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Conditioned facilitation of the unconditioned reflex after classical eyeblink conditioning

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Abstract

The present study ($N=40$) investigated the time-course of conditioned facilitation of the unconditioned eyeblink reflex (UR). In a single-cue delay classical conditioning procedure, a tone conditioned stimulus (CS) signaled an airpuff unconditioned stimulus (airpuff US) to the eye. A paired group received 40 trials of CS/US presentations with an interstimulus interval (ISI) of 200 ms. An unpaired group received an equal number of explicitly unpaired presentations of the CS and US. Thereafter, eyeblink reflex facilitation was assessed by presenting 94 dB white noise (noise US) 10, 30, 50, 100, 150, and 1000 ms after CS onset. In the paired group, URs were significantly increased as early as 100 ms after CS onset compared to the unpaired group. This reflex facilitation was correlated with CR magnitude, indicating that conditioned facilitation of eyeblink URs indexes an early, automatic, preattentive stage in CR formation.

Keywords: Classical conditioning; Eyeblink conditioning; Conditioned facilitation; Reflex facilitation

1. Introduction

Several studies have found that unconditioned reflexes (URs), elicited in the presence of a conditioned stimulus (CS), are facilitated compared to URs elicited in the absence of the CS (Flaten 1993; Flaten and Hugdahl 1991; Weisz and McInerney 1990; Wikgren et al. 2002). The present study investigated the time-course of conditioned facilitation of URs.

It has been hypothesized that conditioned facilitation of the UR indexes an early stage of eyeblink CR formation (Flaten 1993; Weisz and McInerney 1990). Flaten (1993) used an airpuff to the eye as the US, and found eyeblink UR facilitation to a noise US after only four CS/US pairings, prior to the occurrence of eyeblink conditioned responses (CRs). Similar findings were made by Weisz and McInerney (1990) and Wikgren et al. (2002). It was argued that conditioned UR facilitation was the result of a developing associative process that would eventually produce the eyeblink CR. The process underlying UR facilitation could be CS-induced facilitation of the motoneuron pool in the facial nerve that is responsible for the eyeblink (e.g., Guyton and Hall, 2006). After CS presentation, it is hypothesized that the motoneuron pool is gradually more facilitated until a CR is elicited or not. Consequently, when a reflex-eliciting stimulus is presented to an already facilitated motoneuron pool, the magnitude of the reflex increases. The degree of increase of the reflex would depend on the number of facilitated neurons and the amount of facilitation or depolarization.

There is disagreement on the role of voluntary responding and awareness of the CS/US contingency in eyeblink classical conditioning. (Clark and Squire 2004; Manns et al. 2001; Smith et al. 2005) argued that single-cue delay classical conditioning is independent of awareness of the CS/US contingency. However, their measure of awareness was criticized by Lovibond and Shanks (2002a) for underestimating awareness of the CS / US contingency. Lovibond and Shanks (2002b) argued that the claim that automatic, non-conscious processes played a role in human conditioning had not been proven. Rather, a conscious, propositional system could explain the development of the CR. One central point in this debate is the claim by Lovibond and Shanks (2002b) that almost every study of the role of consciousness in classical conditioning used inadequate measures of

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awareness, and that most measures tend to underestimate awareness, or that the experimental procedures used may lead to underestimated awareness.

The present study bypassed the problem of awareness and voluntary responding by investigating CR and UR facilitation at points in time after CS onset that were too short for conscious processes to play a role. The participants in the paired group first received 40 trials of eyeblink conditioning with a short interstimulus interval (ISI) of 200 ms. The short ISI was used to reduce the role of voluntary responding in the CR data. In the unpaired group, an equal number of CSs and USs were presented in an explicitly unpaired manner. Thereafter, UR facilitation was tested by presenting eyeblink reflex-eliciting noise immediately after CS presentation. The stimulus onset asynchronies (SOAs) between CS onset and noise onset were chosen to investigate the latency and development of the process of UR facilitation. A positive correlation between CR amplitude and UR facilitation was expected if the latter could be related to a process underlying CR production. Evaluations of CS and US valence and arousal were obtained to investigate whether emotional processes could be related to UR facilitation.

2. Materials and methods

2.1. Subjects

Forty subjects (23 females and 17 males, age range 19–37, mean age 25.9 years) participated in the study. The participants were randomly assigned to one of two groups (paired group or unpaired group), each containing 20 participants. All participants were in good health and did not report any previous serious disease or injury. All participants had auditory thresholds of 30 dB or less at 1000 Hz in both ears. The project was approved by the Regional Committee of Medical Research Ethics in Health Region IV in Norway (Project 29/2000). The participants were instructed to refrain from drinking caffeinated beverages and use nicotine-containing substances for 3 hours prior to the start of the study. Written informed consent was obtained from all participants, who where paid 2 lottery tickets (equivalent to 50 NOK) for their participation.

2.2. Apparatus and stimuli

The experiment took place in an electrically and acoustically shielded chamber (C. A. Tegnèr), where the temperature was kept at about 20 °C. Hearing was tested with a Grason-Stadler 17 audiometer. A Bruel and Kjær 2235 Sound Level Precision Meter measured intensity of auditory stimuli. Programs written by the last author in ASYST 3.1 and run on an ALR 486 PC, controlled presentation of experimental stimuli and data acquisition.

There were two different unconditioned stimuli (US) in the experiment. The airpuff US was paired with the conditioned stimuli (CS). The noise US was deployed to elicit eyeblink UR and was not paired with a CS. The noise US had an intensity of 94 dB, instantaneous rise time and a duration of 50 ms. The CSs were 1000-Hz tones with intensity of 60 dB, and a rise time of 20 ms. The duration of the prepulses were the same as the stimulus onset asynchrony (SOA) between the tone prepulse and the eyeblink-eliciting noise. The stimuli were delivered through Sennheiser HD 250 headphones.

The airpuff US was directed to the corner of the right eye. The airpuffs had an intensity of 20 kPa, as indicated at the pressure regulator on the air-pressure bottle, and a duration of 50 ms. The airpuffs were delivered by apparatus described in Flaten et al. (1989).

Eyeblink electromyographic (EMG) responses were recorded from the right orbiculari oculi with two Ag/AgCl Sensor Medics miniature electrodes (2 mm diameter) filled with Ultra Phonic conductivity gel. Inter-electrode distance was 1.5–2.5 cm. The ground electrode was placed centrally on the forehead. The EMG signal was amplified by a factor of 60,000 and filtered (passing 90–250 Hz) by a Coulbourn S75-01 bioamplifier. The signal was rectified and integrated with a Coulbourn S76-01 contour-following integrator with a 10-ms time constant, and the output sent to the PC via a Keithley interface. Sampling on each trial began 200 ms prior to onset of the first stimulus and continued for 200 ms after onset of the US. The sampling rate was 10 Hz prior to onset of the first stimulus and 1000 Hz after stimulus onset.

Emotional valence and arousal elicited by the CS and airpuff US was recorded with the Self Assessment Manikin (SAM) (Bradley and Lang 1994). General stress and arousal was recorded with the O'Neill and Parrott (1992) stress and arousal scale.

2.3. Procedure

After arrival at the laboratory the subjects sat down in a desk chair and read and filled in the Informed Consent Form. Level of hearing was tested before the participants were lead into the experimental chamber and seated in a reclining chair. The subjects were informed of the general purpose of the study, about the stimuli and procedure, and that they could withdraw from the study without giving any reason at any time. The skin below the participants' right eye was cleaned with a swab containing alcohol and pumice, and the electrodes for measurement of the eyeblink electromyography (EMG) were attached. The headgear containing the airpuff-presentation equipment and the headphones were attached, and then the subjects filled out the O'Neill and Parrott (1992) stress and arousal scale. The door to the experimental chamber was closed, and after a brief pause the experimental procedure was in effect.

The experiment consisted of two phases (see Fig. 1). In the conditioning phase, the paired group received 40 trials of single-cue classical conditioning with the 1000 Hz tone as the CS and the airpuff to the eye as the US. The interstimulus interval (ISI) from CS onset to US onset was 200 ms. The unpaired group received 40 explicitly unpaired presentations of the CS and US.

In the noise UR phase, UR facilitation to the noise US was investigated by presenting the CS at SOAs of 10, 30, 50, 100, 150, and 1000 milliseconds relative to onset of the eyeblink eliciting noise. Each SOA was presented four times. The noise US was also presented four times alone, so a total of 28 trials were presented. The SOAs were presented in semi-random