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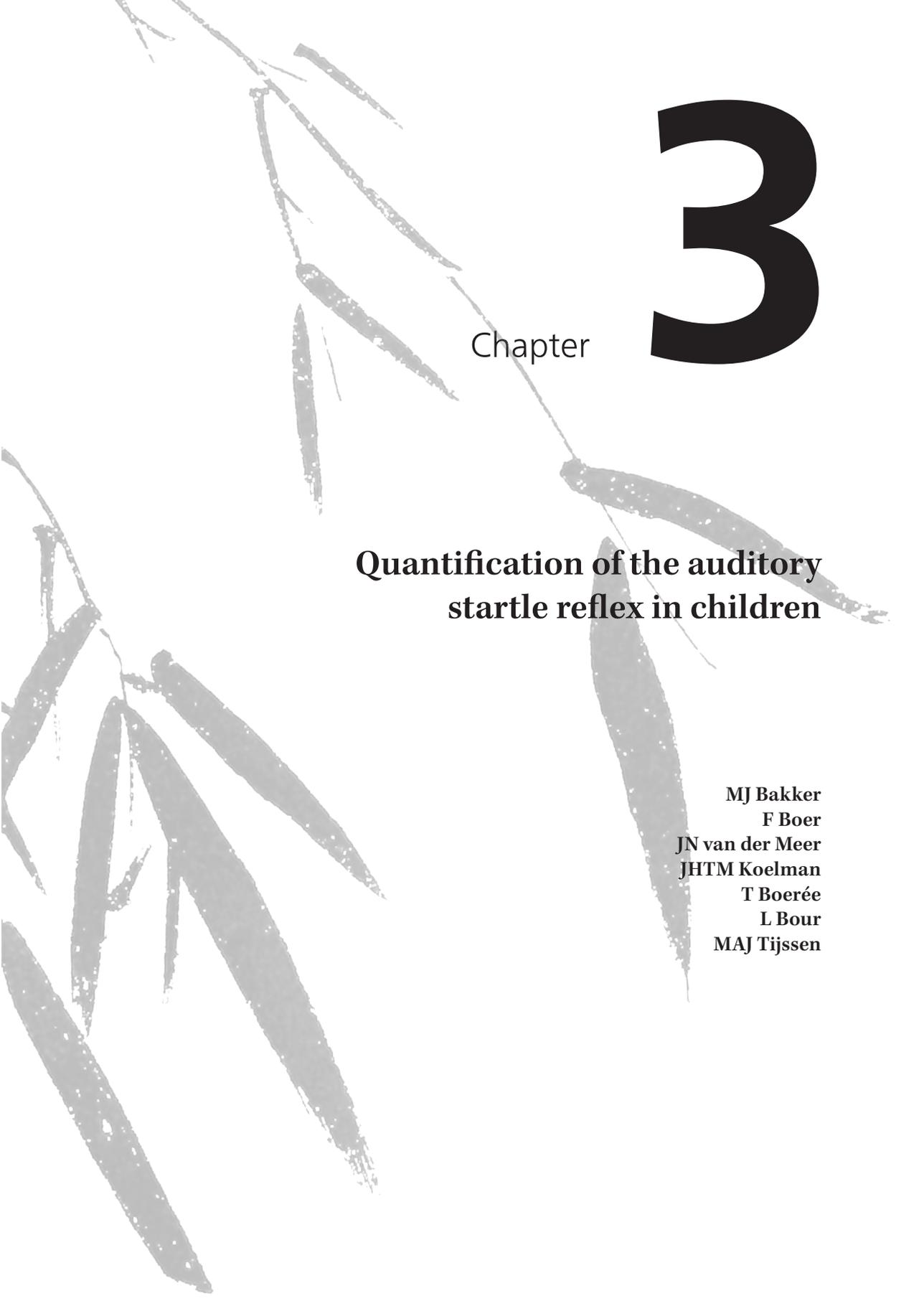
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# 3

Chapter

## **Quantification of the auditory startle reflex in children**

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## Abstract

**Objective:** To find an adequate tool to assess the auditory startle reflex (ASR) in children. **Methods:** We investigated the effect of stimulus repetition, sex and age on several quantifications of the ASR. ASRs were elicited by eight consecutive auditory stimuli in 27 healthy children. Electromyographic activity of orbicularis oculi, masseter, sternocleidomastoid, deltoid, abductor pollicis brevis, quadriceps muscles and the sympathetic skin response were recorded. ASR parameters (response probability in % and magnitude in area-under-the-curve) were: 1) combined response of all six muscles 2) blink response. **Results:** Response probabilities were 78 % in orbicularis oculi (median latency 41 ms), 17 % in sternocleidomastoid (median latency 66 ms), 10 % in masseter (median latency 66 ms) and lower in other muscles. The ASR combined response probability and the sympathetic skin response significantly decreased with the repetitive stimuli, but the blink response probability did not. The magnitude (area-under-the-curve) of both the blink response and the combined response did not decrease with the repetitive stimuli. There were no sex or age effects. **Conclusion:** As in adults, the blink response and the combined response of multiple muscles show different habituation patterns in children. **Significance:** Investigation of multiple muscles seems appropriate to quantify the ASR in children.

## Introduction

The auditory startle response (ASR) is generated by the caudal brainstem.<sup>78</sup> The motor component of the ASR includes facial grimacing, neck flexion and shoulder activity.<sup>45</sup> Moreover, in its most extensive form, the ASR consists of a response including arms, elbows, trunk, hips and knees.<sup>47</sup> Rapid habituation with repetitive stimulation is considered a typical clinical feature of the ASR.<sup>45, 72, 217</sup> In adults, the ASR habituates within two to six trials<sup>47</sup>, depending on the nature of the stimulation. The autonomic component of the ASR, measurable as a slow reflex depolarization of the skin<sup>218</sup> is also known to decrease rapidly with repetitive auditory stimuli.<sup>219, 220</sup>

The ASR can be measured with polymyographic electromyogram (EMG) investigating the activity of multiple muscles.<sup>45, 47, 221</sup> Neurophysiologists consider the multichannel EMG ASR quantification more appropriate to index the ASR than just recording the activity of the blink response in the orbicularis oculi muscle.<sup>47, 54, 63</sup> In contrast, in psychophysiological ASR studies only the blink response is measured.<sup>37, 64, 65</sup> The literature on blink response modulation, including elaborative measurement guidelines<sup>65</sup>, makes it abundantly clear that this response is sensitive to a variety of mental and other stressors and triggers.<sup>50</sup> However, there are doubts whether it is the best parameter to measure the ASR.<sup>47, 79</sup> The orbicularis oculi or blink response consists of two different EMG responses which differ in size and latency.<sup>45, 47, 47, 66-71</sup> The early orbicularis oculi response is likely to be due to a simultaneously induced physiological blink reflex; a protective brainstem reflex specifically to protect the eye.<sup>47</sup> This reflex is considered to be modulated by polysynaptic neural networks<sup>222</sup> involving the substantia nigra pars reticulata and the superior colliculus via tecto-bulbar projections.<sup>223-225</sup> The second part of the orbicularis oculi response (latency > 60 ms) is part of the true ASR.<sup>47, 226</sup> The ASR is also a brainstem reflex but originates in the nucleus reticularis pontis caudalis<sup>47, 53</sup> and is mediated by basal ganglia circuitry by means of the pedunculo-pontine tegmental nucleus.<sup>81</sup> As the two components of the blink response in the orbicularis oculi muscle extensively overlap, it is usually difficult to distinguish them.<sup>67, 68</sup>

In children the habituation of the blink (orbicularis oculi) response has been investigated.<sup>48</sup> In addition, the magnitude and latencies of the ASR measured over multiple muscles in up to five children (8-15 years old) has been described.<sup>82, 89</sup> However, we are not aware of studies in children specifically addressing the magnitude and habituation of the multiple muscle ASR, the effect of sex and age on the multiple muscle ASR or the comparison of the multiple muscle ASR and the blink response ASR (as it is quantified in psychophysiological studies). The aim of the present study is to find an adequate tool to assess the ASR in children. The effect of stimulus repetition, sex

and age on the ASR in various facial, neck and extremity muscles will be investigated. Advantages and disadvantages of both the single muscle (orbicularis oculi) and multiple muscles ASR will be discussed.

## **Methods**

### **Subjects**

We investigated 27 healthy, non-medicated subjects between 8 and 17 years old (15 girls). Mean age of girls was 12.3 (SD 2.52), mean age of boys was 11.5 (SD 2.88). All subjects but one boy and one girl were right-handed. Exclusion criteria comprised a neurological or a psychiatric disorder. The Anxiety Disorders Interview Schedule<sup>227</sup> for DSM-IV disorders<sup>36</sup>, Child and Parent Versions (ADIS-C/P) were administered by the researcher (MB, clinical psychologist). This clinical interview establishes anxiety and mood disorders and screens for other psychopathology. Subjects with neurological abnormalities were excluded by a neurologist (MT). Of the 30 control children tested, 3 had to be excluded due to presence of an anxiety disorder (2) or evidence of a neurological disorder in the past (1). All subjects were non-smokers.

### **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.<sup>65</sup> Following a digital trigger, the tones were generated by an audio stimulator (Walter, Messelelektro) and were presented binaurally through stereo headphones. The triggers of auditory stimuli were stored in the computer synchronously with the EMG data. The stimuli were presented with varying time intervals (1.5-2.5 minutes)<sup>58, 90</sup> which were similar for all subjects.

### **Data collection**

Physiological data, consisting of bipolar left orbicularis oculi, masseter, sternocleidomastoid, deltoid, abductor pollicis brevis, quadriceps EMG and sympathetic skin response measures were recorded employing Biosemi's Active System ([www.biosemi.nl](http://www.biosemi.nl)). After skin preparation (Abrasive gel, NuPrep, D.O.Weaver and Co, Aurora, CO, USA), the cutaneous silver-silver chloride flat (11 mm width, 17 mm length, 4.5 mm height) active surface electrodes (equipped with pre-amplifiers to improve the signal/noise ratio and reduce the movement artefact) were filled with conductive paste (Ten20 Conductive, D.O.Weaver and Co, Aurora, CO, USA). The electrodes were attached 2 cm apart (except the electrodes for sympathetic skin response) with adhesive collars, in accordance with recommendations by the Psychophysiology Committee.<sup>65</sup> Of the electrodes attached to the orbicularis oculi muscle, one was placed straight below the eye and the other 2 cm

towards the ear and slightly higher. The paste cavity of the flat electrodes was designed to reduce motion artefacts. The sintered electrode pallet (4 mm in diameter) provides very low noise, low offset voltages and very stable DC performance. Impedance of the electrodes ( $<10\text{ k}\Omega$ ) 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<sup>219, 228</sup>, but first the skin surface was cleaned with abrasive gel. The isolated ground electrode was placed on the temple. The signal was analogue filtered high-pass (1<sup>st</sup> order; -3 dB at 0.16 Hz) and low-pass (5<sup>th</sup> order anti-aliasing; -3 dB at 3500 Hz). Filtered data were continuously digitized with a sample frequency of 16,384 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. After the electrodes were attached, the subjects sat on a bed in an upright position and were asked to sit quietly and 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 min'. Subsequently the headphones were placed and the stimulation software was started. The preparation and experiment took 45 min in total. The study protocol and informed consent forms (signed by both the subjects and their parents) were reviewed and approved by the Institutional Review Board of the Academic Medical Center, Amsterdam.

## Data analysis

Off-line data analysis was performed with Brain Vision Analyzer 1.05 (Brain Products, GmbH, Munich, Germany). Orbicularis oculi signals were digitally filtered at 100 Hz high-pass 12 dB/oct to minimise DC offsets and slow eye drifts.<sup>229, 230</sup> The cut-off frequency of the high-pass filtering of the other muscles was 20 Hz. The reason for different 100 Hz filtering (high-pass) of the orbicularis oculi muscle is that superimposed on the orbicularis oculi EMG activity is the cornea-retina potential (EOG). When a blink occurs also the eye rotates which leads to a change of the potential coming from the EOG and the frequency content can be up to 70 Hz.<sup>231, 232</sup> This frequency is higher compared to the frequency content of artefacts occurring in other muscles, which, therefore, were filtered at 20 Hz (high-pass). Due to the high sample rate employed (16,384 Hz) the low-pass analogue filter could be chosen at 3500 Hz. The 14 raw unipolar signals were converted into seven pairs of bipolar derivations. All seven channels were segmented into parts of 1000 ms after the stimulus. Baseline correction was applied using the mean of the 0-20 ms time interval. Subsequently, the signals were rectified.

Four different parameters were used to quantify the magnitude of the motor ASR: (1) The combined response probability of all muscles in % (2) The response probability of the blink response in % (3) Combined magnitude of all muscles in EMG area-under-the-curve (4) Magnitude of the blink response in EMG area-under-the-curve. The response probability represents the chance (%) that a certain muscle responds following stimulation.<sup>58</sup> The combined response probability was defined as the average of the response probabilities (expressed in % chance of a muscle response following stimulation) of all six muscles.<sup>58</sup> The response probability of the blink response was defined as the chance (%) of the occurrence of an orbicularis oculi response following stimulation. The magnitude parameters are expressed in area-under-the-curve of the EMG signal of the responses. Quantification of the EMG burst in area-under-the-curve (rectified integrated signal, expressed in  $\mu\text{V}\cdot\text{ms}$ ) is the most direct translation of the raw EMG into measurement units and a commonly used ASR quantification method.<sup>58, 65</sup> The area-under-the-curve will be calculated conform the psychophysiological literature.<sup>65</sup> The reason for choosing this method is that we wanted to compare our data with not only neurological but also psychophysiological studies.

The EMG activity of all muscles after stimulation was visually inspected by the investigator (MB) with Brain Vision Analyzer to determine which trials could be considered as valid responses. A 'response' was scored if EMG activity increasing from baseline occurred in either of the six simultaneously recorded muscles at an appropriate latency.<sup>45, 47, 89</sup> 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  $\mu\text{V}$ ) from baseline (2) the response onset (20-200 ms following stimulation) was marked at the baseline (thus at the start of the  $\mu\text{V}$  increase) (3) all responses were scored by the same investigator (4) all responses were scored at the same screen sensitivity (200  $\mu\text{V}$  on the screen, 100  $\mu\text{V}$  below baseline and 100  $\mu\text{V}$  above baseline). The response probability of the individual muscles was determined by counting the occurrence of these 'responses' and dividing this value by the total amount of recorded traces, usually 216 (27 subjects times 8 stimuli), times 100. Latency was defined as the time from stimulus presentation until the start of the 'response' at the EMG baseline. Response onset (latencies) and response offset were manually marked by the investigator. Trials considered as artifacts (e.g., heartbeat, loose electrodes) were marked as such and not included in the analysis. The combined response area-under-the-curve of all muscles and the blink response area-under-the-curve were based on the psychophysiology guidelines for human startle eye blink electromyographic studies.<sup>65</sup> 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. Subsequently, the EMG area-under-the-curve's of the trials marked by the investigator as response (see earlier) were included in the analysis. For the blink response, only responses with onset latencies between 20 and 100

ms were included in the analysis.<sup>65</sup> For the other muscles, onset latencies were between 20 and 200 ms. The combined area-under-the-curve was defined as the summated transformed EMG area-under-the-curve of multiple muscles.<sup>58</sup> A log transformation was performed to normalize the area-under-the-curve values of the different muscles. The sympathetic skin response<sup>219, 233</sup>, or the related skin conductance response<sup>234</sup>, is usually defined as the largest increase from baseline within 4<sup>234</sup> or 5 sec<sup>233</sup> following stimulation. Further, because it is highly variable in its amplitude, it is recommended to standardize the response intra-individually.<sup>235, 236</sup> In the present study the baseline was defined as the mean  $\mu\text{V}$  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<sup>218, 233</sup>). Subsequently, the peak  $\mu\text{V}$  during the interval 900-4000 ms following stimulation was identified. The sympathetic skin response was defined as the difference in  $\mu\text{V}$  between the baseline and the identified maximum, standardized to the intra-individual maximum. That is, the  $\mu\text{V}$  difference score of a trial was divided by the maximal difference score of that subject (of all eight trials) and then multiplied by 100, giving values ranging from 0 % to 100 %. The intra-individual maximum often was the response following the first stimulus, but not always. The sympathetic skin responses were visually inspected to exclude movement artefacts or artefacts caused by loose electrodes.

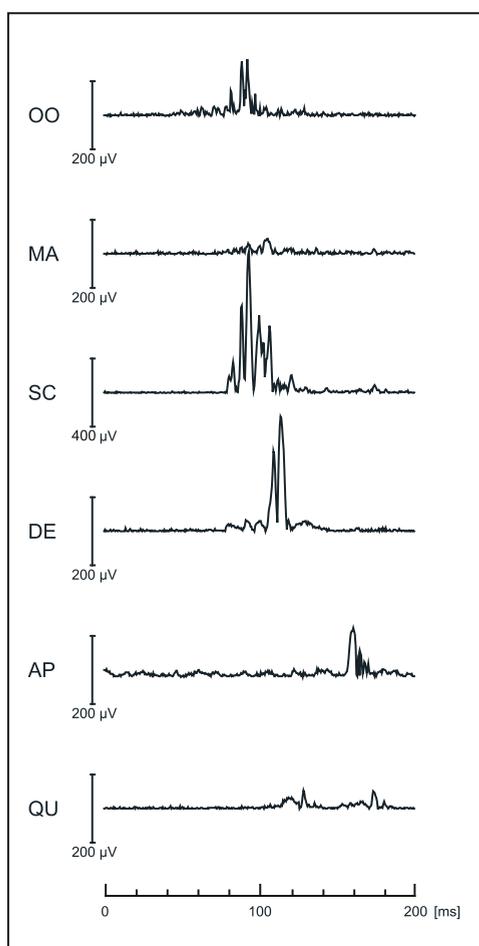
Age and sex effects were investigated by splitting the group in two. The age cut-off was 12, with the younger subjects ranging from 8 - 12 years old and the older subjects from 13 to 18 years old. The older group consisted of 13 subjects (6 boys, 7 girls) and the younger group consisted of 14 subjects (6 boys, 8 girls).

Statistical analysis was performed with SPSS (15.0). A linear mixed-model analysis (type III tests of fixed effects) (compound symmetry so similar to a Repeated Measures Analysis of Variance) was used to examine the effect of the repeatedly presented stimuli, sex and age on the blink and combined response probability, the blink area-under-the-curve and the sympathetic skin response. The log transformed blink area-under-the-curve and the sympathetic skin response showed a normal distribution (log transformed blink area-under-the-curve: Kolmogorov-Smirnov  $Z=0.835$ ,  $p=0.485$ , sympathetic skin response Kolmogorov-Smirnov  $Z=1.32$ ,  $p=0.060$ ). The response probability (combined: 16.6 %, 33 %, 50 %, 68.8 %, 80 % or 100 %) was converted to number of responding muscles (combined: 0, 1, 2, 3, 4, 5 or 6 muscles), which did not follow a normal but a Poisson distribution. When data follows a Poisson distribution the mean and standard deviation are of interest and not the median and range. Therefore, the mixed-models analysis could be performed. 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). The effect of the repeated stimuli on the non-parametric

combined response area-under-the-curve was tested with a General Linear Model with a Greenhouse-Geisser correction. A p-value  $\leq 0.05$  was considered significant.

## Results

Following the auditory stimulation, EMG responses were elicited in orbicularis oculi (78.2 %), sternocleidomastoid (16.7 %), masseter (10.2 %), deltoid (2.8 %), abductor pollicis brevis (3.4 %) and quadriceps (2.8 %) muscles. In Table 1 the response probabilities, area-under-the-curve's and latencies of the individual muscles responses are shown. A representative EMG of a subject following auditory stimulation is shown in Figure 1.



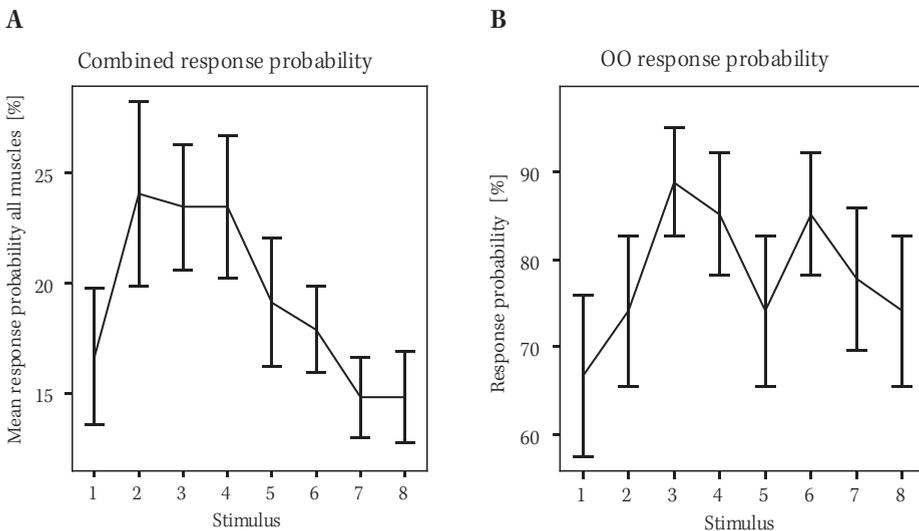
**Figure 1.** EMG (single trial) muscle responses of 13 year old boy following 104 dB tone OO = orbicularis oculi (latency 45 ms), MA = masseter (latency 69 ms), SC = sternocleidomastoid (latency 71 ms), DE = deltoid (latency 75 ms), AP = abductor pollicis brevis (latency 154 ms), QU = quadriceps (latency 102 ms). The latency of the AP muscle is disproportionately long.

**Table 1.** Characteristics of muscle responses in children following eight 104 dB tones. OO = orbicularis oculi, MA = masseter, SC = sternocleidomastoid, DE = deltoid, AP = abductor pollicis brevis, QU = quadriceps.

	<b>Response probability</b>	<b>Area Responses</b>	<b>Latency</b>
	(Mean and SD)	(Median and Range)	(Median and Range)
OO	78.2 % (40.0)	601 $\mu$ V.ms (60 - 4760)	41.3 ms (20 - 100)
SC	16.7 % (37.4)	1820 $\mu$ V.ms (101 - 23600)	66.2 ms (23 - 181)
MA	10.2 % (30.3)	630 $\mu$ V.ms (175 - 16600)	66.2 ms (20 - 126)
DE	2.8 % (16.5)	1240 $\mu$ V.ms (285 - 23200)	76.6 ms (38 - 150)
AP	3.4 % (18.1)	1380 $\mu$ V.ms (171 - 6190)	75.7 ms (66 - 123)
QU	2.8 % (16.5)	430 $\mu$ V.ms (136 - 1300)	154.0 ms (91 - 176)

### ASR response probability

The combined response probability (mean 19.3 %, SD 15.1) was obtained by averaging the response probabilities of all six muscles. There were no missing values. The combined response probability significantly decreased with the repetitive stimuli,  $F(7, 189)=3.54$ ,  $p=0.001$  and  $\chi^2(7)=24.3$ ,  $p=0.01$  (Figure 2A). However, the blink response probability did not significantly change with the repetitive stimuli,  $F(7,189)=1.91$ ,  $p=0.069$  and  $\chi^2(7)=13.1$ ,  $p=0.071$  (Figure 2B).

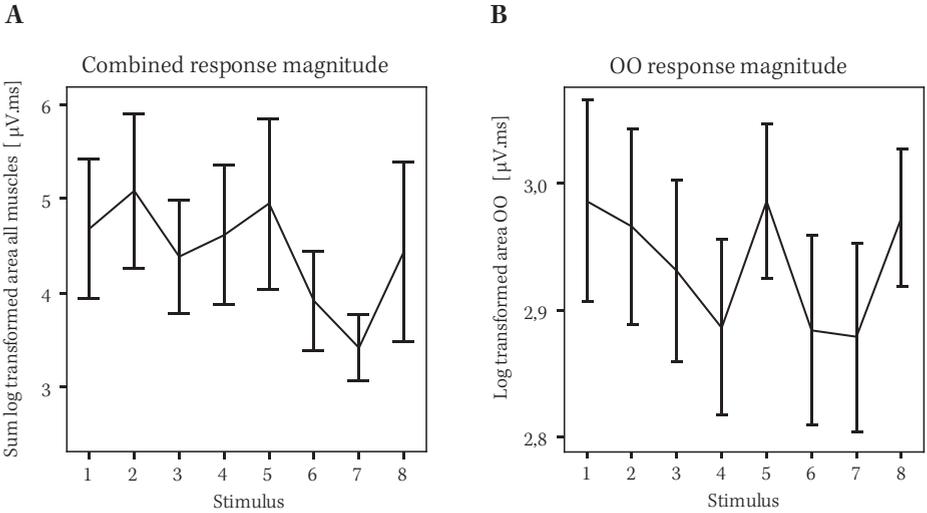


**Figure 2.** ASR occurrence in children following eight 104 dB tones A. The mean response probability (%) of all six muscles B. The response probability (%) of the orbicularis oculi (OO) muscle (blink response). The dots represent the mean, the bars standard error of means.

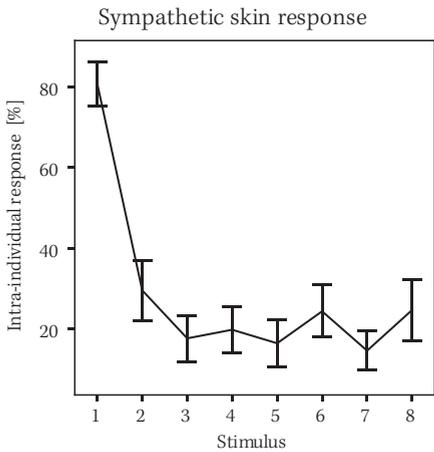
### ASR magnitude

The combined area-under-the-curve was obtained by summing the log transformed area-under-the-curves of the individual muscle responses (based on 177 of the 216 trials; 82%). The combined area-under-the-curve did not significantly decrease over the

repetitive stimuli,  $F(7, 153.7)=0.986, p=0.443$ ) (Figure 3A). The blink area-under-the-curve was based on 169 of the 216 trials (78 %). There was no significant effect of the repetitive stimuli on the blink area-under-the-curve,  $F(7, 144.8)=0.810 p=0.580$  (Figure 3B).



**Figure 3.** ASR magnitude in children following eight 104 dB tones A. The summated log transformed area-under-the-curve ( $\mu V.ms$ ) of all six muscles. B. The log transformed area-under-the-curve ( $\mu V.ms$ ) of the orbicularis oculi (OO) muscle (blink response). The dots represent the mean, the error bars the standard error of means.



**Figure 4.** Sympathetic skin response in children following eight 104 dB tones The difference in  $\mu V$  between the baseline and the identified maximum, standardized to the intra-individual maximum (%). The dots represent means, the bars standard error of means.

## Sympathetic skin response

The mean sympathetic skin response was 28.5 % (SD 36.4). Of the 216 trials, 20 trials were missing. Repetition of the auditory stimulus significantly reduced the sympathetic skin response,  $F(7,171.2)=12.5$ ,  $p=0.000$  (Figure 4).

## Sex and age effects

Age did not have a significant effect on the combined response probability ( $F(1,25)=0.258$ ,  $p=0.121$ ), the blink response probability ( $F(1,25)=0.348$ ,  $p=0.560$ ), the combined area-under-the-curve ( $F(1,24.9)=2.28$ ,  $p=0.143$ ), blink area-under-the-curve ( $1,23.6$ )=2.66,  $p=0.116$ ) or the sympathetic skin response ( $1,23.1$ )=0.004,  $p=0.948$ ).

Sex did not have a significant effect on the combined response probability ( $F(1,25)=0.202$ ,  $p=0.657$ ), the blink response probability ( $F(1,25)=0.878$ ,  $p=0.358$ ), the combined area-under-the-curve ( $F(1,25.11)=1.65$ ,  $p=0.209$ ), blink area-under-the-curve ( $1,23.6$ )=2.66,  $p=0.116$ ) or the sympathetic skin response ( $1,22.9$ )=1.29,  $p=0.723$ ).

## Discussion

### The auditory startle reflex in children

Response frequencies were 78.2 % in orbicularis oculi, 16.7 % in sternocleidomastoid, 10.2 % in masseter and lower in other muscles. This means a blink response in the orbicularis oculi muscle was seen most of the time, regardless of the presence of a more generalized ASR. The low response frequencies in other muscles than the orbicularis oculi may be caused by the moderate stimulus intensity, 104 dB SPL (A), and/or the young age of the subjects (a younger age is correlated with a smaller ASR in adults<sup>58</sup>). For comparison, in adults in a supine position masseter and sternocleidomastoid muscles responded to about 80 % of auditory stimuli presented with a similar interstimulus interval and an intensity randomly differing from 90 to 110 dB.<sup>58</sup> Although the different stimulus characteristics of the studies may have caused the ASR differences, children may exert more inhibitory influence on complex brainstem reflexes compared to adults.<sup>58</sup>

As described previously<sup>89</sup>, the ASR in children shows a similar recruitment pattern compared to adults.<sup>45, 47</sup> The response probability decreases with increasing distance of the segmental muscle innervation to the caudal brainstem, as is the case in adults.<sup>45, 47</sup> Despite the variation in latency of the muscle responses (like in adults all with a non-normal distribution), the overall pattern to the response to sound was distinctive. The latency to onset of the blink response (median 41.3) was much shorter than to the response in the sternocleidomastoid (median 66.2). A distinctive characteristic of the ASR pattern in adults is the relatively delayed hand-muscle response<sup>47</sup>, which also occurred in the

young subjects investigated in this study (illustrated in Figure 1). However, this was not evident in the abductor pollicis brevis latency median (Table 1). This protocol was not tested in adults limiting a proper comparison of the multiple muscle ASR in children and adults. However, the EMG onset latencies of the subjects seem similar compared to adults.<sup>45, 47, 89</sup> This is remarkable because especially the more distal muscles could be expected to be shorter in children. This finding may be explained by the infrequency of EMG activity in the distal muscles (which makes these results less reliable). Evidently facial and neck muscles are close enough to the startle generator (brainstem) resulting in similar onset latencies in children and adults.

### **Habituation pattern of the ASR in children**

As in adults, the blink response following auditory stimuli shows a different habituation pattern from the response as it occurs in the other muscles. The habituation of the ASR combined response was faster compared to the habituation of the ASR blink response. The combined response probability significantly decreased with repetitive stimuli, whereas the blink response probability did not. The magnitude (area-under-the-curve) of both the blink response and the combined response did not show a significant decrease with repetitive stimuli. However, as illustrated in Figure 3, the decrease of the combined response EMG magnitude was stronger compared to the decrease of the blink response EMG magnitude. The difference in habituation patterns of the blink response compared to the other muscles involved in the ASR was previously found in adults.<sup>47, 59, 67, 69, 71, 237</sup> The expected physiologically rapid habituation following repetitive stimuli<sup>47</sup> is present in the combined response but not in the blink response.

### **The sympathetic skin response of the ASR in children**

The autonomic sympathetic skin response showed significant habituation with repetitive stimulation, in accordance with earlier studies in both children and adults.<sup>48, 220</sup> The different pattern of habituation with repetitive stimuli between the skin conductance response and the blink response has been reported before.<sup>48</sup>

### **Sex and age effects**

In contrast to adults<sup>221</sup>, ASR magnitude differences between male and female subjects were not detected. Sex-specific variations of central processing in the brainstem centers involved in the generation of the ASRs<sup>221</sup> may develop after childhood. Age effects present in adults<sup>58</sup> were also not found. However, the relatively small range of ages included in the study could have influenced this outcome.

## Limitations

There is no gold standard of measuring the ASR. Different outcomes of different ASR quantifications are therefore difficult to interpret. Further, the amount of elicited muscle responses in the subjects was low. Although this may be characteristic of younger subjects, it does limit the reliability of the latency and area-under-the-curve results of the more distal muscles. Finally, the interstimulus interval and amount of presented stimuli we used were conform the neurophysiological ASR paradigm<sup>59, 90, 221</sup> but are lower than those advised by the Psychophysiological Committee Guidelines, limiting a full comparison of the current ASR paradigms to the psychophysiological ASR paradigms.

## Implications

The continuing occurrence of the blink response while other muscles involved in the ASR decrease to respond, confirmed in this study for children, has been suggested to imply that the blink response does not reflect the ASR.<sup>47, 67</sup> Like in adults, the results of this study therefore suggest that investigation of multiple muscles is appropriate to quantify the ASR in children. This ASR quantification shows the physiologically expected habituation following mild auditory stimuli. In addition, as abnormal amounts of distal muscle contractions may be seen in startle syndromes<sup>74</sup>, including distal muscle responses may be crucial. However, the advantage of the blink response is the high response frequency of the orbicularis oculi muscle. Ethical concerns and health regulations constrain the use of higher auditory stimulation in this age group, especially when the young subjects are affected with psychiatric disorders. Other types of stimulation like airpuffs directed to the larynx, described to be less aversive for children than auditory stimulation<sup>238</sup>, may be more successful to elicit sufficient responses in muscles other than the orbicularis oculi while being ethically acceptable.

In both blink response and multiple muscle clinical ASR studies the findings are used to elucidate the pathophysiology of the illness, including associations with ASR modulating structures like the reticular activating system or the amygdala. However, similar conclusions can only be made if the measurement is identical. In accordance, over the last few years the quantification of the ASR using both multiple muscles and the blink response have shown different kinds of abnormalities in adult patients with various neurological disorders.<sup>70, 74, 75, 125, 237, 239, 240</sup> Therefore, a gold standard to assess the ASR is necessary.