EFFECT OF EMOTIONAL PICTURE VIEWING ON CLASSICAL EYEBLINK CONDITIONING

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EMOTIONAALISIA TILOJA HERÄTTÄVIEN KUVIEN KATSELUN VAIKUTUS KLASSISESSA SILMÄNISKUEHDOLLISTUMISESSA

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Klassisessa ehdollistumisessa ehdollistumisprosessin on todettu vaikuttavan ehdottomaan reaktioon myös ehdottoman ärsykkeen puuttuessa. Tämä tunnetaan refleksimodifikaationa ja sen on todistettu korreloivan vahvasti oppimisen määrän kanssa. Myös emotionaalisella tilalla on vaikutusta reflekseihin niin, että negatiivinen emotionaalinen tila voimistaa refleksejä verrattuna positiiviseen tai neutraaliin emotionaaliseen tilaan. Tämän tutkimuksen tarkoituksena oli selvittää onko emotionaalisella tilalla vaikutusta oppimiseen klassisessa ehdollistumisessa sekä refleksimodifikaatioon. Ilmapuhallus silmän ulkonurkkaan toimi ehdottomana ärsykkeenä ja ääni ehdollisena. Lihassähkökäyrää (EMG) käytettiin silmänräpäytyksen voiman mittarina. Emotionaalinen tila aiheutettiin katsomalla joko miellyttäviä, epämiellyttäviä tai neutraaleja kuvia. Jokainen 31 koehenkilöstä katsoi vain yhden kategorian kuvat. Tulokset osoittivat, että oppiminen oli merkittävintä neutraalissa ryhmässä ja alhaisinta epämiellyttäviä kuvia katsoneessa ryhmässä. Refleksimodifikaation osalta eroja ryhmien välillä ei ollut. Refleksin aiheuttavan ärsykkeen voidaan katsoa toimineen uutena ärsykkeenä neutraalilla ryhmällä ja siten tehostaneen oppimista, kuten Rescorlan ja Wagnerin teoriassa esitetään.

Avainsanat: Silmäniskuehdollistuminen, Refleksimodifikaatio, Emootio, Vireystila

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Abstract

In classical conditioning, the conditioning process has proved to have effects on an unconditioned response (UR) also without the conditioned stimulus (CS). This is known as a reflex modification and its magnitude has been found to have strong correlation with degree of learning. Emotional state also has an impact on reflex amplitude, for example, negative emotional state has been shown to augment reflexes compared to the positive or neutral state. In the present experiment, the aim was to study whether the emotional state has an impact on learning rate in classical conditioning and reflex modification. An airpuff to the outer corner of the eye acted as an unconditioned stimulus (US) and a tone as a CS. Electromyographic (EMG) activity was used as a measure of the eyeblink magnitude. Emotional state was caused by viewing either appetitive, aversive or neutral pictures. Each of the 31 subjects viewed pictures of only one category. Results showed that learning were largest within a neutral group and lowest within an aversive group. No differences between the groups were found in the amount of reflex modification. The reflex-eliciting stimlus is discussed to serve as a more novel stimlus in neutral group, because of the low arousal compared to the other two groups, and causing this way faster learning as predicted by Rescorla and Wagner model of classical conditioning.

Keywords: Eyeblink conditioning; Reflex modification; Emotion; Arousal

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1. Introduction

In classical conditioning a conditioned response (CR) is expected to emerge because of the affect of pairing a conditioned stimulus (CS) - an unconditioned stimulus (US). In other words, after the pairing of the CS and US, a response to the CS alone is expected to appear. In the human eyeblink conditioning, a small air puff in the corner of the eye is used as a US, while a light or a tone acts as a CS. Because of an aversive nature, an eyeblink conditioning has been used in fear conditioning both in humans and mammals (e.g. Grillon, 2002; Neufeld & Mintz, 2001). The eyeblink reflex has proved to be useful in studying learning (Gormezano, Schneiderman, Deaux & Fuentes, 1962).

In addition to the previous, in animal studies classical conditioning has noted to have effects on an unconditioned response (UR) in the absence of the CS (Schreurs, Oh, Hirashima & Alkon, 1995). This phenomenon is known as reflex modification and it is assumed to represent the plastic changes leading to learning. Harvey, Gormezano & Cool-Hauser (1985) found a strong correlation (+0.86) between the degree of reflex facilitation and the degree of learning. Reflex modification specific to the conditioning is proved to be a reliable and sensitive indicator of classical conditioning processes in human subjects (Flaten & Powell, 1997).

There is also evidence that the UR can be modified as a function of CS-US pairings and not just as the function of experience (Schreurs et al. 1995), which indicates there to be more than one type of facilitation of the reflex. Wikgren, Korhonen and Ruusuvirta (2002) track three types: an experience-related, a CR-related and a CS-mediated reflex facilitation. Experience-related reflex facilitation emerges in mere exposure to the experimental setting without a relation to the forward CS-US pairing. CR-related reflex facilitation is seen when CR emerge and it is linked to the UR in US-alone trials, but only after a robust level of conditioning is reached. Interesting type of reflex facilitation is the CS-mediated one found even before a robust level of CR is achieved. This type is linked to the temporal closeness of the CS, causing greater UR in paired trials as compared with US-alone trials. The CS-mediated reflex facilitation is assumed to relate to a conditioned emotive state (Wikgren et al. 2002). Buck, Seager and Schreurs (2001) found conditioning-specific reflex modification to be general phenomenon but might being depending on the aversiveness of the US.

Emotional state of the subject has also an impact on a reflexive behavior. Eyeblink magnitude has proved to be smallest during viewing of pleasant pictures, intermediate during neutral pictures, and greatest during unpleasant pictures (Greenwald, Bradley, Cuthbert, Lang, 1998; Hamm & Stark, 1993). Impact of an emotional state is too seen in heart rate acceleration and an increased skin conductance while watching negative content pictures (Bradley, Cuthbert & Lang, 1996, Dimberg, 1987). Moreover, brain potentials are shown to be specifically enhanced for more emotionally intense pictures (Cuthbert, Schupp, Bradley, Birbaumer & Lang, 2000).

Although, emotional state is a complex unity, it can also be understood in terms of two primitive strategic dispositions, valence and arousal. Valence refers to positive or negative value of the stimulus and arousal the energy of force in which an organism is reacting. This classification is supported by the outcome, where a startle magnitude is directly related to the affective valence of cognitive processing (Lang, Bradley &

Cuthbert, 1990). Further, presentation of a series of affective pictures of similar valence produces emotional reactions that are either maintained or sensitized and they do not habituate (Bradley, Cuthbert & Lang, 1996).

Nitschke et al. (2002) suggests that emotional effects enhancing in startle potentiation is universal phenomenon and not to be modulated by individual differences. He and his colleagues found no difference between anxious and normal subjects (Nitschke, Larson, Smoller, Navin, Pederson, Ruffalo, Mackiewicz, Gray, Victor & Davidson, 2002). But contrary, Sloan, Bradley, Dimoulas and Lang (2002) found difference in EMG activity between the dysphoric and normal persons when watching unhappy or happy facial expressions. An experiment merely with normal subjects found emotional state to affect on processing of the reflex-eliciting stimulus and in this way affecting learning (Niinivirta, 2003).

In the present study the aim was to quest if the emotional context affects learning rate and if so, does this happen because of reflex modification. In the light of previous studies, where emotional state has proved to affect UR amplitude (e.g. Lang, Bradley & Cuthbert, 1990) and a stronger US leading to enhanced learning (Buck, Seager & Schreurs, 2001), it was hypothesized that learning would be different under different emotional states and this effect would also be seen divergences in reflex modification. Furthermore, according to Lang, Bradley and Cuthbert (1990) emotional state modifies responsiveness to the stimuli eliciting reflex, which is shown as a larger response to the reflex-eliciting stimulus in aversive content than in positive content. This lead to an assumption that learning would be better while watching aversive pictures compared to pleasant or neutral picture watching.

2. Methods

2.1 Participants

Thirty-one (27 female) healthy volunteers, approximate age range 18-53 years (Mean = 21.37; Median = 20) were studied. All subjects were students of the University of Jyväskylä and they did not get any payment for their participation. Each participant was randomly assigned to one of three experimental groups (appetitive, neutral or aversive).

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[1]Valence and arousal ratings.
Appetitive, female: (Arousal: 5.00 - 7.00; mean 5.72 and valence: 6.74 – 8.62; mean 7.58.)
Appetitive, male: (Arousal: 5.05 – 7.72; mean 6.39 and valence: 6.84 – 8.39; mean 7.51.)
Neutral: (Arousal: 1.72 – 3.29; mean 2.77 and valence: 4.03 – 5.93; mean 4.99.)
Aversive: (Arousal: 5.04 – 7.26; mean 6.20 and valence: 1.80 – 2.42; mean 1.97.)

2.2 Design

Emotional state was manipulated by presenting pictures of pleasant, neutral or unpleasant content from International Affective Picture System (IAPS; Lang, Greenwald, Bradley & Hamm, 1993). Each group contained 72 emotional pictures selected from the IAPS based on their valence and arousal ratings[1] (Lang, Bradley & Cuthbert, 2001). Female appetitive pictures differed from male appetitive pictures[2].

Appetitive contents included pictures example of babies, sport events and erotic couples, neutral pictures included example of household objects and neutral faces and aversive pictures included mutilations, guns, etc. Each picture was presented 30s in random order with no delay between them. Pictures were seen at a 17" computer screen placed about 1,5 m in front of the participant.

Eyeblink caused by an airpuff to the outer corner of the right eye acted as an unconditioned stimulus. The airpuff proceeded through a 5 mm diametric plastic tube attached in a cycling helmet. It was aimed about 1 cm side the eye and set approximately 1 cm distance from the surface of the skin. Intensity of the airpuff was 0.5 bar (7.25 psi) and duration 100ms. Eyeblink magnitude was measured by recording electromyographic (EMG) activity with two disposable Ag/AgCl electrodes filled with electrolyte pasta. These electrodes were placed over the orbicularis oculi (see Lang et al. 1990), further a third electrode in participant's forehead worked as a ground. The acoustic stimulus (CS) was a 1 kHz tone presented binaurally though stereo headphones. It lasted 350 ms and its intensity measured 1cm distance from the surface of the headphones was 77db.

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[2]The IAPS slide numbers were as follows:
Appetite 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, 8034, 8041, 8080, 8090, 8162, 8170, 8185, 8190, 8200, 8210, 8350, 8370, 8380, 8420, 8460, 8470, 8490, 8496, 8500, 8501, 8502, 8503, 8531, 8540;
appetite for male: 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

In this delay classical conditioning procedure CS-US interval was randomised from 20 to 30s. In order to have a control base, five CS-alone and US-alone trials were presented before and after the slide show. The conditioning phase contained sixty paired CS-US stimulus within pseudorandomly presented six CS-alone and six US-alone trials. The structure of the session can be seen in table 1.

Table 1. The structure of the delayed paired session.

	no pictures										**IAPS picture slide show**														
Trial:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Type:	cs	us	cs	us	cs	us	cs	us	cs	us	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	us	cc	cc	cc	cc
Trial:	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
Type:	cc	cc	cc	cc	cc	cc	cc	us	cc	cc	cc	cc	cc	cc	cs	cc	cc	cc	cc	us	cc	cc	cc	cc	cc
Trial:	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
Type:	cs	cc	cc	cc	cc	cc	us	cc	cc	cc	cc	cc	cc	cs	cc	cc	cc	cc	us	cc	cc	cc	cc	cc	cc
											no pictures														
Trial:	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95					
Type:	cs	cc	cc	cc	cc	us	cc	cs	us	cc	cs	us	cs	cc	us	cs	cc	us	cs	us					

cs = conditioned stilmulus, us = unconditioned stimulus, cc= cs+us

2.3 Procedure

After arrival at the laboratory, participants read and signed an informed consent form. Possible fobic or panic disorder diagnoses were ruled out in this phase. After this, participants were told that a series of slides, which could be pleasant, neutral or unpleasant, would be presented. The electrodes were attached and participants were told that during the experiment they would get an occasional airpuff to their right eye corner and a tone from the headphones. It was instructed to not a care about these and to concentrate on the pictures.

Participants were seated in a small dimly lit room were the cycling helmet and the headphones were placed on their heads. It was made clear that they would be free to stop the experiment at any time. The willingness to continue was asked in the middle of the experiment, which also was told to participants beforehand. Nobody wanted to stop the experiment in any phase. After the experiment participants had a small debriefing.

2.4 Data scoring and analysis

The data was collected by the BRACE© computer program and divided into the six blocks by the SPSS 11.0 for Windows program, by which the statistical analyses were performed. The six blocks were divided so that first one consisted of first ten trials, the four next blocks consisted of trials during the slide show and the last sixth block consisted of last 13 trials.

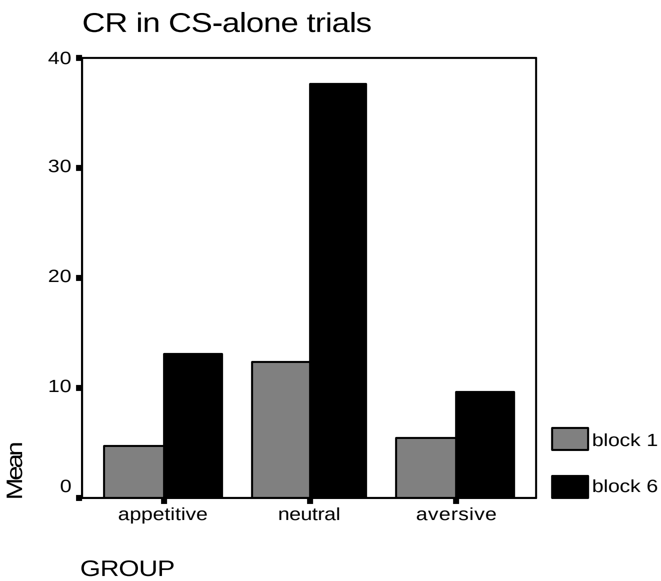
The EMG signal measure was baseline corrected and rectified after the amplification (gain of 25000) and filtering from 60 to 500 Hz. The signals were then digitally low-pass filtered with a 30 Hz cut-off. Averaged signals over the subject were calculated from the 250 ms period before the CS onset continuing to 750ms. A maximum value of averaged eyeblink amplitudes 150 ms after the CS was defined as CR, and 250 ms after the US as UR.

Statistical analyses were conducted using analyses of variance (ANOVA) with repeated measures and for planned comparisons of means were made with independent samples t-tests. Degrees of freedom were corrected (Greenhouse-Geisser) if necessary. Changes in CR in CS-alone trials likewise in UR in US-alone trials were assessed in 3 × 2 (Group × Block 1 and 6). Differences in UR between US-alone trials and paired trials in block 6 were assessed in 2 × (US-alone and paired trial) Group (appetitive, neutral, aversive). Further, the impact of the CS to the US was assessed in 3 × 2 (Group × Block).

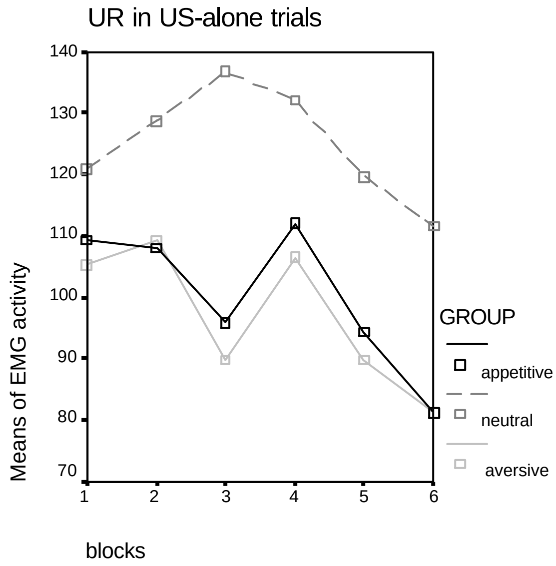
3. Results

Conditioning was inferred from significant difference in reactions to the CS between block 1 and block 6 in all groups, $F = 17.06$, $p < .05$. Also, UR differed significantly among the groups, $F(2,28) = 4.58$, $p < .05$. Reactions to the CS were significantly greater in block 6 in neutral than appetitive group, $t(19) = 2.27$, $p < 0.05$ (one tailed) likewise with neutral and aversive group, $t(19) = 2.57$, $p < .05$ (one tailed). Noteworthy is that almost as equal difference was presented already before the conditioning, $t(19) = 2.14$, $p < .05$, Between the appetitive and aversive group the differences were not significant, Figure 1.

Figure 1.



Compared first block to last block in US alone trials there was a significant difference in all groups, $F(1,28) = 14.26$, $p < .05$ but the interaction was not significant, Figure 2. In other words learning related reflex modification did occur, but the effect of the US was same in all groups. In blocks 1 and 3 the main effect of US was not significant, $F(1,28) = .57$, $p > .05$, when interaction between the groups was $F(2,28) = 3.15$, $p < .05$.



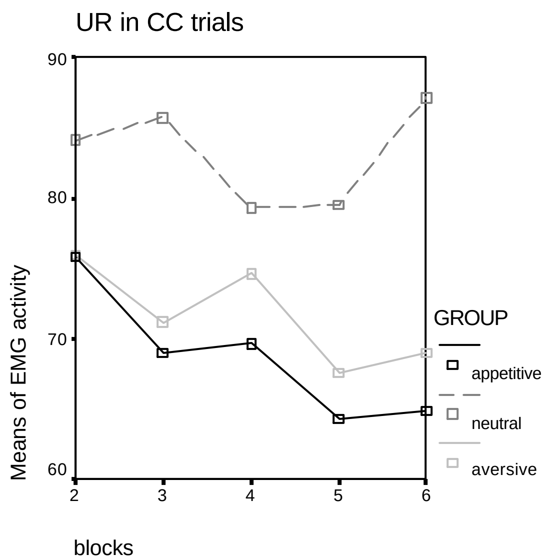
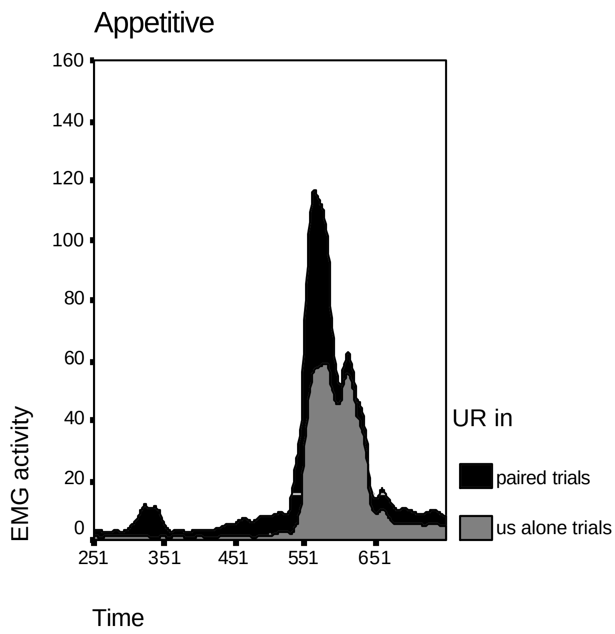
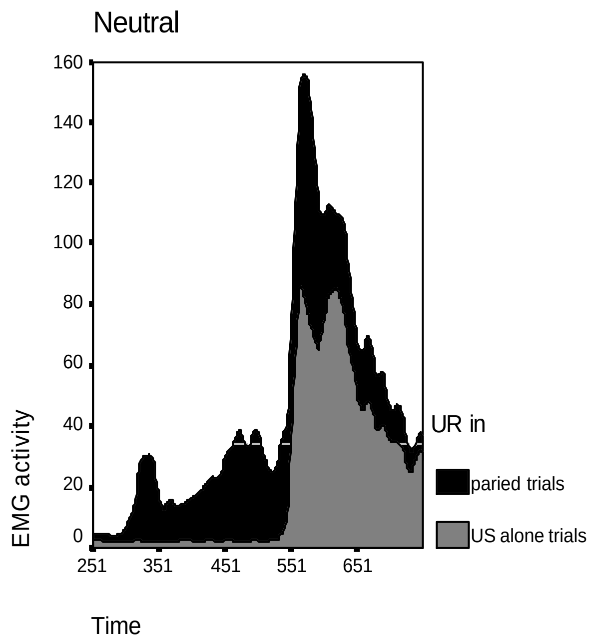


Figure 2. On the upper picture there is seen mean of the EMG-activity after the US-alone trials in every six blocks. The lower picture presents mean of the reactions after the paired trials in every block. The difference in the output level between the neutral and the two other groups is seen in both pictures. Note the difference on EMG scales.

Differences in UR period in paired trials were significant, $F(1,28) = 19.76$, $p < .05$. The impact was though similar in all groups $F(2,28) = .85$, $p > .05$, Figure 2. The impact of the pictures was closely regarded by comparing the last block with pictures (block 5) to block with no pictures (block 6). Significant difference in UR was seen in all groups, $F(1,28) = 4.74$, $p < .05$, but again it was similar in all groups.





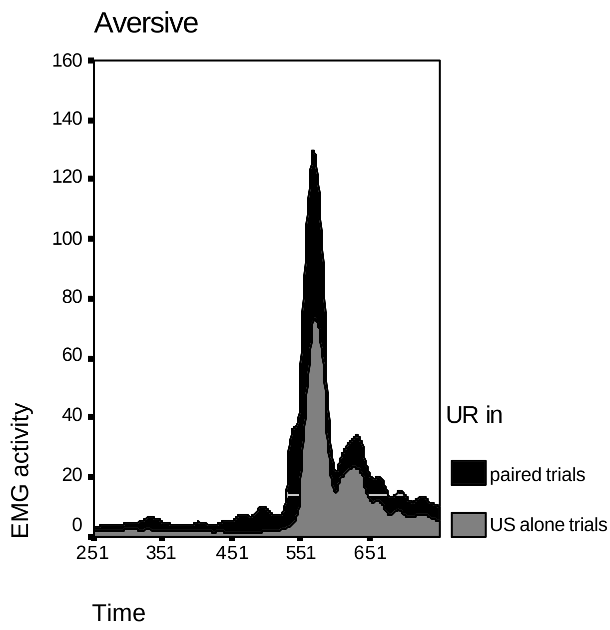


Figure 3. EMG activity after the conditioning phase (block 6). There were no significant differences between the groups in the amount of change of the UR, $F(2,28) = .85$, $p > .05$. In paired trials there were also no significant difference between the groups $F(2,28) = .87$, $p > .05$.

4. Discussion

Learning occurred in all groups, but reactions were largest within neutral group and lowest within aversive group. This is an opposite result compared to other studies (Greenwald, Bradley, Cuthbert & Lang, 1998; Hamm & Vaitl, 1996) and contrary to what was expected. Changes in the UR after US-alone trials were significantly different in all groups. Though, there were no differences between the groups. The unconditioned reaction to the US grew from first to the third block in neutral group, while in appetitive and aversive group it fell down. This may be caused by the differences in arousal of the pictures. With paired trials the differences in UR were significant, but the interaction between the groups was not seen. The impact of pictures was same in every group.

Differences between the neutral and other two groups could be explained because of the difference in arousal. With neutral group mean arousal of pictures was 2.77, while with aversive group 6.20 and with males 6.39 and females 5.72 in appetitive group. Eyeblink reflex is proved to modulate by emotional arousal, though results were opposite compared to the present experiment. In Lang, Bradley and Cuthbert's study (1998) startle response was greater during aversive arousal and smaller during appetitive arousal. Similar results came from Grillon's (2003) study, where the rate of CR was smaller in the appetitive group and greater in the aversive group, compared to the neutral group. But according to Grillon's study, physiological arousal measured from the skin conductance had only little effect on eyeblink conditioning. In the light of this result it could be concluded that arousal does not explain different learning results in the present experiment.

On the other hand, according to Rescorla and Wagner's theory (Thompson, Thompson, Kim, Krupa & Schinkman, 1998), the degree of learning depends on the surprisingness of the reaction-eliciting stimulus. In general that is to say, that when the surprise value of the US is great, learning occurs faster compared to the lower surprise value. In this experiment, it can be concluded that the surprise value of the US has been greatest in neutral group because of their lowest arousal rate. Arousal refers to the readiness to act, so there is an expectation that something will happen. With neutral group, arousal, as well as, expectation of surprising occurrence was low, when the airpuff was reacted as unexpected and surprising. This could explain better learning in neutral group compared to the appetitive and aversive group. It also explains why the reactions to the US grew in neutral group at the onset of the picture watching and why the reactions were opposite with appetitive and neutral group. Moreover, in group with aversive pictures the novelty of the stimulus, while being uncomfortable in nature, was lower compared to the group with appetitive pictures, which explains why learning was lowest in aversive group.

However, this does not explain why the results were not consistent with the previous studies. There are some differences in experimental settings, which might explain the different outcomes. First, the slide duration varied in previous studies between 2 s (Grillon, 2003) to 8 s (Hamm, Greenwald, Bradley & Lang, 1993), where interslide intervals varied between 20s and 35s in the latter experiment. In the current experiment the slide duration was 30 s without an interval, which means that the stimulation has been much more intense than before. Second, in Greenwald et al. (1998) and Hamm et al. (1993) studies each subject exposured each of the three valence categories, whereas in this study, subjects were randomised in different groups. This may have had something to do with the results of arousal having no effect on reflex modification, when in Grillon's study subjects were also randomised in different groups and the results were negative on behalf of arousal effecting on conditioning.

Awareness also seems to be important factor in associative learning. A recent study of anxiety in humans resulted that only subjects aware of the CS-US –pairings showed differential CR (Grillon, 2002). A lack of this experiment is a short of measurement of the awareness, so I have no comprehension of the awareness of the subjects or the differences in awareness between the groups. If I had this information, I could regard whether it would have had an impact on a learning process and if so, could it explain the unexpected differences between the groups. Even though in Grillon's study (2003) there were no differences between the awareness and the group (pleasant, neutral, unpleasant), this kind of information would have been interesting.

In the current experiment subjects were informed to concentrate on the pictures, when awareness of the conditioning process is supposed to be lower than in other case. Blink facilitation is proved to be larger during task-relevant than during task-irrelevant stimuli (Lipp, Siddle & Dall, 1997). Perhaps concentrating on more arousal pictures have effected on the awareness of the conditioning, when results on behalf of learning are divergence. Also regarding very intense picture flow in this experiment and possible lack of awareness in aversive and appetitive group could have lead to symptoms of anxiety, especially with aversive group.

In addition, the conditioning had an effect on responses and they differed between the groups so that in neutral group reflexes were largest and in aversive group they were smallest. This may be caused by the experimental differences compared to the previous studies, with opposite results. Results can also be interpreted according to Rescorla-Wagner's theory, by which stimulus surprisingness leads to the greater CR.

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