

EFFECT OF EMOTIONAL STATE ON EYEBLINK CLASSICAL CONDITIONING IN HUMANS

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The emotional state affects reflexive eyeblink to a startle probe. In startle probes the unconditioned eyeblink reflex to an aversive stimulus is augmented when the subject is in an emotionally negative state comparing to the positive state. The present study examines how the emotional state affects classically conditioned eyeblink to a tone. In startle probes the performance of the reflex is modified by emotional state. On the other hand, in classical eyeblink conditioning the intensity of the stimulus affects learning in a way, that more intense stimulus enhances learning. Question is, whether emotionally negative state enhances performance of the reflex, or also processing of its eliciting stimulus, in which case learning would be different depending on the emotional state. It is hypothesized, that the conditioned eyeblink response to a tone is augmented when the subject is in an emotionally negative state comparing to the response in a positive state. Three groups of participants (N = 30) were shown either appetitive, neutral or aversive pictures in continuous series during eyeblink classical conditioning. Electromyographic activity was measured to assess the eyeblink response. The augmentation of the conditioned eyeblink response to the tone was weaker with the appetitive pictures than with neutral pictures. This effect is proposed to be result of stronger motivational conflict between the pleasant motivational state and the state caused by the aversive startle stimulus. Surprisingly, the eyeblink to the tone was not augmented with the aversive pictures. This effect is suggested to reflect anxiety in the subjects.

1. Introduction

Eyeblink classical conditioning (EBCC) paradigm has proven to be reliable and valuable tool for studying associative learning phenomena in mammals including humans (e.g., Steinmetz, 2000; Thompson, Thompson, Kim, Krupa & Shinkman, 1998; Anderson & Steinmetz, 1994; Harvey, Gormezano & Cool-Hauser, 1985). In classical eyeblink conditioning, association between the neutral conditioned stimulus (CS; e.g., a tone) and an aversive eyeblink reflex producing unconditioned stimulus (US; e.g., an airpuff to the eye) is developed. When the conditioned stimulus and the unconditioned stimulus are frequently presented temporally near to each other, the association between the stimuli is achieved relatively soon. Thus, the presentation of the CS eventually evokes a conditioned response (CR) that is similar to the unconditioned response (UR) followed after the US presentation. One of the reasons why the eyeblink classical conditioning has been under constant focus over the past decades is that once the basic ideas of the learning functions are understood, this provides better base for understanding the more complicated issues of human behavior (Lavond, Kim & Thompson, 1993).

The most remarkable results of the animal models of classical conditioning have been achieved in the search of the engram; that is, the localization of the memory trace. The

engram was first discovered in Richard F. Thompson's laboratory in the early 1980's (McCormick & Thompson, 1984). The engram was localized to the deep cerebellar interpositus nucleus after lesion studies ipsilateral to the conditioned eyelid responses. Since then a great deal of the neural circuit necessary for the discrete conditioned eyelid response has been clarified (e.g. Thompson et al., 1998; Anderson & Steinmetz, 1994). Thompson and his associates (1998) have, however, proposed that the neural circuit responsible for the discrete conditioned eyeblink reflex *per se* follows the Rescorla-Wagner rule, according to which the US loses its salience (i.e. novelty value) when the CS is invariably presented prior to it, and that the other aspects of the stimuli are processed in other parts of the brain. One critical site repeatedly proposed for processing the emotional content of the stimuli is the amygdala (Weisz, Harden & Xiang, 1992; LeDoux, 1993; Mintz & Wang-Ninio, 2001).

In the study of the human EBCC a remarkable amount of research has focused on clinical issues like Alzheimer's disease, Down's syndrome, autism and dyslexia regarding to the modulation of the CS-US association (e.g., Steinmetz, Tracy & Green, 2001; Nicolson, Daum, Schugens, Fawcett & Schulz, 2000). Another focus of the research has been the aging related issues of the human EBCC (e.g. Flaten & Powell, 1998; Woodruff-Pak, Romano & Papka, 1996). These views have left the study of associative learning among normal, healthy subjects to receive somewhat less attention recently.

The animal models of EBCC provide possibilities and advantages for study of associative learning that cannot be reached by using human subjects. The reversible lesions are one such advantage by which it is possible to specify the phenomena of the associative learning (e.g. Chapman, Steinmetz, Sears & Thompson, 1990; Wikgren, Korhonen & Ruusuvirta, 2002). The study of human emotions can account from these animal models of conditioning as well (Cardinal, Parkinson, Hall & Everitt, 2002). There are, however, more direct possibilities to study emotions.

According to Peter Lang and his associates, emotions are organized biphasically between appetitiveness and aversiveness on the emotional valence axis, and can be investigated in humans by using the eyeblink eliciting startle probe (Lang, Bradley & Cuthberg, 1990; Vrana, Spence, & Lang, 1988). Their view is that emotions are action dispositions and the startle reflex can be manipulated by motivational priming (Lang et al., 1990; Bradley, Cuthberg & Lang, 1996; Lang, Greenwald, Bradley & Hamm, 1993). Motivational priming hypothesis suggests that when an organism is at a certain motivational state, responses to the cues of the same valence are primed (Lang, Bradley & Cuthberg, 1997). Thus, the motivational state of an organism affects reflexive behavior. For example, if a person is in an emotionally unpleasant state, the defensive reflex to an aversive stimulus, like an airpuff to the eye, is augmented (Hytönen, 2002). Vice versa, in an emotionally pleasant state the reflex to the unpleasant stimulus is decreased. This suggestion has been tested reliably and successfully in many studies (e.g. Vrana et al., 1988; Lang et al., 1993; Bradley, Cuthberg & Lang, 1996b; Dichter, Tomarken & Baucom, 2002). However, these experiments fail to answer to the question, whether emotional state affects processing of the stimulus, as they reliably prove emotional state's ability to modify performance of the reflex.

The human EBCC and the startle probe share the same behavioral outcome, that is, the eyeblink reflex. In startle probes conducted with electrical stimulations of neural

structures of rats, it is demonstrated the acoustic startle circuit consisting of connections from cochlear root neurons via reticular pontis caudalis to the motor neurons responsible for the startle reflex, and that stimulation of the amygdala, which also has connections to the reticular pontis caudalis, enhances fear-potentiated startle reflex (Rosen & Davis, 1990). Conditioned component of the eyeblink response is mediated from the cerebellum through red nucleus and the trigeminal complex to the facial motor nucleus responsible for the eyeblink elicitation (Steinmetz, 2000; Clark & Lavond, 1996). On the other hand, the basic neural circuit responsible for processing the emotional content of the stimuli during conditioning consists of the projections from the sensory areas of the thalamus to the neocortical areas and to the amygdala (LeDoux, 1993). It has also been proposed that the trigeminal complex might interact with afferent connections to the amygdala (Mintz & Wang-Ninio, 2001; Neufeld & Mintz, 2001). Based on the studies accomplished with rats, it has been suggested that the essential neural substrates responsible for the fast emotional conditioning and the slower discrete motor conditioning are different, but interact with each other, although the behavioral outcome is the same (Mintz & Wang-Ninio, 2001). Thus, the reflexive outcome of the EBCC is modulated by two factors: the conditioned discrete motor reflex, and the processed emotional content of the stimuli presented (Weisz et al., 1992; Mintz & Wang-Ninio, 2001; Neufeld & Mintz, 2001).

The present study was designed to investigate in humans whether there are interactions between classically conditioned eyeblink reflex and the emotional state. This is carried out by examining whether the motivational priming, accomplished by looking at affective pictures during the eyeblink classical conditioning phase, affects the discrete conditioned eyeblink responses. Further, it is hypothesized that the emotional state affects the amplitude of the learned responses in different fashion between the three groups, which are aversive, neutral and appetitive. The groups of the present study are ordered according to either unpleasant, neutral or pleasant content of the pictures shown to the subjects during the conditioning phase. Since aversive motivational priming has been demonstrated to enhance unconditioned reflexive behavior to a startle probe and appetitive priming reducing it, and further, the behavioral outcome of the unconditioned and conditioned eyeblink reflex is the same, it is investigated whether the effects of the emotional state on conditioned eyeblink responses can be found in classical eyeblink conditioning in humans. If so, it can be concluded that emotional state, not only affects the performance of the reflex, but also processing of its eliciting stimulus.

2. Method

2.1. Participants

The volunteers of the experiment (N = 30; 25 female, 5 male) were normal, healthy people ranging in age from 18 to 53 years (Mean = 21.37; Md = 20). The volunteers did not receive any credit for their participation in the experiment.

2.2. Design and Materials

The EBCC design was a standard delay procedure with intertrial interval varying randomly from 20 to 30 s (Mean ITI = 25 s). Five CS-alone and five US-alone trials were presented before the conditioning phase. The conditioning phase consisted of 60 paired CS-US trials and six CS-alone and six US-alone trials which were presented

pseudorandomly among the paired trials. Three paired CS-US trials were presented randomly among the last ten unpaired test trials. Thus, the total number of the trials was 95.

The CS consisted of a 1 kHz tone presented binaurally by the headphones. The intensity of the tone was 77 db measured 1 cm distance from the surface of the headphone. The US consisted of an airpuff to the outer corner of the right eye approximately 1 cm distance laterally from the eye. The intensity of the airpuff was 0.5 bar (7.25 psi) delivered via a plastic tube (5 mm diameter), which was set at approximately 1 cm distance from the surface of the skin.

The duration of the CS was 350 ms and the US 100 ms. In the paired CS-US trials the delay between the stimuli was 250 ms, thus the stimuli overlapped 100 ms and then co-terminated. The EMG measurement began 250 ms prior to the CS onset and continued for 750 ms.

The participants were randomly assigned to one of three experimental groups; appetitive, neutral or aversive. In each group, 72 pictures selected from the International Affective Picture System¹ (IAPS) (Lang, Bradley & Cuthberg, 2001) formed the slide show used as the foreground stimuli. The pictures were selected according to their affective valence and arousal ratings. Of these two dimensions the affective valence refers to the pleasantness of the picture, ranging from pleasant to unpleasant. Arousal dimension refers to the activation level of either aversive or appetitive system ranging from calm to excited. In the IAPS, the ratings are scored such that 1 represents a low rating of both dimensions (i.e. unpleasant and low arousal) and 9 a high rating of both dimensions (i.e. pleasant and high arousal) (Lang, Bradley & Cuthberg, 2001). The subjects were shown pictures of the same valence according to the group she/he was assigned to. The appetitive pictures were chosen separately for female and male participants. The mean value of valence was 7.58 for appetitive female pictures and 7.51 for appetitive male pictures. The mean values of arousal of the female and male

¹ The IAPS picture codes:

Appetitive for female: 1340, 1811, 2050, 2058, 2071, 2150, 2160, 2208, 2209, 2216, 2224, 2344, 2345, 2352, 4520, 4532, 4535, 4538, 4572, 4599, 4603, 4606, 4610, 4614, 4623, 4624, 4626, 4640, 4641, 4660, 5260, 5270, 5460, 5470, 5480, 5621, 5623, 5910, 7220, 7230, 7260, 7270, 7330, 7400, 7430, 7502, 8030, 8033, 8134, 8041, 8080, 8090, 8162, 8170, 8185, 8190, 8200, 8210, 8350, 8370, 8380, 8420, 8460, 8470, 8490, 8496, 8500, 8501, 8502, 8503, 8531, 8540
Appetitive for men: 1710, 1811, 2030, 2340, 2391, 4002, 4141, 4142, 4150, 4180, 4210, 4220, 4232, 4235, 4240, 4250, 4255, 4275, 4279, 4290, 4300, 4302, 4310, 4320, 4607, 4608, 4611, 4626, 4641, 4656, 4658, 4659, 4660, 4664, 4670, 4680, 4683, 4687, 4690, 4800, 5260, 5460, 5470, 5480, 5621, 5623, 5660, 5700, 5910, 5982, 7230, 7270, 7502, 7580, 8030, 8080, 8120, 8170, 8180, 8185, 8190, 8210, 8300, 8340, 8341, 8370, 8380, 8400, 8420, 8470, 8502, 8510
Neutral: 2190, 2200, 2210, 2220, 2381, 2393, 2440, 2480, 2495, 2499, 2570, 2580, 2620, 2840, 2850, 2870, 2880, 2980, 5120, 5130, 5390, 5500, 5510, 5520, 5530, 5533, 5534, 5731, 5740, 7000, 7002, 7004, 7006, 7009, 7010, 7020, 7025, 7030, 7031, 7034, 7035, 7038, 7039, 7040, 7041, 7050, 7060, 7080, 7090, 7100, 7140, 7150, 7160, 7161, 7170, 7175, 7185, 7187, 7205, 7217, 7224, 7233, 7234, 7235, 7490, 7500, 7700, 7705, 7950, 9210, 9360, 9700
Aversive: 2095, 2800, 3000, 3010, 3015, 3030, 3051, 3053, 3060, 3061, 3062, 3063, 3064, 3068, 3069, 3071, 3080, 3100, 3101, 3102, 3110, 3120, 3130, 3140, 3150, 3168, 3170, 3180, 3181, 3230, 3261, 3266, 3301, 3350, 3400, 3500, 3530, 6212, 6230, 6243, 6313, 6315, 6350, 6360, 6415, 6540, 6560, 6570, 9006, 9040, 9140, 9181, 9252, 9253, 9300, 9301, 9405, 9410, 9420, 9421, 9433, 9435, 9560, 9570, 9571, 9800, 9810, 9910, 9911, 9921, 9340, 9500

appetitive pictures were 5.72 and 6.39. The aversive pictures' mean values were 1.97 for valence and 6.20 for arousal. The same values for the neutral pictures were 4.99 and 2.77.

Figure 1 illustrates examples of the pictures the subjects viewed during the conditioning phase. The appetitive slides included pictures of opposite-sex nudes, smiling children and appetizing food, and the neutral slides household objects, neutral faces and neutral nature pictures - for example mushrooms. The aversive slideshow consisted of pictures of mutilated faces, weapons and frightening animals – for example snakes and spiders.

2.3. Signal processing

The two electrodes used to measure the electromyographic (EMG) activity of orbicularis oculi below the outer canthus of the right eye were disposable Ag/AgCl electrodes filled with electrolyte pasta. A ground electrode was located at the midline of the participant's forehead (see Lang et al., 1990).



Figure 1. Examples of the pictures shown to the subjects during the conditioning phase. The first picture is an example of the appetitive (valence = 8.34; arousal = 5.41), the second of the neutral (valence = 4.88; arousal = 2.33) and the third of the aversive (valence = 1.91; arousal = 6.76) pictures. In each group 72 pictures were shown continuously through the conditioning phase.

The experiment was controlled and timed by the BRACE[®] computer program on an IBM 386 computer. The raw signal measured was amplified by a gain of 25000 and band-pass filtered from 60 to 500 Hz. The filtered signal was then sampled at 1000 Hz beginning at 250 ms prior to the CS onset and continuing 750 ms. The signal obtained was baseline corrected and rectified before digital low-pass filtering with a 30 Hz cut-off. The signal was then averaged over subject and the group for calculating the values for analyses.

2.4. Procedure

After arrival to the laboratory, the volunteers read and signed an informed consent agreement, in which they were also asked about possible diagnostic phobic or panic disorder. Volunteers with such disorder would have not been accepted to take part to the experiment. However, none of the volunteers were excluded. The participants were then verbally given a brief description of the experiment before placing the electrodes on and guiding them into the experiment chamber.

The experiment chamber was a dimly lit room where the participants sat with a 17" computer screen approximately 1,5 m in front of them. The participants were told to

focus to the pictures on the screen and try not to mind the tones and the airpuffs presented. The participants were also informed that they could abort the session whenever they felt like it. The airpuff nozzle and the headphones were placed and the electrodes were wired to the amplifier before the participants were randomly assigned to one of the three experimental groups; aversive, neutral or appetitive. The slide show was started by the experimenter at the onset of the conditioning phase, that is, after the initial unpaired trials. The slide show was aborted before the onset of the test trials after the conditioning phase.

The slide show consisted of pictures of the same valence shown randomly each for 30 seconds. There was no delay between the pictures. The experimenter aborted the slide show at the end of the conditioning phase. Debriefing was accomplished after the experiment.

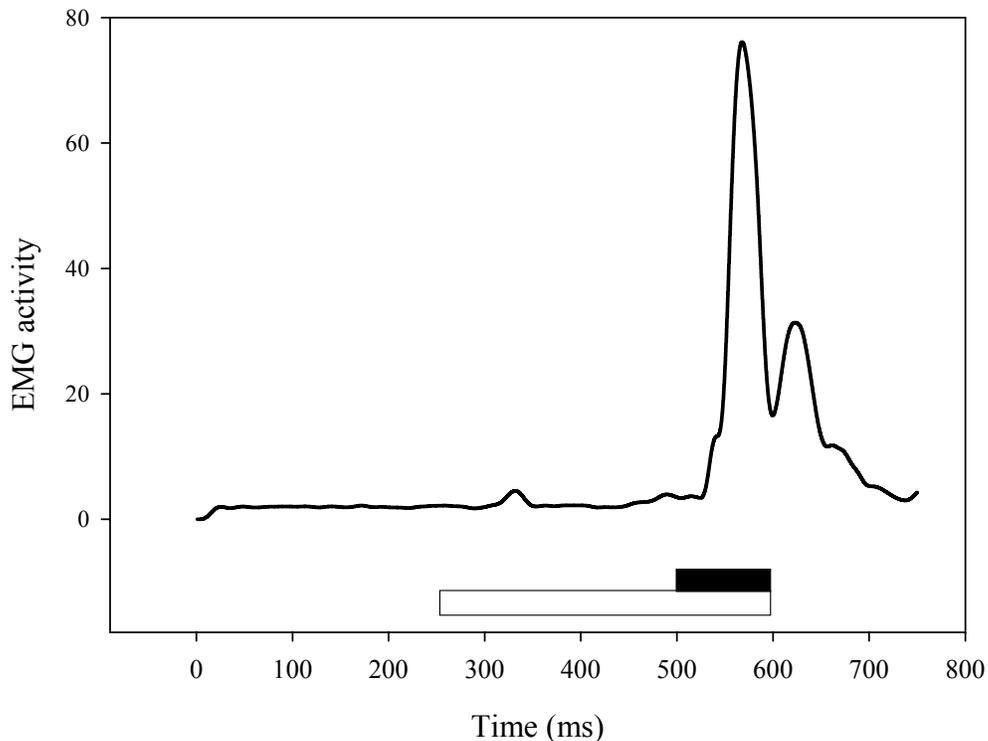


Figure 2. An illustration of a typical EMG activity of an individual subject over a paired trial drawn from filtered data. The CS (white rectangle) was presented at the point of 250 ms and the US (black rectangle) at the point of 500 ms. The stimuli overlapped 100 ms and then co terminated.

2.5. Data analysis

The statistical analyses were performed by the SPSS 11.0 for Windows program. The data was divided into six blocks, of which the first consisted of the initial ten trials and the next four blocks of the trials during the conditioning phase (18 trials in each block). Thus, the sixth block, that is the test trials, consisted of the last 13 trials.

In the CS-alone trials the CR was defined as the maximum value of the mean amplitudes of eyeblink between 401 ms and 750 ms.

The effects of the conditioning and the slide show content on CR amplitude was analyzed by using the analysis of variance (ANOVA) for repeated measures and the paired samples t-test for planned comparisons. The statistical design for the initial trials and the test trials was a 3 (group: appetitive, neutral, aversive) X 2 (blocks: first, sixth). The design for the conditioning phase was a 3 (group: appetitive, neutral, aversive) X 4 (blocks: 18 trial blocks).

Degrees of freedom were corrected by the Greenhouse-Geisser correction if necessary. The significance level was .05 in all analyses.

3. Results

Figure 2 demonstrates a graphic illustration of a typical sample of EMG activity in a paired trial. The temporal presentations of the stimuli are also shown.

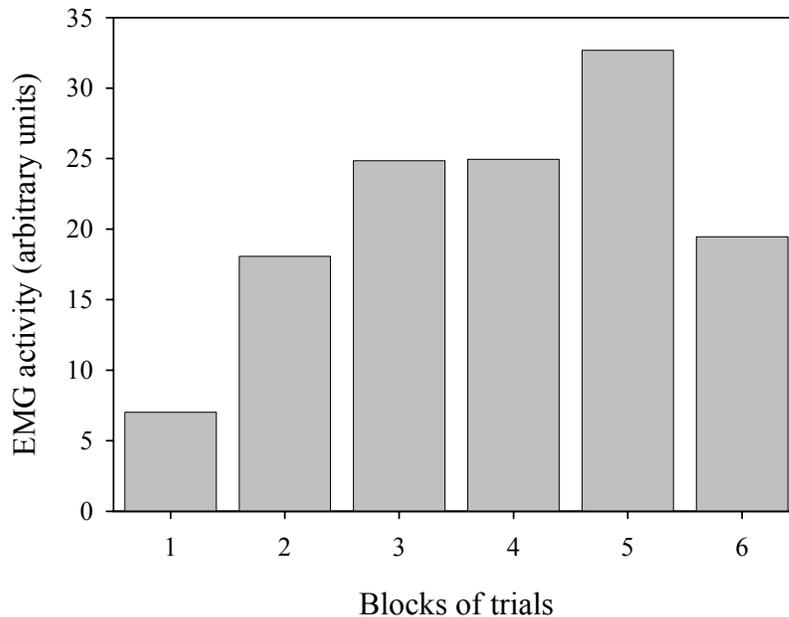


Figure 2. The EMG activity to the tone increased from the first to the last block in the CS-alone trials. The first block consists of the ten initial trials. The conditioning phase consists of the blocks from 2 to 5. The last block consists of the 13 test trials. The pictures were shown during the conditioning phase only.

As seen in Figure 3, the delayed EMG-response to the tone increased from the first to the last block. ANOVA for repeated measures showed a significant difference in amplitude of the CR between the blocks $F(5, 145) = 8.88; p < .001$. More detailed comparison revealed that there was also statistically significant interaction between the blocks and the groups $F(2, 27) = 4.02; p < .05$. These results indicate that the conditioning had an effect, and that this effect was different between the groups.

The difference in the augmentation of the CR amplitude between the groups is demonstrated in Figure 4. Planned comparisons of the first and the last block revealed

that the conditioned eyeblink response was augmented the most within the neutral group $t(9) = 2.77; p < .05$. There was also significant augmentation of the CR amplitude within the appetitive group $t(9) = 5.05; p < .001$. Interestingly enough, there was no significant difference in the CR amplitude between the first and the last block within the aversive group.

The conditioning phase had an effect on the CR amplitude $F(3, 81) = 4.91; p < .05$. Comparing the means revealed that the mean amplitudes of the CR were highest within the neutral group and lowest within the aversive group throughout the conditioning phase. Furthermore, during the conditioning phase, there was no significant interaction between the blocks and the groups. The only significant difference between the groups was found in the fifth block of trials $F(2, 27) = 3.38; p < .05$.

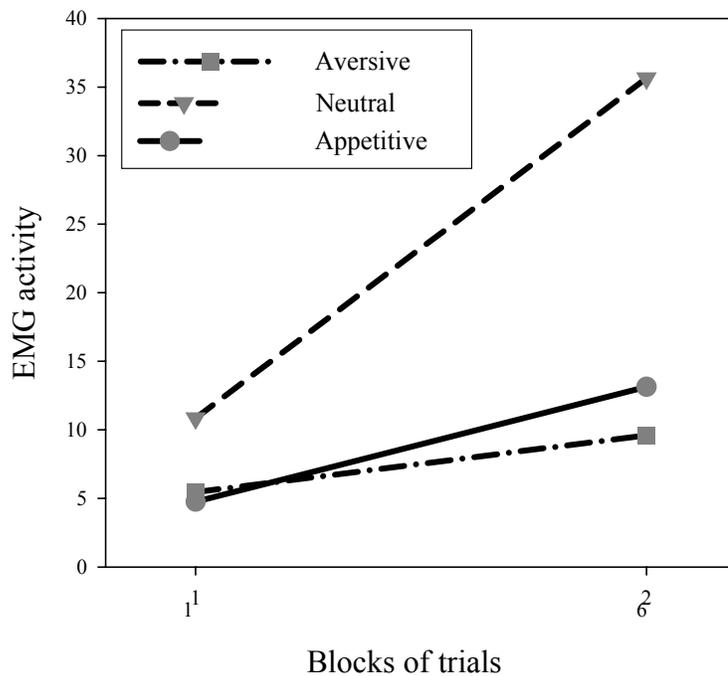


Figure 4. The EMG activity increased the most within the neutral group. Within the aversive group the difference between the blocks was not significant

4. Discussion

The amplitude of the eyeblink response to the tone was increased from the first block of trials to the last block of trials. In line with several other studies conducted with human subjects this result indicates that the conditioning had an effect over the session (Flaten & Powell, 1998; Woodruff-Pak, 1993; Nicolson et al., 2002).

The results of the present study also confirm the hypothesis that emotional state affects learning. As hypothesized, the augmentation of the eyeblink reflex to the tone was inhibited within the appetitive group as compared with the neutral group. This is in line with the motivational priming hypothesis, according to which stronger motivational

conflict between the foreground stimulus and the reflex-eliciting stimulus leads to diminution of the response (Lang et al., 1997). The pleasant pictures viewed during the conditioning phase together with the aversive US, that is the airpuff to the corner of the eye, resulted in stronger motivational conflict among the participants comparing to the neutral pictures and the airpuff, thus diminishing the learned responses. In the startle probe experiments conducted with human subjects, motivational priming is reported to affect the UR (e.g. Lang et al. 1990, Vrana et al., 1988). However, in the current study, motivational priming within the appetitive and the neutral group affected the CR in the same fashion as it affects UR in the startle probes, indicating that emotional state affects processing of the stimulus. Surprisingly, the amplitude of the learned responses was lowest within the aversive group, which is in contrast with the hypothesis of the present study, as the smaller motivational conflict between all the stimuli during conditioning phase was hypothesized to enhance learning. Closer analysis revealed that there occurred no significant learning when the subjects were looking at the unpleasant pictures.

In the present study, the aversive context lead to the inhibition of the learned responses, which is in contrast with results of emotional conditioning probes. Namely, it is found that aversive preconditioning enhances the conditioned responses when the same CS is used. Neufeld and Mintz (2001) conducted an experiment with rats, in which the CS was first presented with a periorbital electrical shock, and after the rats were learned the unpleasant context the CS was presented in, the same CS was used in classical conditioning, thus resulting in an enhancement of learning. Mintz and Wang-Ninio (2001) hypothesize on the basis of emotional conditioning probes, that amygdala and cerebellar motor CR are in close interaction, so that acquired motor CR diminishes amygdalar activation. It is argued that lesioned cerebellum prevents the development of the motor CR, which in turn prevents diminution of the amygdalar activation (Mintz & Wang-Ninio, 2001). It is however possible, in the light of the results of the present study, that summation of the amygdalar activation produced by the foreground stimulus and the aversive US affects the learned motor CR in different fashion than it affects the UR. This derives from the finding that smaller motivational conflict between the context and the aversive stimulus with the aversive group compared to the neutral and appetitive group did not produce augmentation of the eyeblink response. This idea is promoted by the suggestion that the hippocampal emotional context interacts with the US in the amygdala, thus affecting the conditioning (LeDoux, 1993). Thus, the interaction between the emotional CR and the motor CR may be more complicated than Mintz and his associates suggested.

In the current study, the finding that there was no significant learning within the aversive group can be affected by three issues. Firstly, it may partly be a result of the experimental settings. The foreground stimulation presented to the subjects was of very high intensity, because the pictures were shown continuously through the session. In the startle probe studies the intensity of the foreground stimulus has not been as high. In those studies the pictures have been shown to the subjects 6 seconds each with interpicture intervals varying from 6 to 24 seconds (e.g. Vrana et al., 1988; Bradley et al., 1996). Continuous aversive context in the current study might have lead to anxiety among the participants, which in turn could have disturbed learning. The exact nature of how the foreground stimulus of lower intensity affects the amplitude of the learned eyeblink responses especially in an aversive context remains to be studied, for instance,

by replicating the current study with the pictures shown during the conditioning trials only for six seconds each with an interpicture interval same as the intertrial interval, in order to achieve lower intensity of the foreground stimulus.

Secondly, the results of the current study can be interpreted in terms of orienting model proposed by Öhman (1979). According to this model incoming emotional stimuli are processed in a central capacity limited channel after automatic preattentive state and before interpretation to emotional states (Öhman, 1987). Further, it is suggested that fear-relevant stimuli are processed at a non-aware level but are able to affect behavior (Öhman, 1992). In terms of information processing model it is therefore possible that in the current study the fear-relevant aversive emotional foreground stimulus of high intensity occupied the central channel capacity among the subjects in the aversive group, thus leading orientation towards the unpleasant context and interfering with the information processing required for parallel associative learning. The orienting model can also explain the finding that the eyeblink to the tone was augmented more in the neutral group comparing to the appetitive and aversive group, because it could be argued that the neutral pictures were not as complex or interesting as were the pleasant and the unpleasant pictures, thus producing less attentional competition towards the CS and US.

Thirdly, it is possible that the interstimulus interval of the current study was not optimal. Common ISI in the human delayed eyeblink classical conditioning procedures has been 400 ms (e.g. Woodruff-Pak et al., 1996; Woodruff-Pak, 1993; Flaten & Powell, 1998). The interstimulus interval (250 ms) in the current study was thus relatively short. It is possible that a longer delay between the CS and the US would affect the amplitude of the CR. Namely, it has been demonstrated that the ISI has an optimal range for reflex facilitation in rabbits (Weisz et al., 1992). There is also evidence that the interstimulus interval has an optimal range between 250 ms and 500 ms also for the conditioning in rabbits (Steinmetz, 1990, Steinmetz, 2000). Since the length of the interstimulus interval has an effect on reflex facilitation, and on the other hand the optimal ISI for classical conditioning in rabbits is shorter than in humans, it is reasonable to consider that lengthening the ISI could affect the amplitudes of the learned responses.

One might argue that the 250 ms ISI of the current study could have resulted in the prepulse inhibition effect to the reflex eliciting airpuff. Prepulse inhibition (PPI) is seen to be an indicator of nonaware preattentive state during which an organism's ability to filter rapid relevant information is enhanced, and is defined as suppression of the startle reflex when it is preceded by a stimulus of lower intensity (Postma, Kumari, Hines & Gray, 2001). However, there is evidence that the interstimulus interval of the current study is too long to be able to produce PPI effect. Namely, Postma and her associates (2001) found that lengthening the prepulse stimulus and the pulse stimulus onset asynchrony, that is the interstimulus interval, from 120 ms to 200 ms significantly reduced PPI from 75% to 14%. The optimal ISI in their study was 120 ms (Postma et al., 2001). Other scientists have also reported that the optimal ISI for prepulse inhibition is approximately 100 ms (e.g. Swerdlow, Shoemaker, Stepany, Wasserman, Hyun & Geyer, 2002). Further, in the current study the learned reflexes were suppressed only within the aversive group while the responses within the appetitive and the neutral group were not. To my knowledge, there is no evidence that motivational priming would enhance the PPI.

In summary, the responses to the tone in the present study were augmented from the first to the last block of trials indicating that conditioning had an effect. In the appetitive

group the augmentation of the conditioned eyeblink response was weaker than in the neutral group. This effect is suggested to be result of the stronger motivational conflict between the emotional context, elicited by pictures, and the unconditioned stimulus in the appetitive group. Surprisingly, in the aversive group the responses to the tone were not significantly augmented indicating possibly anxiety in the subjects. This is in contrast with the hypothesis, as the smaller motivational conflict between the stimuli during the conditioning phase was hypothesized to augment the learned responses. However, since learning was different between the groups, it can be concluded that emotional state affects processing of the reflex eliciting stimulus, thus affecting learning.

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