Psychophysiology, 42 (2005), **–**. Blackwell Publishing Inc. Printed in the USA.

DOI: 10.1111/j.1469-8986.2005.00271.x

Committee report: Guidelines for human startle eyeblink electromyographic studies

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Abstract

The human startle response is a sensitive, noninvasive measure of central nervous system activity that is currently used in a wide variety of research and clinical settings. In this article, we raise methodological issues and present recommendations for optimal methods of startle blink electromyographic (EMG) response elicitation, recording, quantification, and reporting. It is hoped that this report will foster more methodological validity and reliability in research using the startle response, as well as increase the detail with which relevant methodology is reported in publications using this measure.

Descriptors: Startle, Blink, Electromyographic (EMG), Human

Due to the dramatic increase in the use of the startle blink response in research and clinical settings, Gregory Miller, then Editor of *Psychophysiology* (2001), appointed a committee to consider guidelines for startle blink research in humans. The result is this article, the aim of which is to propose a series of suggestions that might guide researchers in the collection and reporting of data based on the blink component of the startle response. Due to space limitations, this article will not deal with several areas of interest to startle researchers, such as affect, attention, psychopathology, and prepulse inhibition, but will instead focus on the fundamental methodology applied when startle blink electromyographic (EMG) data are used to investigate any research question. One goal of this article is to bring a higher degree of both reliability and validity to this research area by summarizing recent research in which alternative methods have been compared and by providing criteria for choosing among them. Another goal is to encourage the reporting of relevant methodological details in publications in this area of research. We hope that this article will serve as a guide for researchers new to the area of startle, showing them the potential ramifications of deciding to do things one way rather than another. Moreover, experienced researchers may benefit from a review of the methodological advances that have been made in this area over the past few years, and may even reconsider some of their current practices.

This committee was chaired by Terry Blumenthal, to whom comments should be directed at the address below; the order of authorship was determined alphabetically. Diane Filion was partially supported by NIH grant MH061614. We thank Craig Evinger and Chris Patrick for helpful discussions of several points.

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Blink as a Measure of Startle

The startle response consists of several components, including the eyeblink reflex, one of the first measures developed in experimental psychology (Exner, 1874; see Dawson, Schell, & Böhmelt, 1999, for a brief historical background). Whereas most of the measurement methods used in the early studies, such as the high speed camera images employed by Landis and Hunt (1939) or Dodge's pendulum-photochronograph method (Gormezano, 1966), have been resigned to the museum of psychological methodologies, several different approaches to the measurement of blink responses are still in use. Some of these are used to measure eyelid movement, including potentiometric, photoelectric, vertical electrooculographic (vEOG), and magnetic search coil methods. Others are used to measure action potentials generated within the orbicularis oculi muscle (the muscle that closes the eye during a blink), with surface or needle electromyographic (EMG) recording electrodes. Currently, surface EMG is the most frequently used measure in human startle blink research. It has been shown, however, that alternative methods provide very similar results in most cases (see Clarkson & Berg, 1984, comparing vEOG and potentiometric [mechanical] recordings; Flaten, 1993, comparing EMG and photoelectric measures; and Gehricke, Ornitz, & Siddarth, 2002, comparing EMG and vEOG measures).

For precise measurement of lid movement, there is general agreement among oculomotor physiologists that magnetic search coils constitute the best available technology (e.g., Evinger & Manning, 1993). However, most psychophysiologists record blinks not to understand their kinematics and underlying motor physiology, but to investigate reflex elicitation and modification by psychologically interesting factors and manipulations. For this purpose, EMG has become the generally preferred method. EMG offers several experimental advantages over the magnetic search coil method, such as requiring less expensive equipment and less obtrusive sensors. Further, EMG is more sensitive to blink activity than are measures of actual lid movement (Flaten, 1993), because EMG recordings can detect weak contractions of orbicularis oculi that are not sufficient to overcome the inertia of the eyelid. In addition, EMG is capable of portraying distinct subcomponents of muscle activation (e.g., the R1, R2, and R3 components of the trigeminally elicited blink; Kimura et al., 1994; Penders & Delwaide, 1973) as well as the silent period that follows intense orbicularis oculi activation.

Participant Preparation

Blink responses are measured by placing two electrodes on the skin surface overlaying the orbicularis oculi muscle, with the EMG signal then conducted to the recording equipment. It is crucial that this EMG signal be measured with as much sensitivity and fidelity as possible, a process that begins at the surface of the skin (Fridlund & Cacioppo, 1986).

Skin Preparation

Because startle blink EMG is a rather small biosignal (amplitude is rarely more than a few hundred microvolts), recording conditions should maximize the flow of current from the skin surface to the conductive surface of the electrodes. The goal of skin preparation is to reduce the impedance between skin surface and electrode gel, by removing makeup, skin oil, and dead skin cells without causing undue discomfort and potential risk to the participant or experimenter. Although there are many techniques for preparing the blink EMG recording site, the most common methods involve rubbing the skin briskly with a gauze pad and cleansing the site with either soap and water or alcohol (with the participant's eyes closed to minimize eye irritation caused by evaporating fumes). Some researchers then massage a thin layer of nonabrasive electrode gel into the recording site. Excess gel that remains on the skin surface should be wiped off, because residual gel may create a conductive bridge between the two electrodes, creating an electrical shunt that would weaken or eliminate the recorded EMG signal. Use of abrasive gels or pads is not recommended, because they may be too harsh for the sensitive skin around the eyes, and because the startle response is sensitive to negative affect. Any abrasive preparation that poses the risk of skin penetration should be conducted following the Society's Guidelines for Reducing the Risk of Disease Transmission in the Psychophysiological Laboratory (Putnam, Johnson, & Roth, 1992).

Electrode Preparation, Location, and Attachment

Orbicularis oculi is a striated sphincter muscle encircling the orbital fissure, with distinct fast- and slow-twitch portions (Gordon, 1951). Although small EMG electrodes placed at the base of the upper eyelid are optimal for isolating reflexive muscle contractions from other activity, practical difficulties such as intrusive skin preparation, electrode weight, and motion artifacts during lid movement render upper lid EMG measurement impractical. In most settings, recording electrodes are placed below the lower lid, overlaying the orbital slow-twitch portion of the orbicularis oculi.

Figure 1 shows the electrode placement employed in the majority of studies that record blink EMG from the orbicularis oculi muscle. A typical configuration consists of one electrode placed below the lower eyelid in line with the pupil in forward gaze, a second electrode placed approximately 1–2 cm lateral to the first (center-to-center), and a signal ground electrode (also referred to as an isolated ground, not to be confused with an earth ground) attached at an electrically inactive site such as the forehead, mastoid, or temple. It should be noted, however, that facial anatomy varies widely across individuals and that optimal placement of the electrodes relative to the muscle may require individual adjustments of electrode placement. Most published reports state that electrodes are attached under the left eye (probably because the majority of researchers are right-handed, and the left side of the participant's face is more accessible for electrode placement). With binaural stimulation, laterality effects are not significant (Bradley, Cuthbert, & Lang, 1996; but see Hawk & Cook, 1997).

The preferred electrodes are Ag/AgCl miniature electrodes, in which the contact surface (diameter of less than 5 mm) is recessed within a plastic casing having an external diameter of less than 15 mm.Electrodes should be filled with a high-conductivity electrode gel and attached with double-sided adhesive collars. These collars can be trimmed with scissors so that the electrodes can be placed very close to the lower eyelid without interfering with

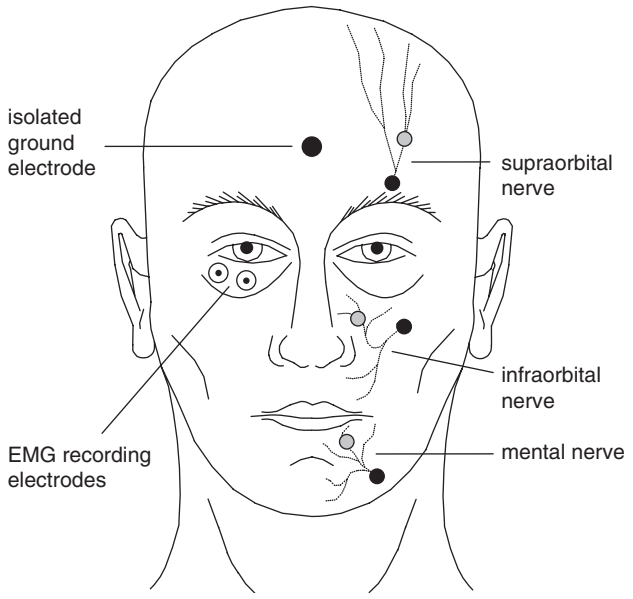

Figure 1. Left: Placement of EMG recording electrodes over the lower orbital portion of the orbicularis oculi muscle. The isolated ground electrode is placed on the forehead. Right: Electrodes for electrical stimulation of the three chief cutaneous branches of the trigeminal nerve. Solid black circles indicate cathodes placed over the nerve branches at the site of their emergence through the supraorbital, infraorbital, and mental foramina. Gray circles indicate anodes.

natural eyelid movement and without touching the lower lid or eyelashes. Trimming of the collars also allows the electrodes to be placed closer together, although collars should not overlap, because this may allow mechanical artifacts due to skin movement. If disposable electrodes that are prepackaged with recording gel are used, caution must be taken because these electrodes can dry out, a problem that can be solved by adding a little conductive gel to the electrode. Reusable electrodes should be cleaned thoroughly and disinfected before they are used with a different participant (see Putnam et al., 1992), avoiding removal of any portion of the AgCl layer.

Electrode impedance should be checked with each recording electrode in a circuit with the signal ground electrode, to confirm good and equal contact across the measurement electrodes. High impedance (above 5000 Ω; Cacioppo, Tassinary, & Fridlund, 1990), and unequal electrode impedances are to be avoided as they attenuate the recorded signal and limit its quality by permitting intrusion of electromagnetic interference. If electrode impedances are not satisfactory, then electrodes may be removed and skin preparation and electrode attachment repeated.

After checking electrode impedances, the EMG signal should be checked for noise artifacts that would obscure the blink reflex. These noise sources include intrusions from physical sources such as interference from power lines (50 or 60 Hz interference), from the startle eliciting stimulus itself, or from other biosignals such as ECG or EMG from other facial muscles. An ideal recording should have a minimal amount of tonic noise and show patterns of orbicularis oculi activation during spontaneous and voluntary blinks that are clearly distinguishable from ongoing tonic activity. High noise levels or a failure to detect spontaneous blinks may require a change of electrode placement (although the possibility of a defective electrode should also be considered). A complete absence of adequate recording of spontaneous or voluntary blinks suggests a problem with the recording equipment. In any case, the participant in such a situation should not be labeled a nonresponder, a classification that should be reserved for participants from whom spontaneous and voluntary blinks are clearly apparent, but who fail to exhibit a startle response on a predetermined number of presentations of the startle stimulus (see the section "Rejection of Trials and Participants" below).

EMG is differentially amplified (see the section "Amplification" below), which means that the amplifier outputs the difference between the signals that reach the two active electrodes and rejects signals that are common to the two electrodes (common mode rejection). Although modern amplifiers are very good at removing noise, other precautions, such as recording in an electrically shielded environment (Faraday cage), use of equipment that emits low levels of electromagnetic noise, or use of notch filters to reduce power line interference (50 or 60 Hz), can enhance the quality of the EMG recording. However, notch filters have the disadvantage of suppressing both the EMG signal and noise in the 50 or 60 Hz range. Thus, it is preferable to record under conditions that minimize 50/60 Hz noise intrusions so that a notch filter is not required. If this is not possible, a filter can be used, but it is likely to result in an underestimation of EMG activity to the extent that there is signal in the frequency range of the notch filter. Noise intrusions can also be reduced by the use of shielded electrode wires or by loosely braiding the electrode wires together, which makes it more likely that both electrodes pick up noise equally (which would be eliminated during differential amplification).

The Data Acquisition Session

Participants are usually asked to sit quietly and to refrain from moving. To minimize movement artifact, participants are typically asked to look toward some object or general area in front of them. The choice of object or area (fixation point, picture, silent movie, a wall or door) depends on the nature of the experiment, because attentional manipulation may affect startle or its modification (Filion, Dawson, & Schell, 1998). A second approach is to ask participants to close their eyes during the recording session (Hawk, Stevenson, & Cook, 1992; Sanes, 1984), although this may lead to drowsiness and falling asleep. A third approach is to record eye movements explicitly with vEOG and hEOG (horizontal EOG), and to exclude contaminated trials. Finally, the experimenter may wish to observe the participant on a closed-circuit video monitor, noting overt movement and state changes during the testing session.

Startle Elicitation

Acoustic Stimulation

Stimulus properties. The blink reflex can be influenced by several parameters of a single eliciting stimulus, including bandwidth, intensity, rise time, and duration (see Berg & Balaban, 1999). When multiple eliciting stimuli are presented, the response can also be influenced by stimulus number and interstimulus interval. The method by which the stimulus is generated and presented to the subject can also influence the startle responses that are recorded. Therefore, it is recommended that researchers report relevant aspects of stimulus composition, generation, and presentation in appropriate detail.

With regard to stimulus bandwidth, the most commonly used acoustic startle stimulus is broadband (white) noise, which is generated to contain frequencies in the 20 Hz to 20 kHz range (although a narrower range of frequencies may actually be presented, due to limitations in frequency responsiveness of the sound production equipment). When other parameters are equal, noise is a more effective startle stimulus than is pure tone (Blumenthal & Berg, 1986a; Blumenthal & Goode, 1991), with responses following noise stimuli resulting in higher response magnitude, probability, and amplitude, and shorter onset latency (see the section "Response Quantification" below for a definition of these response measures).

In general, increasing the intensity of acoustic startle stimuli has the effect of increasing response magnitude, probability, and amplitude and decreasing response onset latency. This effect has been found in studies focusing on parametric variations in simple presentations of startle stimuli (e.g., Blumenthal, 1988, 1996; Blumenthal & Berg, 1986a), as well as startle stimuli presented in the context of a foreground task (Cuthbert, Bradley, & Lang, 1996). Berg (1973), using a psychophysical threshold determination procedure and measuring lid movement with a mechanical recording device (lid potentiometer), reported that the 50% probability threshold for a blink response was 85 dB(A) SPL. In part due to this result, a preponderance of acoustic startle studies have employed intensities in the range of 100 dB(A) SPL or more. However, Blumenthal and Goode (1991) demonstrated that startle responses could be obtained with broadband stimuli in the range of 50 to 70 dB(A) SPL. This implies that prepulses and other lead stimuli in this intensity range may elicit startle blink responses on some trials (Dahmen & Corr, 2004). This also implies that very high stimulus intensities may not be necessary

for the reliable elicitation of the blink response. A moderately intense stimulus would be expected to produce a response that is intermediate between the floor and ceiling of the dynamic range of the startle response, allowing for maximal sensitivity of the response to a variety of experimental factors.

An advantage of using less intense eliciting stimuli is the minimization of risk to participants from unnecessarily high acoustic stimulus intensities. The United States Occupational Safety and Health Act standards (OSHA standard number 1910.95) state that, at a stimulus intensity of 105 dB(A) SPL, hearing protection is not required unless the sound is continuous for 1 h. However, this refers to continuous stimulation, not to impulse stimuli, such as those used to elicit startle. Although a 50-ms-duration stimulus at 105 dB SPL would still be well below the level that OSHA regards as unsafe, less intense stimuli are likely to be less aversive for most subject groups. The comfort of the participant is certainly a relevant concern, given the sensitivity of the startle response to negative affect (Bradley, Cuthbert, & Lang, 1999).

Startle responses are also influenced by stimulus rise time, a measure of how quickly the stimulus reaches its full, steady-state amplitude. Startle stimuli with shorter rise times elicit responses with higher probability, larger magnitude and amplitude, and shorter onset latency (Blumenthal, 1988), presumably because startle is specialized for the detection of sudden change in the environment (Blumenthal & Berg, 1986a; Graham, 1992). In principle, even the fastest rise time must have some finite value, although many researchers report the rise time as ''instantaneous'' when the output of the white noise generator is connected directly to the audio amplifier. A problem that occurs with very fast rising stimuli is the onset transient, a ''frequency splatter'' of sound energy that may be more intense, and of wider bandwidth, than the actual stimulus being used (Berg & Balaban, 1999). Therefore, researchers should report whether stimulus onset is uncontrolled or is controlled with an electronic switch, which can reduce some of the frequency splatter.

The duration of the stimulus also affects startle responding. Longer duration stimuli, up to approximately 50 ms, are associated with larger response magnitude and amplitude, and higher response probability (Blumenthal, Avendano, & Berg, 1987; Blumenthal & Berg, 1986b; Putnam & Roth, 1990). This duration effect has also been found for low-intensity startle stimuli (Blumenthal & Goode, 1991), and reflects the summation of energy in the auditory system (Graham, 1979; Zwislocki, 1969). Based on these results, a 50-ms stimulus duration will typically be sufficient for startle elicitation. Presenting two or more brief startle eliciting stimuli with onsets separated by less than 50 ms can also result in temporal summation, or greater responding than to a single brief stimulus (Blumenthal & Berg, 1986b).

Another factor to consider is the level of noise in the testing environment, which may either mask a prepulse in a startle modification study or act as prepulses (Blumenthal, 1999). Many studies, especially those investigating prepulse inhibition in clinical samples, utilize a steady 65–75 dB background noise during the testing session, which masks less intense environmental noise. Background noise at 70–80 dB increases the startle response in rats relative to a ''silent'' background (Hoffman & Fleshler, 1963), as does background stimulation with 65–85 dB pure tones (Yamasaki & Miyata, 1982). Also, prepulse inhibition in rats is less pronounced in the presence of a 60-dB background noise than a 50-dB background noise (Miyazato, Skinner, & Garcia-Rill, 1999), possibly due to the increase in startle reactivity just mentioned. A parametric test of the impact of background noise that is on throughout the testing session on human acoustic startle has not yet been reported. However, the evidence from both animal and human research suggests that the use of background noise to mask environmental sounds may not be as effective as decreasing those uncontrolled sources of noise or isolating the participant from that environmental noise.

Stimulus creation. Acoustic stimuli can be created by commercially available tone and noise generators or by computer software and sound cards. Software-generated stimuli may allow for more precision of frequency composition and also allow the researcher to anchor pure tone onset to a zero crossing. If software-generated noise stimuli are used, an output frequency of at least 40 kHz is recommended to adequately represent the high-frequency components of the noise stimulus. If the frequency composition of the eliciting stimulus is of interest, the signal being sent to the speaker or headphones can be directed to a data acquisition program with a high sampling rate (40 kHz), and this sampled signal can then be subjected to Fourier analysis to determine the relative frequency components in the stimulus. Further, the output of the speaker or headphones could be directed to a microphone or sound level meter whose output can be sampled by the data acquisition system, with a Fourier analysis being used to identify the frequencies that are presented to the participant (although the limitations imposed by the microphone must be considered in this instance). By sampling and recording the stimulus output as if it were an input line during data collection, a researcher can be certain of the timing of stimulus onset relative to response onset.

Stimulus presentation. Acoustic startle stimuli can be presented either with loudspeakers or headphones, both of which should have a wide range of frequency and intensity responses. With speakers, calibration of stimulus intensity is accomplished by use of a sound level meter placed at the level of the participant's head or with the aid of an artificial head or artificial ear. With headphones, the shape of the earphone should allow the fitting of the appropriate adapter of a sound level meter in order to calibrate stimulus intensity. Some sound level meters include settings for the measurement of transient (impulse) signals, although most researchers report the intensity of a steady-state signal using the dB(A) SPL scale.

The decision of speakers versus headphones depends on a number of factors. In general, it is possible to ensure a more uniform and reliable signal intensity by using headphones as long as the earphones are properly aligned with the auditory canal. Also, calibration of signal intensities is difficult to accomplish as precisely with speakers; this is particularly true when pure tone stimuli are used, due to the occurrence of standing waves. However, speakers may be preferable when headphones might interfere with electrodes, with other sensors mounted on or near the head (e.g., in magnetoencephalogram recordings), or with head-mounted virtual reality displays. There may also be some populations where, due to age, psychopathology, or other factors, the less intrusive nature of speakers makes them preferable.

Visual Stimulation

There are two, apparently unrelated, blink responses to visual stimuli. The effective stimulus for the photic blink reflex is a sudden increase in illumination, whereas the Cartesian blink

reflex (or "blink to visual threat") is triggered in response to a rapidly approaching stimulus. It is unclear whether either of these reflexes is a component of startle; however, the photic blink seems a more likely candidate as it is unlearned and subcortically mediated, whereas the Cartesian blink develops only with experience and requires an intact neocortex (see Hackley & Boelhouwer, 1997). Studies of the photic blink reflex should report the following eliciting stimulus parameters: peak intensity (luminance), duration, rise/fall time, predominant wavelength (if the light is not white), size, and position relative to fixation (in degrees of arc; Hopf, Bier, Breuer, & Scheerer, 1973; Manning & Evinger, 1986), as well as ambient viewing conditions.

When weak, brief, or nonfoveal light flashes are used to elicit the photic blink reflex, two bursts of orbicularis oculi EMG activity can be distinguished (reviewed in Hackley & Boelhouwer, 1997), referred to either as the R50 and R80, to indicate their typical onset latency in milliseconds, or as R2 and R3, to parallel the nomenclature used for the trigeminal blink reflex (see the section "Electrical, Magnetic, and Mechanical Stimulation" below). To avoid contamination of the early EMG component by the electroretinogram (ERG; Hackley & Johnson, 1996), at least one of the following precautions should be taken: (1) Set the low frequency cutoff for EMG recording at 28 Hz to eliminate lower frequency ERG components (van Boxtel, Boelhouwer, & Bos, 1998) and reject blinks with an apparent onset latency of less than 40 ms, because a high frequency burst of ERG is elicited 10 to 40 ms following stimulus onset. (2) Use a short interelectrode distance, because the retina is farther away from the electrodes than is the orbicularis oculi muscle. (3) Optimally, cover one eye with an opaque plastic eyepatch and record EMG from this occluded eye, because the response is the same on both sides (bilaterally equivalent; Hackley & Johnson, 1996).

Electrical, Magnetic, and Mechanical Stimulation

Blink reflexes can be elicited by stimulation of trigeminal cutaneous nerve fibers with transcutaneous electrical or magnetic stimuli (circumventing cutaneous receptors) or by mechanical stimulation of trigeminal skin areas with discrete taps or airpuffs. Response probability is generally higher with electrical or magnetic stimulation, due to the synchronous firing of afferent nerve fibers. Mechanical stimuli are somewhat less effective because they induce short asynchronous trains of afferent impulses. However, both methods elicit blinks that are more resistant to habituation than responses to acoustic or visual stimulation.

Electrical and magnetic stimulation. Electrical stimuli are usually applied via two Ag/AgCl electrodes filled with electrolyte gel. Stimulus electrodes should have similar levels of impedance to reduce stimulation artifacts caused by capacitive coupling between stimulation and recording electrode leads (McGill et al., 1982; Merletti, Knaflitz, & De Luca, 1992; for recommendations regarding electrode application see the section "Skin Preparation" above). Blink reflexes can be elicited by stimulating cutaneous branches of each of the sensory divisions of the trigeminal nerve (supraorbital, infraorbital, and mental nerve; Gandiglio & Fra, 1967; see Figure 1), although the supraorbital nerve is most commonly used. The cathode is placed over the supraorbital foramen above the eyebrow, where the supraorbital nerve emerges from the skull, and the anode is placed about 2 cm higher and slightly more laterally (see Figure 1). To obtain large and stable reflex responses, electrode contact area diameter should be more than 5 mm and interelectrode distance (between the edges of contact areas) should be at least 15–20 mm. Small contact areas and interelectrode distances can produce a high local current density, thereby increasing excitation of superficially located nociceptive Aδ fibers, which mediate sensations of pain and temperature, rather than deeper lying Aß fibers, which mediate touch (Kaube, Katsarava, Käufer, Diener, & Ellrich, 2000). With larger electrodes that are spaced sufficiently, electrical stimuli within the range of effective blink-eliciting intensities are generally not painful. Nevertheless, application of electrical stimulation may be threatening and the affective consequences of this stimulation method should be considered.

Electrical elicitation of blink reflexes is normally performed using a monophasic rectangular current pulse delivered by an electrically isolated stimulator, with pulse intensity being inversely related to pulse duration for pulses producing threshold excitation (McNeal, 1976). A stimulus duration of 0.1 ms and an intensity between 4 and 8 mA is usually adequate to elicit blink reflexes without pain. If necessary, the duration can be prolonged to 0.2 or 0.5 ms. Although the optimal stimulus intensity varies considerably between individuals, it is generally higher than the sensation threshold and lower than the pain threshold (Ellrich, Katsarava, Przywara, & Kaube, 2001).

Stimuli with nonpainful intensities elicit a brief ipsilateral biphasic, triphasic, or polyphasic EMG response with a latency of 9–12 ms (the R1 component), followed by a bilateral polyphasic EMG burst with a latency of 25–35 ms (the R2 component, which is most often reported in startle research). Ipsilateral and contralateral R2 components show decreasing latency and increasing duration with increasing stimulus intensity (Berardelli et al., 1985). Following the R2 component, a bilateral polyphasic component with a latency of 70–90 ms, R3, is sometimes observed. R3 latency decreases with increasing stimulus intensity, resulting in a merging of R3 onset with the tail of R2 (Rossi, Risaliti, & Rossi, 1989). R3 habituates very quickly and is reported to be abolished when the participant pays attention to the stimulus (Rossi et al., 1993).

Magnetic stimulation, inducing an electrical current in the underlying nerve tissue, is an alternative to electric stimulation. Magnetic stimuli are less likely to be painful and, therefore, may be better tolerated. Due to the rapidly growing popularity of transcranial magnetic pulse stimulation (TMS), the technology is readily available. EMG responses elicited in orbicularis oculi by magnetic stimulation of the supraorbital nerve using a small circular coil with an outer diameter of 70 mm show strong similarities with those obtained electrically, an early ipsilateral R1 and a late bilateral R2 (Bischoff, Liscic, Meyer, Machetanz, & Conrad, 1993).

Mechanical stimulation. Blink reflexes can be elicited by discrete taps or airpuffs to skin areas innervated by the trigeminal nerve. These stimuli activate low-threshold mechanoreceptors innervated by nerve fibers in the Aß fiber range (Johansson, Trulsson, Olsson, & Westberg, 1988; Mizobuchi et al., 2000). Mechanical stimulation parameters (intensity, duration, waveform) are generally less effectively controlled than are electrical stimulation parameters. Another problem with mechanical stimuli is the occurrence of acoustic artifacts, via air or bone conduction, that may contribute to the blink reflex response (Flaten & Blumenthal, 1998). However, airpuffs offer a useful alternative in the study of populations with impaired hearing.

The blink reflex can be elicited by a brisk tap on the skin over the lower, medial part of the forehead between the eyebrows (the

glabella) or the supraorbital region (Gandiglio & Fra, 1967; Shahani & Young, 1972). Solenoid and pneumatic devices, which have replaced manual tapping, produce more standardized stimuli (Beise, Kohllöffel, & Claus, 1999; Hoffman & Stitt, 1980; Snow & Frith, 1989). Mechanical or electrical stimulation of the glabella produces bilateral R1 and R2 responses (Shahani & Young, 1972; Snow & Frith, 1989), whereas lateralized stimulation of the supraorbital skin elicits an ipsilateral R1 response and a bilateral R2 response (Beise et al., 1999; Gandiglio & Fra, 1967; Rossi et al., 1989). Mechanical R3 responses can be found with painful taps to the supraorbital skin (Beise et al., 1999). Similar to the electrically elicited R3, the mechanical R3 habituates quickly and is strongly reduced during attention to the stimulus. The response latencies of mechanical R1, R2, and R3 responses are 6–7 ms longer than the latencies following electrical stimulation, reflecting the longer activation times of mechanoreceptors and the less synchronous discharges of the afferent nerve fibers.

Airpuff stimuli directed to the skin anywhere in the upper part of the face elicit blink reflexes as effectively as do electrocutaneous stimuli but, as noted above, may be less anxiogenic. For a description of the stimulation apparatus, see Haerich (1998) or Berg and Balaban (1999). Airpuffs of sufficient intensity elicit R1 and R2 responses with onset latencies longer than those of electrically elicited blinks due to the delay introduced by mechanoreceptor activation time. Airpuff stimuli are very effective when directed to the forehead (e.g., Grillon & Ameli, 1998) or the anterior part of the temporal region between the outer canthus of the eye and the anterior margin of the auditory meatus (e.g., Haerich, 1994; Hawk & Cook, 1997). Spreading of air flow to the cornea should be avoided because this can cause discomfort.

The intensity of the airpuff stimulus will vary with air pressure, the diameter of the air tube's orifice, and the distance from the orifice to the skin. With an orifice of 0.5–1 cm at a distance of 1 cm from the skin lateral to the outer canthus, a stimulus with an outflow air pressure in the range of 5–35 kPa is usually sufficient to elicit a robust blink reflex (Berg & Balaban, 1999; Haerich, 1998; Hawk & Cook, 1997). Stimulus duration is usually in the range of 100–300 ms. Airpuffs regulated by solenoid-operated valves (Haerich, 1998) may have slow rise and fall times, which cause uncertainty about the temporal relationship between the stimulus and the activation of cutaneous receptors. A high-speed air control system that delivers brief airpuffs (duration 1.2 ms, rise time 0.5 ms) with high peak pressures (maximally 122 kPa) to small areas of skin (2.5 mm^2) avoids this (Hashimoto, 1987; Mizobuchi et al., 2000). Stimulus duration, rise time, and transmission time should be reported by recording the output of a microphone positioned at the orifice of the air tube.

Electrical noise and clicks produced by a solenoid-operated valve can be avoided by shielding and insulating the valve or by placing the equipment in a separate room. The flow of air itself causes an acoustic artifact via air or bone conduction (Flaten & Blumenthal, 1998; Haerich, 1998), which summates with the tactile stimulation and may contribute to the blink reflex. The acoustic component can be masked by presenting background noise through the headphones (Miller, Curtin, & Patrick, 1999), although the possible impact of this noise on startle reactivity must be considered (see the section "Stimulus Properties" above). Alternatives include the use of sound-attenuating headphones or earplugs or lowering the intensity of the airpuff (such as by using tubing with a smaller internal diameter; Flaten & Blumenthal, 1998; Haerich, 1998). The intensity and temporal characteristics of the acoustic stimulus component should be measured and reported.

Amplification, Filtering, and Integration of the EMG Signal

Like all EMG measures, eyeblink EMG is measured as a relatively high-frequency signal that oscillates in positive and negative directions around a zero-voltage level (Figure 2). The conditioning of this signal may include various combinations of analog and digital operations (Figure 3). The raw EMG must first be amplified. Second, the signal is filtered to minimize noise

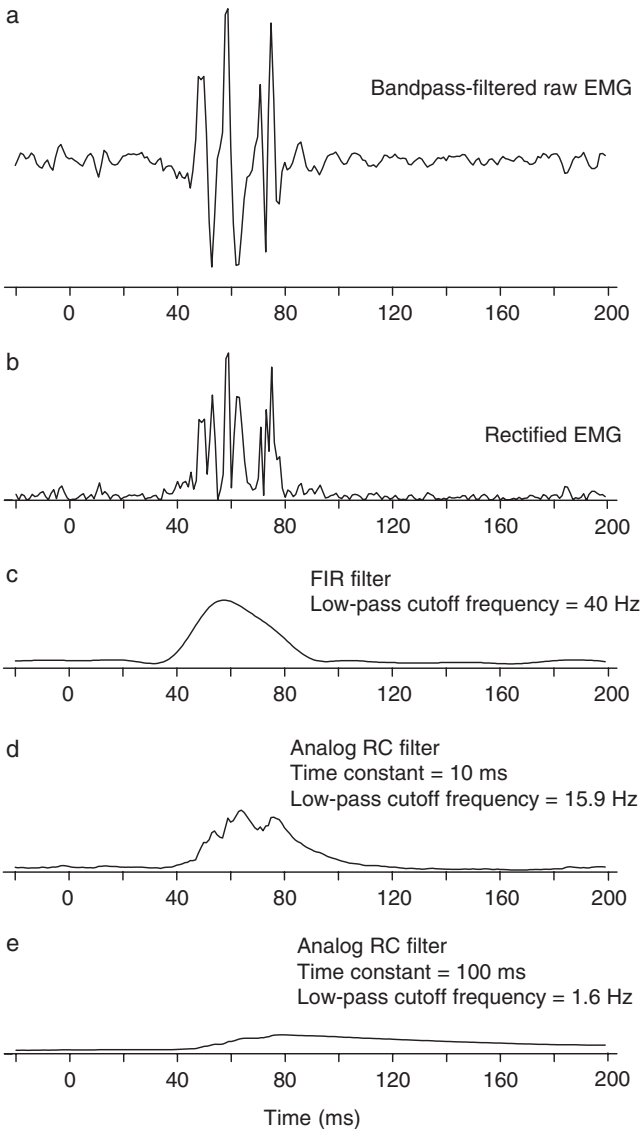

Figure 2. a: A typical acoustically elicited eyeblink EMG response that was digitally filtered (28–500 Hz passband) and sampled at 1000 Hz.The eliciting stimulus (presented at 0 ms) was a 95 dB(A), 50 ms duration broadband noise burst with a rise/fall time shorter than 1 ms, presented via headphones (AKG, Model K100). In (b), EMG was rectified. This signal was then smoothed, either with (c) a variable-weight FIR filter (101 coefficients, low-pass cutoff frequency 40 Hz), or (d and e) a digital implementation of an analog resistor-capacitor (RC) filter (time constant 10 ms or 100 ms).

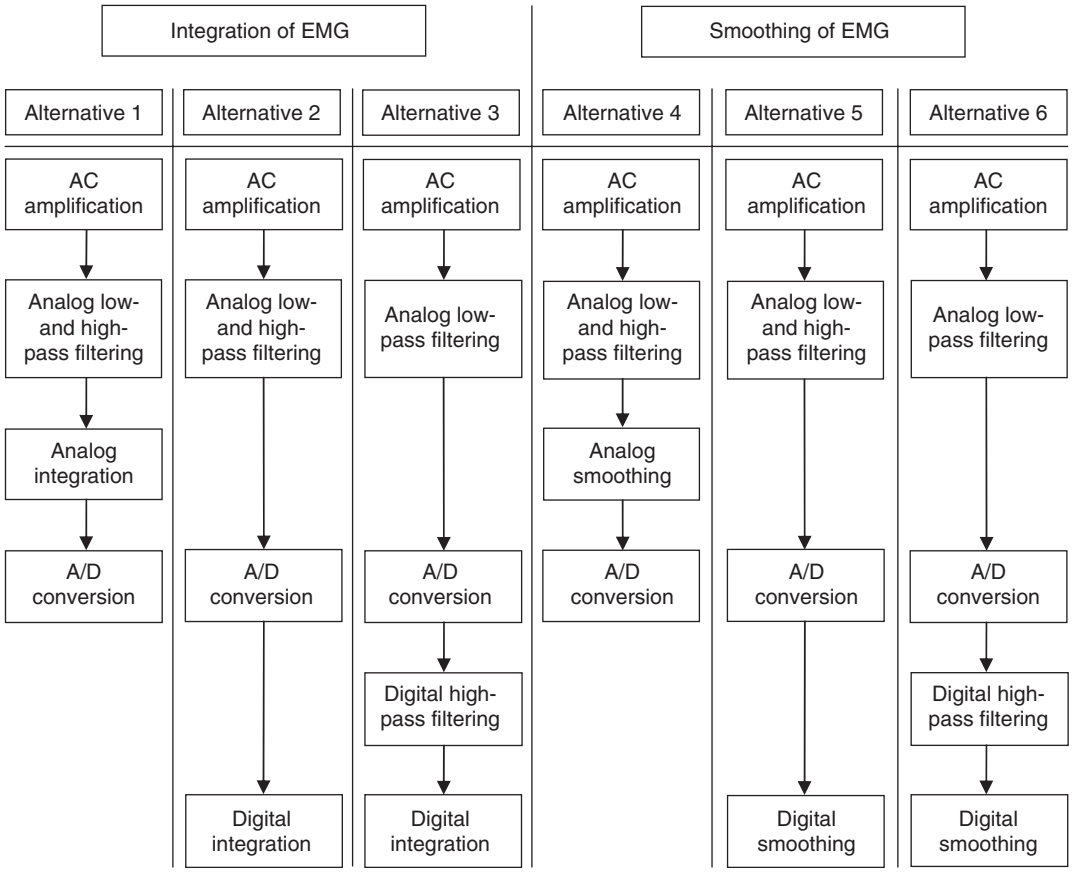

Figure 3. Different procedures for analog and digital processing of the eyeblink EMG response signal. Analog or digital smoothing consists of rectification and low-pass filtering of the EMG signal.

that is above and below the EMG signal frequency band. Third, because the negative and positive components of the waveform could cancel each other out in subsequent processing, the signal is rectified (conversion of data points into absolute values). Finally, the signal is either integrated or smoothed. The following sections provide guidelines for each of these steps.

Amplification

The eyeblink EMG signal is differentially amplified, preferably with an isolated AC-amplifier with a high input impedance (>100 MΩ), high common-mode rejection ratio (>100 dB), and low input noise (<1 μV RMS in the frequency range of 10–500 Hz). Because of the large dynamic variations in blink EMG response amplitude across participants, stimulus conditions, and trials, the amplification factor demands special attention. Too much amplification can cause the signal to exceed the input voltage range of the analog-to-digital (A/D) converter (signal clipping). Too little amplification can result in an inability to detect small responses, particularly when A/D converter resolution is low (e.g., 256 or 4096 digital units associated with an 8-bit and 12-bit converter, respectively). The range of amplification can be explored by eliciting a few blink reflexes prior to the experimental session, if this is not incompatible with the research paradigm. However, these problems can be avoided by using a high-resolution A/D converter (16 or 24 bits), which will preserve sufficient resolution even when only a limited portion of the input voltage range is utilized. Another advantage of high-resolution A/D conversion is that the need for manual gain adjustment for each recording channel and participant is eliminated, as is the subsequent calculation required to correct for such changes during data scoring and analysis.

Filtering

Following amplification, the EMG signal must be conditioned to maximize the signal-to-noise ratio, thereby increasing the fidelity with which the actual blink response is discriminated from the background. Although a tutorial on filtering (e.g., Cook & Miller, 1992) is beyond the scope of this article, a few definitions are provided here. A high-pass filter removes frequencies below some designated cutoff frequency, whereas a low-pass filter removes frequencies that are higher than the cutoff frequency. Input frequencies beyond the cutoff frequency are not completely eliminated from the output. Rather, the further the input signal frequency is beyond the cutoff frequency, the more the output signal is attenuated. The steepness of this ''rolloff'' function depends on the filter design, and is generally specified in dB per octave (where an octave is the doubling or halving of the cutoff frequency). The passband refers to the range of frequencies that a filter will pass without substantial attenuation, the stopband is the range of frequencies in which little energy is passed, and the transition band is the range of frequencies in which gain is intermediate.

A variety of signal conditioning procedures may be used, consisting of analog and digital operations (Figure 3). Within each procedure, the eyeblink EMG signal is high-pass filtered to remove low-frequency artifacts (e.g., motion artifacts and

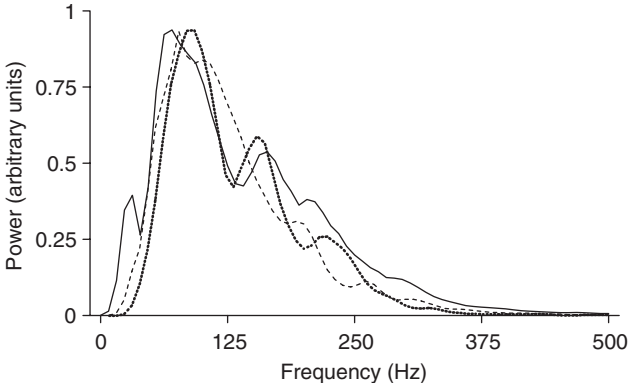

Figure 4. Typical power spectra of acoustically elicited eyeblink EMG responses in three different persons. EMG was digitally filtered (28–500 Hz passband). A data record of 128 ms duration, starting at acoustic stimulus onset, was sampled at 1000 Hz, tapered by means of a Kaiser-Bessel window, and subjected to power spectral analysis.

potentials from other biological sources), and low-pass filtered to reduce wide-band noise (e.g., instrumentation noise, electrode–skin noise) and to remove specific high-frequency components caused by electromagnetic interference (e.g., radio waves, harmonics of the power line frequency). Adequate low-pass filtering also prevents aliasing of digitized signals (see the section "Analog-to-Digital Conversion" below).

The EMG signal may be contaminated by low-frequency potentials from different sources, the most important of which are motion potentials associated with the contraction of orbicularis oculi. These potentials are largely caused by stretching of the skin under the electrode, causing changes in the potential between the electrolyte gel and the skin (Ödman & Öberg, 1982). Eyeblink EMG responses can also be affected by other low-frequency artifacts, such as those generated by eye movements, overlapping electrode collars, retinal potentials, and activity of other facial muscles. Low-frequency artifacts can be minimized by high-pass filtering of the EMG signal, with a cutoff frequency high enough to achieve adequate artifact removal but not so high that a substantial portion of the EMG signal is filtered out. This point is particularly important in research paradigms in which small blink responses may occur, such as after response habituation. Using a digital high-pass filter with an infinite impulse response (4th order Butterworth filter, rolloff 24 dB per octave), van Boxtel et al. (1998) determined that adequate artifact rejection, while preserving real EMG signal components, could be accomplished by a filter with a -3 dB cutoff frequency of 28 Hz (Figure 4). This frequency was found to be optimal for acoustic, photic, and electrocutaneous blink reflexes recorded with varying interelectrode distance (12 and 36 mm, center-to-center). The 28-Hz high-pass cutoff frequency should be considered as an approximate guideline rather than as an absolute standard, because the cutoff frequencies investigated were incremented in steps of 8 Hz, implying that the optimal frequency might actually reflect a value in the range of 24–32 Hz. Also, when using a filter with a rolloff of less than 24 dB per octave, the cutoff frequency should be higher than the recommended frequency and, conversely, the cutoff frequency can be lower with a rolloff of more than 24 dB per octave.

Low-pass filtering reduces high-frequency components caused by instrumentation noise and electromagnetic interference (e.g., radio waves, harmonics of the power line frequency). For all stimulus modality and interelectrode distance conditions, van Boxtel et al. (1998) found that a low-pass cutoff frequency of 400–500 Hz appeared to be adequate because there was a negligible contribution of higher frequency components to the EMG signal (Figure 4). In general, a low-pass filter with a steep rolloff (24 dB per octave or greater) is recommended for EMG signals (Clancy, Morin, & Merletti, 2002). Adequate low-pass filtering also prevents aliasing of digitized signals (see the section "Analog-to-Digital Conversion" below).

Although high-pass filtering is necessary for reliable measurement of the acoustic blink reflex and its electrocutaneous and visual counterparts, it may cause problems when measuring the magnitude of the biphasic or triphasic electrocutaneous R1 component. The R1 component may be contaminated by the response of the high-pass filter to the electrical stimulation artifact shortly preceding R1. Apart from taking measures to reduce stimulation artifacts (McGill et al., 1982; Merletti et al., 1992), van Boxtel et al. (1998) recommend measuring R1 amplitude using a parallel recording of the EMG signal on a separate channel with a lower high-pass cutoff frequency (e.g., 0.5 or 1 Hz).

The blink reflex EMG signal may be high-pass filtered on-line, using an analog filter (Figure 3, alternatives 1, 2, 4, and 5), or off-line, using a digital filter (Figure 3, alternatives 3 and 6). Digital filtering can also be performed on-line if computer processing capacity is sufficient. The primary advantage of digital filters is that they show no limitation in settings and may be repeatedly applied off-line to stored EMG data with modified settings, whereas analog filtering is usually associated with limited settings and irreversible results. In any case, storage of the minimally filtered EMG data is recommended so that the original signal can be filtered digitally in a different way at a later time, if the need arises. Another advantage of digital filters is that they can be exactly specified and are completely consistent in their operations, whereas analog filters do not always conform to their specifications and may show some variability at different times or across different recording channels. Therefore, analog filters need to be calibrated periodically to ascertain that their characteristics have not changed. Finally, a narrow transition band (a steep rolloff) can be more easily obtained with digital filters than with commonly available analog filters (Cook & Miller, 1992; Nitschke, Miller, & Cook, 1998).

A disadvantage of analog filters and digital infinite impulse response (IIR) filters is that they may introduce frequency-dependent phase shifts in the EMG signal, which can result in increased response onset latency and distortion of the input waveform. Symmetrical digital finite impulse response (FIR) filters do not cause phase shifts. Phase shifts and the accompanying increased latencies are less problematic in within-participants designs because they are consistent across experimental conditions (under the reasonable assumption that the frequency characteristics of the EMG signal do not change). If distortion of the signal waveform would be problematic, an analog filter with linear phase shift (a Bessel type filter), which does induce a time shift but produces minimum signal distortion, can be applied.

Rectification and Integration or Smoothing

The next stage of processing involves rectification and integration or smoothing of the signal (Figure 2). Rectification (conversion to absolute values) can be accomplished either with an analog circuit designed for this purpose or digitally. In either case, it is necessary to check whether the output DC level of the

amplifier is centered on zero; if it is not, the usual procedure is to subtract the average unrectified signal amplitude calculated for a prestimulus baseline period from each data point of the rectified poststimulus signal.

Conditioning of the EMG signal may be completed by integration or smoothing. Real (i.e., mathematical) integration involves computing the area under the curve of the EMG signal during a certain time interval (Herzog, Guimaraes, & Zhang, 1999). The outcome, expressed in microvolt · seconds, is related to the tension or force exerted by the muscle (De Luca, 1997; Winter, 1990). Integration can be performed with an analog device (Figure 3, alternative 1) or a digital routine (Figure 3, alternatives 2 and 3). Analog integrators deliver a signal proportional to the value of the integral during an internally or externally controlled time interval; this signal is typically digitized prior to data quantification. However, integration is most often performed using a digital routine, wherein the digitally calculated integral (or response area) is the product of the mean rectified voltage during the integration interval and the duration of the interval.

A more common procedure, as illustrated in Figure 3 (alternatives 4–6), is smoothing of the EMG signal (Winter, 1990), which involves passing the rectified EMG signal to a low-pass filter. This can be performed on-line using an analog device (Figure 3, alternative 4) or off-line using a digital routine (alternatives 5 and 6). Such an analog device, consisting of a precision rectifier and a low-pass filter, is often called a "contour-following integrator" (e.g., Fridlund, 1979), so that the term "integration" has often been used in the literature when "smoothing" would have been more accurate. The low-pass filter usually consists of a simple resistor-capacitor (RC) circuit. The -3 dB cutoff frequency (f_c) of such a filter can be derived from its time constant (τ, in seconds): $f_c = 1/(2\pi\tau)$, with higher time constants representing lower cutoff frequencies. As illustrated in Figure 2d,e, increasing the time constant reduces the impact of high frequency fluctuations in the rectified EMG signal (both during the baseline period and the blink response). However, increasing the time constant also leads to attenuation of the output signal relative to the input signal (Blumenthal, 1994, 1998). At long time constants, this may result in a failure to detect small responses, potentially overestimating the proportion of participants with subthreshold blink responses ("nonresponders"). Blumenthal (1994) found that using a time constant longer than 10 ms decreases the probability that small or brief responses will be detected.

As with analog filters used to condition the raw EMG signal, the low-pass RC filter may introduce frequency-dependent phase shifts, as in Figure 2e. This problem can be avoided by using an analog Bessel filter rather than a simple RC filter. A higher order Bessel filter would also provide a much steeper rolloff, and would, therefore, better suppress random fluctuations in the rectified EMG signal.

Smoothing of the EMG signal can also be performed digitally (Figure 3, alternatives 5 and 6). An IIR filter that acts as an RC circuit (Bendat & Piersol, 2000, p. 401), or simulates other types of analog filters, may be used. Also, a variety of symmetrical FIR filters (preventing phase shifts) can be implemented. A commonly used FIR filter for smoothing is the moving average, or *boxcar*, filter. In fact, this fixed-weight filter attenuates all frequency components in the EMG signal except 0 Hz.The primary advantages of this filter are its simplicity and speed of computation, although it has several limitations that can be avoided by using a more sophisticated variable-weight FIR filter (Nitschke et al., 1998). This type of filter, illustrated in Figure 2c, has several advantages in comparison with analog RC filters. Besides avoiding phase shifts, it also avoids the multiple peaks as observed in the output of RC filters with a short time constant, as well as the strongly attenuated output of RC filters with a long time constant.

Analog-to-Digital Conversion

The sampling rate for analog-to-digital (A/D) conversion of raw or smoothed EMG depends on the highest frequency component of interest in the signal, and should be sufficiently high to enable unique reconstruction of the original signal. Because at least two samples per sine wave cycle are needed for reconstruction of both phase and amplitude, the highest frequency component in the original signal that can be detected is half the sampling rate. This frequency component is called the *Nyquist frequency* or *folding frequency*. Frequency components in the original signal above the folding frequency will not be lost from the signal, but will be folded back into the frequency range from the folding frequency down to 0 Hz (a process called *aliasing*; Bendat & Piersol, 2000, pp. 366–369). This implies that high signal frequencies that are not of interest, and that may be artifacts rather than actual EMG components, can contaminate the real EMG signal. Therefore, it is recommended that the frequency range of the original analog data be restricted with an analog low-pass filter prior to A/D conversion (Figure 3) so that frequencies beyond the highest relevant signal frequency are removed (anti-aliasing filtering). An analog filter is suggested because, once the signal is digitized, the contaminating effects of aliasing are irreversible. Because no analog filter has an infinitely steep rolloff, it is customary to set the anti-aliasing filter cutoff frequency at a lower value than the folding frequency, depending on the rolloff of the filter (Bendat & Piersol, 2000, pp. 368–369). Low-pass filtering with a cutoff frequency within the range of 400–500 Hz to prevent aliasing is adequate for raw EMG signals (van Boxtel et al., 1998), if sampling rate is 1000 Hz or more. Sampling the analog smoothed EMG (Figure 3, alternative 4) requires a much lower sampling rate because the high-frequency components are already removed from the signal. Nevertheless, a higher sampling rate might be necessary for the precise determination of response onset latency and peak latency. For this reason, we recommend that both the raw and the smoothed EMG signal be sampled at a rate of at least 1000 Hz.

Measurement Units

As indicated above, integrated blink EMG magnitude is expressed in microvolt · seconds, whereas the peak amplitude of the smoothed EMG response is expressed in microvolts. Eyeblink EMG amplitude (or magnitude) has in the past often been reported in analog-to-digital units, or arbitrary units, neither of which can be directly compared across research settings. Conversion to microvolts or, in the case of integrated EMG, microvolt · seconds, would facilitate comparisons across experiments and laboratories. This is the norm in all other branches of electrophysiological research and adoption of this standard by startle researchers is recommended.

Comparability across research settings would also be facilitated by reporting the details of a microvolt-level calibration procedure. Specifically, a calibration signal can be generated by the computer's digital-to-analog converter or other equipment for transmission to the coupler or preamplifier of the EMG

recording system. (Some differential bioamplifiers may not operate properly when the input is a single-ended output from a D/A converter. In this case, the D/A output may need to be routed to the next stage in the processing sequence, which will typically be single ended rather than differential.) Using this procedure, the impact of data processing can be quantified by comparing the original calibration signal to the resulting signal after amplification, filtering, smoothing, and so forth. These calibration procedures and the ratio of output to input could be reported along with the experimental data, allowing startle researchers to compare their own results with results from other research laboratories.

Because response measures can be influenced by the decisions made regarding recording and calibration methodology, investigators are encouraged to report relevant aspects of EMG signal processing, including cutoff frequencies and rolloffs for analog and digital filters, integration parameters or smoothing coefficients, A/D converter resolution (bits), and sampling rates.

Quantifying the Startle Blink EMG Response

Response Quantification

Response quantification involves identifying and measuring EMG parameters such as onset latency and peak amplitude (Berg & Balaban, 1999). During the scoring process, startle blink responses must be distinguished from background EMG activity and from voluntary and spontaneous blinks. The inclusion of these other blinks can be minimized by limiting acceptable reflex responses to blinks with response onset in a narrow latency window following eliciting-stimulus onset. Common response onset latency windows include 21–120 ms for acoustically elicited blinks (suggested by Balaban, Losito, Simons, & Graham, 1986) and 21–150 ms for visually elicited blinks (Graham, 1975). However, these values were based on norms for a very wide age range. The relatively low standard error of onset latency measurement (e.g., Blumenthal, Elden, & Flaten, 2004) suggests that a narrower window (e.g., 21–80 ms) might be more appropriate for adult acoustic startle experiments, particularly when a short EMG smoothing time constant is used.

The nature of the EMG waveform being quantified has a significant impact on the startle parameters that will be obtained. For example, long smoothing time constants produce a significant reduction in indices of response size (amplitude, magnitude, area). Similarly, indices of response speed (onset latency, peak latency, response duration) are most accurately obtained from the raw EMG waveform (e.g., Blumenthal, 1994, 1998). Because the time constant of EMG integration (or the degree of smoothing of the signal) can delay the peak, measures of peak latency should be interpreted with caution.

Data scoring can be done manually or with computer-assisted scoring or with fully automated procedures, and each method is based on user-defined scoring parameters. Manual scoring involves the scorer's selection of response onset, peak, and so forth, on a trial-by-trial basis. With computer-assisted scoring, the program identifies response parameters based on user-defined criteria, but each response is visually inspected and an acceptance or override decision is made. In the case of either manual or computer-assisted scoring, the procedure should be done blindly with respect to experimental condition, the parameter identification and measurement rules should be reported, and, ideally, interrater reliabilities for subjective aspects of these procedures should be documented. For reasons of speed, cost, and consistency, some investigators prefer fully automated systems. However, visual inspection allows a researcher to accept or reject trials based on his or her seasoned judgment. Whatever criteria or methods are used to score EMG data, these should be applied in a consistent manner across data sets and across data scorers. If more than one person scores the data from a study, interscorer reliability should be maximized by adequate training in the scoring methods used.

For each trial, the researcher or scoring program must first decide whether a response could have been seen had one occurred. If the baseline period is contaminated with noise, movement artifact, and so on, or if a spontaneous or voluntary blink begins before the minimal onset latency value, then a stimulus-elicited blink cannot be accurately quantified on that trial. Thus, the trial should be rejected. On trials that are not rejected, the next decision is whether or not the criterion for response onset has been met. If not, then this trial represents a failure of the stimulus to elicit a response, that is to say, a nonresponse trial (also called a "flat response" or a "zero response").

Identification of the onset of a blink response can involve searching forward in the EMG waveform from the time of stimulus onset for a significant increase or change in slope of the waveform or, alternatively, searching backward from the peak of the response to this initial point of change (although this method assumes that a response is seen on this trial). Because there is always noise in the EMG signal, the change in slope can be thought of as a change in slope of the line of best fit, which can be established statistically or by visual estimation. A variety of response onset criteria have been used in previous research, such as the first point that is two standard deviations above the baseline mean (Ornitz, Hanna, & de Traversay, 1992); or the first point that is at least three times the mean of the baseline (Grillon & Davis, 1995) or the first point at which the slope of the EMG signal changes by some number of microvolts (or arbitrary units) within some number of milliseconds (or samples; Blumenthal, 1995; Hamm, Greenwald, Bradley, & Lang, 1993; Vrana, 1995). All of these require that the EMG signal exceed the baseline EMG activity by some factor. The optimal method of onset latency determination is still an open question (Brinkworth & Turker, 2003; Leader, Boston, & Moore, 1998; van Boxtel, Geraats, van den Berg-Lenssen, & Brunia, 1993).

Similar to the procedure for determining response onset, identifying the peak of a startle blink response involves examination of the EMG waveform within a particular time window (e.g., from 20 to 150 ms after stimulus onset for acoustic blinks). The most common method for determining response peak is to simply identify the maximal EMG value within this specified window. If multiple peaks occur, as is often the case when short smoothing time constants are used, the maximum value is still identified as the peak, unless the EMG response line has returned to baseline for a long enough time that the later peaks are clearly not components of the stimulus-elicited response. The definition of these criteria is still an open question, and the researcher should report whatever decision is made regarding multiple peaks. The minimum acceptable response size (response criterion) should also be reported. Note that a criterion that is too low can result in nonresponses being scored as responses, whereas too high a setting can cause "true" blinks to be missed and scored as nonresponses.

With the onset and peak of a response determined, indices of response size can be computed. Response amplitude is typically

computed as the difference between the EMG value at response peak and either the EMG value at response onset or the average EMG value during a baseline period preceding or immediately following eliciting-stimulus onset. This baseline period should, of course, end before the beginning of the response onset window.

Once response amplitude has been computed for each trial, two options for averaging across trials include computation of mean response amplitude and probability or combining these into mean response magnitude. The terms *amplitude* and *magnitude* are interchangeable when describing the size of a single response, but they differ when describing the average across trials. In that case, the term magnitude is traditionally used when the average includes values of zero for nonresponse trials, whereas the term amplitude is used if the average is computed with nonresponse trials excluded. Response probability refers to the total number of detected responses divided by the total number of eliciting stimuli presented (after adjusting for trials contaminated by artifact), within each stimulus condition. The mean response magnitude for a set of trials is the product of the mean amplitude within that condition and probability for that condition ($mM = mA \times P$; Blumenthal & Berg, 1986a). This implies that, as response probability increases, mean response magnitude approaches mean response amplitude. The term magnitude should be used, rather than amplitude, to describe the size of signal averaged EMG (see the section "Signal Averaging versus Analysis of Single Trials" below), because trials with no response are normally included in the computation of these averaged waveforms.

The startle blink parameters described thus far are computed using two data points in the EMG signal, response onset and peak. Response peak may provide information about the activation of the largest motor unit in close proximity to the electrodes, although it could be based on the simultaneous activity of several smaller motor units. To examine the activity of all recruited motor units, measures of response duration and area may be computed using the points of response onset and recovery to baseline. Response *duration* is defined as the time from response onset to response recovery and reflects the duration of agonist muscle activation during the blink response. Response *area* is defined as the area under the curve of a response waveform and reflects the entire muscle activation. Blumenthal (1998) reported high correlations between measures of response magnitude and response area, using data derived both from raw EMG and from rectified and smoothed EMG. However, it is better to measure response area and duration from raw, rather than smoothed, EMG, because the tail of the smoothed response reflects, in part, the recovery of the smoothing process.

One final step of processing is frequently performed before blink data are subjected to statistical analyses. For reasons as yet largely unknown, wide individual differences in absolute blink magnitude are observed, and this variation is often unrelated to the experimental phenomena of interest. Accordingly, the use of absolute blink magnitudes can result in a small number of subjects with unusually large blinks disproportionately affecting the outcome. For this reason, many experimenters standardize blink magnitudes in some way, such as using all blinks for a given subject as the reference distribution and reporting the results as z or T (mean = 50, $SD = 10$) scores. An alternative is to use only blinks obtained during intertrial intervals, or other nontask parts of the session (control blinks), as the reference distribution (e.g., Bonnet, Bradley, Lang, & Requin, 1995). In this manner, the extent of blink modulation due to the experimental conditions can vary freely in relation to the reference, because they are not part of this distribution's variance. However, this method relies on a sufficient number of control blinks obtained throughout the course of the session (to represent habituation effects adequately) to form a valid reference distribution. An alternative approach is to eliminate participants or individual trials that are considered outliers (e.g., 3 *SD*s from the mean). Although no preferred method for standardization or rejection of outliers has emerged, any such data transformation should be reported in detail. (The reader is referred to Blumenthal et al., 2004, who discuss similar issues in the context of quantification of prepulse modification of the blink response.)

Signal Averaging versus Analysis of Single Trials

As discussed above, the most common method of analyzing blink EMG data is to measure the amplitude, onset latency, and probability in single trials, and then calculate the condition means for use in inferential statistics. An alternative approach is to signal average across trials within a condition, measure the resulting waveforms, and then submit these measurements to statistical analysis. The latter technique is identical to that used to extract event-related potentials from EEG, except that EMG signals must be rectified prior to signal averaging. Rectification is necessary because, at any given point in time following stimulus onset, the EMG electrodes are as likely to record a positive as a negative spike, and the sign of the wavelet is generally not an important consideration in differential recording (Melkonian, Blumenthal, & Meares, 2003). If unrectified data were averaged across trials, the positive and negative spikes would cancel out, resulting in a more-or-less flat waveform.

Signal averaging is necessary for the extraction of event-related potentials from EEG because these potentials are smaller than the background activity. This is not the case for EMG blink responses, where the signal is usually considerably larger than the noise. However, there are several advantages to signal averaging of (unsmoothed) EMG: (1) It allows components of the response (e.g., the R50 and R80 components of the photic blink reflex) to be readily distinguished. (2) Differential effects on these components, or on portions of a single component, can be distinguished in the signal averaged waveforms. For example, prepulse inhibition reduces the amplitude of the peak and trailing edge of the photic R50 component but has no effect on the leading edge of that component (Burke & Hackley, 1997). (3) Signal averaging permits responses that are smaller than the background activity to be detected, provided that these small responses do not vary too much in onset latency. (4) The silent period that commonly follows large EMG bursts can be investigated. (5) Event-related potential or event-related desynchronization data can be collected and analyzed concurrently with startle, to maximize comparability across measures.

The principle disadvantages of signal averaging compared to conventional single-trial analyses are: (1) Stochastic versus amplitude-modulation effects cannot be distinguished. For example, analysis of signal averaged data cannot reveal whether prepulse inhibition produces a reduction in response probability, a reduction in response amplitude, or both. (2) Signal averaging introduces a bias such that the response onset latencies more strongly reflect minima than means. The point of response onset in an averaged waveform, for example, would be primarily determined by the fastest responses of the fastest participants. To reduce or eliminate this bias, the point at which the leading edge of the response first reaches 50% of peak amplitude can be

substituted for response onset latency (Smulders, 1993). (3) Significant variations in the onset latency of responses result in a phenomenon known as "latency jitter," in which the averaged response peak is lower than any individual peak because the individual peaks occur at different points in time, compromising measures of response magnitude.

If signal averaging is employed, the investigator should report the analog-to-digital conversion rate (in hertz), the type of epoch segmentation (i.e., stimulus- vs. response-locked), the length of the epoch (in milliseconds), and artifact rejection criteria (e.g., blink in progress at the time of stimulus delivery). Waveforms for contrasting conditions should be overlaid in the figures, but no more than four conditions should be superimposed, in the interest of clarity. Time and amplitude scales should be indicated in the figure itself (e.g., with calibration bars) rather than in the caption.

Rejection of Trials and Participants

Because the orbicularis oculi reaches very low levels of noise at rest, the signal-to-noise ratio for startle is generally very high, and what little noise does exist can be reduced considerably by proper skin preparation and electrode placement. In addition to video monitoring of the participant, recording complimentary channels of physiological information such as vEOG, ECG, EEG, and EMG from other pericranial muscles can be useful for identifying artifacts due to motion or other sources. However, spontaneous blinks should also be considered artifacts when reflex blinks are the response of interest. Gehricke et al. (2002) suggest that reflexive and nonreflexive blinks can be distinguished by simultaneous recording (coregistration) of EMG and vEOG. However, because vEOG is also sensitive to movements of the eyeball, it is not possible to distinguish EOG activity caused by lid movement from that caused by eyeball rotation. The alternative to coregistration is adherence to strict latency criteria for response onset, with the realization that a narrow window of acceptable onset latencies will decrease the probability of artifact being mistaken for true responding.

The most common procedure for reducing the impact of artifacts on the data is to exclude any trial in which there is excessive noise in the EMG signal, or in which a spontaneous blink occurs either in the period immediately preceding stimulus onset or in the interval between stimulus onset and the minimal blink onset latency. Optimal criteria for rejecting a trial will vary depending on the nature of the waveform and the quality of the recording. Regardless of the strategy adopted, the criteria for trial exclusion and percentage of trials lost using these criteria should be reported. Finally, a rejected trial should not be considered equivalent to a trial on which there is no response.

Participants as well as trials sometimes must be rejected, often because the individual exhibited blink responses on too few trials (i.e., a floor effect), or none at all. Such participants are typically labeled "nonresponders" and are excluded from experimental analyses. Attrition rates are typically higher for studies using acoustic and visual eliciting stimuli than for those using electrocutaneous or mechanical reflexogenic stimuli. The problem also increases as stimulus intensity decreases. An informal survey of current practices (C. Patrick, pers. comm.) indicates acoustic nonresponder rates of approximately 5–10% for healthy young adult participants, whereas rates reported for clinical populations, children, and the elderly are somewhat higher. Participants may also be rejected for other reasons, including a restricted range of responding within a specific experimental condition or outlier status based on extreme amplitude or latency values for a given experimental condition. However, the nonresponder category should be used exclusively for participants who fail to exhibit a sufficient number of reflexive responses. The criteria for defining a participant as a nonresponder should be clearly stated, including the minimum number of responses required for inclusion in the study and the response criterion (minimum response detectable with the measurement system used).

Because nonresponding and artifacts can produce a substantial loss of data, it is essential that the experimental design include several trials per condition. Inclusion of "control trials" (e.g., in a prepulse modification study, trials with only a startle stimulus) across the experimental session can provide a gauge of response habituation over trials. The order of stimulus conditions also needs to be counterbalanced to accommodate reduced responding across the session due to habituation. This can be accomplished by presenting blocks of trials, with each block containing one or two trials in each stimulus condition, in counterbalanced or randomized order. The control trials in each block can be used to quantify habituation across the session. In addition, two to four blink-inducing stimuli are often presented at the beginning of the session (in advance of any experimental procedures), with these trials excluded from analysis. These initial blinks are often exaggerated in size, after which habituation follows a more gradual course.

Missing data result in empty cells, and these empty cells are more likely for measures of response amplitude and latency, for which zero is not an acceptable value, than for measures of response magnitude or probability, for which zero is a possible value. Magnitude and probability will be missing only when a trial is contaminated by artifact; amplitude and latency will be missing under these conditions, but also when a response could have been seen but none occurred. This problem becomes more pertinent as the probability of a response decreases, such as when lower intensity stimuli are used, or after the response habituates or when a prepulse inhibits the startle response. Empty cells cause most ANOVA programs to delete the entire subject, decreasing the *N* and, thereby, the power of the analysis. These reduced *N*s should be reported for each analysis.

When a given trial is determined to meet criteria for rejection, the investigator must then decide whether to exclude that trial from calculations of the mean in that condition or to select a replacement value. Missing data can be estimated based on valid responses, either of the participant in question (e.g., the average of the preceding and following trials in the same condition or the mean for that subject for that condition) or based on the mean across subjects for that condition. Such an approach is appropriate if the estimates are performed conservatively (i.e., if there is a bias, it would tend to favor the null hypothesis). However, it is often preferable to reject the data of a participant rather than to attempt statistical salvage operations. In either case, the strategy selected to deal with rejected trials should be clearly described.

Conclusions

The present article makes recommendations about specific aspects of startle blink research, illustrating the different outcomes that result from making different decisions on a particular methodological issue. It is acknowledged that decisions about methodology may be constrained by limitations imposed by the equipment used in a particular research setting, and that chang-

ing methodology may limit the extent to which data collected before and after the change can be compared. However, a researcher setting up a new laboratory or modifying an existing one should consider recording data in as raw a fashion as possible, and then manipulate those data with software rather than hardware, to circumvent some of these equipment-imposed limitations. Independent of the decisions made regarding methodology, it is our recommendation that relevant procedures be fully described in a published report, to assist the reader in evaluating the methodology.

Startle is a sensitive measure that can provide a wealth of information across species and ages, in a variety of areas in the broadly defined fields of psychophysiology and neuroscience. Improving the methodological rigor with which startle data are gathered, analyzed, and reported will enhance the interpretability of these studies, increasing the potential contribution of research using this measure. We hope that the guidelines offered in this article will help to decrease the error variability of startle blink data, thereby decreasing noise and increasing precision in the use of this measure in research and clinical applications.

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(Received August 12, 2003; Accepted September 2, 2004)