To obtain the area-under-the-curve (μV·ms) of the 20-200 ms time interval, we did not smoothe the EMG trials, but we removed background noise (estimated at three times the median of the interval 500 to1000 ms following stimulation)\textsuperscript{229} For both parameters, we obtained the combined response by summating the log transformed values of all 6 muscles.

Finally, we assessed the autonomic sympathetic skin response (left hand). The sympathetic skin response, comparable to the skin conductance response\textsuperscript{234}, is an easy test to analyze the sympathetic nervous system function.\textsuperscript{219, 228, 257} Any arousal stimulus, i.e. a stimulus that is perceived to be novel, surprising, intense, significant, or emotional\textsuperscript{1258}, can be used to activate sympathetic sudomotor nerve fibers and thereby change skin resistance.\textsuperscript{228} With repetitive auditory stimuli the sympathetic skin response decreases.\textsuperscript{219, 220} The sympathetic skin response is usually recorded from the palm of the hands, with the reference electrode on the dorsum of the hands.\textsuperscript{219, 228} We defined the baseline as the mean μV during 0-900 ms following stimulation (this could be chosen following the stimulus because the onset of the hand sympathetic skin response is 1500 ms following stimulation).\textsuperscript{218, 233} Subsequently, we identified the peak μV during the interval 900 - 4000 ms following stimulation by software. We defined the sympathetic
skin response as the difference in μV between the baseline and the identified maximum, standardized to (relative to; giving values ranging from 0 to 100 %) the intra-individual maximum.\textsuperscript{250} It is recommended to standardize the sympathetic skin response intra-individually because it is highly variable.\textsuperscript{235, 236} The intra-individual maximum often was the response following the first stimulus, but not always. We visually inspected the sympathetic skin responses to exclude movement artefacts or artefacts caused by loose electrodes.

**Statistical analysis**

We performed statistical analysis with SPSS (15.0). We performed a power analysis based on a previous study in which ASR latency abnormalities were found.\textsuperscript{185} With a mean orbicularis oculi latency of 28 and a standard deviation of 8, a minimum of 20 participants is needed for 80 % power to detect a clinically relevant change of 25 %. We used a linear mixed-model analysis (type III tests of fixed effects) to test group differences in parameters over the repeatedly presented stimuli. We performed the analyses under the assumption of compound symmetry for the covariance structure, which is equivalent to the classical approach to repeated measures ANOVA. In some parameters, adjustments to the denominator degrees of freedom were needed (e.g. area-under-the-curve of the blink response because only the area-under-the-curve’s of the responses were included, sympathetic skin response and fixed time interval parameters because of missing values). The response probability parameters revealed a Poisson distribution. We log transformed area-under-the-curve parameters to reduce skewness of the data and to standardize the area-under-the-curve values of the different muscles (different in size and strength). We then summated them combined area-under-the-curve calculation. As the latency distributions were not normally distributed, we performed Wilcoxon-Mann-Whitney tests to assess group differences in latency. We performed a Student’s t-test to assess general characteristics. We considered a p-value ≤ 0.05 to be significant in all tests.

**Results**

Following auditory stimulation, EMG responses were elicited in orbicularis oculi, sternocleidomastoid, masseter, deltoid, abductor pollicis brevis and quadriceps muscles (Table 2). Two representative EMG’s of a control participant and a patient are shown in Figure 1.
Table 2. ASR characteristics of individual muscle responses. P-values are given with a * for a significant difference. nd=not done

<table>
<thead>
<tr>
<th>Frequency of response (%; mean and SD)</th>
<th>Patients</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orbicularis oculi</td>
<td>69.5 (SD 46.1)</td>
<td>76.5 (SD 42.5)</td>
<td>0.487</td>
</tr>
<tr>
<td>Sternocleidomastoid</td>
<td>34.0 (SD 47.5)</td>
<td>15.0 (SD 35.7)</td>
<td>0.021 *</td>
</tr>
<tr>
<td>Masseter</td>
<td>34.5 (SD 47.6)</td>
<td>10.5 (SD 30.7)</td>
<td>0.011 *</td>
</tr>
<tr>
<td>Deltoid</td>
<td>15.5 (SD 36.3)</td>
<td>2.0 (SD 14.0)</td>
<td>0.025 *</td>
</tr>
<tr>
<td>Abductor pollicis brevis</td>
<td>19.5 (SD 39.7)</td>
<td>3.6 (SD 18.8)</td>
<td>0.016 *</td>
</tr>
<tr>
<td>Quadriceps</td>
<td>8.5 (SD 27.9)</td>
<td>1.0 (SD 9.9)</td>
<td>0.110</td>
</tr>
<tr>
<td>COMBINED RESPONSE</td>
<td>30.3 (SD 28.2)</td>
<td>18.2 (SD 14.6)</td>
<td>0.027 *</td>
</tr>
</tbody>
</table>

Figure 1. Representative ASR EMG's of a patient (left) and a control participant (right). OO=orbicularis oculi (eye; blink), SC=sternocleido-mastoid (neck), MA= masseter (jaw), DE=deltoid (shoulder), AP= abductor pollicis brevis (hand) and QU= quadriceps (leg) muscles
Multiple muscle ASR

The multiple muscle response probability was significantly enlarged in patients compared with controls (patients mean 30.25 % SD 28.2, controls mean 18.2 % SD 14.6; F(1,50)=5.16, p=0.027) (Figure 2, Table 2). In addition, the response probabilities of all individual muscles except the orbicularis oculi and quadriceps muscles were significantly enlarged in patients compared with controls (Table 2).

The combined area-under-the-curve of all muscles was significantly enlarged in patients compared with controls (patients mean 7.5 and SD 5.4, controls mean 4.4 and SD 3.4, F(1,50)=7.05, p=0.011) (Table 2). In Table 2 the area-under-the-curve’s of the individual muscles are shown: none of these were significantly different between groups.

Blink response

The blink response (orbicularis oculi muscle) probability was 69.5 % (SD 46.1) in the patients and 76.5 % (SD 42.5) in the controls (Table 2), a difference which was not significant, F(1, 50)=0.512, p=0.47. The blink area-under-the-curve the-curve also was not significantly enlarged after log transformation (controls median 601 μV·ms, range 60-4760, patients median 783 μV·ms, range 52-16700, F(1,42.8)=3.47, p=0.069) (Table 2).

Fixed time interval peak amplitude and area-under-the-curve

The peak amplitude between 20 and 200 ms of all EMG trials was significantly enlarged among the patients compared with the controls, F(1,47.8)=4.37, p=0.042. The area-under-curve of all EMG trials was not significantly enlarged, F(1,42.5)=2.11, p=0.150. The peak amplitude of all orbicularis oculi trials, F(1, 47.8)=0.284, p=0.59, and the area-under-the-curve of all orbicularis oculi trials, F(1,47.5)=0.66, p=0.79, were not significantly different between groups.
Sympathetic skin response

The sympathetic skin response was significantly enlarged in patients compared with controls (patients mean 36.8 % SD 34.2, controls mean 27.4 % SD 36.6); F(1, 47.7)=8.2, p=0.006) (Figure 3).

Figure 2. Multiple muscle ASR. A. The multiple muscle ASR combined response probability (the mean of the six response probabilities) is significantly enlarged in anxiety disorder (AD) patients compared with controls but not in siblings compared with controls. B. The course of the multiple muscle ASR with the repetitive stimuli is shown for the anxiety disorder (AD) patients and controls. Bars represent means; error bars standard error of means.

Figure 3. Sympathetic skin response. A. The sympathetic skin response (maximal change in µV standardized to the intra-individual maximum) is significantly enlarged in anxiety disorder (AD) patients and unaffected siblings compared with controls. B. The course of sympathetic skin response with the repetitive stimuli is shown for the anxiety disorder (AD) patients and controls. Bars represent means, error bars standard error of means.

Sympathetic skin response

The sympathetic skin response was significantly enlarged in patients compared with controls (patients mean 36.8 % SD 34.2, controls mean 27.4 % SD 36.6); F(1, 47.7)=8.2, p=0.006) (Figure 3).
ASR pattern
The ASR of both patients and controls showed a similar recruitment pattern. The response probability decreased with increasing distance of the muscle to the startle generator, the caudal brainstem. Furthermore, the pattern of onset latencies of the different muscles of the ASR was normal in patients compared with controls (Table 2). The ASR latencies were not significantly different in patients compared with the controls in orbicularis oculi (U=202, Z=-1.15, p=0.24), masseter (U=24, Z=-1.70, p=0.08), sternocleidomastoid (U=87, Z=-0.146, p=0.88), deltoid (U=9, Z=-0.612, p=0.54), abductor pollicis brevis (U=26, Z=-0.170, p=0.86) and quadriceps muscles (U=0, Z=-1.7, p=0.08) (Table 2). Particularly in the distal muscles the latencies of the control participants are not reliable because there were not many trials in which responses occurred (Table 2).

Siblings
In the non-affected siblings of the patients with anxiety disorders, the combined response probability of all muscles was not significantly different compared with controls, F(1, 30)=2.16 p=0.15 (Figure 2). However, the sympathetic skin response was significantly enlarged in the siblings compared with controls, F(1, 27.9)=5.52, p=0.026 (Figure 3).

Sex and age effects
There was no significant effect of sex on the multiple muscle response probability (F(1,23)=1.1 p=0.30), combined area-under-the-curve (F(1,23)=1.5, p=0.69), blink response (F(1, 19.0)=0.78, p=0.38) or the sympathetic skin response in the anxiety disorder patients (F(1, 23.1)=2.0, p=0.16). Similarly, in patients with anxiety disorder there was no significant effect of age on the multiple muscle response probability (F(9,15)=0.70, p=0.69), combined area-under-the-curve (F(9,15.6)=0.29, p=0.96), blink response (F(8,12.6)=0.24, p=0.97) or sympathetic skin response (F(9,14.3)=0.64, p=0.74).

Discussion
To our knowledge, ours is the first study to show that the ASR is abnormally enlarged in young patients with anxiety disorders. The multiple muscle ASR (response probability and EMG area-under-the-curve) or whole-body ASR was enlarged among patients compared with controls of the same age and sex. In contrast, the blink response (response probability and EMG area-under-the-curve) showed no abnormalities in patients compared with controls. The autonomic reactivity (as assessed by the sympathetic skin response) in young patients with anxiety disorders was also enlarged compared with controls. The results
were not due to an investigators bias, as investigation of fixed time window parameters showed similar results.

The ASR measured over multiple muscles is thought to be a better tool for detecting ASR abnormalities in young patients with anxiety disorders. The failure in the present study to find enlarged blink responses is in accordance with similar negative findings in young patients with anxiety disorders, highly anxious children and children with a behaviorally inhibited temperament (a vulnerability factor for the development of anxiety disorders) and with the results of several studies in adults. The contrasting results of the multiple muscle ASR (whole-body ASR) and blink response in our study confirm that, in children, the blink response cannot be simply equated to the ASR, which occurs in the whole body. The measurement over multiple (distal) muscles gives a different, more complete representation of the ASR than the blink response alone.

Both patients with anxiety disorders and their non-affected siblings had a significantly enhanced autonomic response (as assessed by electrodermal activity of the sympathetic skin response) compared with controls. Autonomic hyperreactivity in children with anxiety disorders has been reported in previous studies. As our sample of siblings was small, the results give only preliminary evidence of a shared characteristic of patients and siblings, which may be a vulnerability factor or endophenotype for anxiety disorder. However, previous studies support the findings. At-risk offspring of parents with anxiety disorders have been reported to show enlarged electrodermal activity when exposed to fear stimuli. Both children with conduct disorders and their fathers have shown a reduced sympathetic skin response (similarly quantified in the present study) following auditory stimuli. Finally, twin studies have shown a significant heritability for phasic electrodermal activity. However, other studies investigating children at-risk children and young patients with anxiety disorders did not find enlarged autonomic reactivity following auditory stimuli. In these studies, the electrodermal activity was not standardized to the intra-individual maximum or mean like in our study, which could explain these findings. The ASR (multiple muscle response probability) was almost as large in siblings as in patients, but the sample was too small to statistically establish that it was abnormally enlarged in siblings compared with controls. Several blink response studies have shown enlarged orbicularis oculi responses in non-affected offspring of patients with anxiety disorders but not always.

Like in our previous study in healthy children, in the present sample we did not find sex and age effects on the ASR that are present in adults. As most participants included in our studies are children and not adolescents, the explanation that sex- and age-specific variations of the ASR may develop after childhood is plausible.
Our study has several implications. First, our extensive investigation of the ASR in anxiety disorder patients strengthens the association between enlarged ASRs and pathological anxiety in humans. The results point towards a hypersensitive central nervous system in childhood anxiety disorder. More specifically, as the amygdala (modulated by the prefrontal cortex) is a key structure involved in pathological anxiety and associated with fear-potentiated ASRs in animals and thus humans, abnormal functioning of the amygdala or its modulating structures may explain the enlarged ASRs. Second, our findings provide evidence for a preattentive hyperresponsiveness to auditory stimuli in young patients with anxiety disorders -- an early (within 200 ms poststimulus) sensorimotor processing alteration at the brainstem level. Similarly, in adults with anxiety disorders excessive amygdala and medial prefrontal cortex/anterior cingulated cortex activity was found during the processing of emotional stimuli without awareness. This supports the idea that patients with anxiety disorders have an increased response of brain arousal systems to (emotional) sensory stimuli early in the time course (between 100 and 150 ms poststimulus). Thirdly, the multiple muscle ASR is recommended to assess ASR abnormalities in patients with anxiety disorders. Finally, ASR abnormalities serve as candidate endophenotypes for anxiety disorders in children.

Limitations
Our sample was of a moderate size. In addition, the comparison of the current ASR paradigm to the psychophysiological ASR paradigms was limited as the interstimulus interval of the current study was longer and the amount of presented stimuli was lower. That is, if more stimuli would have been presented, as is usual in blink response studies, perhaps the blink response would also have been abnormally enlarged in the present sample of children with anxiety disorders. In spite of this limitation, our results show that the ASR measured over multiple muscles is more sensitive to detect ASR abnormalities in anxiety disorder patients compared with the blink response.