Psicothema

Eyeblink conditioning with a noise burst as unconditioned stimulus

José Luis Marcos Malmierca[1] and Azahara Marcos de Vega[2]
[1] Universidad de A Coruña and [2] Clínica La Luz (Madrid)

Abstract

Background: The aim of this study was to determine if a white noise burst could be used as an effective unconditioned stimulus (US) to produce differential conditioning of eyeblink responses that were recorded as EMG activity of the orbicularis oculi. **Method:** Two fear-relevant stimuli served as conditioned stimuli (CS). An angry woman's face (CS+) was consistently followed by a white noise burst (US) with 100 dB intensity and 100 milliseconds in duration. A fearful face of the same woman (CS-) was not followed by the US. CS duration was 500 milliseconds (ms) for 18 participants (long interval group), and 250 ms for 19 participants (short interval group). The US was presented in both groups immediately after terminating CS+. **Results:** The results showed acquisition of differential conditioning in the long interval group, but not in the short interval group. **Conclusions:** These results suggest that a white noise burst as US could be used in one single experimental procedure which was capable of simultaneously producing conditioning in neural, autonomic and somatomotor response systems.

Keywords: Eyeblink conditioning, EMG activity, acoustic unconditioned stimulus.

Resumen

Condicionamiento de la respuesta de parpadeo con una explosión de ruido como estímulo incondicionado. **Antecedentes:** el objetivo de este trabajo era determinar si una ráfaga de ruido blanco podía ser utilizada como un eficaz estímulo incondicionado (EI) para producir condicionamiento de la respuesta de parpadeo, que era registrada como actividad electromiográfica (EMG) del músculo orbicularis oculi. **Método:** dos estímulos relevantes de miedo servían como estímulos condicionados (EC). Un rostro enfadado de mujer (EC+) se presentaba siempre seguido por una explosión de ruido blanco (EI) con una intensidad de 100 dBs y 100 milisegundos de duración, mientras que otro rostro de la misma mujer con expresión de miedo (EC-) nunca iba seguido del EI. La duración del EC era de 500 milisegundos (ms) para 18 participantes (grupo de intervalo largo) y de 250 ms para otros 19 participantes (grupo de intervalo corto). El EI era presentado en los dos grupos inmediatamente después de la terminación del EC+. **Resultados:** los resultados mostraron adquisición de condicionamiento diferencial en el grupo de intervalo largo, pero no en el grupo de intervalo corto. **Conclusiones:** estos resultados sugieren que es posible utilizar una explosión de ruido blanco como EI en un único procedimiento experimental, que sería capaz de producir simultáneamente condicionamiento de respuestas del sistema nervioso central, autónomo y somático.

Palabras clave: condicionamiento palpebral, actividad EMG, estímulo incondicionado acústico.

Undoubtedly, classical conditioning is the most prominent area of research regarding associative learning. Ever since Pavlov discovered this type of learning, a considerable body of scientific evidence has been amassed on the laws, principles, models, and parameters in research on both humans and animals. Notwithstanding, a key controversial issue refers to whether classical human conditioning is a low level process, or otherwise dependent on higher order cognitive processes such as contingency awareness between conditioned stimulus (CS) and unconditioned stimulus (US).

The empirical data substantiating unconscious conditioning have been obtained mainly from eyeblink conditioning studies (e.

g., Clark, Manns, & Squire, 2001; Manns, Clark, & Squire, 2002) and sporadic studies of electrodermal (Esteves, Parra, Dimberg, & Öhman, 1994; Knight, Nguyen, & Bandettini, 2006; Schultz & Helmstetter, 2010) and neural (Merz et al., 2012; Wong, Bernat, Snodgrass, & Shevrin, 2004) response conditioning. However, several authors have suggested that awareness of the stimulus contingency is a prerequisite for conditioning to occur (Mitchell, De Houwer, & Lovibond, 2009; Singh, Dawson, Schell, Courtney, & Payne, 2013). A plausible explanation for the contradictory results may be that the conditioning procedures used in the studies involved different response systems. The empirical data have shown that the effects of conditioning parameters and cognitive factors on conditioned responses (CR) were closely linked to the response system under investigation (e. g., Powell & Levine-Bryce, 1988; Prescott, Durkin, Furchtgott, & Powell, 1992).

Eyeblink conditioning is the most important model of a somatomotor conditioning response system. Usually a tone or flashing light is presented as a CS, whereas the US consists typically of a corneal airpuff to elicit an eyeblink as an unconditioned

response (UR). As a result of repeated CS/US pairings the CS elicits a conditioned eyeblink response. The interval between CS onset and US onset (interstimulus interval: ISI) is the most widely assessed parameter in eyeblink conditioning studies. Several studies have found optimal ISI in human eyeblink conditioning ranged from 400 to 500 milliseconds (ms) in young adults (Kimble, 1961), and this interval was found to increase with age (Prescott et al., 1992; Woodruff-Pak & Finkbiner, 1995). Moreover, as many pairing trials were required for eyeblink conditioning to occur, most experiments on eyeblink conditioning used more than 100 trials. Most research on eyeblink conditioning has focused on delay and trace conditioning. In delay conditioning the CS is presented and remains on until the US is presented, whereas in trace conditioning a silent interval (the trace interval) is interposed between CS termination and US onset. Behavioural and neurobiological research suggests that awareness of stimulus contingency is necessary for CR acquisition during trace, but not for delay eyeblink conditioning (Clark et al., 2001; Manns et al., 2000, 2002; Smith, Clark, Manns, & Squire, 2005).

Classical autonomic conditioning is among the most extensively researched models of associative learning owing to the nexus between the autonomic nervous system and arousal and emotions. Moreover, studies on autonomic conditioning have primarily focused on electrodermal conditioning, where a neutral stimulus is repeatedly paired with an US (usually an electric shock or a loud white noise), and skin conductance response (SCR) is recorded as CR. Electrodermal conditioning parameters differ significantly from eyeblink conditioning. CS duration frequently lasts for more than 5 secs (e.g., Knight et al., 2006; Schultz & Helmstetter, 2010) to register the CR for each pairing trial before presenting the US. Moreover, only a small number of CS/US pairing trials are required to achieve electrodermal CR acquisition, i.e., numerous experiments have shown 7 to 8 pairing trials were sufficient to produce conditioning. Furthermore, several studies have corroborated the notion that contingency awareness is essential for the expression of electrodermal CR (Dawson & Furedy, 1976; Shanks, 2010; Singh et al., 2013; Sevenster, Beckers, & Kindt, 2014).

Conditioning with central nervous system (CNS) responses is uncommon, thus, its parameters remain largely unknown. Like autonomic conditioning, neural conditioning uses neutral stimuli as CSs and electric shock (Merz et al., 2012; Tabbert et al., 2011), or white noise bursts (Wong et al., 2004) as US to elicit a neural response. In contrast to electrodermal conditioning, these studies have found evidence of neural CRs (activation of several brain structures and event evoked potentials) independent of contingency awareness.

The discrepancies in the findings suggest conditioning mechanisms vary according to each response system. Unfortunately, no systematic studies are available to compare conditioning in the three response systems. This would require a conditioning procedure using a US capable of activating all three response systems at the same time to achieve simultaneous conditioning of eyeblink, skin conductance, and neural responses using the same stimuli and parameters. Currently, no such US with these properties has been described in the literature. As previously mentioned, a noise burst is commonly used in electrodermal conditioning and has proven to be an effective US in neural conditioning experiments (Wong et al., 2004). In spite of that, no systematic study on eyeblink conditioning with acoustic stimuli has been undertaken. However, white noise bursts with short rising time (< 5 ms) and duration of around 50 ms were used to elicit the acoustic startle reflex. This reaction consisted of the coordinated movement of several muscle groups, the eyeblink response being the most reliable, fastest, and most resistant to the habituation component of the startle reflex (Landis & Hunt, 1939). The startle eyeblink response is recorded as EMG activity of the orbicularis oculi (for a review, see Dawson, Schell, & Böhmelt, 1999). These studies stress that a noise burst could be used as a satisfactory US for eyeblink conditioning.

Thus, the aim of this study was to determine if a white noise burst could be used as an effective US in differential eyeblink conditioning. As stated above, ISI is a key variable in eyeblink conditioning, with optimum ISI ranging from 400 to 500 ms in human conditioning. However, a few studies have suggested the equivalent effects of ISIs of 250 and 500 ms for older participants (Carrillo, 1996). Moreover, studies on animal conditioning have set the optimum interval ranging from 250 to 300 ms (e. g., Tseng, Guan, Disterhoft, & Weiss, 2004; Weiss, Bouwmeester, Power, & Disterhoft, 1999). Bearing in mind the results of these studies, in the present study one group of participants underwent conditioning trials with a 500 ms ISI, whereas the other group 250 ms ISI.

Method

Participants

Thirty-seven undergraduate volunteers (22 women and 15 men; age range 18 to 25 years) participated in this study. Three additional subjects were excluded from later analysis owing to a large amount of electrical noise on the EMG recording. The 37 participants were randomly assigned to one of the two groups. The long ISI group consisted of 18 subjects (10 women and 8 men) and the short ISI group consisted of 19 subjects (12 women and 7 men). All had normal or corrected-to-normal visual acuity, and none reported a history of neurological or hearing disorders. All participants were unaware of the purpose of the experiment and gave their informed consent.

Instruments

Since Seligman (1971) hypothesized that evolutionary prepared learning occurs easily to fear relevant stimuli, there has been considerable experimental evidence showing that certain stimuli such as snakes, spiders, and angry or fearful faces have evolutionary salience that can easily elicit fear CRs in comparison to neutral or non-relevant stimuli (see Öhman & Wiens, 2001). Thus, two 317 × 460 pixel pictures of a woman face showing either an angry or a fearful expression served as CSs. The pictures were taken from Ekman and Friesen (1975, figure 20D and figure 38A). The CS+ (the angry face) was followed by the noise burst (US), whereas the other CS- (the fearful face) was presented alone. The assignment of the pictures to CS+ and CS- was counterbalanced in the two groups and conditioning was inferred when eyeblink responses were greater to CS+ than to CS-. The CS duration was 500 ms for the long ISI group, and 250 ms for the short ISI group. The US was a white noise burst with instantaneous rising time, 100 dB intensity, and 100 ms in duration. This stimulus was delivered binaurally through Woxter PC 975 headphones. Stimuli presentation and intertrial intervals were controlled by Psych Toolbox software (Brainard, 1997), using a PC computer.

Eyeblink EMG responses were recorded from the left orbicularis oculi muscle with two Ag/AgCl electrodes (4 mm diameter) filled with conductive gel (Signa, Parker). An electrode was placed approximately 1 cm under the pupil and the other 1 cm below the lateral canthus. The ground electrode was positioned centrally on the forehead. The EMG signal was amplified by a factor of 1000 and band-pass filtered (90-250 Hz) by an EMG Biopac amplifier. The sampling rate was 1000 Hz and a 50-Hz notch filter was used to reduce the interference of the electrical noise. The signal was offline rectified and integrated (3-ms time constant).

Procedure

The experiment consisted of two parts.

1. Adaptation phase: Participants were seated in front of the computer screen, at a distance of approximately 60 cms, and an alcohol swab was used to clean the areas where the electrodes were placed in order to eliminate any substances that could hinder electrical conductance. Once the electrodes had been attached, participants were informed about the aims of the study, the stimuli, the procedure, and were explicitly informed they could freely abandon the experiment at any moment without having to give any reason. Subjects were also reminded of the importance of focusing on the centre of the computer screen in order to detect the stimuli given that their duration would be very short. At this point, two demonstration trials with only the noise burst were presented to check the electrodes were correctly attached, and that changes in EMG activity were correctly elicited and recorded. Thereafter, CS+ and CS- were presented twice in random order to allow the subject to become familiar with the parametric characteristics of the CSs, and learn to identify the differences between these stimuli. Then, adaptation continued for 2 to 3 minutes to allow EMG activity to stabilize and to lower subject activation levels.
2. The conditioning phase consisted of 50 conditioning trials presented in a pseudorandom trial sequence of 25 CS+ and 25 CS-, with the restriction that no more than three consecutive CSs could be the same. Presentation of CS+ was followed by a noise burst US of 100 ms duration. The conditioning trials were separated by random intervals of 15, 20, and 25 secs.

Data analysis

Conditioned and unconditioned EMG responses were scored during the acquisition phase. CR was defined as the difference in microVolts (μV) between the mean of a 200 ms pre-stimulus baseline and the maximum EMG level in the window from 125 ms after CS onset to US onset. This interval was chosen to ensure alpha responses could not be mistakenly scored as CRs. These eyeblinks are typically terminated between 100 and 120 ms after CS onset and are considered reflexive responses to this stimulus. Voluntary responses are another form of non-associative responses that may occur in the same latency window as CR. These responses usually exhibit a broad spectrum of amplitudes, and long duration (Carrillo, 1996). To avoid these responses, scores with standard deviations 2.5 above the subject mean were discarded from later analyses. URs were scored as the difference between the mean EMG level for the last 200 ms prior to CS onset and the maximum amplitude of the

EMG response in the window from 20 to 140 ms after US onset of the same trial (Asli & Flaten, 2008). To evaluate the reliability of effects on EMG response amplitude, ANOVAs were calculated. Greenhouse-Geisser epsilon corrections were used in the ANOVAs to adjust probabilities for repeated measures effects (Jennings, 1987). A region of $p<0.05$ was used for all main effects and interactions.

Results

Mean conditioned EMG responses of the trial blocks of the acquisition phase were subjected to a 2 (ISI: long vs. short) × 2 (CS: CS+ vs. CS-) × 5 (blocks) mixed model ANOVA with repeated measures on the last two factors. The analysis revealed that the main effect of CS was significant [$F_{(1/35)} = 24.79$, $p<0.01$, $\eta_p^2 = 0.42$], showing higher EMG response amplitude to CS+ (M = 40.93; SD = 25.84) than to CS- (M = 34.80; SD = 22.26). There was also a significant effect of ISI [$F_{(1/35)} = 28.43$, $p<0.01$, $\eta_p^2 = 0.45$], given that EMG responses were greater in the long ISI (M = 53.59; SD = 24.49) than in the short ISI group (M = 22.14; SD = 7.65). Furthermore, the analysis revealed that the CS × ISI interaction was also significant [$F_{(1/35)} = 4.30$, $p<0.05$, $\eta_p^2 = 0,11$], given the higher EMG responses to CS+ than to CS- in both the long ISI group [$t_{(17)} = 3.77$, $p<0.01$, d = 8.68], and the short ISI group [$t_{(18)} = 3.57$, $p < 0.01$, d = 3.57], but the long ISI group exhibiting larger CS+/CS- discrimination. The ISI × CS × blocks interaction was also statistically significant [$F_{(4/140)} = 3.94$, $p<0.01$, $\eta_p^2 = 0.10$]. Post-hoc t-tests showed significantly higher EMG responses to CS+ than to CS- in block 3 [$t_{(17)} = 2.03$, p = 0.05, d = 9.51], block 4 [$t_{(17)} = 3.17$, $p<0.01$, d = 19.03], and block 5 [$t_{(17)} = 3.16$, $p<0.01$, d = 13.25] in the long ISI group. In contrast, the pattern of CS+/CS- discrimination in the short ISI group revealed significant CS+/CS- discrimination only in block 2 [$t_{(18)} = 2.66$, $p<0.05$, d = 7.76]. The remaining factors and interactions were not significant.

A similar 2 × 5 (ISI × blocks) ANOVA was calculated to evaluate the reliability of effects on the amplitude of unconditioned EMG responses. This ANOVA showed a significant main effect of blocks [$F_{(4/140)} = 7.36$, $p<0.01$, $\eta_p^2 = 0.17$], revealing a general decline in UR amplitude over trials in the two ISI groups. The main effect of ISI did not reach significance and the ISI × blocks interaction was not significant either.
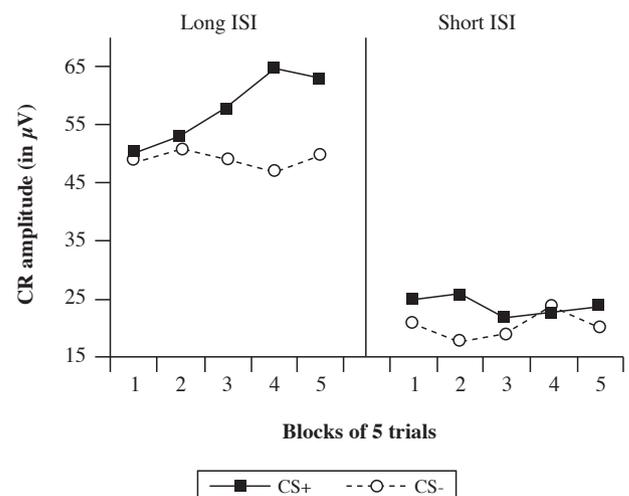

Figure 1. EMG response amplitude to CS+ and CS- over trial blocks in the Long ISI and Short ISI groups
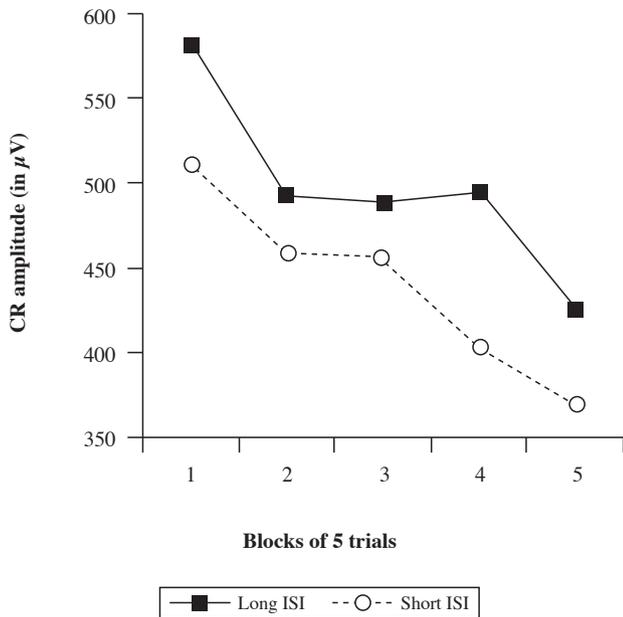
Figure 2. UR amplitude over trial blocks in the Long ISI and Short ISI groups

Discussion

The results of the present study substantiate that discriminative eyeblink conditioning occurred, as indicated by the higher conditioned EMG responses to CS+ than to CS- in the long ISI group. The interactions between the factors and the follow-up analyses provided the relevant data to characterize this conditioning. These analyses found higher CS+/CS- discrimination in the long ISI group than in the short ISI group, which would suggest higher eyeblink conditioning when a long ISI is used during acquisition. This supposition is confirmed by comparing the response pattern over the trial blocks in both ISI groups. A followed-up *t-tests* of the long ISI group found no significant differences in responses to CS+ and CS- in the first two blocks, discrimination reached the limit of significance in the 3rd block, and was highly significant in the last two trial blocks. As seen in Fig. 1, the amplitude of eyeblink responses to CS- remained quite stable over the five blocks, whereas amplitude response to CS+ increased significantly from the third trial block onwards. This response pattern was consistent with the standard acquisition process of differential conditioning. Moreover, the differential conditioning procedure used in this study excluded the potential effects of voluntary EMG responses, or any other non-associative factor since these effects were presented in the same manner to the CS+ and CS-. In short, it may be concluded that authentic differential conditioning had occurred in the long ISI group.

Furthermore, the post-hoc *t-tests* for the short ISI group revealed significant CS+/CS- discrimination only in the 2nd block. Strikingly, as seen in Fig. 1, the response pattern for the short ISI group did not fit the usual acquisition process whereby CS+/CS- discrimination was expected to increase over trial blocks, from non-significance in the first trial blocks to significance in subsequent trial blocks. This unexpected response pattern cast serious doubts as to whether real eyeblink conditioning had actually occurred in the short ISI group. As revealed by the data analysis, EMG

responses to CSs showed lower amplitude in the short ISI than in the long ISI group. This disparity in response may be due to the recording interval for CR, which was only 125 ms in the short ISI group (250 ms CS duration minus the first 125 ms corresponding to alpha responses), whereas the window was amplified to 375 ms in the long ISI group (500 ms CS duration minus 125 ms alpha responses). Thus, it was conjectured that the recording interval for CRs for the short ISI group was too short to allow for adequate expression of eyeblink conditioning. This, explanation would be consistent with the findings of several studies with other eyeblink conditioning experimental procedures that found an optimal ISI of 0.5 secs in young human adults (e. g., Kimble, 1961; Prescott et al., 1992; Woodruff-Pak & Finkbiner, 1995).

An alternative explanation could be that participants in the short ISI group had less eyeblink responsiveness. However, this assumption was not consistent with the results on unconditioned EMG responses showing no difference in amplitude between the two ISI groups. As shown in Fig. 2, UR amplitude showed a similar pattern of decline over blocks in both groups, revealing a typical habituation process. This habituation can be considered moderate given that UR amplitude decreased 27% in the long ISI, and 28% in the short ISI over acquisition trials. Given that the same interval was used for recording UR amplitude in both ISI groups and no significant differences were observed between them, it would be plausible to believe that the lower CR amplitude obtained in the short ISI group may be due to the shorter interval used to record conditioned EMG responses.

In short, the results of this study have shown that a noise burst could be used as an adequate US to produce eyeblink conditioning using an ISI of 500 ms. The finding that a burst of white noise could be used as an effective US in eyeblink conditioning is significant given that this stimulus can be used as US for autonomic and neural responses conditioning (Wong et al., 2004). The results of this study suggest that a noise burst US could be used for simultaneously eliciting and conditioning all three response systems with one single experimental procedure. This procedure will allow the elucidation of crucial theoretical issues such as whether each response system represents different aspects of conditioning, or if contingency awareness is a necessary condition for learning to take place.

Eyeblink conditioning provides the main evidence underpinning unconscious learning. This evidence suggests that contingency awareness is necessary for trace but not for delayed conditioning (Clark et al., 2001; Manns et al., 2000, 2002; Smith et al., 2005). In contrast, most studies on electrodermal conditioning have found contingency awareness of a CS/US relationship was a requisite for conditioning (Lovibond & Shanks, 2002; Mitchell et al., 2009; Sevenster et al., 2014; Shanks, 2010; Singh et al., 2013; Thorwart, Glautier, & Lachnit, 2010). The contradictory findings regarding the effects of contingency awareness on the expression of CR are probably due to the specific nature and characteristics of the different response systems involved in eyeblink and electrodermal conditioning, as well as to the different parameters, stimuli, and techniques used in each of the procedures to control contingency awareness between CS and US. As indicated above, bursts of white noise have frequently been used as an US in antonomic (for a review, see Boucsein, 1992) and neural (Wong et al., 2004) conditioning. Moreover, the present study has corroborated the efficacy of this stimulus as US for eyeblink conditioning. This emphasises the fact that a noise burst can be used as a US to

elicit eyeblink, neural, and autonomic responses in one single experimental procedure to compare conditioning in the three response systems using identical stimuli, parameters, and control procedures for CS/US contingency awareness.

Finally, this study has shown a noise burst as US can substantially facilitate research in human eyeblink conditioning because this experimental procedure is much simpler and easier to set up than the corneal airpuff or trigeminal stimulation. Moreover, the results have revealed that unconditioned EMG responses exhibited relatively little habituation over trials, and many pairing trials may be administrated without the manifestation of the response disappearing. These potential advantages highlight the benefits of using this acoustic US, and the need for further research to assess procedures to determine optimum parameters as well as to evaluate conditioning simultaneously in all three response systems.

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