The startle reflex in children with neuropsychiatric disorders
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Increased auditory startle reflex in children with functional abdominal pain

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MA Benninga
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Abstract

Objective: Visceral hypersensitivity has been demonstrated in children with abdominal-pain related functional gastrointestinal disorders, such as irritable bowel syndrome and functional abdominal pain syndrome. Rather than just a local phenomenon in the gut, these patients could suffer from a general hypersensitivity for sensory stimuli. This is investigated by examining the auditory startle reflex (ASR) measured over multiple muscles. **Patients and methods:** ASR’s were elicited by consecutive tones (104 dB, interstimulus 2 minutes) in 20 children classified according to Rome III classifications: 13 irritable bowel syndrome, 7 functional abdominal pain syndrome (mean age 12.4 years, 15 girls), 23 controls (14 girls, mean age 12.3 years) and 25 patients with anxiety disorders (17 girls, mean age 12.0 years). The activity of six left-sided muscles was measured by an electromyogram (EMG). Furthermore, the sympathetic skin response was obtained. The muscle response probability (%) and magnitude of the EMG response (μV.ms) of the combined response of six muscles (multiple muscle ASR) and separately the blink response (μV.ms) were investigated. **Results:** The multiple muscle ASR’s (response probability and EMG magnitude) and the blink response (EMG magnitude) all proved to be significantly increased in patients with functional abdominal pain compared with controls. No increase of the autonomic sympathetic skin response was found. Comorbid anxiety disorders (7 patients; 6 irritable bowel syndrome and 1 functional abdominal pain syndrome) or Rome III classification did not significantly affect these results. There were no significant ASR and sympathetic skin response differences between the patients with abdominal pain and patients with anxiety disorders. **Conclusions:** This is the first study demonstrating a hyperresponsivity to non-gastrointestinal stimuli in children with irritable bowel syndrome or functional abdominal pain syndrome. These findings suggest that these children suffer from a generalized hypersensitivity of the central nervous system.
Chapter 6
Startle reflex in children with anxiety disorders

Introduction

Irritable bowel syndrome and functional abdominal pain syndrome in childhood are abdominal-pain related functional gastrointestinal disorders and represent the vast majority of consultations to pediatric gastroenterology outpatient clinics. In both childhood irritable bowel syndrome and functional abdominal pain syndrome lowered sensory threshold (by self-report) to rectal balloon distension has been described. Furthermore, there is high comorbidity of non-gastrointestinal functional diseases and psychiatric disorders, such as anxiety disorders and depression, in these children. Lacking a well-defined biomarker abdominal-pain related functional gastrointestinal disorders are conceptualized as a manifestation of a disturbance in the brain-gut axis.

Regarding adult irritable bowel and functional abdominal pain syndromes there is an ongoing debate whether primarily peripheral (visceral) or central (brain) deficits underlie these disorders. Support for the latter is provided by the findings that anxiety and stress increase symptoms in irritable bowel syndrome patients. There is also the observation that gastrointestinal stimulation in adult irritable bowel syndrome patients leads to abnormal activity in emotional processing brain areas (amygdala, anterior cingulate cortex, prefrontal cortex). However, this does not prove that brain abnormalities are the main origin of the abnormal gut sensations. Similarly, previous research has failed to demonstrate a clear-cut intestinal dysfunction.

The investigation of reactivity in patients with functional gastrointestinal disorders to extra-visceral stimuli has been used to identify more general sensory processing alterations. Studies of pain thresholds to cutaneous heat or cold pain stimuli suggest that (some) adult irritable bowel syndrome patients are hypersensitive to a range of somatic stimuli wider than just visceral. However, other studies described a normal or higher perceptive or pain threshold to somatic stimuli in these patients. Unfortunately, most of these studies are based on verbal patient report, limiting their reliability. Response parameters independent of verbal report of the patients would offer more objective evidence of altered processing of sensory stimuli in functional gastrointestinal disorders patients.

The Auditory Startle Reflex (ASR) is a pre-attentive primitive emotional response and an index of neuronal excitability. It is not prone to informants’ bias or under volitional control. A recent study showed that adult female patients with irritable bowel syndrome have an increased ASR as measured by the blink response. To our knowledge, the ASR had not yet been investigated in pediatric irritable bowel syndrome or functional abdominal pain syndrome. In addition, measuring the ASR over multiple muscles
(multiple muscle or whole-body ASR) is considered more appropriate to assess the ASR than measuring the blink response alone. The aim of our study is to investigate whether the ASR measured over multiple muscles, extended by the autonomic response to the auditory stimuli as expressed by the sympathetic skin response, is enlarged in children with the most common abdominal-pain related functional gastrointestinal disorders (irritable bowel syndrome and functional abdominal pain syndrome) compared with controls of comparable age and sex. In addition, the young patients with functional abdominal pain will be compared with similarly young patients with anxiety disorders (in which the ASRs were shown to be enlarged compared with controls).

**Methods**

**Subjects**

Children with chronic abdominal pain were referred by general practitioners, child psychologist, general pediatricians or pediatric gastroenterologists to a pediatrician specialized in childhood gastrointestinal disorders (MBe) at the Pediatric Outpatient Clinic of the Academic Medical Center in Amsterdam between August and October 2006. All patients aged between 8 and 17 years old who fulfilled the childhood/adolescent Rome III criteria for either irritable bowel syndrome or functional abdominal pain syndrome were invited to participate in the study. Of the 26 invited patients with abdominal pain, 20 could be included in the study. Two patients did not want to participate, one patient suffered from a hearing defect and three patients were excluded after initial inclusion/invitation (H. Pylori, mechanical problems during testing). These 20 patients fulfilled the Rome III criteria for irritable bowel syndrome or functional abdominal pain syndrome, amongst others based on a 2-week abdominal pain diary which was kept as part of the intake procedure. In this diary pain intensity was scored using the Facial Affective Scale with emotional faces showing no pain at all (face 1, happy face) to faces showing severe pain (face 9, crying face). If intensity was scored as 5 (sad face) or higher more than twice a week, patients were eligible for the study (all patients were eligible).

No structural or biochemical abnormalities were found upon physical examination and tests of blood [Hb, leukocytes, CRB, BSE, ASAT/ALAT, amylase, phadiatop, IgE, IgG, IgA, IgM, Coeliakie AS], urine [sediment, glucose] and stool samples [Haema, TFT, H.Pylori] in these children.

The patients with anxiety disorders (aged between 8 and 17 years old) were referred to the Child Psychiatry Outpatient Clinic of the Academic Medical Center in Amsterdam where they presented with an anxiety disorder. They are described in more detail elsewhere. Most patients met criteria of more than one anxiety disorder diagnosis but primary diagnoses were: social anxiety disorder (13), generalized anxiety disorder (9), phobia (2) and panic disorder (1).
Controls, described previously, were excluded if they met criteria for psychopathology. As 12 of the originally described 27 controls were boys (44%) and only 5 of the 20 patients with abdominal pain were boys (25%) and 8 of the 25 patients with anxiety disorders were boys (32%), four control boys (selected on the basis of their age) were excluded to make the control and patient groups optimally similar in terms of number, sex and age. All subjects meeting criteria for a major depression disorder, neurological disorder, mental retardation or schizophrenia or other psychotic disorders, were excluded from the study. All subjects were screened for sedative medication, a hearing defect, or another major somatic disorder (which may cause anxiety and/or abdominal pain). All subjects were non-smokers. The controls and patients with anxiety disorders were free of recurrent abdominal pain, as was screened by the Abdominal Pain Index.

Psychiatric assessment
The Anxiety Disorders Interview Schedule (ADIS) was used to formally establish or exclude anxiety disorders. The ADIS is a semi-structured interview based on DSM-IV classification of psychopathology and includes both a child and a parent interview (ADIS-C/P).

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. Following a digital trigger, the tones were generated by an audiostimulator and were presented binaurally through stereo headphones. The stimuli were presented with varying time intervals (1.5 – 2.5 minutes) which were similar for all subjects.

Data collection
Physiological data, consisting of bipolar left orbicularis oculi, masseter, sternocleidomastoid, deltoid, abductor pollicis brevis, quadriceps electromyography (EMG) and sympathetic skin response measures were recorded employing Biosemi’s Active System. Details of the data collection were conform a previous study. After skin preparation, the cutaneous silver-silver chloride flat active surface electrodes were filled with conductive paste and attached 2 cm apart with adhesive collars. Impedance of the electrodes (<10 kΩ) was checked before recording. The sympathetic skin response was recorded from the palm of the hands, with the reference electrode on the dorsum of the hands. The signal was analogue filtered high-pass (1st order: -3dB at 0.16 Hz) and low-pass (5th order anti-aliasing: -3dB at 3500 Hz). Filtered data were continuously digitized with a sample frequency of 16384 Hz per channel using a 24-bit A/D converter.
Procedure
All subjects were asked to refrain from caffeinated beverages on the day of testing. The subjects sat on a bed (with backrest) in an upright position and were asked to sit relaxed. The subjects were given 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 the headphones were placed and the stimulation software was started. In total (including preparation) the experiment took 45 minutes. The study protocol and consent forms were reviewed and approved by the Institutional Board of the Academic Medical Center.

Data processing
Details of the data processing were previously described. Several parameters were obtained. The multiple muscle ASR response probability was defined as the average of the response probabilities of the six muscles. To determine the muscle response probability, the EMG activity of all muscles after stimulation was visually inspected by the investigator (MB). A ‘response’ was scored if an increase of EMG activity from baseline occurred in either of the six simultaneously recorded muscles at an appropriate latency. Strict rules were defined before scoring the responses: (1) a response was defined as a clear increase (duration increase at least 30 ms, magnitude response at least 30 μV) from baseline (2) the response onset (20-200 ms following stimulation) was marked at the baseline (thus at the start of the μV increase) (3) all responses were scored by the same investigator (4) all responses were scored at the same screen sensitivity (200 μV on the screen, 100 μV below baseline and 100 μV above baseline). In each group the response probability of the individual muscles was determined by counting the total occurrence of responses and dividing this value by the total amount of recorded traces (amount of subjects times 8 stimuli), and multiplying this value by 100. Latency was defined as the period between stimulus onset and the start of the response at the EMG baseline. Response onset (latencies) and response offset were manually marked by the investigator. Trials considered as artefacts (e.g. heart beat, loose electrodes) were marked as such and not included in the analysis. The multiple muscle ASR magnitude of the EMG signal was defined as the summated log transformed EMG area-under-the-curve of the individual muscles, with the area-under-the-curve as quantifier (expressed in μV.ms) of the EMG signal between response onset and offset. Although scoring was performed conform strict rules, it was logistically impossible to keep the investigator completely blind for group membership. To rule out a possible investigator’s bias we also analyzed parameters using fixed time intervals. We chose to determine the peak amplitude and area-under-the-curve of all EMG trials (6 muscles times 8 trials is 48 trials per subject) between 20 – 200 ms following stimulation. The peak amplitude (μV) of the smoothed
(with a 40 Hz filter low-pass filter\(^ {65}\)) EMG trials was identified by software between 20 and 200 ms following stimulation and these amplitudes were averaged per muscle. To obtain the EMG area-under-the-curve (\(\mu V\cdot ms\)) of the 20-200 ms time interval the EMG trials were not smoothed but background noise (estimated at three times the median of the interval 500 to 1000 ms following stimulation) was removed.\(^ {229}\) For both parameters the combined response was obtained by summatting the log transformed values of all six muscles. The sympathetic skin response was recorded from the palm of the hands, with the reference electrode on the dorsum of the hands.\(^ {219, 228}\) The sympathetic skin response was defined as the difference in \(\mu V\) between the identified maximum (the peak \(\mu V\) during the interval 900 - 4000 ms following stimulation) and the baseline (mean \(\mu V\) during 0-900 ms following stimulation) standardized to (relative to; giving values ranging from 0 to 100 %) the intra-individual maximum.\(^ {235, 236}\)

**Statistical analysis**

A linear mixed-model analysis (type III tests of fixed effects) was used to test group differences in parameters over the repeatedly presented stimuli. The response probability parameters did not show a normal but a Poisson distribution. Therefore, in addition a Poisson regression model was fitted (while accounting for the repeated measures per subject by calculating robust standard errors using a General Estimates Equation method). EMG area-under-the-curve parameters were log transformed to reduce skewness of the data and to standardize the area-under-the-curve values of the different muscles (different in size and strength) after which they were summated in the combined area-under-the-curve calculation. As the latency distributions were not normally distributed, Wilcoxon-Mann-Whitney tests were conducted to test group differences in latency. Student’s t-tests were used to test general characteristics. A p-value \(\leq 0.05\) was considered significant in all tests.

**Results**

**General and clinical characteristics**

A total of 13 patients with irritable bowel syndrome and 7 patients with functional abdominal pain syndrome (15 girls, 75 %, mean age 12.4), 23 controls (15 girls, 65 %, mean age 12.3) and 25 patients with anxiety disorders (17 girls, 68 %, mean age 12.7) were included in the study. Of the 20 patients with abdominal pain 8 (40 %) suffered from a comorbid anxiety disorder (7 irritable bowel syndrome, 1 functional abdominal pain syndrome)(7 girls and 1 boy).
Multiple muscle ASR

Both the response probability and the magnitude of the EMG response of the multiple muscle ASR’s (the combined response of six muscles) were significantly enlarged in the patients with abdominal pain compared with controls (response probability F(1,39.2)=9.9 p=0.003; \( \chi^2 \)(1)=5.6, p=0.017), EMG area-under-the-curve F(1,38.7)=6.6 p=0.014)(Table 1) (Figure 1). Compared with the patients with anxiety disorders the multiple muscle ASR parameters of the patients with abdominal pain were not significantly different (response

Table 1. Muscle response characteristics. The multiple muscle ASR represents the average of all response probabilities and the sum of all log transformed EMG area-under-the-curve’s. P-values are given with a * for P ≤ 0.05, ** for P ≤ 0.01 and *** for P ≤ 0.005.

<table>
<thead>
<tr>
<th>Muscle Response Characteristics</th>
<th>Abd. Patients</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probability of response (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orbicularis oculi</td>
<td>90.0 ± 30.1</td>
<td>77.7 ± 41.7</td>
<td>P=0.156</td>
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<td>Sternocleidomastoid</td>
<td>41.3 ± 49.4</td>
<td>19.5 ± 39.7</td>
<td>P=0.033*</td>
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<td>Masseter</td>
<td>29.7 ± 45.8</td>
<td>10.3 ± 30.5</td>
<td>P=0.037*</td>
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<tr>
<td>Deltoid</td>
<td>19.3 ± 39.6</td>
<td>3.2 ± 17.8</td>
<td>P=0.005**</td>
</tr>
<tr>
<td>Abductor pollicis brevis</td>
<td>22.0 ± 41.6</td>
<td>3.9 ± 19.6</td>
<td>P=0.006**</td>
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<tr>
<td>Quadriceps</td>
<td>15.5 ± 36.4</td>
<td>3.2 ± 17.8</td>
<td>P=0.045*</td>
</tr>
<tr>
<td>Multiple Muscle</td>
<td>36.4 ± 26.8</td>
<td>20.0 ± 15.8</td>
<td>P=0.003**</td>
</tr>
</tbody>
</table>

**Figure 1.** Auditory Startle Reflex (ASR) A. The multiple muscle ASR (response probability, 0 – 100 %), shown for the eight repetitive stimuli, is significantly enlarged in patients with abdominal pain (n=20) compared with controls (n=23), but not compared with patients with anxiety disorders (n=25). B. The multiple muscle ASR (EMG magnitude; area-under-the-curve in µV.ms), shown for the eight repetitive stimuli, is significantly enlarged in patients with abdominal pain (n=20) compared with controls (n=23), but not compared with patients with anxiety disorders (n=25). Bars represent means; error bars standard error of means.
probability $F(1,41.1)=0.703$, $p=0.407$; $\chi^2(1)=0.85$, $p=0.356$, EMG area-under-the-curve $F(1, 41.5)=0.133$, $p=0.717$). In Table 1 the response probabilities, EMG area's-under-the-curve and latencies of all individual muscles are illustrated.

The blink response

The magnitude of the EMG of the blink response (Table 1) was significantly enlarged in patients with abdominal pain compared with controls after log transformation (EMG area-under-the-curve $F(1, 38.1)=8.2$, $p=0.007$) but compared with the patients with anxiety disorders it was not significantly different (EMG area-under-the-curve $F(1,38.5)=1.1$, $p=0.306$).

Fixed time interval parameters

The magnitude of all EMG trials without selection between 20 to 200 ms post-stimulus were significantly enlarged in the patients with abdominal pain compared with controls, peak amplitude $F(1,38.8)=7.8$, $p=0.008$ and EMG area-under-the-curve $F(1,33.2)=8.3$, $p=0.007$, but not significantly different compared with children with anxiety disorders, peak amplitude $F(1,41.1)=0.834$, EMG area-under-the-curve $p=0.367$, ($F(1,38.4)=2.6$, $p=0.115$).

Sympathetic skin response

The sympathetic skin response was larger in patients with abdominal pain (35.5 %; SD 37.9) compared with controls (26.3 %; SD 35.6), but this difference did not reach significance ($F(1,36.4)=4.0$, $p=0.052$; Figure 2). Compared with the patients with anxiety disorders (mean 36.8 % SD 34.2) the sympathetic skin response of the patients with abdominal pain was not significantly different ($F(1,40.0)=0.75$, $p=0.786$; Figure 2).
Comorbidity anxiety disorders and Rome III subcategory

The presence of a comorbid anxiety disorder did not significantly affect the multiple muscle ASR (response probability $F(1,16.0)=0.120$, $p=0.733$, EMG area-under-the-curve $F(1,16)=0.007$, $p=0.935$) or sympathetic skin response ($F(1,40.2)=2.5$, $p=0.120$) of the patients with abdominal pain (Figure 3). Similarly, the Rome III subtype (irritable bowel syndrome and functional abdominal pain syndrome) also did not significantly affect the multiple muscle ASR (response probability $F(2,15.0)=0.388$, $p=0.685$, EMG area-under-the-curve $F(2,15.2)=0.096$, $p=0.909$) or the sympathetic skin response ($F(1,19.3)=0.00.92$, $p=0.347$) of the patients with abdominal pain.

Sympathetic skin response

The sympathetic skin response was larger in patients with abdominal pain (35.5 %; SD 37.9) compared with controls (26.3 %; SD 35.6), but this difference did not reach significance ($F(1,36.4)=4.0$, $p=0.052$; Figure 2). Compared with the patients with anxiety disorders (mean 36.8 % SD 34.2) the sympathetic skin response of the patients with abdominal pain was not significantly different ($F(1,40.0)=0.75$, $p=0.786$; Figure 2).
Discussion

This is the first study to demonstrate a general hypersensitivity for sensory stimuli in children with abdominal-pain related functional gastrointestinal disorders; irritable bowel syndrome and functional abdominal pain syndrome. We found that these patients show increased responses following sensory stimuli which are not presented to the viscera. All auditory startle reflex (ASR) quantifications used in this study showed a significant increase in patients with abdominal pain compared with controls.

The enlarged ASR’s converge with results of studies in adult irritable bowel syndrome patients providing evidence of an auditory or somatic hypersensitivity by using objective measures (independent of the subjective report of the patient). Auditory stimuli resulted in increased blink responses in adult female irritable bowel syndrome patients. Further, alterations of brain processing like increased event-related potentials and abnormal cerebral activity (in the limbic system) were found in adult irritable bowel syndrome patients following both neutral and emotional stimuli. Somatic stimuli administered to adults with irritable bowel syndrome resulted in increased brain activity in the thalamus and anterior cingulate cortex.

The findings point towards an abnormally sensitive central nervous system in children with abdominal-pain related functional gastrointestinal disorders. Auditory stimuli are transmitted directly to the brain via the eighth cranial nerve; without peripheral involvement. A hypersensitive central nervous system in childhood abdominal pain-related functional gastrointestinal disorders is compatible with the increasing evidence for aberrant central nervous system processing in adult irritable bowel syndrome patients. Like in adults, a generalized increase in emotional and sensory sensitivity may explain the current findings. The human ASR originates in the reticular formation of the medial caudal brainstem. As the reticular formation is known as the brain’s arousal system, the enlarged ASR’s may be due to hyperarousal in these patients possibly related to emotional aspects of the auditory stimuli (sudden, loud stimuli). An abnormal arousal circuit (including the amygdala, locus coeruleus and anterior cingulate cortex) via altered activity of the central noradrenergic modulatory systems has been suggested to underlie irritable bowel syndrome pathophysiology in adults. Further, an enhanced emotional sensitivity is consistent with the similarly enlarged ASR’s in children with anxiety disorders and the described augmenting effect of stress on the ASR in animals. Interestingly, unlike in adults, the presence of comorbid anxiety disorders in the young patients with abdominal pain did not contribute to the augmentation of the response to the auditory stimuli, suggesting that the demonstrated ASR abnormalities are independent of comorbid anxiety symptoms. However, the
enlarged ASR’s could still represent a cognitive-affective common pathophysiology of abdominal-pain related functional gastrointestinal disorders and anxiety disorders in children; it may be a characteristic which is not indexed by the traditional anxiety measures (DSM-IV).\textsuperscript{36} This is in agreement with the hypothesis that children with abdominal-pain related functional gastrointestinal disorders are hypersensitive to stress but tend to express it somatically rather than verbally.\textsuperscript{316}

The sympathetic skin response was, in contrast to our expectation, not enlarged in patients with abdominal pain compared with controls, although a trend was observed. This negative finding was surprising because as the visceral system is part of the autonomic system, it is plausible that the autonomic nervous system as one of the primary gut-brain communication pathways plays an important role in pediatric abdominal-pain related functional gastrointestinal disorders. Although there are discrepant findings in the literature, alterations of the sympathetic or parasympathetic system were demonstrated in adult patients with irritable bowel syndrome (see\textsuperscript{317}).

The current study does not give conclusive evidence about the primary origin of the abdominal symptoms in functional gastrointestinal disorders patients; it does not allow judgment about the differential contribution of afferent and efferent processes in the brain to the present findings. An underlying central dysfunction may be responsible for the abdominal pain symptoms as well as the increased ASR’s, or the abdominal pain symptoms may have sensitized the central nervous system which triggered the increased ASR’s. Although there is no absolute proof of gastrointestinal defects there is some evidence pointing at the contribution of inflammation, prior injury or infection, and changes in the local factors of the gastrointestinal tract in abdominal-pain related functional gastrointestinal disorders (for a review see\textsuperscript{40}). Obviously, it will take further research to further identify the underlying cause, or more likely: multiple causes of the identified central nervous system hypersensitivity to sensory stimuli in pediatric abdominal-pain related functional gastrointestinal disorders.

**Conclusions**

Hypersensitivity to auditory stimuli, demonstrated by an enlarged ASR, indicates that hypersensitivity to sensory stimuli in children with abdominal-pain related functional gastrointestinal disorders is not restricted to visceral sensations. These findings suggest a generalized hypersensitivity in children with abdominal-pain related functional gastrointestinal disorders.