The Optimal Method and Parameter to Quantify Emotion-Modulated Startle EMG in Schizophrenic Patients

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Preface
This study was performed as final examination of the Masters course of Biological and Cognitive Psychology at Erasmus University Rotterdam, The Netherlands. It was conducted at the Department of Psychiatry at Erasmus Medical Centre, Rotterdam.

First, I would like to thank Roelie Hempel and Joke Tulen for introducing me into the field of psychophysiology. The subject was quite complex and relatively new to me, causing me to acquire a wide variety of practical and theoretical skills in the past seven months. I would not have achieved this without your daily coaching. Also, I would like to thank Jan van Strien. Although we did not have much contact during the course of my traineeship, I truthfully appreciate the critical reviewing of my paper.

Finally, and most importantly, I would like to express my gratitude to my closest family and my dear Lester, for their encouragement and always believing in me. From the beginning of my study until the last words of this thesis, you were there for me!

Johanna Glimmerveen,
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Abstract
In electromyographic (EMG) research, different parameters and signal conditioning methods are used to quantify the EMG signal. In the present study, several methods (integration, smoothing, envelope) and parameters (magnitude, relative amplitude, blink area, onset latency, amplitude latency, response duration) were investigated by means of startle eyeblink EMG. Because previous studies have shown schizophrenic patients to have a disturbed emotional modulation of the startle reflex, 38 schizophrenics and 53 controls were subjected to an emotion-modulated startle paradigm, in which sudden noisebursts were presented while pictures with emotional content (negative, neutral, positive) were viewed. The results revealed that optimal discrimination between response patterns of patients and controls was obtained with the combination of the envelope method and the relative amplitude parameter. The present results are of great value for future studies in this area, offering new directions for the use of EMG in psychophysiological research.
Table of contents

1. Introduction 1
   1.1. Startle eyeblink EMG 1
   1.2. Modulation of startle magnitude 2
   1.3. Startle reflex in schizophrenia 4
   1.4. EMG signal conditioning methods 5
   1.5. EMG parameters 6
   1.6. Main research question and hypotheses 7

2. Methods 9
   2.1. Subjects 9
   2.2. Stimuli 9
   2.3. Procedure 10
      2.3.1. General procedure 10
      2.3.2. EMG measurement 11
      2.3.3. Startle task 12
   2.4. Data reduction and analysis 13
      2.4.1. Signal conditioning 13
      2.4.2. Computation of EMG parameters 16
   2.5. Statistical analyses 17
3. Results

3.1. Intensity parameters

3.1.1. Magnitude

3.1.2. Relative amplitude

3.1.3. Blink area

3.2. Latency parameters

3.2.1. Onset latency

3.2.2. Amplitude latency

3.2.3. Response duration

4. Discussion

4.1. Comparison of methods and parameters

4.2. Differences between startle responses of patients and controls

4.3. Limitations and recommendations

4.4. Conclusions

References

Appendix
1. Introduction

1.1 Startle eyeblink EMG

The electromyogram (EMG) is an important and commonly used measure in psychophysiological research. With the EMG, muscle contractions can be measured by detecting changes in muscular electrical potential. Often, it is used to investigate clinical populations, but the EMG signal is also appropriate to study ‘normal’ psychophysiological responses. The most widespread application of the EMG is directed towards measurements of the eyeblink response. The eyeblink EMG makes it possible to measure action potentials that are generated in the orbicularis oculi muscle (Blumenthal, 2005). This striated muscle closes the eye during an eyeblink and surrounds the orbital fissure. The orbicularis oculi muscle is directed by the nucleus of the seventh cranial nerve, the facial motor nucleus, which makes it possible to monitor activity of the nervous system by means of measuring blink reflexes (Blumenthal, 1998). For example, if stimuli activate blink reflexes, these stimuli must generate neural activity that eventually activate or facilitate the facial motor nucleus or projections to this nucleus. One advantage of EMG registrations is that very weak contractions of the orbicularis oculi can be detected, without actually having to close the eye. The blink reflex is a fairly small biosignal, which rarely exceeds a few hundred microvolts. In addition, with the EMG it is possible to report distinct subcomponents of muscle activation, which is another great advantage of this technique (Blumenthal, 1998).

The blink reflex is part of a complex response called the startle reflex. The startle reflex is a response to sudden and intense stimuli with rapid risetimes and can be evoked by stimuli in the acoustic, visual and tactile modalities (Neumann, Lipp & McHugh, 2004; see Blumenthal et al., 2005, pp. 3-6, for a review). Many researchers investigating the startle reflex present acoustic startle-eliciting stimuli, which results in the strongest and most robust startle response as compared to stimuli in other modalities (e.g., Zeigler, Graham, & Hackley, 2001). In the experiment of Zeigler et al. (2001), the effect of cross-modal warning signals on the startle reflex was investigated. The results from the unwarned control trials revealed that when subjects passively perceived the startle stimuli, acoustic stimuli resulted in the highest blink amplitude and the shortest blink
Methods and parameters for quantifying startle eyeblink EMG

latencies, as compared with visual and cutaneous startle stimuli. In addition, subjects rated acoustic startle stimuli as more intense than stimuli in the other modalities.

Studies of the acoustic startle response in rats have suggested that the reflex is mediated by a primary brain stem circuit through the nucleus reticularis pontis caudalis (Davis, 1986). The physiological effects of the startle response can be characterized by increased heart rate, increased electrodermal activity (skin conductance), higher blood pressure and increased frequency and intensity of eyeblinks (Turpin, Schaefer & Boucsein, 1999). Turpin et al. (1999) suggested that, in attentional terms, startle causes an interruption of an activity one is engaged in, directing attention towards the startle-eliciting stimulus. From a motivational perspective, Turpin et al. (1999) considered startle to reflect withdrawal, preparing the subject to ‘fight or flight’. The startle reflex is generally used to study states of anxiety, affective disturbances, sensitization, and motivational states. For these investigative purposes, startle is very useful, but application of startle as a diagnostic tool is not recommended (Grillon & Baas, 2003), because pathological startle response patterns have not yet been identified.

1.2 Modulation of startle magnitude

The startle reflex is modulated by attentional and emotional mechanisms. Attentional modulation of the startle reflex is most obvious when a prepulse is presented, which is a relatively weak sensory event occurring before presentation of the startle-inducing stimulus. Presentation of the prepulse has an inhibitory influence on the magnitude of the startle response, a process called prepulse inhibition (PPI). PPI occurs when the interval between the prepulse and the startle stimulus has a duration of 30 – 500 ms, and when prepulse and startle stimulus are presented in either the same or different modalities (Braff, Geyer & Swerdlow, 2001). Commonly, PPI is viewed as a measure of ‘sensorimotor gating’, a process in which irrelevant sensory information is filtered or ‘gated’ out of awareness to maintain the focus of attention on important aspects of the environment. Incoming sensory events, such as the startle-inducing stimulus, are suppressed until perceptual analysis of the prepulse is completed. This explanation is also referred to as the attentional resource theory, which states that the magnitude of the
startle reflex is determined by the amount of attentional resources allocated to the modality of the startle-eliciting stimulus (Filion, Dawson & Schell, 1998).

The magnitude of the startle reflex is also modulated by emotional context. Affective modulation occurs when the interval between prepulse and startle-inducing stimulus has a duration of at least 800 ms. Depending on the emotional context, affective modulation can have inhibitory as well as facilitating effects. Vrana et al. (1988) discovered a linear relationship between emotional category (negative versus positive) and startle magnitude in a startle experiment in which emotional pictures were viewed. Compared to neutral pictures, positive pictures reduced the amount of startle, while negative pictures resulted in a larger startle response. This effect was replicated in several studies. For example, Bradley, Cuthbert and Lang (1993) obtained the same linear effect when startles were presented during emotional picture viewing, an effect also found by Dichter, Tomarken and Baucom (2002). In addition, emotional modulation of the startle reflex occurs in response to different kinds and modalities of lead stimuli affecting the emotional state of the subject. For example, variations in lead stimuli include aversive and pleasant odors (Ehrlichman et al., 1995), unpleasant and appetitive film clips (Jansen & Frijda, 1994) and naturally occurring sounds provoking emotional reactions (Bradley & Lang, 2000).

Affective modulation of the startle reflex is often explained using the motivational priming hypothesis (Lang, Bradley & Cuthbert, 1990). According to this hypothesis, reflexive behaviour is determined by two factors. The first factor is the classification of the reflex as approach or as withdrawal, while the second factor reflects the affective valence of the ongoing emotional state of the subject. Reflexes reflecting approach behaviour will be facilitated by positive emotional states, while avoidant reflexes are enhanced by negative emotional states of the individual. The magnitude of the startle reflex is therefore determined by the affective valence of the stimulus preceding the startle-eliciting stimulus, with negative lead stimuli causing facilitation of the startle reflex and positive stimuli inhibiting the startle response. Support has been found for the motivational priming hypothesis. For example, Hamm et al. (1997) found that individuals with specific phobias showed larger startle potentiation when viewing pictures of their
feared objects (i.e. experiencing a negative emotional state) than non-phobic subjects viewing the same or other aversive pictures.

1.3 Startle reflex in schizophrenia

Schizophrenia is a psychiatric illness that affects the cognitive and emotional system of the patient. Schizophrenia has a worldwide incidence of 2 – 4 per 10,000 people, with a lifetime prevalence of approximately 1% (Mueser & McGurk, 2004). Information processing difficulties are part of the affected cognitive system, which is often explained by a deficit in sensorimotor gating. The patient is less capable of selecting input from the environment, resulting in an overload of stimuli into awareness (Braff, Swerdlow & Geyer, 1999). Deficits in emotional information processing include difficulties with emotional learning and recognizing emotional facial expressions, with negative emotions being the most difficult for this patient group to process (Edwards et al., 2001; Exner et al., 2004). Schizophrenic patients exhibit a different startle response pattern than healthy individuals. In healthy subjects, a positive emotion inhibits the startle reflex, while it is facilitated by a negative ongoing emotion (Blumenthal, Elden & Flaten, 2004; Bradley et al., 1993; Dichter et al., 2002). Several studies have shown that in patients with schizophrenia, the disturbed processing of cognitive and emotional information leads to a prolonged and more intense startle response than in healthy subjects (Meincke et al., 2004; Weike et al., 2000) and, in addition, emotional modulation of the reflex occurring to later presented startle probes than in healthy individuals (Volz et al., 2003).

Three studies have investigated affective modulation of the startle reflex in schizophrenic patients. The first of these studies was performed by Schlenker, Cohen and Hopmann (1995). In this study, subjects were presented acoustic startle stimuli while viewing slides depicting negative, neutral and positive scenes. Startle probe onset, measured from slide onset, varied between 3700 to 5100 ms. During negative pictures, schizophrenic patients showed less startle facilitation than healthy subjects. In addition, within the patient group no significant difference was found between startle amplitude during negative slides and startle amplitude during neutral slides. However, both patients and controls showed a reduction in startle response when viewing positive pictures. This effect can be explained by the altered negative affect schizophrenic patients are suffering,
leaving positive emotional modulation intact. Volz et al. (2003) replicated this effect, but only when using startle stimuli with an early onset. When using late-onset startle probes, which varied between 800 and 3800 ms, no difference in startle potentiation was found between patients and controls. This suggests that schizophrenic patients need more cognitive evaluation of negative sensory input to obtain a ‘normal’ startle response pattern as compared with healthy subjects. This pattern in responses to late-onset stimuli has also been found in the third study that investigated affective modulation of the startle response in schizophrenic patients, which was performed by Curtis et al. (1999). These researchers also used emotional pictures and acoustic startles to investigate differences between patients and controls, resulting in indistinguishable startle responses of patients and controls. Using startle probe onset latencies between 2000 and 5000 ms after picture onset, both groups showed the ‘normal’ pattern, in which the startle amplitude is potentiated by negative pictures and attenuated by positive pictures. However, Schlencker et al. (1995) and Volz et al. (2003) did find differences between patients and healthy subjects when using late-onset and early-onset probes, respectively. These deviating response patterns to different emotional picture types suggest that the EMG signal resulting from the eyeblink reflex seems an important source of information about the emotional system associated with the schizophrenic syndrome.

1.4 EMG signal conditioning methods
The raw EMG signal cannot be analysed and interpreted immediately after it is obtained from the subject. Instead, it has to be conditioned to make it possible to detect peaks and to compute the variables of interest. At present, two signal conditioning methods are generally accepted by psychophysiological researchers in the field. The most classical method is integration, but currently the majority of investigators apply the smoothing method (Blumenthal et al., 2005). The third way of signal conditioning, the envelope method, is relatively new and designed by a researcher of Erasmus MC, Rotterdam.

With integration, the area under the curve of the waveform is computed for each time interval. The second method, smoothing, softens the shape of the waveform, removing spikes that result from extreme values. The envelope method shapes the signal into a mirror image, called the envelope. The envelope is the result of the Hilbert
Transform algorithm, which does not affect the original values of the signal as much as the first two methods do.

1.5 EMG parameters

Three measures of response intensity that are used by the majority of psychophysiological investigators are peak amplitude, peak magnitude and blink area. Peak amplitude is the maximum value in a scoring window, so it reflects the point with the largest accumulation of energy within a response. Blink area is the region below the curve of the response waveform, summing all values within that scoring window. Because blink area reflects the total amount of muscle activation during the response, it provides a more accurate estimation of the magnitude of the muscle contraction than a measure of peak amplitude (Blumenthal, 1998). Response magnitude is computed as the product of the mean amplitude within a condition and the response probability of that condition. This probability is the total amount of detected responses divided by the number of presented startle-eliciting stimuli, causing the mean magnitude to approach the mean amplitude when response probability increases.

Three commonly used measures concerning the temporal component of the response are onset latency, peak latency and response duration. Onset latency is the time needed to initiate the response, while peak latency refers to the time needed to reach response peak, both measured from stimulus onset. Peak latency, as well as amplitude, provides information about activation of the largest motor unit that is close to the electrodes, but it is also possible that it reflects the simultaneous activation of several smaller motor units. Blumenthal (1998) proposes therefore to investigate all possible involved motor units, by computing response duration and blink area. Response duration is the time from response onset until the recovery to baseline value, and reflects the total duration of muscle activation during the blink response.

At present, there are no clear and uniform guidelines for choosing a particular variable or method, although in the past few years some researchers have presented general but extensive recommendations for the use of the EMG signal in psychophysiological research (Blumenthal, 1998; Blumenthal et al., 2004; Blumenthal et al., 2005). It seems that in most cases a particular variable or method that gives the best
results of the study is chosen, while choosing a measure that fits the purpose and design of the experiment is a necessary contribution to the validity of the study. Blumenthal (1998) already found high correlations between peak magnitude and blink area, within and across the signal conditioning methods of smoothing and integration. However, these investigations were primarily focused on startle experiments without emotional modulation, and without comparing particular clinical populations. The present study therefore tries to integrate, on the one hand, research focusing on the more general application of EMG methods applied to measure the startle eyeblink reflex, and, on the other hand, the specific use of EMG to study schizophrenic patients and the emotional modulation of the startle reflex.

1.6 Main research question and hypotheses

The purpose of the present study is to decrease the ambiguity in the analysis of the EMG signal, in order to discriminate schizophrenic patients from controls in an emotion-modulated startle paradigm. Stated specifically, the aim of the study is twofold: first, to find the optimal method and the optimal variable, second, to investigate which combination of method and variable is optimal in discriminating between patients and controls. The present study can contribute to the foundation of a guideline directing the choice of the appropriate fashion to analyse the EMG signal when the investigative aim is to differentiate those groups. Researchers using the EMG signal to discriminate between schizophrenic patients and controls will profit from the results of this study, because this knowledge is vital to get to a correct interpretation of results. The group of schizophrenic patients will consequently benefit from the investigations these researchers perform, because more knowledge about the schizophrenic syndrome will improve treatment and care during a psychotic episode and eventually will improve the quality of life of these patients.

The main research question results from this need for unequivocality in the analysis of the EMG signal. It is focused on which signal conditioning method and which EMG parameter is optimal for discriminating the eyeblink reflexes of schizophrenic patients and controls during an acoustic startle task in which emotional pictures modulate the acoustic startle eyeblink.
It is hypothesized that the EMG outcome measures can be divided into two categories: one reflecting response intensity, and one reflecting response latency. Reflecting the total amount of muscle activation, blink area is expected to be the optimal measure of response intensity in its ability to discriminate between the startle reflexes of schizophrenic patients and controls. Response duration, reflecting the total duration of muscle activation, is hypothesized to be the measure of response latency that is optimal for differentiating the startle reflexes of schizophrenic patients from those of controls.

It is expected that there will be a difference between the three signal conditioning methods in their ability to distinguish the eyeblink reflexes of schizophrenic patients and controls in an emotion-modulated startle task. The signal that is conditioned with the envelope method will be superior in showing possible differentiating effects, because the envelope method has the least distorting effect on the original signal as compared with the other two methods.

Considering the startle responses of the subjects to the pictures of the different emotional categories, it is difficult to formulate an hypothesis, due to the small amount of available literature on the subject. At present, only three studies have investigated emotional modulation of the startle reflex in schizophrenic patients, all using acoustic startle probes and pictures as emotional stimuli (Schlenker et al., 1995; Curtis et al., 1999; Volz et al., 2003). Because the aim of this study is to investigate emotional modulation, the startle probe onset latencies that are found to be most robust for this purpose (1300 and 3800 ms) will be used, and not the early probe onset latencies that measure prepulse inhibition, which is mainly an attentional process. In two of the three previous performed studies, no differences between patients and controls were found in the pattern of startle responses on the emotional categories when these onset latencies were used for analysis (Curtis et al., 1999; Volz et al., 2003). These results lead to the expectation that patients will show the same startle response pattern as controls. However, based on the attentional resource theory, it is hypothesized that patients will have lower startle amplitudes than controls in all emotional categories, because of their lack of attention to process both startles and pictures at the same time.
2. Methods

2.1 Subjects

In the present study, 38 patients participated (32 males; mean age = 22.71; SD=5.10), who were admitted to the psychiatry ward of Erasmus MC, Rotterdam. All patients were diagnosed with a first episode psychosis indicating schizophrenia or the schizo-affective syndrome according to DSM-IV (American Psychiatric Association, 1994) criteria, as assessed by a senior psychiatrist. In addition, the severity of the disorder was scored using the Positive and Negative Syndrome Scale (PANSS; Kay, Fiszbein & Opler, 1987), which resulted in a mean score of 72.45 (SD = 14.64). Controls were 53 psychology students and medical students (40 males, mean age = 22.15, SD = 3.44) from Erasmus University, Rotterdam. Controls were defined as persons not meeting the criteria for an axis I or axis II disorder, nor for substance abuse, and were free of medication at the time of testing. Patients participated voluntarily, while controls received course credit or a monetary reward for their participation. All participants provided written informed consent before participation. The study was approved by the Medical Ethical Committee of Erasmus MC Rotterdam.

2.2 Stimuli

The pictures with emotional content were selected from the International Affective Picture System (IAPS; Center for the Study of Emotion and Attention, 1999)\(^1\), with a priori assigned values for valence and arousal for each picture, resulting in three emotional categories: negative, neutral, and positive. Positive pictures depicted thrilling sports or erotic scenes, neutral pictures showed furniture or nature scenes, and negative pictures displayed scenes of threat and mutilation. Trials presenting same-sex pictures were excluded from further analysis to control for gender differences in responses to sexually arousing pictures\(^2\). The visual stimuli were presented using a Dell Dimension

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\(^1\) Negative IAPS pictures: 3000, 3010, 3060, 3069, 3080, 3102, 3120, 3170, 6200, 6212, 6230, 6260, 6300, 6313, 6350, 6550; Neutral IAPS pictures: 5120, 5260, 5510, 5530, 5535, 5711, 5731, 5740, 5900, 7000, 7002, 7004, 7006, 7009, 7010, 7020, 7025; Positive IAPS pictures: 4220, 4290, 4490, 4520, 4608, 4660, 4670, 4680, 5260, 5470, 5621, 5910, 8030, 8170, 8490, 8501.

\(^2\) Removed pictures for men: 4490, 4520; Removed pictures for women: 4220, 4290.
M200a Personal Computer with a Pentium processor and a 17-inch Samsung SyncMaster monitor.

The startle probe was a discrete white noise burst of 100 dB, lasting 50 ms, with an instantaneous rise and fall time, presented binaurally through headphones. The acoustic stimuli were presented through Sennheiser HD 265 linear headphones. The experiments were programmed using E-Prime (Psychology Software Tools, Inc., 2002).

2.3 Procedure

2.3.1 General procedure
The present study was embedded in a larger study; a short description of the total experimental procedure is provided here. The experiment was always performed between 09.00 and 11.00 in the morning. Subjects were seated in a comfortable chair in a dimly lit room, which was sound-attenuated. When subjects arrived at the laboratory, they first completed a paper and pencil questionnaire about their current mood, by means of a Dutch translation of the Profile of Mood States (POMS I-1; Wald & Mellenbergh, 1990). After this, the subject was asked to provide saliva in a capped plastic vessel for cortisol measurements. After taking the salivary sample, blood pressure was measured manually. Consecutively, all electrodes were attached to the participant: three ECG electrodes on the chest, one respiratory chest strain gauge around the waist which was connected to two electrodes, two EMG electrodes beneath the left eye and one on the forehead serving as a grounding electrode, two electrodes attached to the second and the fourth finger of the nondominant hand for measuring Skin Conductance Level, and one cuff around the middle finger of the nondominant hand for measuring blood pressure continuously.

When all electrodes were attached, the participants performed three computerized tasks. The startle task, which provided data for the present study, was performed first. Subjects viewed pictures with emotional content on a computer screen, while acoustic startles were presented irregularly through headphones. Next, subjects performed a probe detection task. Finally, to control for deviations from the a priori assigned values of the pictures, subjects completed the Self Assessment Manikin (SAM; Bradley and Lang, 1994). This is a digital questionnaire assessing participants’ subjective judgments about
the pictures that were presented during the startle task, measuring the dimensions of valence and arousal.

2.3.2 EMG measurement

The EMG was measured using two cup electrodes (Ag/AgCl) that were filled with electrolyte paste (Spectra® 360; Parker Laboratories, Inc.). The electrodes were located on the skin below the left eye of the participant, over the orbicularis oculi muscle (see Figure 1). One electrode was placed 2 cm under the pupil when the subject was looking straightforward, the second electrode was placed 1 cm lateral from the first. A third electrode was placed on the forehead, serving as an isolated ground electrode. The EMG was sampled at 1024 Hz. The signal was stored in A/D units and filtered using a lowpass filter with a cutoff frequency of 500 Hz and a time constant of 30 ms. All data was sampled and stored on a flashcard by means of a portable digital recorder (Vitaport™ System, TEMEC Instruments B.V., Kerkrade, The Netherlands). After completion of the recording, all physiological data was imported to and processed on a Dell Optiplex GX270 Personal Computer with a Pentium 4 processor, by means of a Vitascore™ software module (TEMEC Instruments B.V., Kerkrade, The Netherlands).

![EMG electrodes](image)

**Figure 1.** EMG electrodes are placed over the lower portion of the orbicularis oculi muscle (mirror image). The isolated ground electrode is located on the forehead. *Picture is partly adopted from Blumenthal et al. (2005).*
2.3.3 Startle task

Subjects were seated at a distance of approximately 70 cm from the computerscreen. All participants were told to sit as relaxed as possible, and that they were about to see a series of pictures evoking positive, neutral, or negative feelings. They were instructed to watch all pictures quietly. They were also told that occasional noises would be heard through the headphones, but that these noises should be ignored.

First, five startles were presented without picture presentation, making participants accustom to the noise bursts and to decrease the impact of habituation effects during the experiment. Consecutively, pictures from three emotional categories (negative, neutral, positive) were presented randomly for a duration of 6 s each. Each picture category contained 16 pictures. During 12 pictures of each of these valence groups, startles were presented at Interstimulus Intervals (ISI’s) of 300, 800, 1300, and 3800 ms after stimulus onset, in such a way that each ISI was presented three times within the valence category. However, because previous investigations have demonstrated that affective modulation of the startle response is most robust when startle stimuli are presented at 1300 and 3800 ms after picture onset (e.g., Bradley et al., 1993), only the 1300 and 3800 ms startles were used for analysis in the present study. The 4 remaining pictures in each category were presented without startles, serving as baselines for investigative purposes other than in this study. In addition, 12 startles were presented during the Intertrial Intervals (ITI’s), which varied between 10 to 25 s. Responses to these startle probes were also not analysed in the present study. An overview of all manipulations is presented in Table 1.

<table>
<thead>
<tr>
<th>Pictures</th>
<th>Startle</th>
<th>ISI’s</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 positive</td>
<td>+12</td>
<td>3 x (300, 800, 1300, 3800 ms)</td>
</tr>
<tr>
<td>12 neutral</td>
<td>+12</td>
<td>3 x (300, 800, 1300, 3800 ms)</td>
</tr>
<tr>
<td>12 negative</td>
<td>+12</td>
<td>3 x (300, 800, 1300, 3800 ms)</td>
</tr>
<tr>
<td>4 positive</td>
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<td>4 neutral</td>
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<td>4 negative</td>
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<tr>
<td>- 12 x</td>
<td></td>
<td>Randomly during ITI’s</td>
</tr>
<tr>
<td><strong>48 pictures</strong></td>
<td><strong>48 startles</strong></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Experimental manipulations
2.4 Data reduction and analysis

2.4.1 Signal conditioning

The EMG signal was sampled with a sample frequency of 1024Hz and was stored in A/D units. The first filter applied to the EMG signal was a zero-phase bandpass finite impulse response (FIR-) filter with \( \cos^2 \)-slopes. The -6dB cutoff frequencies of the passband were defined at 28Hz and 500Hz, with a steepness at both slopes better than -20dB per octave. This frequency range was chosen in order to attenuate the low and high frequency artifacts. For example, below 28Hz there is the risk of contamination by movement artifacts, while above 500Hz there are influences of instrumentation noise and electrode-skin noise. A schematic presentation of actions performed on the signal is provided in Figure 2. The effects of these actions on the graphical nature of the signal are depicted in Appendix A.

Next, to perform integration or smoothing, the bandpass filtered signal was rectified, converting all data points into absolute values (Blumenthal et al., 2005; De Luca, 2003; Fridlund & Cacioppo, 1986). The third step when applying integration was performed using a boxcar filter. With a boxcar filter, the area under the curve of the signal during a certain time interval is computed, which is called the integral. The boxcar computes the integral one timepoint ahead, overlapping the previously computed time interval minus the first timepoint of the previous interval. The width of the time interval can be adjusted by the researcher. The time interval is of great influence on the precision of the computation of the integral, with a small time interval being much more accurate than a wider time interval. This is because with a small time interval, values at more time points are taken into account, while a wider time interval involves the risk of reducing extremes to mean values. In this study, a boxcar with a time interval of 24 ms was applied to compute the integral.

To complete the smoothing method after the signal was rectified, a lowpass filter was applied with a -6dB cutoff frequency defined at 40Hz. This technique is also called a “contour-following integrator”, because extremes are removed from the signal. Similar to the bandpass filter, the lowpass filter was a zero-phase FIR-filter with a \( \cos^2 \)-slope, which had a steepness of better than -20dB per octave.
To apply the envelope method, the EMG signal was not rectified after application of the 28-500 Hz bandpass filter. To explain the envelope method, it is necessary to describe some properties of the EMG signal. This signal is a biological signal with a rapid component and a slow component. A signal with a rapid component and a slow component can be uniquely written as a complex signal $A(t)e^{j\phi(t)}$, in which $A(t)$ is the slow component and $e^{j\phi(t)}$ is the rapid component (see figure 3). A complex signal is
composed of a real signal and an imaginary signal, in which the real part is the original signal. To use the envelope method, the imaginary part was computed from the original signal by means of the digital Hilbert Transform. Computing the absolute value of each number of the complex signal resulted in the signal $A(t)$, which is the slow component. This is called the envelope of the original signal. Subsequently, a lowpass filter was applied (40 Hz.).

![Waveform](image_url)

**Figure 3.** Waveform with a rapid component (depicted in green) and a slow component (depicted in red). The envelope is computed from the slow component.

The EMG-recording of each participant was examined visually for possible baseline contaminations (e.g., noise, movement artifacts) and spontaneous or voluntary blinks before the minimal onset latency value. This procedure was performed separately for the three signal conditioning methods. In case of such distortions, the blink response could not be accurately quantified on that trial, resulting in trial-rejection. In consecutive analyses, rejected trials were excluded from calculations of the mean of the particular condition in which they occurred. Peaks had to exceed an amount of two standard deviations above baseline value to be further analysed as a blink response. In case no peak within the 20-100 ms scoring window satisfied this requirement, the response was scored as missing (or ‘nonresponse’), while the amplitude with the highest voltage was determined as peak of the response when multiple peaks within the 20-100 ms scoring window existed.
2.4.2 Computation of EMG parameters

After conditioning the signal, the different variables concerning the intensity component (blink magnitude; relative blink amplitude; blink area) and the temporal component (onset latency; amplitude latency; response duration) of the startle response were computed. Because there is one value for each method, this resulted in three values for each variable. In Figure 4, the location of all investigated parameters within the waveform is graphically presented. Response amplitude was computed as the amount of voltage of the highest peak in the 20-100 ms time window after startle stimulus onset. To compute response magnitude, first the probability of a response in each condition (negative vs neutral vs positive) was computed, by means of dividing the total amount of detected responses by the number of presented startle probes. Response magnitude was then computed as the product of mean response amplitude within each condition and the probability of a response in that condition. Blink area was computed as the sum of all values within the 20-100 ms time window.

Response latency was computed as the time from startle stimulus onset to response onset, while peak latency was computed as the time to the highest value within the 20-100 ms scoring window, also measured from startle probe onset. Finally, response duration was computed as the time from response onset to recovery to baseline value. Baseline value was defined as the mean value in the 50 ms time window before startle probe onset. Trials were rejected when the blink response occurred outside the 20-100 ms time window. Subjects were excluded from further analysis in case the number of rejected trials exceeded 33% of all trials.

![Figure 4. Startle parameters.](image-url)
2.5 Statistical analyses

Because the investigated parameters were measured in different units, separate factorial Analyses of Variance (ANOVAs) were conducted for each parameter to investigate the optimal method and parameter for discriminating the startle responses of patients from those of controls. In each ANOVA, the dependent variable was one of the six investigated parameters. Group, containing two levels (patients vs. controls) was assigned as independent between-group variable. Method, composed of three levels (envelope vs. smoothing vs. integration), and Picture type, containing three levels (negative vs. neutral vs. positive) served as independent within-group variables.

Three main effects were of interest. First, a main effect of Group, to examine the parameters’ ability to discriminate between the startle responses of patients and controls. In addition, a main effect of Picture Type, to investigate the ability of the parameter to differentiate between the responses to startles presented during different emotional picture types. Furthermore, a main effect of Method, to investigate differences between the values of the parameter of interest, when the signal is conditioned with the three different methods.

Also, different interaction effects were examined. First, an interaction effect of Method and Picture Type, to investigate which combination of method and parameter leads to the best differentiation between responses to startles presented during different emotional picture types. Moreover, an interaction effect of Method and Group, to determine the best combination of parameter and signal conditioning method to discriminate between the startle responses of patients and controls. In addition, an interaction effect of Group and Picture type, to examine which emotional picture type leads to the greatest differences between responses of patients and controls, for each particular parameter. Finally, a three-way interaction between Method, Picture type and Group, to determine if there are differences between the three methods in their ability to discriminate between patients and controls, based on the differentiation between responses to different emotional picture types, for each parameter.

In case the assumption of sphericity was violated according to Mauchly’s test, multivariate test statistics were used to determine significant effects. To indicate the locus of differences in case of significant effects, contrasting statistics (simple) were consulted.
In case of a significant interaction effect between Method and Picture type, a simple effects analysis was conducted on Picture type. In this procedure, each method is analysed separately for the parameter of interest, to determine which method is optimal in discriminating responses to the different emotional pictures within the use of that particular parameter.

3. Results
The maximum percentage of missing values was exceeded in seven patients and three controls, causing these subjects to be excluded from further analysis. For each significant effect, degrees of freedom are provided. Means and standard deviations of responses for each combination of parameter, method, and picture type are depicted for the intensity and latency parameters in Figure 5 and Figure 6, respectively.

3.1 Intensity parameters
3.1.1 Magnitude
The ANOVA’s revealed a significant main effect of Method on blink magnitude, meaning that the values of blink magnitude differed significantly between the three methods ($\lambda = .47, F(2,69) = 39.61, p < .001$). Contrasting statistics demonstrated that differences were significant between all possible combinations of the three methods, with the largest mean blink magnitude being created using the envelope method, and the smallest magnitude resulting from the integration method. A summary of statistical values of all significant contrasts is provided in Table 2 and Table 3. In addition, a significant main effect of Picture type on blink magnitude was found ($F(2,140) = 4.76, p = .01$), meaning that the blink magnitudes of responses to the three emotional picture types differed significantly from each other. Contrast analyses showed that the magnitude of responses to negative slides was smaller than those to neutral and positive slides. Also, a trend towards significance was found for the main effect of Group on blink magnitude ($F(1,70) = 3.69, p = .059$), suggesting that it is possible to discriminate the responses of patients from those of controls when using blink magnitude as response measure, with patients having smaller response magnitudes than controls.
Methods and parameters for quantifying startle eyeblink EMG

Figure 5. Means and standard deviations of intensity parameters during startle blinks when viewing negative, neutral, and positive pictures, for both groups.

3.1.2 Relative amplitude
The factor Method showed a significant main effect on relative blink amplitude ($\lambda = .38$, $F(2,69) = 56.11$, $p < .001$). All methods differed significantly from each other, as indicated by contrasting statistics, with the largest amplitudes resulting from the envelope method and the smallest amplitudes resulting from the integration method. Furthermore, the main effect of Picture type on relative amplitude was significant ($F(2,140) = 7.24$, $p = $
Positive slides resulted in lower amplitudes as compared with the other picture categories. In addition, there was a significant interaction effect between Method and Picture type in relative blink amplitude ($\lambda = .87$, $F(4,67) = 2.58$, $p < .05$). This interaction indicates that, when relative amplitude is used as response measure, the extent to which it is possible to discriminate a certain pair of picture types, depends on the method that was used to condition the signal. Contrast analyses showed that there were significant differences between the integration and envelope method in the way that the relative amplitude of responses to negative and positive pictures were differentiated. The envelope method resulted in the best discrimination, with larger differences between means of responses to these picture types. The integration and envelope method also differed in the way they differentiated the relative amplitude of responses to neutral and positive pictures, again showing the envelope method to be the best discriminator.

Finally, the integration method differed from the smoothing method in discriminating the relative amplitude of startle eyeblink responses to negative and positive slides. The smoothed signal showed greater differences between the mean amplitudes of responses to negative and positive slides than the integrated signal.

A simple effects ANOVA on relative amplitude demonstrated a significant interaction between Group and Picture type ($F(2,158) = 3.40$, $p < .05$). This interaction indicates a difference between the two groups in the way the relative amplitudes of their responses to neutral pictures differed from those to the other two picture types, when the signal was conditioned with the envelope method. Patients showed the largest startle response when viewing negative pictures, while positive pictures provoked the smallest response, as compared with the other two picture types. Controls also showed the smallest response to positive slides, but their largest startle response was evoked by neutral pictures. A trend to this effect was present within the relative amplitude of the smoothed signal ($F(2,158) = 2.60$, $p = .08$). Considering between-subjects effects, there was a significant main effect of Group on relative amplitude ($F(1,70) = 4.21$, $p < .05$), in which patients had smaller amplitudes than controls.
3.1.3 Blink area

The main effect of Method on blink area was significant ($\lambda = .49$, $F(2,69) = 35.49$, $p < .001$). Significant contrasts existed between all methods, with the envelope method creating the largest blink areas and the integration method resulting in the smallest blink areas. The main effect of Picture type on blink area was also significant ($F(2,140) = 9.14$, $p < .001$), with positive slides resulting in the smallest blink area. In addition, Group had a significant main effect on blink area ($F(1,70) = 4.25$, $p < .05$), in which patients had smaller blink areas than controls.

### Table 2. Statistical values of significant contrasts in effects of method, picture type and method by picture type interaction, for all intensity parameters.

- **Magnitude**
  - F(1,70) = 70.50, $p < .001$
  - F(1,70) = 78.29, $p < .001$
  - F(1,70) = 74.92, $p < .001$
  - F(1,70) = 8.45, $p < .01$
  - F(1,70) = 4.00, $p < .05$

- **Relative amplitude**
  - F(1,70) = 71.14, $p < .001$
  - F(1,70) = 87.16, $p < .001$
  - F(1,70) = 112.81, $p < .001$
  - F(1,70) = 12.20, $p < .01$
  - F(1,70) = 8.58, $p < .01$
  - F(1,70) = 10.70, $p < .05$
  - F(1,70) = 4.00, $p < .01$
  - F(1,70) = 9.11, $p < .01$

- **Blink area**
  - F(1,70) = 71.93, $p < .001$
  - F(1,70) = 64.20, $p < .001$
  - F(1,70) = 7.09, $p < .001$
  - F(1,70) = 13.55, $p < .01$
  - F(1,70) = 10.89, $p < .01$
  - F(1,70) = 10.89, $p < .05$
  - F(1,70) = 4.00, $p < .05$
  - F(1,70) = 9.11, $p < .01$

*Note. Env = Envelope, Smo = Smoothing, Int = Integration, Neg = Negative, Neu = Neutral, Pos = Positive.*
3.2 Latency parameters

3.2.1 Onset latency

There was a significant main effect of Method on onset latency ($\lambda = .14$, $F(2,69) = 214.56, p < .001$). The integration method differed significantly from the smoothing and envelope method, as expressed in faster onset latencies of responses. Furthermore, Picture type had a significant main effect on onset latency ($F(2,140) = 5.61, p < .01$). Responses to positive slides had significantly slower onset latencies than responses to negative and neutral slides.

Figure 6. Means and standard deviations of latency parameters during startle blinks when viewing negative, neutral, and positive pictures, for both groups.
3.2.2 Amplitude latency

For the amplitude latency parameter no significant effects were found.

3.2.3 Response duration

Analysis of response duration revealed a significant main effect of Method ($\lambda = .35$, $F(2,69) = 62.99, p < .001$), in which the integration method led to longer response durations than the smoothing and envelope method. In addition, a main effect of Picture type on response duration was present ($F(2,140) = 8.84, p < .001$), with shorter durations of responses to positive slides, as compared with responses to negative and neutral slides.

<table>
<thead>
<tr>
<th>EFFECT</th>
<th>Parameter</th>
<th>Method</th>
<th>Picture type</th>
<th>Method * Picture type</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Onset latency</td>
<td>Env vs. Smo</td>
<td>Neg vs. Pos</td>
<td>Env vs. Int * Neg vs. Pos</td>
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<td><img src="image" alt="Table 3" /></td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td><img src="image" alt="Table 3" /></td>
<td>Response duration</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Note.* Env = Envelope, Smo = Smoothing, Int = Integration, Neg = Negative, Neu = Neutral, Pos = Positive.
4. Discussion

4.1 Comparison of methods and parameters

Despite extremely high standard deviations in the values of the intensity parameters that were the result of interpersonal variability, it was still possible to find significant effects concerning these parameters. For all parameters, except amplitude latency, there was a difference between the values that result from the three different signal conditioning methods. In these parameters, the integration method seemed to reflect weaker responses, faster onsets and longer response durations than the other two methods. Furthermore, for the intensity parameters, it was found that the smoothed signal showed weaker responses than the signal conditioned with the envelope method. It was expected that blink area and response duration would be optimal in discriminating abilities, because they reflect the total amount and total duration of muscle activation, respectively (Blumenthal, 1998). The present results did not confirm this hypothesis.

Responses to negative and neutral pictures could not be differentiated by any of the investigated parameters. On the contrary, all parameters except amplitude latency found differences between responses to positive pictures and responses to the other two picture types. Positive slides evoked weaker responses, slower response onsets and shorter response durations than neutral and negative slides. These results are not in complete agreement with the data of Curtis et al. (1999), Schlencker et al. (1995), and Volz et al. (2003). Those investigators found emotional modulation of all three picture types when using late startle probe onsets. In the present study, only startle inhibition caused by positive slides was found.

There was an interaction between signal conditioning method and picture type in relative amplitude. The extent to which negative and positive pictures could be differentiated with the relative amplitude of the integrated signal, was much lower than it was with the relative amplitude of the signal conditioned with the smoothing or envelope method. In addition, the relative amplitude of the signal conditioned with the envelope method was more sensitive for differences between responses to neutral and positive pictures than the amplitude of the integrated signal was. Stated differently, the envelope method showed the largest differentiating effects between responses to the different picture types, as was expected. When using the envelope method, the original signal is not
as much affected as with the other two methods, which is a possible explanation for its superiority in discriminating abilities. The Hilbert Transform does not reduce fluctuations in voltages within a response as much as happens with rectification, which tends to reduce extremes and flatten the signal in the direction of baseline value. More peaks can be discriminated with the envelope method, resulting in much clearer response patterns.

Finally, when the signal was conditioned with the envelope method, relative amplitude was the only parameter capable of differentiating patients from controls, based on the responses to the different picture types. Controls showed the highest response amplitudes to neutral pictures, while negative pictures evoked the highest amplitudes in the patient group.

To summarize, the envelope method results for almost all parameters in the clearest and most robust response, as compared with integration and smoothing. The responses of the two groups can be discriminated only when using intensity parameters, and not when using the investigated latency parameters. Of these intensity parameters, amplitude appears to be optimal. Because interactions between method and picture type and between group and picture type only existed within the relative amplitude of the signal, this parameter is considered optimal in its sensitivity for differences between the groups. In combination with the optimal method, the envelope, this parameter is optimal in discriminating responses to different emotional pictures, as compared with all other investigated combinations of parameter and method. In addition, and most importantly, discriminating the two groups based on the way they respond to the different emotional pictures is only possible when the signal is conditioned with the envelope method in combination with the use of relative amplitude as response measure.

Because this is the first study comparing these parameters in schizophrenic patients, and no other studies were conducted comparing the envelope method with other signal conditioning methods, it is not possible to review other investigations as a comparison for the present results. Only Blumenthal (1998), Blumenthal et al. (2004), and Blumenthal et al. (2005) approached this subject of research, but the amount of identical investigative methodologies is too limited to consider these studies as comparable with the present study. The current investigations concerning EMG
4.2 Differences between startle responses of patients and controls

Patients differed from controls in the values of the intensity parameters, showing patients to have less intense startle eyeblink responses than controls. This is in agreement with the expectations. These overall less intense responses of patients, as compared with controls, can be explained with attentional resource theory. The deficit in sensorimotor gating causes attentional resources to be distributed among much more loci than in healthy individuals, leaving less attention available for the processing of pictures and noises. However, the used startle onset latencies in the present study were thought to activate emotional modulation, while the attentional system influences the response at earlier probe onset latencies. Probably, a sharp distinction between these modulating systems based on probe onset time is not possible and depends on the type of schizophrenic patient group. For example, patients suffering an acute psychosis may have a different emotional modulation of their startle reflexes than patients with chronic schizophrenia.

In addition, concerning emotional picture processing, patients had the most intense response to negative pictures, while controls responses were largest to neutral pictures. This does not corroborate the hypothesis which stated that patients would have the same response pattern as controls. The deviating pattern as exhibited by the control group, in which startle probes presented during neutral pictures led to the largest responses, is possibly due to anticipation fear evoked by an attentional bias towards the upcoming startle probe. Because attention is not attracted to the picture as much as with negative and positive slides, it is possible that subjects prepare themselves for the subsequent noiseburst during picture viewing. In addition, some control participants stated after the experiment that neutral pictures sometimes led to fearful thoughts. For example, a slide depicting leaves and sand on the ground in a forest caused participants to think that a corpse might be buried underneath. This phenomenon leads to the suggestion that for these subjects, the neutral pictures involved in these negative thoughts are processed in the same way as negative pictures. Investigation of the subjective ratings as assessed by the SAM (Bradley and Lang, 1994) would be appropriate to control for these
Methods and parameters for quantifying startle eyeblink EMG

deviating judgments and to enhance validity of the experiment. However, due to the limited amount of time available for this study, it was impossible to analyse these data.

The current data are inconsistent with the results of Volz et al. (2003) and Curtis et al (1999). Using standardized scores of magnitude, Volz et al. (2003) failed to find differences between schizophrenic patients and controls when using late-onset startle stimuli. However, in the present study group differences were found with the use of magnitude. It is possible that the use of standardized scores had diminished the effect in the study of Volz et al. (2003), because this procedure reduces values of extreme responses. Their suggestion that schizophrenic patients need more cognitive evaluation of negative sensory input, could not be confirmed by the present study, because in the present study group differences existed in those specific probe onset latencies, for all intensity parameters. Furthermore, it is unclear which signal conditioning method was used in the study of Volz et al. (2003). They state in their article that the signal is rectified, but this procedure is necessary for both integration and smoothing. Nevertheless, it is clear that they did not use the envelope method, because with this method the signal is not rectified at all. The present data show the envelope method to be most optimal in discriminating abilities, raising the possibility that Volz et al. (2003) would have found differences if they had used the envelope method.

The results of Curtis et al. (1999) also showed no differences between schizophrenic patients and controls when using very late startle onset latencies (2000 ms and 5000 ms). However, Curtis et al. (1999) did not measure EMG amplitude in microvolts, but in arbitrary digital units that were not further specified in the paper describing their study. It is possible that this conversion of data leads to different effects than when ‘original’ parameters are used. In addition, scores were standardized by means of range correction, leading to loss of specific information in responses. Furthermore, Curtis et al. (1999) used the integrated signal. The results of the present study show this method to have the worst discriminating potential, and provide another possible explanation for the absence of group differences in the study of Curtis et al. (1999).

In the present study, no differences between responses to neutral and negative scenes were found in both groups. Schlenker et al. (1995) found this lack of differences only in their patient group. Those investigators explained the pattern exhibited by their
patient group with the affected negative emotional system that is part of the schizophrenic syndrome, but the current data suggest this pattern to be present in the general population. Schlenker et al. (1995) partly used longer startle onset latencies, but this methodological difference cannot explain the differences between results. In addition, these investigators used the integrated signal for their analyses. The present study shows this method to result in the largest reduction of values of the intensity parameters, but applying this method should lead to less significant effects instead of more. Schlencker et al. (1995) did find a significant difference between responses to probes presented during negative and neutral pictures in their control group. This was not achieved in the present study, not even with the signal that was conditioned with the envelope method. Again this cannot explain conflicting results, but the application of the integration method does show methodological weaknesses in the study conducted by Schlencker et al. (1995). Furthermore, parameters used by Schlenker et al. (1995) were response latency and converted amplitudes. Referring to the present results, it is clear that latency measures provide no means of discriminating responses. In addition, converted values always deviate from original fluctuations within subjects, decreasing the extent to which those results can be generalized to these particular populations.

4.3 Limitations and recommendations

Although the difference in intensity of responses between patients and controls was significant, there is dramatic variability between subjects in the absolute values of the intensity parameters, as showed by standard deviations. Using standardized scores would have solved this problem, but because extremes are removed with this procedure, the effect would be diminished. Still finding significant differences between groups, despite these high standard deviations, proves the effect to be very strong. However, less variability between subjects is always preferred.

Due to limitations in time and resources, the SAMs (Bradley and Lang, 1994) that were completed by all subjects, could not be analysed to serve as control option in the present study. In future investigations, it is recommended to include SAM data, resulting in increased validity of results and offering more explanations for perhaps deviating outcomes.
Methods and parameters for quantifying startle eyeblink EMG

Concerning participants, better matching of patients and controls would improve the research design. Especially preferred are comparable educational levels of both groups, which was definitely not achieved in the present study. While all controls were in the course of an academic study, patients were generally lower educated or quit their studies prematurely. Besides educational level, it is recommended to include all important demographic variables as covariates. In addition, types of medication used by the patients should be included in analyses.

4.4 Conclusions
This research was focused on finding an optimal response measure and signal conditioning method to quantify and analyse the EMG signal, investigating several parameters and methods that are used interchangeably across investigations. Of the three investigated methods, the envelope method was superior in discriminating groups and picture types. Peaks were most easy to identify when the signal was conditioned with the envelope method, leaving the original signal relatively unaffected. Investigators conducting future studies in this area are strongly recommended to consider the use of the envelope method for conditioning of the EMG signal.

Because no group differences on the latency parameters were found, it is proposed that those measures should not be consulted in research using an emotion-modulated startle paradigm to discriminate people with a psychosis from healthy individuals. All investigated intensity measures are capable of differentiating these groups, but relative blink amplitude seems to have the largest effect and is therefore strongly recommended to be used for this purpose. In addition, relative amplitude turned out to be most optimal for differentiating responses to different emotional pictures as well. Currently, blink magnitude is commonly used as primary outcome measure, but the inability of this parameter to discriminate patients from controls based on response patterns to different emotional pictures in this study indicates a lack of validity in the use of magnitude. It is suggested that future studies applying startle EMG should use more similar methodologies, leading to more meaningful and thorough comparisons of results.
Methods and parameters for quantifying startle eyeblink EMG

References
Methods and parameters for quantifying startle eyeblink EMG


Methods and parameters for quantifying startle eyeblink EMG


Methods and parameters for quantifying startle eyeblink EMG

Appendix A. Effects of performed actions in each method on the appearance of the EMG signal.


Methods and parameters for quantifying startle eyeblink EMG

5. Integration, lowpass filter, and peak detection. Method: Integration