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Short communication

Uncertainty of trial timing enhances acquisition of conditioned eyeblinks in anxiety vulnerable individuals

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HIGHLIGHTS

- Human eyeblink conditioning is not affected by extending ITI.
- Behaviorally inhibited individuals exhibit enhanced eyeblink conditioning.
- Temporal uncertainty enhanced acquisition in anxiety vulnerable individuals.

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ABSTRACT

Recent work has found that behaviorally inhibited (BI) individuals exhibit enhanced eyeblink conditioning in omission and yoked training as well as with schedules of partial reinforcement. We hypothesized that spacing CS-US paired trials over a longer period of time by extending and varying the inter-trial interval (ITI) would facilitate learning. All participants completed the Adult Measure of Behavioural Inhibition (AMBI) and were grouped as behaviorally inhibited (BI) and non-behaviorally inhibited (NI) based on a median split score of 15.5. All participants received 3 US alone trials and 30CS-US paired trials for acquisition training and 20CS alone trials for extinction training in one session. Conditioning stimuli were a 500 ms tone conditioned stimulus (CS) and a 50-ms air puff unconditional stimulus (US). Participants were randomly assigned to receive a short ITI (mean = 30 +/− 5 s), a long ITI (mean = 57 +/− 5 s) or a variable long ITI (mean = 57 s, range 25–123 s). No significant ITI effects were observed for acquisition or extinction. Overall, anxiety vulnerable individuals exhibited enhanced conditioned eyeblink responses as compared to non-vulnerable individuals. This enhanced acquisition of CRs was significant in spaced training with a variable long ITI, but not the short or long ITI. There were no significant effects of ITI or BI on extinction. These findings are interpreted based on the idea that uncertainty plays a role in anxiety and can enhance associative learning in anxiety vulnerable individuals.

1. Introduction

Recent work has focused on a learning diathesis model of anxiety disorders involving behavioral inhibition and associative learning [1–4]. Behavioral inhibition or BI is defined as a temperamental tendency to withdraw from or avoid novel social and non-social situations [5,6] In addition to avoidance, BI also includes social reticence and enhanced reactivity to novelty, threat, and uncertainty [7–9]. Uncertainty has been put forth as a major factor in anxiety. It has been hypothesized that anxiety disorders may come about through a maladaptive response to uncertainty producing hypervigilance to stimuli [10].

This hypervigilance may underlie recent classical conditioning findings in behaviorally inhibited individuals. BI has been found to be linked to enhanced associative learning in classical eyeblink conditioning in humans. Eyeblink conditioning involves the pairing of a conditioned stimulus (CS) tone with an unconditional stimulus (US) corneal air puff which results in learning a conditioned response (CR) eyeblink to the previously neutral CS. Behaviorally

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inhibited individuals in an omission protocol (in which a CR to the tone resulted in the US air puff not occurring on that trial) exhibited greater enhanced acquisition of conditioned responses as compared to 100% CS-US paired trials [3]. Partial reinforcement with either CS or US alone trials inter-mixed with CS-US paired trials produced enhanced acquisition of conditioned responses that was greater than that observed with 100% CS-US paired trials [1]. These training protocols resulted in situations where there is some uncertainty about whether the next trial would be a CS-US paired trial or a presentation of the CS or US alone. This uncertainty as to when the next CS-US trial would occur could be a factor in the enhancement of acquisition of conditioned responses found with behaviorally inhibited individuals.

As compared to other forms of learning in which uncertainty effects have been investigated [10], eyeblink conditioning does not require the cortical or limbic systems. The cerebellum is known to form the CS-US association in classical eyeblink conditioning [11]. In the case of delay conditioning (the type of conditioning in the current study) this association can be formed in the absence of the hippocampus [12,13] or cerebral cortex [14]. However, cerebellar learning is modulated by inputs from the septohippocampal system [15] and amygdala [16]. One effect of uncertainty is hypervigilance via heightened amygdala activity [10] which may enhance classical eyeblink conditioning in BI individuals through the enhancement of cerebellar learning.

Another factor that could affect acquisition in behaviorally inhibited individuals is trial spacing. Partial reinforcement schedules with CS or US alone trials spaced the CS-US paired trials across a longer inter-trial interval (ITI) than short delay conditioning with 100% CS-US paired trials. Extending ITIs have been found to produce faster acquisition of conditioned eyeblinks in humans [17–19], as well as slower extinction to CS alone training [18].

In the current study, we tested the hypothesis that spacing trials over a longer period of time by extending the ITI would facilitate learning and slow extinction. We manipulated ITI as a between groups variable, and did not present different ITIs to individual subjects. It was also hypothesized that introducing uncertainty by varying the long ITI would facilitate learning. Finally, we hypothesized that behaviorally inhibited individuals would exhibit greater enhancement of acquisition in the both of the long ITI conditions as compared to the short ITI condition.

2. Materials and methods

2.1. Participants

Eighty nine college-aged students were recruited from the University of Northern Colorado, School of Psychology. Students voluntarily participated to receive class credit or extra credit for psychology classes. Sixty three females and twenty six males with a mean age of 19.7 years (SD = 3.5, range 18–38) and mean education of 12.6 years (SD = 0.95, range 12–15) participated in the study. Informed consent was obtained in accordance with procedures approved by the University of Northern Colorado Institutional Review Board.

2.2. Materials and apparatus

The eyeblink conditioning apparatus and procedures were similar to that previously described [20]. The tone stimulus was produced with Coulbourn Instruments (Allentown, PA, USA) signal generators and passed to a David Clark aviation headset (Model H10–50, Worchester, MA, USA). Sound levels were verified with a Realistic sound meter (RadioShack, Fort Worth, TX, USA). The headset was fitted with a boom placed 1 cm from the cornea that delivered a 5 psi air puff US via sylastic tubing connected to a regulator and released by a computer controlled solenoid valve (Clipper Instruments, Cincinnati, OH). To record the eyelid electromyographic (EMG) signal, pediatric silver/silver chloride EMG electrodes with solid gel were placed above and below the right eye, with the ground electrode placed on the neck. The EMG signal was passed to a medically isolated physiological amplifier (UFI, Morro Bay, CA, USA), low-pass filtered and amplified 10 K. The EMG signal was sampled at 500 Hz by an A/D board (PCI 6025E, National Instruments, Austin, TX, USA) connected to an IBM-compatible computer. Software control of stimulus generation was performed by LabView (National Instruments).

2.3. Psychometric scales

Participants completed the Adult Measure of Behavioural Inhibition or AMBI [21]. The AMBI is a 16-item self-report inventory that assesses current tendency to respond to new stimuli with inhibition and/or avoidance, and has also been shown to be a measure of anxiety proneness. An AMBI cut off score of 15.5 was used to differentiate behaviorally inhibited from non-inhibited individuals based on established methodology [21].

2.4. Conditioning session

Participants provided informed consent and were instructed that the study was going to evaluate responses to tones and air puffs to the eye, that they were to watch a silent video (e.g. a nature video with sound muted), and that they were to remain awake during the testing session. Participants were then fitted with electromyographic (EMG) electrodes and headphones, EMG signal quality was verified, and the conditioning program was started. The program began with 3 US-alone (50 ms, 5 psi air puff) exposures to assess UR quality and magnitude for all participants. The acquisition session began immediately following the US exposures.

Delay training consisted of 30 paired CS-US trials (500 ms/1200 Hz pure tone CS overlapping and co-terminating with the US) followed by 20 CS alone extinction trials. Participants were randomly assigned to one of three possible ITI groups, short, long, or variable long ITI. The inter-trial interval for the short ITI ranged pseudo-randomly between 25 and 35 s with a mean of 30 s. The short ITI is the standard ITI from previous studies [1,3]. The inter-trial interval for the long ITI ranged pseudo-randomly between 52 and 63 with a mean of 57 s. The inter-trial interval for the variable long ITI ranged pseudo-randomly from 25 to 123 s with a mean ITI of 57 s (SD = 30.4). Both of the long ITI conditions were based on the temporal parameters from the previous partial reinforcement protocol [1]. The total length of the conditioning sessions was 25 min for the 30 s short ITI condition and 47.5 min for both the 57 s long ITI condition and the 25-123 s variable long ITI condition.

2.5. Signal processing and data reduction

EMG data were evaluated on a trial-by-trial basis for all participants. Processing of eyeblink responses followed methods previously reported [20]. To determine the occurrence of an eyeblink, EMG activity was first lowpass filtered with a Lowess filter (Stat-Sci, Tacoma, WA, USA) using a time constant of 0.025, and a smoothing interval of 5. For an eyeblink response to be scored, smoothed EMG activity in a 500-ms window beginning at the onset of the CS had to exceed the mean activity, plus 4 times the standard deviation, of the activity in a 125-ms comparator window that immediately preceded the CS window. An alpha (orienting) response was defined as an eyeblink occurring within 80 ms of CS onset, and this trial was not counted as a CR. A CR was defined as