EMOTIONAL STATE MODIFIES EYEBLINK REFLEX ELICITED BY AN AIRPUFF

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Emotionaalisen tilan on todistettu muokkaavan refleksiivistä käyttäytymistä sekä eläimillä että ihmisillä. Tarkemmin sanottuna negatiivisesti virittynyt emotionaalinen tila (esimerkiksi pelko) voimistaa defensiivisiä reflexsejä. Tätä on usein tutkittu ihmisillä mittaamalla kokonaisvaltaisen säikähdysrefleksin silmäniskukomponentin voimakkuutta samalla kun koehenkilöt ovat katselleet emotionaalisia tiloja herättäviä kuvia. Tässä tutkimuksessa oli kaksi koeasetelmaa. Ensimmäisen kokeen tarkoituksena oli tutkia kahta silmäniskurefleksiä, joista toinen aiheutettiin kokonaisvaltaisella ärsykkeellä (ääni) ja toinen paikallisella ärsykkeellä (ilmapuhallus silmäkulmaan). Koehenkilöt katselivat erilaisia emootioita herättäviä kuvia (aversiivisia, neutraaleja ja appetitiivisia) ja heille esitettiin satunnaisesti ärsyke, joka aiheutti silmäniskurefleksin. Toisessa kokeessa käytettiin ainoastaan ilmapuhallusta ja sen voimakkuutta vaihdeltiin. Silmäniskurefleksi, joka aiheutettiin ilmapuhalluksella muokkautui emotionaalisen tilan mukaan samoin kuten Langin ja hänen kollegoidensa aikaisemmissa kokeissa, jossa silmäniskurefleksi on aiheutettu äänen avulla. Silmäniskurefleksin voimakkuus oli pienin kun koehenkilöt katselivat appetitiivisia kuvia ja suurin kun he katselivat aversiivisia kuvia. Ilmapuhalluksella aiheutettu silmäniskurefleksi näytti muodostuvan kahdesta komponentista, joista ensimmäinen muodostui ärsykkeen alkaessa ja toinen ärsykkeen sammuessa. Näistä ainoastaan jälkimmäinen muokkautui emotionaalisia kuvia katseltaessa. Ilmapuhalluksen voimakkuus ei muuttanut kuvien refleksiä muokkaavaa vaikutusta. Tulokset ovat yhteneviä aikaisempien tutkimustulosten kanssa, joissa on tutkittu säikähdysrefleksin silmäniskukomponenttia ja ne tukevat teoriaa, jonka mukaan aversiivisesti virittynyt emotionaalinen tila voimistaa kaikkia defensiivisiä refleksejä.

Avainsanat: EMG; Emootio; Ilmapuhallus; Silmäniskukomponentti; Silmäniskurefleksi; Säikähdysrefleksi

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Emotional state has been proven to have an effect on reflexive behavior in human and non-human animals. More specifically, negative emotional state (e.g. fear) amplifies defensive reflexes. This has been studied in humans usually by measuring startle responses elicited by indefinite stimulus during emotional picture viewing. This study contains two experiments. The purpose of the Experiment I was to examine two eyeblink reflexes: startle reflex elicited by a non-local stimulus (tone) and eyeblink reflex elicited by a local stimulus (airpuff towards the eye-corner). The subjects viewed emotional pictures (appetitive, neutral and aversive) and the eyeblink reflex was elicited by an occasional presentation of a stimulus. In the Experiment II the intensity of an airpuff was varied between groups. The results showed that the eyeblink reflex elicited by an airpuff was modified by emotional pictures in the same way as in the previous studies of Lang and his co-workers in which the eyeblink reflex was elicited by a tone. The reflex amplitude was smallest when the subjects viewed appetitive pictures and largest when they viewed aversive pictures. However, in the eyeblink reflex elicited by an airpuff, there appeared two components: the first at the stimulus onset and the second at the stimulus offset. The latter one was modified by emotional content of pictures, but the stimulus-driven part (immediate response) of the reflex was not modified. The change of the intensity level of the airpuff did not change the modificatory effect of pictures. These results are in accordance with earlier studies in which the startle responses have been elicited by a tone and further supports the theory that any defensively motivated reflex is facilitated by aversive emotional state of the subject.

Keywords: Airpuff; EMG; Emotion; Eyeblink component; Eyeblink reflex; Startle reflex

Introduction

Startle response is a common tool for testing emotion hypotheses because it is a defensive reflex that can be elicited and replicated reliably. According to Landis and Hunt (1939) "an abrupt sensory event will prompt a chained series of rapid flexor movements that cascade throughout the body" (Lang, Bradley, & Cuthbert, 1997, 111). Brown, Kalish, and Farber (1951) were the first who examined the whole–body startle reflex; they compared reflexes and startle probes in shock conditioning experiments in rats (Lang et al., 1997). This startle reaction seems to be a primitive reflex that protects from possible injuries and helps an individual to act in threatening situations (Lang, Bradley, & Cuthbert, 1990).

Primitive defensive reflexes, like approach and avoidance actions, are considered to be activated by motivational systems (Lang, 1995; Lang et al., 1990). These motivational systems called as appetitive and aversive, are a part of a larger network that involves other behavioral and emotional actions as well (Lang, 1995; Lang, Davis, & Öhman, 2000). Therefore measuring reflective actions can be used indexing emotional valence (Bauer, 1998). Because of the short latencies of reflexes, it is possible to examine neural pathways involved in reflex formulation (e.g. Davis, 1998).

The reflex amplitudes vary with the psychological context in which they are evoked. General theory derived from the work of Lang et al. (1990) suggests that the emotional state of an individual modifies their responsiveness to reflex-eliciting stimuli. A change in the startle reflex is a typical example of this kind of modification. According to this theory the amplitude of the startle reflex should be greater when the aversive motivational system is active (Lang et al., 1990; Vrana, Spence, & Lang, 1988).

In humans, a relatively stable behavioral element in the startle reflex sequence is a sudden closure of the eyelids (e.g. Lang, 1995; Lang, Bradley, & Cuthbert, 1998). An acoustic stimulus elicits the reflex 30-50 ms after the onset of the stimulus (Lang et al., 1990). The magnitude and the latency of the blink reflex can be easily measured as EMG activity by placing electrodes over the orbicularis oculi muscle. This muscle surrounds and protects the eye and when stimulating the muscle, it contracts and causes an eye closure (Lang, 1995). The primacy of the eyeblink has been confirmed by numerous studies, which have shown that the eyeblink reflex may occur alone to stimuli which is not strong enough to engage the whole startle reflex (Lang et al., 1990).

The startle reflex mechanisms and motivational systems in the brain are well understood due to the animal research, done mainly with rats and rabbits. These studies have confirmed that the motivational systems behind the reflex involves brain areas above the brain stem (e.g. amygdala and its connections) (Lang, 1995; LeDoux, 1993). Usually the focus has been in defensive systems of the brain, related to fear and anxiety. The studies with animals have suggested the amygdala being in an important role in controlling these defensive systems. The findings have also confirmed that the fear-potentiated startle effect can be modified by inducing a state of fear (e.g. Davis, 1998). The researchers (e.g. Davis et al.) have managed to clear out the rat brain's fear-startle circuit and that way they have been able to find out the primary acoustic startle pathways in human as in non-human brain (Davis, 1998; Lang, 1995; Lang et al., 2000). In emotion studies it is hypothesized that startle blink modulation indicates the extent to which a stimulus engages the motivational systems in the subcortical areas of the brain (Lang et al., 1990).

The local eyeblink reflex has been studied by many researchers (e.g. Gormezano, Schneiderman, Deaux, & Fuentes, 1962) mostly in the context of learning, especially in classical conditioning in rabbits (Steinmetz, 2000; Steinmetz, Tracy, & Green, 2001). The classical eyeblink conditioning in animals has made it possible to study learning both in terms of behavior and neural functions. Using classical conditioning paradigm, the specific areas in the brain (e.g. cerebellum, amygdala) have been confirmed to be involved in reflex modulation (Steinmetz et al., 2001). However, the actual reflex mediation does not involve higher parts of the brain. The primary acoustic startle pathway which mediates reflexes contains cohlear root neurons, neurons in the nucleus reticularis pontis caudalis and motorneurons in the facial motor nucleus. The secondary pathway, which has been suggested to modulate the reflex, contains parts of amygdala and the central gray (Davis, 1998). In conditioning studies with animals the reflex has usually been elicited by a direct stimulation of the eye either by an airpuff (e.g. Steinmetz et al., 2001) or electrical stimulation (e.g. Servatius, & Shors, 1994; Steinmetz et al., 2001). Earlier emotion studies have mostly tested modification of the startle reflex which is elicited by a tone (e.g. Cuthbert, Schupp, Bradley, McManis, & Lang, 1998).

The startle reflex involves whole body movement but, in the contrast to that, the eyeblink reflex caused by an airpuff towards the eye-corner is local and does not elicit fear. The startle and eyeblink reflex elicited by an airpuff do not share the same sensory nuclei, but it can be concluded that they can be modulated in the same way, because they both can be said to be aversive (i.e. defensive) in nature. It is hypothesized by Lang et al. (1990) that **any** aversively motivated reflex can activate neural defensive systems. When activated, the difference of two stimuli evokes different reflexes due to the congruence or discrepancy between stimuli: the reflex should be greater when viewing an aversive picture and smaller when viewing a pleasant picture (Lang et al, 1990). Startle reflex studies, both in rats and humans, have examined how emotional states modify reflexive behavior. An easy way to evoke emotional states in human subjects is picture viewing. Carefully selected pictures have been used as emotional stimuli, because they can be controlled easily in the laboratory yet they provide a visual representation of the real world (Lang et al., 1990; Lang, 1995).

This present study consists of two experiments. First, the effects of two startle stimuli were examined: a tone and an airpuff. Second, the effects of two different intensities of the airpuff stimulus were studied. The purpose was to see whether the eyeblink reflex elicited by a local stimulus is modified by emotional state similarly to the startle eyeblink reflex elicited by a non-local stimulus. Another purpose was to compare the startle reflex and the eyeblink reflex in relation to emotion evoking pictures presentations. If the eyeblink reflex elicited by an airpuff is also modified by the emotional pictures, it can be concluded that the emotional modification of any reflexive actions could have the same neural origins.

EXPERIMENT I

The effects of two different type of stimuli as a startle stimulus were examined: a tone and an airpuff. The purpose was to see if the emotional state of an individual could modify the eyeblink reflexes elicited by a tone or an airpuff.

Method

Subjects

The experiment was carried out in 23 normal healthy volunteers (7 male, 16 female) approximate age ranging from 20 to 31 years. Subjects were students and the staff members of the University of Jyväskylä. Subjects did not get any payment for their participation.

Figure 1. A cycling helmet was used to hold the airpuff nozzle and the EMG activity was measured by using three electrodes of which two were attached beneath the right eye and the third, the ground electrode on the forehead.

Materials and Design

The session consisted of 36 emotional pictures (pleasant, neutral and unpleasant) taken from the internet and 36 presentations of an airpuff or a startle tone. The order of the trials was pseudorandomly arranged so that 10 pictures from each category were presented together with reflex-evoking stimulus (2-5 s from the appearance of the picture).

In order to prevent the subjects from anticipating the stimuli, 6 airpuffs (or startle tones) and 6 pictures (from each category) were presented alone at random points. The intertrial interval varied between 20-40 s. EMG recordings were made only in the case of startle or airpuff stimuli presented during picture viewing.

The startle stimulus consisted of a 100 ms 1 kHz tone, which was presented binaurally by headphones. The loudness of the tone was 95 dB measured from the 0.5 cm distance from the surface of the headphone. The eyeblink reflex was elicited by a 100 ms presentation of an airpuff (2.1 Nm/cm2) to the outer canthus of the right eye. The intensity of the airpuff was 0.5 bar. Airpuffs were presented via a plastic tube (5 mm diameter) attached to a cycling helmet, used to hold the airpuff nozzle (see Fig. 1).

Procedure

After arrival at the laboratory, subjects signed a written informed consent form. The subjects were told that the experiment would contain different emotion evoking pictures, some of which might be considered unpleasant. Subjects were instructed that it was important to focus on every slide during the entire viewing period despite the possible unpleasant content. Subjects were also told that they would get an occasional airpuff to their right eye corner or a loud tone from the headphones and that it might feel uncomfortable. If they would feel any distress they were free to stop the experiment at any time. Nobody wanted to stop the experiment.

After attachment of the electrodes, subjects were seated in a chair in a dimly lit room in front of a computer display (the monitor distance about 1 m). The cycling helmet/ the headphones were placed on their heads.

Signal processing and Data Analysis

The eyeblink component of the startle reflex was measured by recording electromyographic (EMG) activity over the right orbicularis oculi site (see Fig. 1), using two disposable Ag/AgCl electrodes filled with electrolyte pasta (see Lang & al., 1990). A ground sensor was located at the midline of the forehead. The raw signal was amplified by a factor of 25000 and band-pass filtered from 60 to 500 Hz. The filtered signal was sampled at 1000 Hz, beginning at 250 ms before airpuff presentation and continuing until 750 ms after airpuff onset. The experiment was controlled and the data was collected by the BRACE© computer program.

The raw EMG signal was baseline corrected and rectified. Thus obtained signals were then digitally low-pass filtered with a 30 Hz cut-off. The signals were averaged over subject and picture type and the areas and maximum values were then calculated from the 250 ms period after the onset of the airpuff.

The effect of the picture content on startle response elicited by a tone and an airpuff were analyzed first in a 2 X 3 mixed-design analysis of variance (ANOVA) and then separately. Degrees of freedom were corrected using the Greenhouse-Geisser procedure if necessary. A significance level of .05 was used in all analyses.

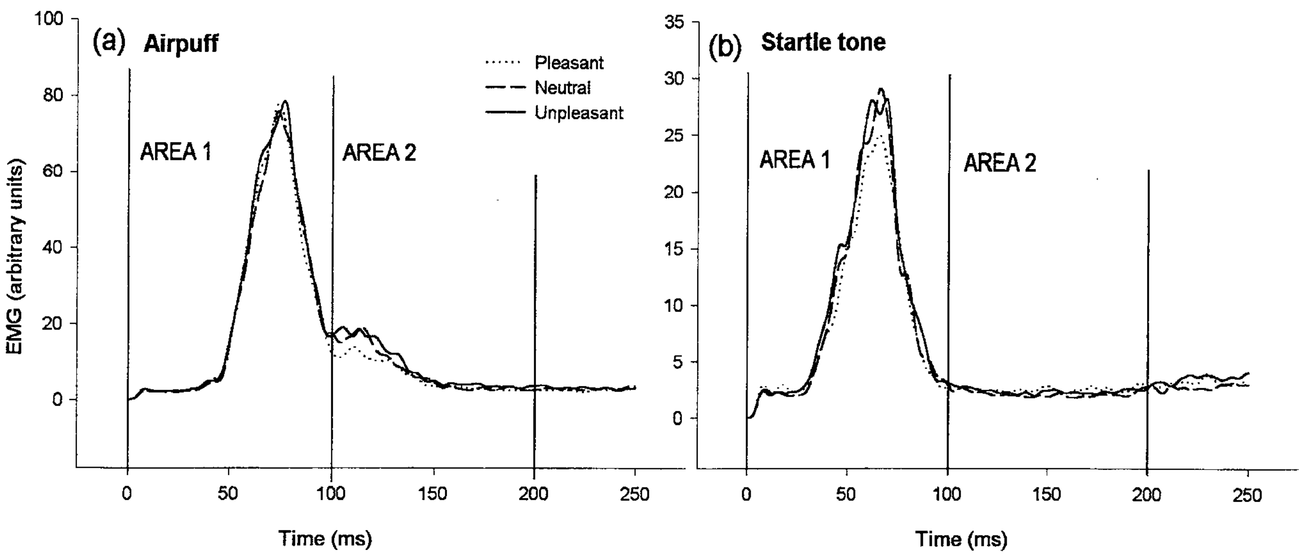
Results

The eyeblink reflex elicited by an airpuff seemed to be composed of two components, one after the stimulus onset and one after the stimulus offset (see Fig. 2a), therefore the two components were examined separately.

In mixed model ANOVA 2 (stimulus type: a tone, an airpuff) X 3 (picture content: appetitive, neutral and aversive), the maximum amplitude of the later component in the case of the airpuff and the maximum amplitude of the startle reflex (in AREA 1) were analyzed (see Fig. 3).

Significant interaction between emotional picture content and stimulus type was not found. The picture content had a significant main effect on maximum amplitudes [$F(2,42) = 10.07$; $p < .001$] but not on area. In both cases the amplitude of reflex was smallest when viewing appetitive pictures and largest during aversive picture viewing (see Fig. 3)

The picture content had no effect on the maximum amplitudes and the area of the first eyeblink reflex component. In contrast, both the maximum amplitude and the area of the second component were modified by the picture content [$F(2,18) = 5.82$; $p < .05$] and [$F(2,18) = 6.44$; $p < .01$], respectively. In the case of a startle tone (see Fig. 2b), picture content had a significant effect on both the maximum amplitude [$F(2,24) = 4.22$; $p < .05$] and the area of the startle reflex [$F(2,24) = 3.42$; $p < .05$].



Figures 2a & 2b. EMG-activity from orbicularis oculi, recorded during a blink reflex elicited by an airpuff and a startle tone. The eyeblink reflex elicited by an airpuff contains two components: one after the stimulus onset and the other after the stimulus offset. Note the different scaling because the airpuff evoked stronger responses.

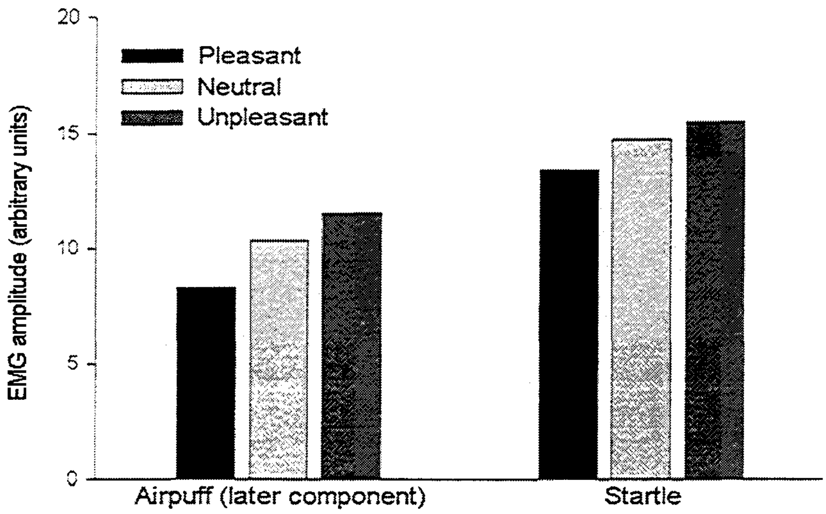


Figure 3. Mean magnitudes (maximum amplitude) of the eyeblink response elicited by an airpuff and a startle tone during pleasant, neutral and unpleasant picture viewing.

Discussion

The aim of this research was to examine two reflexes: the startle reflex elicited by a non-local stimulus (tone) and the eyeblink reflex elicited by a local stimulus (airpuff). The starting point was to see if the eyeblink reflex elicited by an airpuff would be similar to the reflex elicited by a tone as the previous studies of Lang et al. (1990) predicted. The results showed that emotional evoking pictures can modify similarly them both although the airpuff differs in its nature (being local).

The eyeblink reflex elicited by an airpuff seemed to contain two components, one after the stimulus onset and one after the stimulus offset. Picture content did not have an effect on the first component of the eyeblink reflex. Only the latter component was modified by picture content. The reason for this might be a ceiling effect: the immediate reflex was compulsory because it occurred every time almost in the same intensity after the stimulus (airpuff) onset and therefore could not be modified. Therefore, in the Experiment II, the airpuff intensity was lowered to see, whether there would be a difference between the groups. In addition, in Experiment II the pictures were the same that Lang et al. have used, thus the results could more reliably be generalized with international research.

EXPERIMENT II

The purpose of the experiment was to examine the modification of the eyeblink reflex elicited by an airpuff in relation to emotion evoking pictures. The intensity of the airpuff was varied between groups

Method

Subjects

The experiment contained 15 subjects (8 male, 7 female), age range 20-27 and two groups were made (n = 9 and n = 6).

Materials and Design

Fifty-four pictures were chosen from the IAPS[1], International Affective Picture System (Center for the Study of Emotion and Attention, 2001) and the pictures were divided in three different categories. The categories were pleasant (e.g. infants and opposite sex nudes), neutral (e.g. household objects and neutral faces) and aversive (e.g. spiders and mutilations) and consisted of 18 pictures each. Pictures were chosen based on their valence ratings[2] that were taken from the IAPS system in which the picture affect categories are defined (Lang, Bradley, & Cuthbert, 2001).

The slides were arranged in three blocks, such that each block contained six pleasant, six neutral and six unpleasant pictures. The blocks varied in order of the picture content but the proportions remained the same in each block. The block order was also altered between subjects. Each session was about 30 minutes long, after which the purpose of this experiment was explained.

Only the airpuffs were used as eyeblink eliciting stimuli. Airpuffs were presented in the same way as in Experiment I, but because of the possible ceiling effect in the Experiment I, the intensity of the airpuff was varied between two groups (0.5 bar to 0.3 bar). 36 airpuffs were presented at a random time point 2–5 s during the intertrial intervals. Subjects viewed 54 slides. Each slide was presented for 6 s and the airpuff was presented at a random point between 0.5-4 s after the appearance of the picture. Intertrial interval varied from 16 to 24 s.

In both experiments pictures were presented as color slides with the PowerPoint program for Windows. The presentation and timing of stimuli were accomplished under the control of the BRACE© computer program. All the analyses were performed with SPSS 11.0 program for Windows.

Procedure

The procedure was the same as in the Experiment I, but this time only the airpuffs were used as stimuli.

[1] The IAPS slide numbers were: pleasant: 1440, 1460, 1620, 1750, 1920, 2030, 2080, 2091, 4142, 4210, 4290, 4460, 4470, 4490, 4611, 4660, 4680, 7230, 7350, 8080, 8420; neutral: 2303, 2393, 5130, 5500, 5740, 5750, 7002, 7009, 7010, 7020, 7031, 7034, 7100, 7170, 7500, 7550, 7700, 7705; and unpleasant: 1114, 1120, 1205, 1274, 1280, 1300, 2095, 2205, 2683, 2710, 2800, 3000, 3010, 3015, 3030, 3102, 3230, 6230.

[2] Mean ratings were: pleasant: 7.49; neutral: 5.15; and unpleasant: 2.50.

Results

Only the second component of the eyeblink reflex was analyzed, because the pictures did not have an effect on the first component in the Experiment I.

In 2 (airpuff intensity: 0.3 bar, 0.5 bar) X 3 (picture content: appetitive, neutral and aversive) mixed model the picture content had a significant main effect on the area [$F(2,26) = 8.72$; $p < .001$] but not on the maximum amplitude (see Fig. 4). Interaction between picture content and stimulus intensity was not found. In both analyses, using maximum amplitude or the area, the groups differed significantly so that, curiously enough, the weaker airpuff elicited stronger responses [$F(1,13) = 56.43$; $p < .001$] and [$F(1,13) = 33.61$; $p < .001$, respectively (see Fig. 4).

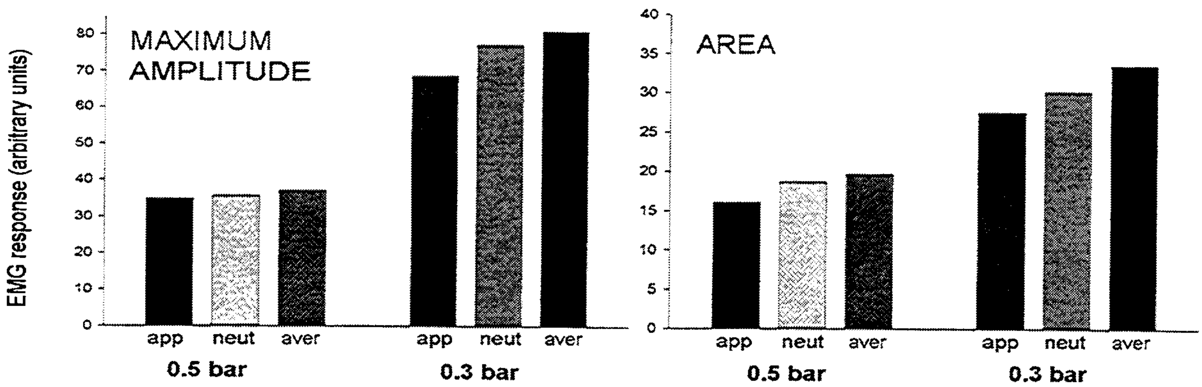


Figure 4. Mean magnitudes of the eyeblink response measured by maximum amplitude and area. The intensity of the airpuff was 0.5 bar in the first group and 0.3 bar in the second group during pleasant, neutral and unpleasant picture viewing.

Discussion

In this experiment the weaker airpuffs (0.3 bar) compared to 0.5 bar airpuffs elicited stronger responses, but the modificatory effect of pictures remained the same between groups when using the area (see Fig. 4). The pictures had an effect on the area but not on maximum amplitude. The reason for this may have been the small group. Though it seems that the area is a better indicator when testing differences in eyeblink reflexes, because the shape of the reflex is a curve not a peak. Therefore the horizontally measured area differentiates mean values better than the vertically measured maximum amplitude.

General Discussion

The results showed that in the startle reflex in which the eyeblink was elicited by a tone, the reflex immediately after the stimulus onset was modified. In contrast, it seems that in these present studies the eyeblink reflex elicited by an airpuff consists of two components. The reflex was modified significantly only after the stimulus offset. The stimulus-driven part of the reflex (immediate response) (see Fig. 2) was not modified. The first component may have been compulsory, because it did occur every time almost at the same intensity after the stimulus (airpuff) onset and could not be modified. The second component can be thought as being modified as a function of the emotional state or the emotional content of the picture, because the magnitude of the eyeblink was stronger when viewing aversive pictures and weaker when viewing pleasant pictures.

These results suggest that the eyeblink reflex elicited by an airpuff is similar to the startle reflex, in a way that it can be modified by emotional pictures although the modification is slightly different. In the startle reflex studies the latency of the eyeblink reflex has been 30-50 ms and in this study it was 30 ms. In contrast the latency of the eyeblink reflex elicited by an airpuff was 50 ms (see Fig. 2a and 2b).

In these experiments when using an airpuff as a reflex-eliciting stimulus the statistically significant differences were found in a smaller number of subjects than in the case of the startle reflex elicited by a tone. From this point of view, the airpuff appears to be more economical when testing human subjects in the context of emotion and e.g. learning though the adjustment of the optimal intensity level of the airpuff requires more experimenting. However, pictures for the Experiment II were chosen based only on their valence ratings. In startle experiments the focus has been both in affective picture valence (pleasant – unpleasant) and arousal (calm – aroused).

Lang et al. (e.g. 1990) have argued that the difference of two stimuli may evoke different reflexes because of the congruence or discrepancy between the stimuli: when viewing an aversive picture, an aversive stimulus evokes a larger reflex because of the congruence between the motivational state and the nature of the stimuli (both aversive). In contrast, during pleasant picture viewing, the aversive stimulus causes the discrepancy between the stimuli and elicits a smaller reflex. An airpuff can be classified as an aversive stimulus as well as a loud tone because they both evoke defensive reflexes. The theory of Lang et al. (1990) has suggested that all defensive reflexes are motivated aversively.

This experiment showed that the eyeblink reflex elicited by an airpuff contains two components. The second component of the eyeblink reflex elicited by an airpuff can be modified by the emotional content of the picture. It would be interesting to study the mechanisms behind the two-component formulation. These results provide new possibilities for future studies when experimenting emotional aspects (especially in learning) by using eyeblink modification in humans. The previous conditioning and learning studies in animals have already provided a lot of information about the brain functions responsible for these mechanisms.

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