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Suvi Karla

To Approach or to Avoid?

Emotional Picture Viewing Effects on Reflexive Responses in Humans

JYVÄSKYLÄN YLIOPISTO

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UNIVERSITY OF JYVÄSKYLÄ

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Cover picture by Veera and Aava Karla. They were asked to draw “emotions”. Veera’s drawing is a bunch of flowers, which are happy, except the black end, which is sad. In Aava’s drawing, there’s a pink ghost with black spots and someone is throwing rock on it. And that makes the ghost feel bad.

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ABSTRACT

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The motivational priming theory implies that emotions engage sensory systems in reflexive level and thus direct attention as well as prepare individuals for action, i.e. to approach or to avoid. This research examined the effects of emotional modulation on, in addition to defensive reflexive responses, responses which can be seen as protective but not reflexive or reflexive but not protective. Thus the aim was to test the generality of the motivational priming theory by extending its scope also to non-defensive and voluntary reactions. Emotional modulation was carried out by emotional picture viewing. The main research focus was to examine the eyeblink response, which, can in general be regarded as an inherently protective response. As a control phenomenon, the effect of emotional modulation was also tested on a reflexive action considered non-defensive, that is, an electrically induced motor response that is known to involve a cortical contribution.

In the first study, the effects of emotional state on classically conditioned eyeblinks were studied. The results indicated that the defensively motivated behavior, even when not reflexive but learned, was modified by emotional valence. In the second study, the effect of emotional state on reflexive and voluntarily generated eyeblink responses was examined. The results showed that only reflexive, not voluntary eyeblinks, were augmented by unpleasant emotional state. In the third study, the non-defensive motor responses (cutaneo-muscular response, CMR) were measured. The responses were also, not expectedly, augmented during the viewing of unpleasant pictures.

To conclude, in unpleasant state, reflexive responses were augmented, irrespective of whether they were inherently defensive or not, whereas voluntary actions were not sensitive to emotional modulation in the same manner as reflexive actions. It seems that motivational priming effect is more straightforward on protective, reflexive behavior. That is, when we are not consciously attending or voluntarily performing our actions. It is also important that should the emotional modulation occur, the time between the stimulus and response is important – as it grows, the effect of emotional modulation diminishes. In addition, the behavioral response needs to have a direction - we need to approach or avoid something.

Keywords: EMG, emotional modulation, motivational priming, reflexive responses, humans

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TIIVISTELMÄ (FINNISH ABSTRACT)

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Lähestyä vai välttää? Emotionaalisten kuvien katselun vaikutus refleksiivisiin reaktioihin ihmisillä
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Motivationaalisen virittymisteorian mukaan emootiot vaikuttavat havaitsemiseen refleksiivisellä tasolla ja valmistavat yksilöä toimintaan, joko lähestymään miellyttäviä, tai välttämään epämiellyttäviä ärsykkeitä. Tässä tutkimuksessa selvitettiin teorian yleistettävyyttä muihinkin kuin refleksiivisiin käyttäytymisvasteisiin ja tutkittiin vaikuttaako emotionaalinen tila myös itse tuotettuun reaktioon ja ei-defensiiviseen reaktioon, eli reaktioon, joka ei varsinaisesti ole lähestymis- eikä välttämiskäyttäytymisen ilmentymä. Emotionaalinen muokkautuminen suoritettiin näyttämällä tutkittaville henkilöille emotionaalisia kuvia. Keskeisenä tutkimuskohteena oli silmänräpäytysreaktio ja kontrollina mitattiin lihasvaste käden lihaksesta.

Ensimmäisessä osatutkimuksessa mitattiin silmän lihasjännityksen muutosta klassisen silmäniskuehdollistamisen aikana. Tutkimuksen tarkoituksena oli selvittää, vaikuttaako emotionaalinen tila jo opittuun käyttäytymiseen. Tutkimuksessa havaittiin, että jo opitut käyttäytymisvasteet voimistuivat epämiellyttävän emotionaalisen tilan aikana. Toisessa osatutkimuksessa selvitettiin emotionaalisen tilan vaikutusta sekä refleksiiviseen, että itse tuotettuun silmänräpäytysreaktioon. Tulosten mukaan epämiellyttävä emootiotila voimisti refleksiivistä reaktiota, mutta ei itse tuotettua reaktiota. Kolmannessa osatutkimuksessa mitattiin ei-defensiivistä reaktiota, käden lihaksesta. Tämäkin reaktio, vastoin odotuksia, oli suurin epämiellyttävän emootiotilan aikana.

Kokonaisuudessaan nämä tutkimustulokset tukevat motivationaalista virittymisteoriaa siinä mielessä, että kaikissa osatutkimuksissa epämiellyttävä emootiotila näytti voimistavan refleksiivisiä reaktioita. Ristiriidassa teorian kanssa oli motorisen, ei-defensiivisen vasteen voimistuminen epämiellyttävien kuvien katselun aikana. Näyttäisikin, että emotionaalinen muokkautuvuus näkyy selvemmin refleksiivisellä tasolla, etenkin silloin, kun emme itse aktiivisesti havaitse, tai tietoisesti kontrolloi käyttäytymistämme. Emotionaaliseen muokkautuvuuteen vaikuttaa ärsykkeen ja reaktion välinen aika - sen kasvaessa emotionaalisen muokkautuvuuden vaikutus näyttää pienenevän. Emotionaalinen muokkautuvuus vaatii myös sen, että käyttäytymisellä täytyy olla suunta, eli jotain miellyttävää on tarkoitus lähestyä tai epämiellyttävää välttää.

Avainsanat: EMG, emotionaalinen muokkautuvuus, motivationaalinen virittyminen, refleksiivinen reaktio, ihmiset

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I would like to dedicate this thesis to my beloved girls Aava and Veera.
Be strong but stay soft!

Jyväskylä 17.2.2015
Suvi Karla

LIST OF ORIGINAL PUBLICATIONS

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Taking into account the instructions given and comments made by the co-authors, the author of the thesis collected the data, conducted the analyses, and wrote the reports of the three publications.

FIGURES

TABLES

CONTENTS

1 INTRODUCTION

The focus of this research was to study the effects that emotional states have on reflexive responses in humans. Emotional modulation is necessary in situations in which individuals have to adapt their behavior. Emotional modulation has generally been thought to affect, in particular, defensively motivated reflexes; however, recent studies imply that the modulation effect is not limited to subcortically driven responses (Konagemaru, Domen, Fukuyama, & Mima, 2012) or to merely biologically prepared behavior (Balderston & Helmstetter, 2010; Knight, Nguyen & Bandettini, 2003; Knight, Waters & Bandettini, 2009; Reagh & Knight, 2013).

Emotional modulation, according to *motivational priming theory* of emotions by Lang and his colleagues (e.g. Lang, 1995; Lang, Bradley, & Cuthbert, 1997), affects, in particular, defensively motivated reflexive responses. Lang and his colleagues have found that the inner state of an individual – motivational and emotional - guides the direction as well as the intensity of actions by directing attention to relevant stimuli in order to sustain life (Lang & Bradley, 2010). Lang and his colleagues refer to emotions incisively as *action dispositions* (Lang, 1995; Lang et al., 1997). From the evolutionary perspective, motivation is developed as an inner system for detecting external stimuli to match individuals' inner needs (Lang & Bradley, 2010).

The motivational priming theory has been most extensively studied by examining emotional effects on acoustic startle eyeblink reflex (ASR). The amplitude of such a defensive response has been shown to be sensitive to certain cognitive or task-dependent factors. Facilitation of the startle eyeblink reflex can be seen as the result of *attentional priming*, e.g. in an unpleasant emotional state the aversive cues are primed and the reflex is augmented (Lang, Bradley, & Cuthbert, 1990). This is due to *congruence* between an ongoing state and task demands. The startle eyeblink itself can be regarded as defensive, as it serves to protect the eye. However, the mechanisms recruiting the elicitation of the eyeblink response can differ, depending on e.g. the context or stimulus-eliciting properties, but the visible motor response remains the same. It is not clear whether emotional modulation holds in reflexively and voluntarily produced eyeblinks. In addition, recent evidence implies that it is not merely defensive actions that are sensitive to emotional state. Emotional modulation has also been shown in motor responses which are not considered as defensive acts (Baumert, Sinclair, McLeod, & Hammond, 2011; Baumgartner, Willi & Jancke, 2007; Coombes, Cauraugh, & Janelle, 2007; Coelho, Lipp, Marinovic, Wallis, & Riek, 2010; Hajcak, Molnar, George, Bolger, Koola, & Nahas, 2007; Koganemaru et al., 2012). The results of e.g. Coombes et al. (2007) imply that emotional state alters motor preparedness as well as the actual motor act. It may be that attention (shift) is not necessarily needed, especially in an aversively motivated state. The studies of (Balderston & Helmstetter, 2010; Knight et al., 2003; Knigh et al., 2009; Reagh & Knight, 2013) have shown that majority of emotional stimuli is unconsciously processed and that processing is not restricted to threat stimuli or biologically important significant stimuli alone (Pessoa & Adolphs, 2010).

In the articles reported in this research, the first aim was to determine whether emotional modulation would be shown for eyeblinks acquired through associative learning in much the same way as for reflexive eyeblinks (Study I). This was expected according to the motivational priming theory of emotions (Lang, 1995; Lang et al., 1997), which hypothesizes that all defensively motivated behavior is affected by the prevailing emotional state. Learned eyeblinks are associated with the aversiveness of the unconditioned stimulus and thus regarded as defensive acts in a same way as unconditioned responses. The general objective in these studies was to examine whether the inherently defensive eyeblink response would be sensitive to emotional state in different situations. Therefore, the emotional modulation of the eyeblink was also tested in a non-threatening situation (Study II), where the eyeblink was voluntarily performed. In addition, emotional modulation was studied in a non-defensive action by measuring a reflexive response of cortical origin (Study III).

The present research sought to provide more information about emotional modulation effects also on non-defensive behavior and discusses the results in the light of motivational priming theory of emotions as well as current views of emotional modulation.

1.1 What are emotions?

Generally, emotions can be viewed as adaptive functions applied to solve basic ecological problems that individuals face on an everyday basis (Frank, 1988; Plutchik & Robert, 1982). Subjectively, feelings refer to inner states of an individual which are relatively hard to define or directly measure, whereas emotions are more objective physiological states with different measurable components (valence, arousal, expression etc.). Some researchers hold that emotions usually involve cognition (see e.g. Lazarus, 1991), whereas others favor the view that emotional responses need no cognition to emerge (Zajonc, 1984). In humans, behavioral responses are more or less influenced by several attributes, such as relevance, pleasantness, interest of the stimuli, etc. The definition of emotions can vary: for example, some may characterize inner affects, like hunger or sexual drive, as emotions, or they can be categorized roughly into positive, neutral and negative emotions.

The history of modern emotional studies goes back to the 19th century, when a theory of emotions by William James and Carl Lange (commonly known as the James-Lange theory of emotion) was first proposed (James, 1884). The theory is based on the idea that emotions depend on evoked bodily responses. This means that an emotional experience does not develop without the experience of bodily responses, i.e. autonomic nervous system (ANS) responses such as a rapidly beating heart, fast breathing, etc. For example, if someone finds himself in danger, this perception prompts different bodily responses, which can be interpreted as fear. In the 20th century, Walter Cannon (Cannon, 1929; later Cannon, 1987) criticized the James-Lange theory for being over explicit. Cannon argued that the emotional experience and bodily changes occur simultaneously and independently from each other, and hence bodily experiences cannot explain the emotional experience. Furthermore, the same bodily changes can be linked to several different emotions, positive and negative. This is known as the Cannon-Bard theory, which Cannon developed together with his student Philip Bard (Cannon & Walter, 1929). The emotional experience contains much more than just ANS responses: for example emotional expression. To some extent in agreement with the James-Lange theory, Antonio Damasio (1994; 1998) proposed a somatic marker theory of emotions. He argued that during an emotional state, the accompanying neuronal changes in the brain lead to changes in bodily functions. Previous events and memories leave *somatic markers* in the brain which will help, for example, in decision-making in the future.

In the 1960s, Stanley Schachter and Jerome Singer found that people tend to interpret the arousal they experience differently, depending on the type of situation they are in. Thus, differentiating emotions is more due to cognitive than psychophysiological factors; consequently, different physiological states are not distinguished by specific emotions (Schachter & Singer, 1962). However, it has been shown that some emotions do, in fact, have significantly different bodily profiles (LeDoux, 1996; for a more recent view, see Nummenmaa, Glerean, Hari, & Hietanen, 2014).

Appraisal theorists take a functional approach to emotion. From their point of view, emotions always involve evaluations of the situations in which they are experienced. Each emotion or feeling has its' own appraisal and that appraisal has a core theme. Appraisal gives rise to attraction or aversion, and emotion is equated with this “felt tendency toward anything intuitively appraised as good (beneficial), or away from anything intuitively appraised as bad (harmful)” (Arnold, 1960). Richard Lazarus (1991) sees appraisals as crucial for emotional experience. Nico Frijda (1986) also sees patterns of action readiness and appraisals important in characterizing different emotions, but he does not characterize these patterns solely in terms of attraction and aversion.

In 19th century, Charles Darwin was interested in the expression of emotions (for principles, see Darwin, Ekman & Prodger, 2002). In animals, emotional expression has been considered crucial in e.g. attack situations, but in humans it is also important in communication generally. Following Darwins' ideas, Paul Ekman investigated "affect programs", of which emotional expressions form part. The Paul Ekman group has proposed a theory of discrete basic emotions (disgust, sadness, happiness, fear, anger, surprise) that lack higher cognitive processes and have evolved to facilitate adaptive behavior in response to specific, recurrent challenges (e.g. Ekman et al., 1987; Ekman, 1992a; Ekman, 1992b).

Thus, emotional phenomena form a diverse fabric of intertwined concepts, theories and observations. However, for the purposes of this research, a simpler viewpoint is adopted. The basic principle of the motivational priming theory by Peter Lang and his colleagues (Lang, 1995; Lang et al., 1997) is that the events and stimuli in one's surroundings induce emotional experiences that can be organized along two dimensions: valence (pleasantness-unpleasantness) and arousal (calmness-exitedness). These experiences then activate, to a varying amount, either the appetitive or aversive drive system. This emotional system is an adaptive system which facilitates action by directing attention appropriately, i.e. to biologically important stimuli. Simply stated, aversive stimuli cause withdrawal behavior and appetitive stimuli approach behavior. In the 1980s, Anthony & Graham (1985) suggested that defensive behavior is modulated by *attention.* They studied the acoustic startle reflex (ASR) and suggested that the modality of the foreground stimulus and the startling stimulus must match in order to augment the response. Mismatch between them would lead to reflex inhibition. The motivational priming hypothesis was developed by Lang and his colleagues to provide an alternative view on these phenomena (Lang, 1985; Lang, 1995; Lang et al., 1997). According to them, attention shift between the different sensory modalities is not an adequate explanation. They suggested that the reason why attention is directed to one stimulus or to another depends on the emotional or motivational content of the stimulus.

In sum, emotions can be understood via physiological processes, perceptions, neuropsychological states, adaptive dispositions, and evaluative judgments. In the present research emotions are considered as measurable changes in individuals' bodily states that can influence their reflexive as well as voluntary behavior. Emotions are categorized as positive (appetitive), neutral and unpleasant (aversive). Behavioral responses to emotional stimuli are quantified as muscle activity by electromyogram (EMG).

1.2 The motivational priming theory

The motivational priming theory states that any defensively motivated behavior is sensitive to emotional state. In an aversively motivated, i.e. unpleasant state, the unpleasant stimuli are primed and behavioral responses to these stimuli are augmented. In contrast, behavioral responses to stimuli at the other end of the valence dimension are inhibited. Biologically, motivation is a built-in system for detecting harmful or useful stimuli and, thus, a motive to act. The changes in the inner state prepare the body for effortless action. Emotions, according to this theory, are seen as *action dispositions* (Lang et al., 1990; 1997). The motivational system that is currently active leads to *attentional priming*, e.g. in an unpleasant emotional state the aversive cues are primed and the reflex is augmented (Lang et al., 1990). This is presumed to be due to *congruence* between an ongoing emotive state and current task demands instead of being due to congruence between sensory modalities, as proposed by Anthony & Graham (1985).

A central question in the present research is whether all defensively motivated behavioral responses are modulated by emotional state as predicted by the motivational priming theory (Lang, 1995; Lang et al., 1997). If so, such modulation is expected to be observed in any defensive, but not non-defensive, responses.

1.3 Viewing of emotional pictures

The human visual system is effective and fast: according to studies (see e.g. Green & Oliva, 2009; Potter, 1975) the gist of pictures is recognized. The emotional picture series, IAPS (International Affective Picture System), developed by Lang et al. (2008) has been widely used in laboratories all around the world. The series contains over a thousand pictures normatively rated according to their *valence*, *arousal* and *dominance*. The pictures represent real-life events and objects, ranging from those eliciting strong emotional reactions to those generally considered to be neutral. According to Davis et al. (1995):

> Static color slides of the IAPS induce an affect that is highly replicable, generally complex but synergically patterned, and relatively weak; and dimensional measures of affect induced by IAPS slides reflect an average computed across categorical responses.

Viewing pictures in the laboratory is a passive situation, as participants are expected to sit still and follow the instructions they are given. However, a novel situation, even passively observed, will most likely sharpen the senses for future events. Such a situation can be regarded as resembling predator-prey encounters in the wild just before each party senses the presence of other (Lang & Bradley, 2013). Obviously, picture viewing in laboratory settings is somewhat compromised in terms of ecological validity, owing to the fact that it takes place outside of the participant's everyday environment. This also means that, despite containing fairly unpleasant as well as very pleasant content, the pictures may not be powerful enough to elicit responses as strong as those elicited in real life encounters. However, viewing pictures in the laboratory is advantageous in its controllability with respect to the rating of emotional valence and arousal, while minimizing the effects of the kinds of extraneous stimuli that are often encountered in real life situations.

1.4 The emotional modulation of eyeblinks and other responses

Blinking is obviously an important defensive act that, among other functions, such as lubricating the eye (Korb et al., 1994), protects our vision. One important feature of the eyeblink response is that it can be elicited by different stimuli, such as acoustic, visual, or tactile, along with variation in the stimulus parameters (Berg & Balaban, 1999). The (startle) eyeblink reflex has been found to be sensitive to emotional and attentional demands and is modulated differently depending on the stimulus properties and intervals, or pre-pulses preceding and post pulses following elicitation of the reflex (e.g. Anthony & Graham, 1985; Clarkson & Berg, 1984; Filion, Dawson, & Schell, 1993; Lipp, Arnold, Siddle, & Dawson, 1994; Lipp & Krinitzky 1998; Valls-Sole, Hallett, & Alvarez, 1996). In addition to reflexive eyeblinks, humans have a strong tendency to blink whenever something approaches the eye, even in the absence of an actual threat (Polewan, Vigorit, Nason, Block, & Moore, 2006).

According to the motivational priming theory (e.g. Lang, 1995; Lang et al., 1997), the active motivational system exerts a modulatory effect on the action programs it is engaged in. This means that reflexes that are functioning *non-defensively* are considered not to be affected by the motivational system and, therefore, not to be modulated by emotional states aroused by this system (Bonnet, Bradley, Lang, & Requin, 1995). The emotional modulation of defensive reflexes, such as the startle reflex, is attributed to valence — the emotional state primes sensory processing in order to approach appetitive stimuli and avoid potentially harmful stimuli (Haerich, 1994; Lang & Bradley, 2010). Although pictures with both high and low hedonic valence values can induce heightened arousal, the startle amplitudes are not affected by arousal (Lang et al., 1990). In contrast, spinal reflexes that are considered to be non-defensive have been found to be augmented by high arousal but not to be sensitive to emotional modulation (e.g. Bonnet et al., 1995).

Emotional modulation has also been recently found in studies where non-defensive motor responses have been elicited by transcranial magnetic stimulation (TMS) (Baumert et al., 2011; Baumgartner et al., 2007; Coelho et al., 2010; Coombes et al, 2007; Hajcak et al., 2007; Koganemaru et al., 2012; van Loon, et al., 2010). In these experiments, motor cortex excitability has been studied during emotional settings, and it has been demonstrated by both visual (e.g Coombes, 2007) and auditory (Baumert et al., 2011) stimuli. However, in the case of emotional modulation, the results have been contradictory. Baumgartner et al., (2007) and Hajcak et al. (2007) observed equivalent effects with pleasant and unpleasant pictures. In turn, the studies of Coombes et al., (2009) and Coelho et al. (2010) demonstrated emotional modulation in such a way that motor excitability was augmented during unpleasant but not during neutral or pleasant picture viewing. However, both positive and negative emotions can increase arousal and signal the need for action, i.e. to approach or to avoid, although negative emotions are more likely to trigger rapid and more straightforward actions. It has been shown that in fact the same brain regions can be activated whether it is appetitive or defensive action that is needed. This observation implies that the two motive systems may not be spatially distinct in the brain but that these systems may differ in neural connections between brain regions (Lang & Bradley, 2013). It has also been suggested that stimulus relevance, whether it is positive or negative, can affect attention (Broeren & Lester, 2013).

Although emotional modulation effects on motor cortex excitability have been examined in several studies (Baumert et al., 2011; Baumgartner et al., 2007; Coelho et al., 2010; Coombes et al, 2007; Hajcak et al., 2007; Koganemaru et al., 2012; van Loon, et al., 2010), comparison between these studies with their conflicting findings is problematic. The research procedures followed differ widely: In some studies participants have had an active role, e.g. in studies measuring reaction times (Koganemaru et al., 2012), whereas in other studies participants have passively looked at pictures (e.g. Coelho et al., 2010). The results indicate that emotional modulation seems to depend on methodological issues, such as the choice of response-eliciting stimuli, task or target muscles, etc. The extent, to which motor excitability is sensitive to emotional arousal on the one hand and valence on the other, also remains unclear.

1.4.1 Acoustic startle eyeblink reflex

The most studied eyeblink response is the acoustic startle eyeblink reflex (ASR) (e.g. Lang et al., 1990; Vrana, Spence & Lang, 1988), which is usually elicited by a burst of noise presented via either headphones or loudspeakers. ASRs are often accompanied by other startle reflexes such as the whole-body startle reaction and its autonomic nervous system correlates. The magnitude of the startle eyeblink reflex is measured by electromyogram (EMG). The ASR consists of three components -R1, R2 and R3- of which the R2 component, with an onset latency of approximately 25-35 ms, is usually the target of interest in startle eyeblink studies (Blumenthal et al., 2005). The acoustic startle pathway is the simplest reflexive pathway, and involves cochlear root neurons, neurons in the nucleus reticularis pontis caudalis, and motoneurons in the facial motor nucleus (pinna reflex) (Davis, Gendelman, Tischler, & Gendelman, 1982). The nucleus reticularis pontis caudalis receives projections from the central nucleus of the amygdala (Lang, Davis, & Ohman, 2000).

The startle eyeblink reflex is sensitive, especially to emotional valence (Lang et al., 1990) and shows a linear trend, the ASR diminishing during a pleasant emotional state and augmenting during the unpleasant state relative to neutral state. Lang and his colleagues have argued that this trend results from *congruence* between the emotional state and the stimulus. The ongoing motivational state directs attention to congruent stimuli. However, several factors can alter this modulation. If during the startle eyeblink reflex procedure a cognitively demanding task is given, modulation of the eyeblink reflex does not occur (see King & Schaefer, 2011; Wangelin, Löw, McTeague, Bradley, & Lang, 2011). In contrast, emotional modulation is absent if the attention is focused on the startle tone (Panayiotou, Witvliet, Robinson, & Vrana, 2011). The ASR is also sensitive to prepulse and postpulse effects (Lipp et al., 1994; Lipp & Krinitzky, 1998; Valls-Sole et al., 1996).

1.4.2 Conditioned eyeblink response

Instead of a reflexive eyeblink reaction to a sudden noise such as a startle tone, emotional modulation can also target associatively learned responses. In classical conditioning, a conditioned stimulus (CS, tone) is forwardly paired with an unconditioned stimulus (US, airpuff). For example, a neutral tone is presented before the airpuff that elicits an unconditioned response (UR). Eventually the tone results in a conditioned response (CR) (e.g. Thompson, Thompson, Kim, Krupa & Shinkman, 1998). Whereas both startle eyeblink and airpuff-induced eyeblinks are immediate reflexes driven by simple neural pathways, the startle eyeblink reflex circuit is simpler than the circuit of the conditioned eyeblink in the sense that it does not involve the cerebellum (e.g. Steinmetz, 2000). The ASR is a one component of the whole-body startle response. The CR is instead a discrete and fairly neutral response (e.g. Thompson et al., 1998). However, after being paired with an aversive US (airpuff), the CS (tone) is also associated with aversiveness. This in turn makes the CR a defensive response. According to the motivational priming theory (Lang et al., 1997), it is expected to be sensitive to emotional modulation.

1.4.3 Voluntary blink

Usually the eyeblink is a reflexive or spontaneous act, but it can, of course, also be produced voluntarily. When blinking voluntarily, the same motor mechanisms are activated as in the reflexive act (Kaneko & Sakamoto, 1999; Korb et al., 1994), with the exception that the voluntary response is also mediated by cortical connections. The reflexive and spontaneous eyeblinks are both sensitive to emotional state, but effects of emotional modulation on voluntary eyeblinks have not been reported.

However, as voluntary eyeblinks are mediated by cortical connections, they are generated with longer latencies than eyeblink reflexes and can probably be affected by “cognitive appraisal”. Therefore, even if the motor output is the same, it is possible that emotional state modulates only those actions that are initiated by biologically meaningful threat signals. In other words, if the voluntary eyeblink is produced in the absence of a threatening stimulus, it may not be modulated by emotional state.

1.4.4 Supraspinal cutaneo-muscular reflex response (CMR)

The cutaneo-muscular response (CMR) is a stretch reflex response that can be measured from the surface of hand muscle, interrosseaus dorsalis (IOD). The CMR consists of two excitatory components (E1 and E2) separated by an inhibitory phase (I 1). The early components are thought to be mediated by oligosynaptic spinal pathways (Carr, Harrison, Evans & Stephens, 1993; Jenner & Stephens, 1982; Tarkka & Larsen, 1986). The latter excitatory component is considered to have cortical connections corresponding to those of the supraspinal pathway of the long latency stretch reflex (e.g. Carr et al., 1993; Tarkka & Larsen, 1986). In light of recent studies (Baumert et al., 2011; Baumgartner et al., 2007; Coelho et al., 2010; Coombes et al, 2007; Hajcak et al., 2007; Koganemaru et al., 2012; van Loon, et al., 2010) showing that emotional modulation effects also has effects on cortically mediated motor responses, it is possible that the cortical component of the CMR would also show similar effects. However, such a result would not be in accordance with the motivational priming theory.

1.5 Aims of the research

The primary aim of the present work was to study the effects of emotional state on reflexive responses that are either defensive or non-defensive. According to the motivational priming theory of emotions (Lang et al., 1997) any type of defensive behavior is affected by emotions such that in appetitive state it is inhibited and in an aversive state it is augmented. However, it remains unclear whether neutral or non-defensive reflexive responses show similar emotional modulation.

In Study I, the effects of emotional state were examined during an associative learning task. Classically conditioned eyeblinks to a weak tone were forwardly paired with a local airpuff towards the eye. As the conditioned response is, like the startle eyeblink reflex, defensively motivated, even though acquired through learning, the interest was in finding out whether it would show similar emotional modulation. The conditioned eyeblink is a very discrete response (e.g. Thompson et al., 1998), and is elicited with a delay rather than immediately in relation to the onset of the conditioned stimulus (CS). Notwithstanding, it was expected, according to the motivational priming theory (Lang et al., 1997), to be sensitive to emotional state.

In Study II the aim was to examine the emotional modulation effects on eyeblink response performed voluntarily in response to a neutral stimulus. Owing to the fact that both are protective actions and share same motor mechanism (orbicularis oculi muscle performing the eyelid closure), it was tested whether voluntary eyeblinks could be modified by emotional states in much the same way as reflexive eyeblinks. Voluntary eyeblinks were generated in response to a written command, and reflexive eyeblinks in response to a startle tone.

Study III investigated whether emotional states exert control over motor functions, as the response was elicited by electrical stimulation (cutaneomuscular response, CMR). Such emotional modulation was not expected to alter motor functions that are non-defensive. However, recent findings suggest that cortically mediated motor responses are also sensitive to emotional state (Baumert et al., 2011; Baumgartner et al., 2007; Coelho et al., 2010; Coombes et al, 2007; Hajcak et al., 2007; Koganemaru et al., 2012; van Loon, et al., 2010).

2 METHOD

Overall, sixty-six University students participated in the research (N = 25, N = 24, N = 17 in Studies I-III, respectively). The eyeblink response, one component of the whole-body startle reflex, was measured in Studies I-II. The amplitude of the eyeblink response was measured by surface EMG with Ag/AgCl miniatures filled with electrode gel (Blumenthal et al., 2005). EMG is an easy method which is sensitive to even weak activity of the muscle (Flaten & Blumenthal, 1998). The eyeblinks were recorded as EMG activity of the orbicularis oculi muscle, the muscle which is responsible for eyelid closure. Active electrode placement, beneath the right (Study I) or left eye (Study II) (see Figure 1a), was aligned with the pupil, and the reference electrode placed 1-2 cm lateral to it. To ensure an effortless flow of current from the skin to the electrodes, the skin was carefully prepared and the impedance between the electrodes was checked. In all the studies, the raw EMG signal was amplified, digitized with ac sampling rate of 1 000 Hz, rectified and digitally off-line filtered with a low-pass (< 20 Hz) filter.

In Study III, the EMG of the interosseus dorsalis (IOD) muscle was recorded from the IOD muscle in the belly of the left hand by using two disposable Ag/AgCl electrodes (for details, see Blumenthal et al., 2005; Tarkka & Larsen, 1986) (see Figure 1b). The reference electrode was attached 25 mm proximal to the active one and the ground electrode placed over the bony area of the left arm. Prior to electrode placement, the skin was carefully prepared. The electrical stimulation was delivered by a pair of flexible ring electrodes to the digital nerves of the index finger by placing the cathode proximal to it. During the CMR recordings, the participants were instructed to maintain a weak isometric contraction of the muscle by stretching a rubber band placed between the middle and index fingers (see Figure 1b). The stretching was maintained during the picture viewing and the participants told that they could relax the muscle whenever the monitor was black. The stimuli were delivered by a constant current stimulator Digitimer LTD. The stimulus intensity was set about 1.5-times the individual sensory threshold, starting from where the stimulation was just noticeable, and hence it was always kept below the pain threshold.

In all studies emotional pictures used were selected from the International Affective Picture System (IAPS) (Lang, Bradley & Cuthbert, 2008). The pictures were pleasant (e.g. food, animals etc.), neutral (e.g. household objects, neutral faces etc.) or unpleasant (e.g. mutilations, spiders etc.). The normative mean valence and arousal ratings and their standard deviations for pleasant, neutral and unpleasant pictures were 7.53 (0.43), 5.04 (0.60), 2.49 (0.71) and 4.90 (1.02), 2.87 (0.42), 6.01 (0.89), respectively. Stimulus presentation was controlled for by E-Prime software.

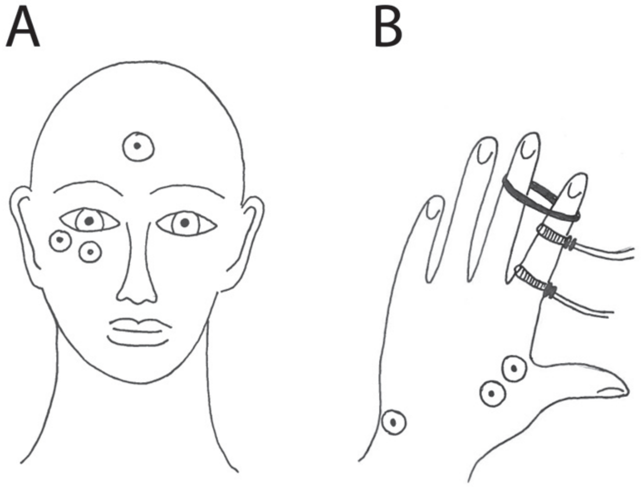

FIGURE 1 A) Placement of EMG recording electrodes over the orbicularis oculi muscle. The electrodes were beneath the right eye in Study I and beneath the left eye in Study II. B) Placement of EMG recording electrodes over the interosseus dorsalis muscle (IOD) (Study III). Rubber band was stretched between index and middle fingers and ring electrodes were used for stimulating the IOD.

3 OVERVIEW OF THE ORIGINAL STUDIES

3.1 Study I: Affective modulation of conditioned eyeblinks

Reflexive behavior is affected by emotional states by being potentiated during unpleasant states and diminished by appetitive states. This was expected to be found (Lang et al., 1990; 1997; 1998) for defensively motivated responses of any kind would be modified in a similar fashion. Study I was conducted to examine whether this expectation holds for associatively learned defensive eyeblinks. It was investigated whether a conditioned eyeblink response (CR) is modified in amplitude by emotional picture viewing. The conditioned eyeblink is not an innate reflexive response but an adaptive response to the tone, and is associated with aversive nature of an unconditioned stimulus.

Twenty-five University students participated in the study. The experiment was a classical conditioning procedure which consisted of an acquisition phase and a test phase (for more details, see the original paper). In the acquisition phase, 80 tone-airpuff-pairs were presented during viewing of neutral pictures. In the test phase, 30 tone-airpuff-pairs were presented during presentation of pictures varying in emotional content (pleasant, neutral, unpleasant, 10 each). The objective was to test whether emotional states modulate the amplitude of defensive CRs to a CS (mild tone) that is itself not aversive. Emotional picture viewing modulated defensive eyeblink-CRs which were highest in amplitude during the viewing of unpleasant pictures. However, the difference between the viewing of pleasant and neutral pictures was not significant. The unconditioned responses (URs, blinks to the airpuff) were not modulated by the pictures.

The results were in line with the previous startle eyeblink reflex studies, suggesting that associatively learned behavioral responses can be modulated by emotional states in much the same way as innate reflexive behavior. However, a linear trend was not observed across the different picture categories: CRs did not differ significantly in amplitude between pleasant and neutral pictures. Modulation or URs were expected, but were not acquired. It is most likely that the intensity of the airpuffs was too high, leading to a ceiling effect. The effect size (Cohen's d) of the results is reported in Table 1.

3.2 Study II: Effect of emotional state on voluntary eyeblinks

Study II addressed the effects of emotional states not only on reflexive eyeblink responses to an aversive stimulus but also on voluntarily generated eyeblink responses to a neutral stimulus. Namely, the voluntary blink was performed whenever the word "BLINK" appeared. We aimed at investigating whether emotional state modulates motor functions that share the same output but differ in input and purpose or context.

Twenty-four University students participated in two procedures: startle eyeblink and voluntary blink procedures. The startle eyeblink procedure consisted of two phases: a habituation phase and an acquisition phase. In the habituation phase, 5 startle tone-alone trials were presented in order to familiarize the participants with the loud tone. In the acquisition phase, 30 trials with the startle tone and randomly selected affective picture content (pleasant, neutral or unpleasant, 10 each), 10 trials with the startle tone alone and 9 trials with affective pictures alone (3 neutral, 3 pleasant and 3 unpleasant) were presented in pseudo-random order. The participants were informed that they would hear occasional tones, but their task was to ignore them.

In the voluntary eyeblink procedure, 30 trials with randomly selected affective picture content (pleasant, neutral or unpleasant, 10 each) with visual stimulus, 10 trials with visual stimulus alone (black background) and 9 affective pictures alone (3 neutral, 3 pleasant and 3 unpleasant) were presented in pseudo-random order. The participants were informed that their task was simply to blink their eyes every time they saw the word "BLINK".

Voluntary eyeblinks were not affected in amplitude by the emotional content of the pictures. The only difference observed in responses to emotional picture content was that the onset latency of these responses was significantly longer when viewing pleasant or unpleasant relative to neutral pictures. This negative finding is in line with the congruence assumption of the motivational priming theory (Lang et al., 1990; Lang, 1995), i.e. the stimulus (the word "BLINK") was not threatening. That is, a defensive action to an aversive stimulus would have been needed for the effect to be observed. The reflexive startle eyeblinks were augmented by the viewing of unpleasant pictures. This result is parallel with result of Study I and partly in line with a number of previous startle eyeblink studies. However, the difference between the responses to pleasant and neutral pictures was not significant.

The findings suggest that the valence-specific emotional modulation is reasonably specific to the reflex generating mechanism as it did not modulate the voluntary eyeblinks. The reflexive and voluntary eyeblinks may therefore recruit neural mechanisms that differ in their sensitivity to emotional states. The effect size (Cohen's d) of the results is reported in Table 1.

3.3 Study III: Unpleasant emotional state augments supraspinal reflex response

Study III examined the effects of emotional states on a cutaneo-muscular response (CMR), more specifically, on the cortical component of this response. The spinal component of this response is expected not to be sensitive to the valence component of emotional modulation but to be augmented by arousal (Bonnet et al., 1995). The CMR was induced by a weak (neutral) electrical stimulus. It was, therefore, not considered as a defensive act, from which it also follows that it was not expected to be modified by emotional states in a similar manner as defensive reflex responses (Lang et al., 1990).

Seventeen University students participated in the experiment. The analysis was eventually performed for ten participants, as robust CMRs were not observed in the remaining seven participants. The EMG of the interosseus dorsalis muscle was recorded from the IOD muscle of the left hand by using two disposable Ag/AgCl electrodes (for details, see Tarkka & Larsen, 1986 and original paper). Each recording consisted of 300 averaged sweeps.

The late component of CMR was found to be augmented by unpleasant emotional states. Against the motivational priming theory (Lang et al., 1997), this non-defensive reflexive response was thus sensitive to the valence component of emotional states.

The result was in line with those of in Study I and Study II. In all three studies, unpleasant states augmented reflexive responses, whereas they were not inhibited by pleasant emotional states. The result of this study also accords with the findings for other cortically mediated motor responses that have shown valence-specific emotional modulation (e.g. Coelho et al., 2010). The effect size (Cohen’s d) of the results is reported in Table 1.

TABLE 1 Effect sizes of all results (Studies I-III). In all studies, the effect size is between small and moderate.

Study I	df	t	*p*	Cohen d
CR amplitude				
pl-ne	24	0.154	0.879	0.010
pl-un	24	2.680	0.013	0.320
ne-un	24	2.822	0.009	0.340
Study II				
Startle amplitude				
pl-ne	23	0.590	0.559	0.030
pl-un	23	1.610	0.122	0.100
ne-un	23	3.080	0.005	0.140
Voluntary blink amplitude				
pl-ne	22	0.580	0.565	0.030
pl-un	22	0.530	0.600	0.030
ne-un	22	0.740	0.468	0.060
Voluntary blink onset latency				
pl-ne	22	2.380	0.030	0.270
pl-un	22	1.460	0.160	0.130
ne-un	22	3.180	0.004	0.390
Study III				
CMR amplitude				
pl-ne	9	0.440	0.668	0.070
pl-un	9	2.840	0.019	0.330
ne-un	9	1.950	0.084	0.440

CR = conditioned response; pl = pleasant, ne = neutral, un = unpleasant; CMR = cutaneo-muscular response

4 DISCUSSION

The present research aimed to examine the effects of emotional states on defensive as well as non-defensive reflexive responses. The focus was on eyeblink responses, as these responses are inherently defensive acts, although, in the context of voluntary responding, they can be non-defensive acts as well. As a reflex, it is a protective response to a potentially harmful stimulus, but when produced voluntarily to a neutral stimulus it can also be non-defensive. However, whether defensive or non-defensive, the actual motor output is the same, the only difference between the two residing certain underlying neural mechanisms. The purpose of the present research was to examine the effects of emotional modulation on these particular differences.

It seems that emotions play a more crucial role in a reflexive level (Studies I-II) by intensifying or inhibiting reflexive actions than in a voluntary level (Study II). In addition, reflexive actions need not to be inherently defensive to be augmented during negatively motivated state, as Study III suggests. The unpleasant state can also augment learned responses that have initially neutral and then associated with an aversive outcome, as in Study I. Overall, the output of a given discrete behavior, like the eyeblink response, can recruit a range of neural systems depending on its environmental and learning context.

4.1 Motivational priming and emotional modulation

According to the motivational priming theory of emotions (Lang, 1995; Lang et al., 1997), emotions ease perceptual processing the nature of which matches the prevailing environmental challenges reflected by the emotions. In doing so, they prepare the organism for fast and favorable behavioral responding. The motivation priming theory proposes that the congruence between the ongoing state and the stimulus determines whether the response is inhibited or augmented (Lang et al., 1990; Lang, 1995). This makes sense from the biological point of view. However, it should be noted that a lot of emotional material is processed unconsciously and that this processing is not focused on biologically important, e.g. fear-eliciting, stimuli alone (Balderston & Helmstetter, 2010; Knight et al., 2003; Knight et al., 2009; Reagh & Knight, 2013). The relevance of the stimulus may also play an important role in directing attention, as the recent study by Broeren & Lester (2013) suggests. The result of their study suggests that attentional biases are not restricted merely to negative stimuli. It seems that associations with relevant stimuli can be learned as threat-related associations (Pessoa & Adolphs, 2010; Purkis & Lipp, 2011).

Recent studies also suggest that motor responses that are generated faster than reflexes and do not involve sensory feedback can be affected by emotional valence or arousal. Studies of transcranial magnetic stimulation (TMS) have shown emotional modulation of cortical excitability (Baumert et al., 2011; Baumgartner et al., 2007; Coelho et al., 2010; Coombes et al, 2007; Hajcak et al., 2007; Koganemaru et al., 2012; van Loon, et al., 2010).

The presentation of negative emotional pictures increases the amplitude of motor potentials (MEPs) in peripheral muscles evoked by TMS applied to the motor cortex (Coombes et al., 2009; Hajcak et al., 2007; van Loon et al., 2010). Similar effects have been found by using emotional stimuli with the auditory modality (Baumert, 2011), indicating that so increased corticospinal excitability by unpleasant emotional states is not limited to the visual channel (see also Oathes, Bruce & Nitschke, 2008). Further studies are needed to decide this issue. Also, it remains unclear whether the excitability is more sensitive to the valence or arousal of the emotion-inducing stimuli.

The results of Studies I-III suggest that unpleasant emotional states augment reflexive responses irrespectively of whether they are defensive or not. Although the findings of Study I and II support the motivational priming theory those of Study III run counter to this theory. It seems that motivational priming effect is more straightforward on protective, reflexive behavior. In addition, should the emotional modulation occur, the behavioral response needs to have a *direction* - we need to approach or avoid something. However, the effect seems not so clear when we are consciously attending or voluntarily performing actions. In that case, the reaction time gets longer, and the effect of emotional modulation diminishes. The *time* between the response and stimulus may be crucial in emotional modulation. That may partly explain the results on studies II-III, as the non-defensive response was augmented by unpleasant emotional state and voluntary eyeblink response was not affected by emotional modulation.

4.2 Pleasantness and unpleasantness

Aversive emotions have been a more predominant interest of reflex studies more than pleasant ones, and therefore the neural basis of aversive emotions, such as fear, is fairly well known (see e.g. fear-circuit and ASR circuit Davis et al. 1982; Davis, 1989; Lang et al., 2000). Although the same neural networks can be active in pleasant and unpleasant contexts (Lang & Bradley, 2013), the processing of negative emotions seems to be more bottom-up directed and recruits a relatively simple neural network to support fast responding in contrast to the widely distributed neural mechanisms supporting cognitive appraisal (Lazarus, 1991), while the processing of unpleasant stimuli is also faster than that of pleasant and neutral stimuli (Globisch, Hamm, Esteves & Öhman, 1999). This view was supported by the results of Studies I-III. During unpleasant emotional states, reflexive actions were augmented compared to neutral or pleasant emotional states. In Study II in which voluntary eyeblink responses were addressed, such augmentation was not observed. As the latency of voluntary eyeblink responses is long relative to reflexive eyeblink responses, the participants were likely to use cognitive appraisal to evaluate the situation.

An automatized negative motive system is evolutionarily rational: it is of vital importance to learn fast about fear-related material in order to avoid harmful aspects of the environment and to survive. Nevertheless, pleasant stimuli are not meaningless; pleasant stimuli influence the inner state of an individual so as to sustain and secure resources (Lang & Bradley, 2010). However, the presence of pleasant stimuli may not cause immediate action. There are several possible explanations for this. First, positive emotions are probably turned on slower than negative ones (Lang & Bradley, 2010). Second, rating stimuli to pleasant e.g. compared to neutral may not be as straightforward as in case of unpleasant stimuli. The control of positive emotions seems more top-down oriented and more influenced by cognitive appraisal and social factors. For example, expressing positive emotions is more subjectively controlled than negative emotions (Lang & Bradley, 2010). In all the studies summarized here, the difference between the viewing of pleasant and that of neutral pictures was failed to show. It is hard to determine, with these data, whether this was due “controlled behavior” by the participants, or whether the pictures were not sufficiently pleasant to inhibit the magnitude of the eyeblink response.

4.3 Limitations and future directions

This research aimed at studying the effects of emotional state on defensive and non-defensive eyeblink responses.

The three studies reported here have their limitations. In Study I, the conditioned responses were augmented during the unpleasant state. This finding is in line with those of previous related studies. However, contrary to our expectations (linear trend of emotional valence across positive, neutral and negative emotional pictures, respectively), the smallest responses were measured during the neutral instead of positive picture viewing. Further studies are needed to explore this issue. Furthermore, URs were not affected by emotional state. It is arguable that this was due to the fact that the airpuffs were rather intense, leading to a ceiling effect. In future research, this issue could be addressed in detail by varying airpuff intensity to see if the same result is replicated with lower intensities or not. In this study, the cerebellum-dependent delay conditioning was used; however, it would be interesting to find out whether the same results would be obtained with trace conditioning paradigms that are mediated by the hippocampus and other 'higher' brain areas. In delay conditioning the CS and US are overlapped and co-terminated, whereas in trace conditioning they are separated with a trace period, which lasts a few hundred milliseconds. This would allow us to test whether the neural mechanisms of emotional modulation are restricted to those supporting delay conditioning or whether they also have access to those recruited by cognitively more demanding trace conditioning.

In Study II, voluntary eyeblinks were merely delayed by a neutral visual command, the word "BLINK" in green font on the viewing screen. The stimulus was in same sensory modality as the pictures for inducing specific emotional states. The results may reflect the arousal component of emotions, i.e. the arousal generated by affective pictures was prone to distract attentional resources away from responding to the command, thereby increasing reaction times. And also, the emotional material disrupts attention and causes "stroop-effect" (Williams, Mathews, & McLeod, 1996). This could be further tested by presenting the command "BLINK" in the auditory modality (auditory command "BLINK") and observing whether the delay is shorter than when presenting the command in the visual modality. In addition, the blink-eliciting stimulus could be rendered more aversive, for example by coloring the letters red, which would signal a stimulus of a warning type, and so allow comparison of the neutral, green, command with its more aversive, red, counterpart. To test attentional effects more precisely, the use of an eye-tracking system which is capable of tracking microsaccades and fixation points as well as pupil size variation of the eye could also be applied in future studies. That is, in order to ensure the viewing time and e.g. measure arousal.

In Study III, with two excitatory components (E1, E2 peaks) of CMR and with its inhibitory phase in between the two (see e.g. Tarkka & Larsen, 1986), the early component (E1) was only observable in a few subjects. Furthermore, the latter component was shown for only 10 out of 17 participants. This could reflect insufficient or weak stimulation to which the participants might have gradually habituated during the experiment due to erroneous electrode placement, weak electrode contacts to the skin, or the use of an insufficient number of sweeps (300). Future studies should pay more attention to these factors and increase the number of sweeps to at least 400-500.

Inhibition by pleasant picture viewing was not found in any of the present three studies. This was surprising as the startle eyeblink procedure was performed with close attention to the relevant guidelines (Blumenthal et al., 2005) and replicating previous startle studies. The possible reasons that were not taken account but might have led to this outcome are: 1) *Personality factors*. Sensitivity to startle stimulus or sensitivity overall varies across humans, as, for example, shown by Corr et al. (1995), where linearity of the startle response occurred only in extrovert participants. Although the EMG baseline activity of each participant is taken account in the analysis, it might be reasonable to add a simple introvert/extrovert personality questionnaire in studies of this kind in the future. 2) *Subjective ratings of emotional stimuli*. In order to reliably observe valence effects, the pictures should be assessed by the participants themselves instead of using normative scales. 3) *The effect of arousal*. Although the present interest was mainly on valence effects, measuring, for example, the skin conductance response (SCR) would have been a valuable indicator of physiological arousal and could have been advantageous for excluding possible arousal effects. 4) *The IAPS picture series*. The IAPS picture series was developed to evoke affective, i.e. pleasant, neutral and unpleasant states, similarly across different cultures (Lang et al., 1998). It is nevertheless possible that cultural differences may still exist, and hence that Finnish participants' experience of the pleasant pictures may have differed from, e.g., those of North Americans. This should be considered in future studies. Furthermore, according to the feedback received, the pictures used were outdated and of poor quality – some pictures being almost unrecognizable. 5) *Evoking (positive) emotions with pictures*. Although the IAPS picture series provides naturalistic scenarios and objects, looking at pictures is a rather passive task and does not necessarily induce imminent need for action, especially in the case of pleasant pictures (Lang & Bradley, 2010). Hence, if the focus is more on action or action preparedness, the experimental setting needs to contain an active task, such as in the study by Thibodeau (2011), in order to test approach and avoidance behavior. The experimental setting could be also improved by selecting culturally more specific and meaningful pictures and setting the quality parameters of the pictures (luminance, object layout, colors etc.) with care. Moreover, to ensure the participants were obeying the instructions and viewing the pictures, an eye-tracking system would have been useful.

The measurements of the oribicularis oculi were obtained from the right eye in Study I, mostly for the technical and practical reasons. This might not be the optimal way to do the measurements, owing to the lateralization of emotional information processing (Bradley, Cuthbert & Lang, 1991; Davidson, Ekman, Saron, Senulis, & Friesen, 1990). In fact, it has been suggested that the right hemisphere processes emotional information more actively than the left hemisphere (e.g. Fox, 1991). In Study II, the response was recorded from the left eye. However, according to results, the side of the measurement did not play a crucial role.

To conclude, the results of the present research indicate that emotional modulation on the reflexive level is largely dependent on motivation and attention. Thus far, the motivation priming theory seems to continue to hold sway. However, the facts that non-defensive motor acts are modulated by emotional state and that the motor system is sensitive to emotional (valence or arousal) modulation, suggest that attention is not always needed. Overall, the augmentation of reflexive and motor acts during the unpleasant state implies that motivational priming effect is clear when avoiding unpleasant stimuli, whether they are fear-related or not. In emotional modulation, the *time* between the stimulus and response (reflexive vs. voluntary act) is important, and also, the direction of the behavior - whether to approach or to avoid.

YHTEENVETO (FINNISH SUMMARY)

Lähestyä vai välttää? Emotionaalisten kuvien katselun vaikutus refleksiivisiin vasteisiin ihmisillä

Emotionaalinen tila suuntaa tarkkaavaisuutta ja valmistaa yksilöä toimimaan tietyissä olosuhteissa suotuisella tavalla. Emotionaalisella tilalla voidaan viitata myös ihmisen sisäiseen motivaatiotilaan. Motivaatio on lajikehityksen myötä syntynyt ihmisen sisäinen systeemi, sisältäen niin neuraalisen aktivaation kuin toimintaan valmistavat sisäelinerityksellisetmuutokset, joiden avulla tarkastelemme ympäristöä ja reagoimme siellä esiintyviin ärsykkeisiin. Motivationaalisen virittymisteorian mukaan ympäristön olosuhteet ja ärsykkeet, jotka ovat miellyttäviä, saavat aikaan lähestymiskäyttäytymistä, kun taas epämiellyttävät ärsykkeet laukaisevat välttämiskäyttäytymisen. Yksinkertainen keino tutkia yksilön lähestymis- tai välttämishalukkuuden voimakkuutta, on mitata käyttäytymistä, kuten silmän sulkeutumisreaktiota, erilaisissa emotionaalisissa tilanteissa. Tyypillisessä ärsykejärjestelyssä, ns. startle-kokeessa, esitetään satunnaisesti voimakas ääniärsyke, joka säikäyttää. Säikähdysreaktio on kokonaisvaltainen kehon puolustusreaktio, joka sisältää useita fysiologisia muutoksia. Silmänräpäytyksen voimakkuus on yksi ensimmäisistä säikähdysreaktion mitattavista komponenteista, ja sen on havaittu heijastavan puolustusreaktion voimakkuutta erilaisissa emotionaalisissa olosuhteissa. Epämiellyttävä emootiotila kasvattaa silmänräpäytyksen voimakkuutta ja miellyttävä emootiotila vaimentaa sitä. Silmän sulkeutuminen on yksi tärkeimmistä puolustusreaktioista, koska se suojelee näköaistia. Silmän sulkeminen voidaan myös tuottaa muutenkin kuin tahattomasti eli refleksiivisesti. Silmän voi sulkea myös spontaanisti tai tahdonalaisesti. Tässä tutkimuksessa oli tarkoituksena tutkia motivationaalisen virittymisteorian yleistettävyyttä muihinkin kuin refleksiivisiin käyttäytymisvasteisiin ja selvittää, vaikuttaako emotionaalinen tila myös itse tuotettuun reaktioon sekä ei-defensiiviseen reaktioon, eli reaktioon, joka ei varsinaisesti ole lähestymis- eikä välttämiskäyttäytymisen ilmentymä.

Ensimmäisessä osatutkimuksessa mitattiin silmän lihasjännityksen muutosta klassisen silmäniskuehdollistamisen aikana. Ehdollisen neutraalin ärsykkeen, äänen, kanssa esitettiin ilmapuhallus, joka aiheutti ehdottoman reaktion, silmänräpäytyksen. Näiden toistaminen sai aikaan se, että silmänräpäytys tuotettiin pelkkään ääneen. Tutkimuksen tarkoituksena oli selvittää vaikuttaako emotionaalinen tila jo opittuun käyttäytymiseen. Aiemmissa tutkimuksissa, jotka pohjautuvat motivationaaliseen virittymisteoriaan, on saatu tuloksia, joiden mukaan puolustusreaktiot muokkautuvat emootiotilan mukaisesti, yleensä emootiotilan kanssa kongruentisti eli samansuuntaisesti. Silmänräpäytys, joka tuotetaan oppimisen seurauksena aiemmin neutraaliin ärsykkeeseen, on sellainen puolustusreaktio, jonka voitiin olettaa muokkautuvan emootiotilan mukaan. Tutkimuksessa havaittiin, että jo opitut käyttäytymisvasteet voimistuivat epämiellyttävän emotionaalisen tilan aikana. Tämä tuki aiempia tutkimustuloksia, joskaan käyttäytymisvasteen voimakkuus ei muuttunut lineaarisesti, kuten aiemmissa startle-kokeissa. Ehdoton reaktio, ilmapuhalluksella aiheutettu silmänräpäytys, ei kuitenkaan muokkautunut emootiotilan mukaan. Kyseessä saattoi olla liian voimakkaan ilmapuhalluksen aiheuttama kattoefekti.

Toisessa osatutkimuksessa mitattiin silmän lihasjännityksen muutosta startle-kokeen aikana sekä itse tuotettuna reaktiona visuaaliseen ärsykkeeseen (BLINK-sana). Käsky räpäyttää silmää BLINK-sanan ilmestyessä pakottaa tekemään puolustusreaktion ilman varsinaista uhkaa. Emotionaalinen tila muokkasi silmänräpäytystä startle-kokeessa aiempien tutkimustulosten mukaisesti, mutta emotionaalinen tila ei vaikuttanut itse tuotetun silmänräpäytyksen voimakkuuteen. Tutkimuksessa kuitenkin havaittiin, että itse tuotetun räpäytyksen latenssi eli ärsykkeen ja reaktion välinen aika, oli suurempi sekä miellyttävän että epämiellyttävän emootiotilan aikana. Tämä saattaa viitata siihen, että vaikka kyseessä on saman reaktion motorinen ilmentymä, emotionaalinen valenssi muokkaa vain refleksiivisesti tuotettua reaktiota. Sen sijaan vireystila vaikuttaa myös itse tuotetun reaktion tuottamiseen, eli pidentää reaktioaikaa, mutta ei muokkaa sen voimakkuutta. Silmänräpäytyksen tuottamiseen vaikuttavat erilaiset hermostolliset järjestelmät riippuen siitä, onko reaktio tahaton vai itse tuotettu. Jälkimmäisen tuottamiseen sisältyy kognitiivista arviointia, mitä automaattisessa refleksiivisessä reagoinnissa ei tarvita. Tulosten mukaan näyttäisi siltä, että emootiotila ei vaikuta reaktion tuottamiseen, kun kyseessä on itse tuotettu reaktio.

Kolmannessa osatutkimuksessa haluttiin tutkia motorista reaktiota, jolla ei olisi defensiivistä ilmentymää, kuten silmänräpäytysreaktiolla. Tutkimuksessa mitattiin supraspinaalista refleksiä ja sen muokkautuvuutta emotionaalisten kuvien katselun aikana. Kyseisen reaktion oletettiin olevan sellainen, että sen toiminta ei liity suoranaisesti motivaatiojärjestelmään, vaan on sinällään neutraali motorinen reaktio ulkoiseen ärsytykseen, jolloin motivationaalisen virittymisteorian mukaisesti emootiotilalla ei olisi siihen vaikutusta. Supraspinaalinen refleksiivinen reaktio aiheutettiin sähköisellä ärsytyksellä, joka oli havaintokynnyksen yläpuolella, mutta selkeästi kipukynnyksen alapuolella. Tämä refleksi sisältää niin spinaalisen kuin kortikaalisen vasteen. Aiemmissa tutkimuksissa on huomattu, että spinaaliset refleksit eivät sinänsä muokkaudu emotionaalisen havainnon mukaan, vaan ne voimistuvat vireystilan voimistuessa. Mutta viimeaikaisten tutkimustulosten mukaan emootiotila muokkaa motorisia reaktioita, joiden tuottamiseen osallistuu kortikaalisia alueita. Tämän vuoksi tutkittiin refleksin kortikaalista vastetta ja havaittiin, että reaktio voimistui epämiellyttävien kuvien katselun aikana. Tämä viittaa siihen, että epämiellyttävä emootiotila vaikuttaa muidenkin kuin pelkästään defensiivisten reaktioiden voimakkuuteen.

Kokonaisuudessaan nämä tutkimustulokset tukevat motivationaalista virittymisteoriaa siinä mielessä, että kaikissa tutkimuksissa epämiellyttävä emootiotila voimisti refleksiivisiä käyttäytymisvasteita. Erona oli, että missään tutkimuksessa ei miellyttävien kuvien ja neutraalien kuvien katselulla ollut merkitsevää eroa. Tämä voi liittyä siihen, että kuvasarjan kuvia ei koettu tarpeeksi miellyttäviksi. Lisäksi kuviin liittyvät kulttuuriset erilaisuudet, kuvien laatu, ihmisten persoonallisuus jne. ovat voineet vaikuttaa yksilöiden kokemukseen, joita ei tässä tutkimuksessa kontrolloitu. Ristiriidassa teorian kanssa oli motorisen, ei-defensiivisen vasteen voimistuminen epämiellyttävien kuvien katselun aikana. Tämä viittaa siihen, että etenkin epämiellyttävän emootiotilan aikana motorinen toimintavalmius kasvaa. Lajikehityksellisesti ajatellen tämä kuulostaa järkevältä; meissä on hyvin nopea sisäinen systeemi, joka ilman vaivalloista kognitiivista arviointia valmistaa meitä välttämään haitallisia ärsykkeitä, olivatpa ne pelkoa herättäviä tai ei. Sitä, johtuuko vaikutus emotionaalisesta valenssista tai viriävyydestä vai niiden yhteisvaikutuksesta, ei valitettavasti tällä tutkimuksella pystytä täysin vahvistamaan. Yhä useammat tutkimukset näyttävät kuitenkin vahvistavan sitä seikkaa, että prosessoimme emotionaalista materiaalia hyvin paljon myös tiedostamattomasti, ja prosessointi ei rajoitu pelkästään biologisesti merkittäviin ärsykkeisiin, tai äärimmäisen epämiellyttäviin, kuten pelkoa herättäviin ärsykkeisiin. Näyttäisikin, että emotionaalinen muokkautuvuus näkyy selvemmin refleksiivisellä tasolla, etenkin silloin, kun emme itse aktiivisesti havaitse, tai tietoisesti kontrolloi käyttäytymistämme. Emotionaaliseen muokkautuvuuteen vaikuttaa ärsykkeen ja reaktion välinen *aika* - sen kasvaessa emotionaalisen muokkautuvuuden vaikutus näyttää pienenevän. Emotionaalinen muokkautuvuus vaatii myös sen, että käyttäytymisellä on *suunta,* eli täytyy olla jotain miellyttävää, jota on tarkoitus lähestyä, tai epämiellyttävää, jota on tarkoitus välttää.

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I

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II

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Effect of Emotional Picture Viewing on Voluntary Eyeblinks

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Abstract

Eyeblinks, whether reflexive or voluntary, play an important role in protecting our vision. When viewing pictures, reflexive eyeblinks are known to be modulated by the emotional state induced thereby. More specifically, the hedonic valence (unpleasantness-pleasantness) induced by the picture has been shown to have a linear relationship with the amplitude of a startle blink elicited during picture viewing. This effect has been attributed to congruence between an ongoing state and task demands: an unpleasant emotional state is assumed to bias our attention towards potentially harmful stimuli, such as startle tones. However, recent research suggests that the valence-specific modulation may not be limited to the sensory parts of the reflexive pathway related to startle responses. Here, we examined the effect of emotional picture viewing on voluntary (in response to a written command) eyeblinks in adult humans. Emotional modulation of startle blinks was also evaluated. We found that when viewing unpleasant pictures, the amplitude of reflexive eyeblinks was augmented, but the amplitude of voluntary eyeblinks was unaffected. Nevertheless, the response latencies of voluntary eyeblinks were found to be delayed during the viewing of pleasant and unpleasant relative to neutral pictures. We conclude that these results support the theory that emotional experience augments sensory processing specific to potentially harmful stimuli. Further, the emotional state seems not to exert an effect on voluntarily elicited motor activity.

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Introduction

Emotional state, particularly its hedonic valence, modulates defensive reflexes [1,2]. The most common way to examine emotional reflex modulation is to assess the effect of emotional picture viewing on the amplitude of the eyeblink component of the acoustic startle reflex. The viewing of pictures with unpleasant content (low hedonic valence) has been found to potentiate and pictures with pleasant content (high hedonic valence) to diminish acoustic startle eyeblink reflexes. According to the motivational priming hypothesis [3], emotional picture viewing mobilizes a motivational system that, in turn, facilitates the accessibility of motor action programs that correspond to the state of this system with respect to the hedonic valence of an emotion. Reciprocally, action programs that do not correspond to the valence become less accessible and, in consequence, are less likely to be activated. Reflexes that are not inherently defensive i.e. protective, are not considered to be engaged to the motivational system and, therefore, not modulated by emotional state [4]. The modulation effect has been specifically attributed to valence. Although pictures with both high and low hedonic valence values induce heightened arousal, arousal does not affect startle amplitudes [3].

The unpleasant emotional state is assumed to bias our attention towards potentially harmful stimuli resulting in enhanced defensive responses. However, this augmentation caused by viewing unpleasant pictures has also been found for behavioral responses elicited artificially by direct stimulation of the motor cortex [5,6]. Furthermore, such a valence-specific increase has even been observed in the accuracy of choice-reaction-time task performance through enhancement of local inhibitory networks and increased plasticity by the GABAergic system and NMDA receptors [6]. What remains an open question is whether valence-specific modulation of eyeblinks by emotional states could operate not only on the reflexive but also voluntary eyeblinks, as voluntary eyeblinks can be regarded as protective actions, even if they are not elicited by threatening stimuli.

To test this notion, we measured, in adult humans, eyeblinks that were generated either by a startle stimulus or as voluntary response to a written command.

These responses recruit the same motor mechanism (motoneurons in the facial motor nucleus or spinal cord) as that which contracts the orbicularis oculi muscle [7,8]. However, eyeblinks are initially driven by different mechanisms, one residing in the acoustic startle pathway (reflexive eyeblinks) and the other lying in the cortex (voluntary eyeblinks). We presented the participants with unpleasant, neutral and pleasant emotional images drawn from the International Affective Picture System; IAPS [9] and measured their voluntary eyeblinks (initiated by a neutral visual stimulus, the word "BLINK") and acoustic startle stimulus-evoked reflexive eyeblinks. We examined whether voluntary eyeblinks are affected by emotional state and, if so, whether the underlying mechanisms could be regarded as the same, or at least as analogous with the mechanisms underlying reflexive eyeblinks, in the sense that they would relate to the valence of an emotion as predicted by the motivational priming hypothesis [3].

Material and Methods

Participants, ethics statement and stimulus material

A total of 24 volunteers (age range 19 to 33; mean age 22, of whom 21 were females) participated in the experiment. As a compensation for their participation, the participants received a ticket to a local cinema.

This study was approved by the research ethics committee of the University of Jyväskylä and was performed in accordance with the Declaration of Helsinki.

White noise startle probes (105 dB, 50 ms, center frequency 1000 Hz, 5 ms rise/fall times) were generated with SoundForge 6.0 software and presented through headphones. Voluntary eyeblinks were elicited by a visual stimulus consisting of a 250-ms flash of the word "BLINK" (lime green colour, bolded Courier New, font size 90) which appeared in the centre of the computer monitor either against a black background or superimposed on an affective picture (Figure 1). The luminosity value of each picture was analyzed with the IrfanView program to ensure the word BLINK was similarly detectable between the emotional categories. The luminosity ratings did not differ significantly between the emotional categories (pleasant, neutral, unpleasant).

The 90 emotional pictures used in the experiment were selected from the International Affective Picture System (IAPS [9]. The pictures were pleasant (e.g. food, animals etc.), neutral (e.g. household objects, neutral faces etc.) or unpleasant (e.g. mutilations, spiders etc.). The normative mean valence and arousal ratings and their standard deviations for pleasant, neutral and unpleasant pictures are reported in Table 1. These categories of pictures significantly differed from each other both in valence, i.e., pleasant vs. unpleasant [$t(29) = 21.33$; $p < 0.001$], pleasant vs. neutral [$t(29) = 20.36$; $p < 0.001$] and neutral and unpleasant [$t(29) = 29.73$; $p < 0.001$], and in arousal, i.e., pleasant vs. unpleasant [$t(29) = 9.60$; $p < 0.001$], pleasant vs. neutral [$t(29) = 15.31$; $p < 0.001$] and neutral and unpleasant [$t(29) = 4.07$; $p < 0.001$]. Stimulus presentation was controlled for by E-Prime 2.0 software.

Orbicularis oculi EMG activity was recorded from beneath the left eye by using two disposable Ag/AgCl electrodes, following the guidelines presented in [10]. The active electrode was attached vertically below the pupil and was referenced to an electrode about 1 cm lateral to it. The EMG data were recorded with a QuickAmp amplifier using BrainProducts Recorder software running on a PC. All off-line signal processing was conducted using Matlab with the Signal Processing Toolbox.

Procedures and Experimental Design

Upon arrival at the laboratory, participants were instructed to read and, if they decided to participate, sign an informed consent form which provided general information on the experiment. During the experiment, the participants were seated in a comfortable chair in a dimly lit room with a 17-inch computer

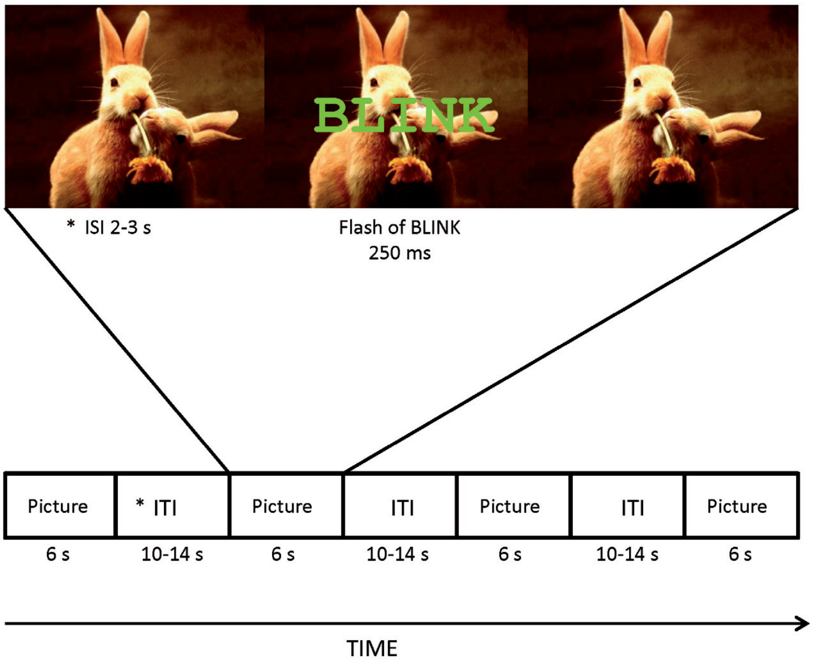

Figure 1. Schematic illustration of the voluntary eyeblink procedure. An example of one trial, using a pleasant picture. *ITI and ISI refer to Inter-Trial-Interval and Inter-Stimulus-Interval, respectively.
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Table 1. The normative mean valence and arousal ratings and their standard deviations.

IAPS pictures	Pleasant	Neutral	Unpleasant
The normative mean (SD) valence ratings	7.53(0.43)	5.04(0.60)	2.49(0.71)
The normative mean (SD) arousal ratings	4.90(1.02)	2.87(0.42)	6.01(0.89)
The mean (SD) luminosity ratings	111.20(41.39)	101.52(36.65)	97.66(39.11)

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monitor located approximately 1 m in front of them. They were instructed to view the pictures shown on the computer monitor and to ignore all irrelevant stimuli in the experimental room. During the experiment, occasional tones or the flash-word BLINK would be presented. Randomly selected examples of the BLINK stimuli both on the affective picture and on the black background were presented to the participants before the experiment. The experiment consisted of two procedures: a startle eyeblink procedure and a voluntary eyeblink procedure. The order of the two procedures was counterbalanced across the participants.

The startle eyeblink procedure

The startle eyeblink procedure consisted of two phases: a habituation phase and an acquisition phase. In the habituation phase, 5 startle tone-alone trials were presented in order to familiarize the participants with the loud tone. In the acquisition phase, 30 trials with the startle tone and randomly selected affective picture content (pleasant, neutral or unpleasant, 10 each), 10 trials with the startle tone alone and 9 trials with affective pictures alone (3 neutral, 3 pleasant and 3 unpleasant) were presented in pseudo-random order. The participants were informed that they would hear occasional tones, but their task was to ignore them.

The voluntary eyeblink procedure

In the voluntary eyeblink procedure, 30 trials with randomly selected affective picture content (pleasant, neutral or unpleasant, 10 each) and the visual stimulus, 10 trials with the visual stimulus alone (black background) and 9 trials with affective pictures alone (3 neutral, 3 pleasant and 3 unpleasant) were presented in pseudo-random order. The participants were informed that their task was simply to blink their eyes once every time they saw the word "BLINK".

During the startle and voluntary eyeblink procedures, the pictures were visible for 6 seconds and the startle tone or the word BLINK was presented either 2 or 3 s from image onset. The inter-trial interval, during which the display was black, was randomly either 10, 12 or 14 s.

Data Analysis

The startle and voluntary blink EMG signal was amplified, digitized (sampling rate 2000 Hz), rectified and digitally off-line filtered with a low-pass (<20 Hz) filter. First, bad trials were visually excluded with the observer blind to the trial type. The exclusion criterion was excessive EMG activity during the 500-ms baseline period. Peak amplitude and the peak and onset latencies over the period 20 to 150 ms after startle tone onset were determined automatically with a Matlab script. The values were then averaged for each type of picture content (pleasant, neutral and unpleasant).

The same measures were determined for the voluntary eyeblink responses. Visually identified bad trials were first excluded by an observer blind to the trial type. The exclusion criteria were i) excessive EMG activity during the 500-ms baseline period or a blink the onset latency of which occurred during the first 100 ms after the BLINK stimulus, and ii) no response up to 1 000 ms after the BLINK stimulus. Onset latencies were visually determined trial-by-trial on a computer display and hand-scored. Peak amplitudes and latencies were determined automatically with a Matlab script for the period of 100 to 1 000 ms after the BLINK stimulus. One participant was discarded owing to very late responses (mostly in excess of 1 000 ms after the BLINK stimulus).

Analysis of variance for repeated measures and additional post-hoc paired samples *t*-tests with Bonferroni-correction were used to analyze the effects of conditioning and image content. Greenhouse-Geisser-corrected degrees of freedom were used if the sphericity assumption was violated.

Results

Peak amplitudes, onset latencies and peak latencies

The peak amplitudes in both eyeblink types (startle vs. voluntary) as a function of image content (pleasant, neutral, unpleasant) were analysed with a 2×3 repeated measures ANOVA, which showed a significant interaction between factors [F(2,44) = 3.36; p<0.05], indicating that the modification effect of image content on blink amplitude was different between the response types. The main effect of response type was also significant [F(1,22) = 4.70; p<0.05], indicating larger peak amplitudes in startle responses than in voluntary blinks. ANOVA performed for each response type separately revealed a significant main effect of image on startle peak amplitude [F(2,46) = 3.23; p<0.05], indicating that emotional picture viewing modified the startle eyeblinks (Figure 2). Pairwise comparisons between pleasant vs. unpleasant [t(23) = 1.61; p = 0.122] and pleasant vs. neutral [t(23) = 0.59; p = 0.559] did not reach significance, but the difference between neutral and unpleasant was significant [t(23) = 3.08; p<0.01], indicating that the largest startle eyeblinks were elicited during unpleasant picture viewing. For voluntary eyeblinks, no main effect of image on peak amplitude was observed [F(2,44) = 0.44; p = 0.647].

Repeated measures ANOVA for a main effect of image content and response type on onset latency showed significant interaction [F(2,44) = 5.89; p<0.01], indicating that image content had a different modulatory effect on the timing of startle blinks compared to voluntary blinks. Both response and image type had a significant main effect [F(1,22) = 425.92; p<0.001 and F(2,44) = 7.06; p<0.01, respectively]. ANOVAs performed for each response type separately showed no significant main effect of image type on onset latency for startle blinks but a main effect was found for voluntary blinks [F(2,44) = 6.62; p<0.01]. Subsequent paired t-tests showed that the onset latencies for both pleasant and unpleasant images were longer than those for neutral images [t(22) = 2.38, p<0.05 and t(22) = 3.18, p<0.01], respectively (Figure 2).

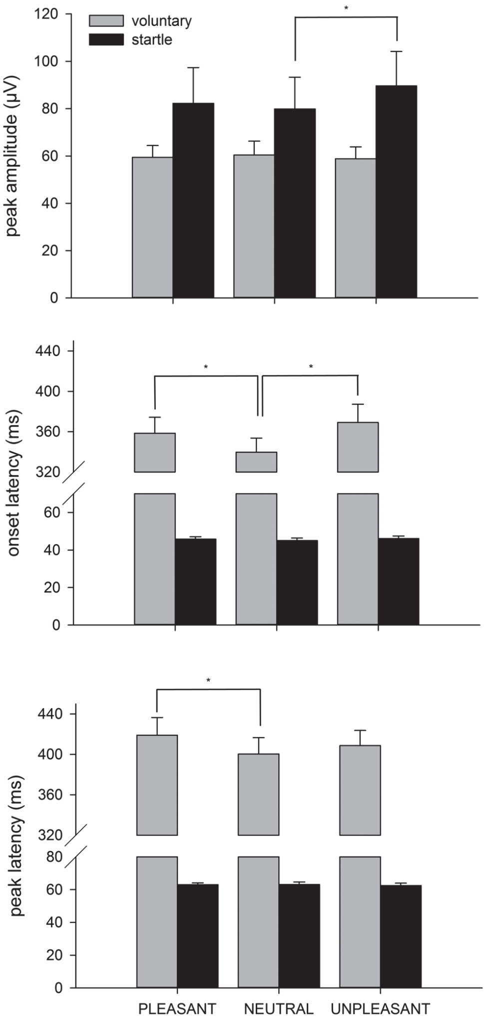

Figure 2. Startle and voluntary eyeblink properties (+ SEM) during affective picture viewing, *$p<0.05$. The amplitudes of the startle eyeblinks were significantly augmented during the unpleasant picture viewing. No such effect on the amplitude of the voluntary eyeblinks was observed. The onset latencies for both pleasant and unpleasant images were longer than those for neutral images and the voluntary eyeblinks during the viewing of pleasant images had significantly longest peak latency.
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Image x response type ANOVA revealed an almost significant interaction [F(2,44) = 2.41, p = 0.10] and main effect of image type [F(2,44) = 3.09, p = 0.056]. Response type had a clearly significant effect [F(1,22) = 508.40, p<0.001]. ANOVAs performed for each response type separately revealed no significant effect of image type on peak latency for startle blinks [F(2,46) = 0.23, p = 0.793] and a nearly significant effect for voluntary blinks [F(2,44) = 2.74, p = 0.076]. Subsequent paired t-tests indicated that voluntary blinks during the viewing of pleasant images had a significantly longer peak latency than those during the viewing of neutral images [t(22) = 2.38, p<0.05] (Figure 2).

Discussion

The present study investigated whether emotional picture viewing similarly affects the amplitude of voluntary and reflexive eyeblinks. In the case of reflexive eyeblinks, the highest amplitude was found with the viewing of unpleasant pictures. In contrast, no such valence effect was found in the amplitude of voluntary eyeblinks. The only effect of emotional picture viewing on voluntary eyeblinks was in onset latency which was longer with pleasant and unpleasant pictures relative to neutral pictures.

The augmentation of reflexive eyeblinks by unpleasant pictures was highly expected (e.g. [1,2]) and is in line with the motivational priming hypothesis [3]. However, no linear trend was observed in startle eyeblink modification, because smallest responses were measured during neutral picture viewing. The reason for this is unclear, but one possibility may be, that even if the IAPS picture series is widely used in several cultures, Finnish participants' experience of the pleasant pictures may differ from e.g. those of North American. In fact, the same pattern was observed in our previous study on the effect of emotional picture viewing on the amplitude of conditioned eyeblink responses [11].

The modification of startle eyeblinks is a phenomenon which is believed to index defensive-protective tendencies. The simplest and fastest pathway for reflexive eyeblinks that is modulated by emotional valence is the acoustic startle pathway. This pathway consists of cochlear root neurons which project to neurons in the nucleus reticularis pontis caudalis, and motoneurons in the facial motor nucleus (pinna reflex) and spinal cord [12–14]. The nucleus reticularis pontis caudalis receives projections from the central nucleus of the amygdala [14,15]. These projections have an important role in mediating fear/defense responses, and are likely to mediate the valence effects of reflexive eyeblinks. The central nucleus of the amygdala also has reciprocal connections to various cortical sites [15], implying that valence-specific reflex modification, as allowed by the amygdala, may also apply to cortically-induced behavioral responses, such as voluntary eyeblinks As both of these mechanisms are accessible by the central nucleus of the amygdala, we reasoned that if valence-specific emotional modulation is not restricted to the reflex generating mechanism, such modulation should be similarly observed in both modes of eyeblink responses. The results, however, did not show this to be the case.

The negative valence of the pictures could be regarded as facilitating the mechanism for reflexive eyeblinks of a defensive nature. In contrast, voluntary eyeblinks did not show similar augmentation. Although these responses are initially driven by different mechanisms, they are both accessible by the central nucleus of the amygdala [15], and thus can be expected to show emotional modulation. However, as voluntary eyeblinks are also mediated by cortical connections, they are generated later than reflexes and are most probably affected by "cognitive appraisal". Therefore, even if the motor output is the same, the present results imply that the valence-specific emotional modulation observed might be more restricted to the reflex generating mechanism and not affect the voluntary eyeblinks. It is possible that emotional state modulates only those actions that are initiated by biologically meaningful threat signals that reach the amygdala. Information about voluntary actions might reach the amygdala differently or to lesser extent, which might explain the lack of emotional modulation of voluntary eyeblinks.

However, voluntary eyeblinks were affected by the emotional picture viewing, as they were significantly delayed during both pleasant and unpleasant images in comparison to neutral images. This effect could be due to either arousal or, more simply, to the fact that the emotion-evoking pictures tend to be more complex. Thus, the relative delay induced by emotional pictures in voluntary eyeblinks may relate to selective attention [2]. Namely, attentional mechanisms might have prioritized pictures inducing high emotional arousal over the written ''BLINK'' command as an emotionally neutral stimulus, thereby delaying the execution of the voluntary blink. This possibility is further supported by previous findings where behavioral responses to a neutral stimulus were delayed by background stimuli of high emotional arousal [16,17].

It might also be suggested that valence played no observable role in modifying the amplitude of voluntary eyeblinks because the visual stimulus inducing these blinks (the word ''blink'') was not of a clearly threatening nature (for the congruency assumption of the motivational priming hypothesis, see [3]). This is, however, not supported by previous findings of responses induced by TMS pulses to the motor cortex augmented by unpleasant relative to pleasant or neutral pictures [5,6], as TMS pulses, directly delivered to the motor cortex, cannot per se be considered as perceptually threatening stimuli. This possibility could, nevertheless, be tested by also making the visual stimulus itself indirectly threatening (for example by using a word that signals a threat, such as ''air puff'', that can be removed by a blink). If such a setting fails to produce valence-specific effects, then it is possible that emotional valence can only modify a behavioral response that is both defensive in nature and an innate reaction to a threat cue.

The sensitivity of reflexive but not voluntary eyeblinks to emotional valence was unlikely to be accounted for by the different stimulus modalities (visual vs. auditory) used to induce them, as startle eyeblinks can be similarly amplitude-modulated by emotions irrespective of whether the eyeblinks are induced by visual, acoustic or tactile stimuli [18,19]. In fact, it would be possible to examine the stimulus modality effect by using an auditory instead of visual command to elicit the voluntary eyeblink and so test whether the voluntary eyeblink remains be non-sensitive to emotional valence.

To conclude, we found augmentation of reflexive eyeblinks to unpleasant pictures and delayed voluntary eyeblinks to both unpleasant and pleasant pictures relative to neutral pictures. This pattern of findings suggests that emotional experience augments sensory processing that is specific to potentially harmful stimuli and that in terms of their sensitivity to emotional states, reflexive and voluntary eyeblinks recruit different mechanisms as described by the motivational priming hypothesis.

Supporting Information

Appendix S1
(DOCX)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: SK JW. Performed the experiments: SK. Analyzed the data: SK JW. Contributed reagents/materials/analysis tools: SK JW. Wrote the paper: SK JW TR.

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III

UNPLEASANT EMOTIONAL STATE AUGMENTS SUPRASPINAL REFLEX RESPONSE

by

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Unpleasant emotional state augments supraspinal reflex response

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Abstract

Emotional states modulate the amplitude of defensive reflexes. More specifically, viewing of images with unpleasant content (low hedonic valence) augments these reflexes whereas viewing of pleasant images (high valence) diminishes them. One view posits that this amplitude modulation occurs when an emotional state (aversive or appetitive) biases sensory processes towards the kind of stimuli that are congruent with the state and thereby facilitating the perceptual stage of reflex generation. However, recent evidence suggests that emotional state also may prime the cortical motor control of reflexes. However, it remains unclear whether it is valence or arousal that is behind the effect. Here, we examined whether emotional picture viewing affects the cortically mediated (supraspinal) component of the cutaneo-muscular reflex (CMR, elicited in the first interosseus dorsalis muscle by electrical stimulation). This response is neither elicited by an unpleasant stimulus nor serves any defensive purpose. We found that viewing of unpleasant pictures augmented the amplitude of CMRs, suggesting that emotional valence affects motor cortical function.

Keywords: emotional state, cutaneo-muscular reflex response (CMR), cutaneous stimulation

Introduction

Innate defensive reflexes, fast motor responses driven by sudden obnoxious stimuli, serve to protect the organism from physical threats. These reflexes, like the startle reflex generated by an abrupt loud acoustic stimulus, are modulated by a system that uses motivational cues to adapt rapidly and with less effort to the prevailing circumstances [1, 2]. Emotionally unpleasant cues prime defensive reflexes and pleasant ones inhibit these reflexes e.g. [3, 4]. The effect of emotional modulation is not limited to whole-body startle responses but also applies to locally induced defensive responses, such as those evoked by airpuffs towards the eye [5] and classically conditioned eyeblink responses [6].

According to the motivational priming hypothesis e.g. [4, 7], the active motivational system exerts a modulatory effect on the action programs in which they are engaged. This hypothesis suggests that emotions are "action dispositions" which direct our sensory systems in a way that enhances proper action. Hence, emotional valence modulation is thought to result from the interaction between the current motivation state and the role of the reflex: The stimuli that are congruent with the current motivated state are primed, e.g. in negatively valenced emotional state the readiness to pick up stimuli that are potentially harmful is facilitated and defensive reflexes are augmented (see [7]). This in turn means that *non-defensively* functioning reflexes are considered not to be affected by a motivational system and, therefore, not modulated by emotional states aroused by such a system e.g. [8]. This view is supported by the finding that the amplitude of the spinal tendinous (T)-reflex, which is sensitive to both defensively and appetitively motivated action, does not change as a function of emotional valence but is augmented by high arousal [8].The emotional modulation of a defensive reflex, such as the startle reflex, is attributed to valence – the emotional state primes sensory processing in order to approach appetitive stimuli and avoid potentially harmful stimuli [2, 9]. Although pictures with both high and low hedonic valence values induce heightened arousal, arousal per se does not affect startle amplitudes [7]. In contrast, spinal reflexes, which are considered to be non-defensive, have been observed to be modulated merely by emotional arousal [8].

Emotional modulation has also been found to affect motor responses of a non-defensive nature when these responses are elicited cortically, for example, by transcranial magnetic stimulation (TMS) [10-14]. However, the findings have been inconsistent and have varied by e.g. stimulus type, task type and the target muscle. Coelho et al. [13] found augmentation in EMG activity of the first interosseous dorsalis muscle elicited by TMS during unpleasant picture viewing, whereas Koganemaru et al. [14] reported that unpleasant picture viewing shortened reaction times during the choice reaction-time task. van Loon et al. [12] also found facilitation of action readiness in a choice task during both unpleasant and pleasant picture viewing, implying that arousal rather than valence played a role. Consistently, Hajcak et al. [10] found evidence of the involvement of arousal in emotional modulation. They found augmentation of muscle activity (the abductor pollicus brevis muscle) during viewing of both pleasant and unpleasant pictures. The results of these studies leave open the question whether cortically mediated responses are more sensitive to emotional valence or whether arousal might play a decisive role. To test this, we measured supraspinal cutaneo-muscular reflex responses (CMRs) generated by mild electrical stimulation. These responses do not serve any motivational purpose and therefore, as low-level reflexes, one can expect them not to be affected by emotional modulation. However, CMR consists of two excitatory components of which the one with longer latency is considered to be a reflex response that is modulated by the cortex via the supraspinal pathway [15, 16]. The transcortical origin of such long latency responses (> 60 ms) are confirmed by studies in patiens with lesions made to corticospinal tract, and also, in infants the maturation of the long-latency component of is noticed to parallel the corticospinal tract maturation [17]. If emotional modulation is observed, it is likely to take place in the cortically modulated part of the response.

We presented the participants with the unpleasant, neutral and pleasant emotional images drawn from the International Affective Picture System; IAPS [18] while measuring their CMRs induced by electrical stimulation of the index finger. We expected that if emotional modulation also targets artificially elicited motor responses of a neutral nature, they might also modulate supraspinal reflexes that are generated cortically.

Material and methods

Participants

A total of 17 volunteers (age range 20 to 51; mean age 25, of whom 12 were females) participated in the experiment. The data analysis was conducted to 10 participants (age range 20 to 51; mean age 25, of whom 7 were females). The study was approved by the research ethics committee of the University of Jyväskylä and was performed in accordance with the Declaration of Helsinki. As compensation for their participation, the participants received a ticket to a local cinema.

Stimulus material

Electrical stimulation was delivered by a pair of wire flexible ring electrodes to the digital nerves of the index finger and hence that the cathode was placed proximally. The stimuli were delivered by a constant current stimulator (Digitimer LTD) at a frequency of 2 Hz and with a pulse duration of 0.1 ms. Simulus intensity was set to about 1.5 times the individual sensory threshold (intensity ranging from 0.37 µV to 1.08 µV) and was always maintained below the level of discomfort.

The emotional pictures used in the experiment were randomly selected from the 90-picture set of the International Affective Picture System (IAPS)[1]. The pictures were pleasant (e.g. food, animals etc.), neutral (e.g. household objects, neutral faces etc.) or unpleasant (e.g. mutilations, spiders etc.). The normative mean valence and arousal ratings and their standard deviations for the pleasant, neutral and unpleasant pictures are reported in Table 1. These categories of pictures significantly differed from each other both in valence, i.e., pleasant vs. unpleasant [$t(29) = 21.33$; $p < 0.001$], pleasant vs. neutral [$t(29) = 20.36$; $p < 0.001$] and neutral and unpleasant [$t(29) = 29.73$; $p < 0.001$], and in arousal, i.e., pleasant vs. unpleasant [$t(29) = 9.60$; $p < 0.001$], pleasant vs. neutral [$t(29) = 15.31$; $p < 0.001$] and neutral and unpleasant [$t(29) = 4.07$; $p < 0.001$]. Stimulus presentation was controlled for by E-Prime 1.2 software.

The CMR recordings were made while the participant maintained a weak isometric contraction of the left first dorsal interosseus muscle (IOD) by stretching a rubber band placed between the middle and index fingers. The electromyogram (EMG) of the IOD was recorded on the muscle belly using two disposable Ag/AgCl electrodes; for details see [15]. The reference electrode was attached 25 mm proximal to the active one and the ground electrode on the proximal bony area of the left arm. Each recording consisted of 300 averaged sweeps (10 per each picture presentation).

EMG data were recorded with a QuickAmp amplifier with a 1000-Hz sampling rate with BrainProducts Recorder software running on a PC. All off-line signal processing was performed using Matlab with Signal Processing Toolbox.

Procedure and experimental design

Upon arrival at the laboratory, participants were asked to read and sign an informed consent form which provided general information on the experiment. During the experiment, the participants were seated in a comfortable chair in a dimly lit room with a 17-inch computer monitor located approximately 1 m in front of them. They were instructed to view the pictures projected via the computer monitor and to ignore all irrelevant stimuli in the experimental room. During the experiment, occasional electrical stimulation would be elicited. Before the experiment, the stimulus intensity was adjusted individually for each participant.

During the experiment, participants viewed pleasant, neutral and unpleasant pictures. Pictures (10 of each type) were visible for 7 s and the train of 10 pulses of electric stimulation was presented during the picture viewing, starting 2-3 s from image onset (Figure 1). The inter-trial interval (offset-to-onset) between images was randomly either 10, 12 or 14 s. While the participants were viewing the pictures, their left forearm was resting on the arm of the armchair. They were instructed to view the pictures and stretch the rubber band during the time that the pictures were visible, and rest their fingers when the monitor was black.

Data analysis

The raw EMG signal was amplified, digitized (sampling rate 1000 Hz), rectified and digitally off-line filtered with low-pass (< 20 Hz) filter. EMG signals were then averaged for each type of picture content (pleasant, neutral and unpleasant). The maximum amplitudes of the supraspinal CMRs were calculated from an electrical stimulation window of 60 to 80 ms (as indicated in [15]).

Analysis of variance for repeated measures and additional paired samples *t*-tests with Bonferroni-correction were used to analyse the effects of condition and image content. Greenhouse-Geisser-corrected degrees of freedom were used if the sphericity assumption was violated.

Results

Seven participants were excluded from analysis because the CMRs were not successfully elicited. In these cases, the stimulation intensity appeared to be too low to properly elicit measurable responses. Therefore, the data analysis was confined to the remaining 10 (out of 17) participants.

A significant main effect of picture valence was found for CMR amplitude [$F(2,18) = 3{,}854$, $p = .040$], indicating that emotional picture viewing modified this amplitude. Follow-up Wilcoxon signed-ranks verified that CMR amplitude differed significantly between unpleasant and pleasant [$Z = 2.293$, $p = .022$] and between neutral and unpleasant picture viewing [$Z = 1.988$, $p = .047$], respectively, whereas it did not differ between pleasant and neutral picture viewing [$Z = .357$, $p = .721$], (see Figure 2).

Discussion

The present study was conducted to investigate the effects of emotional picture viewing on the supraspinal component of the cutaneo-muscular reflex (CMR) (Figure 3). Emotional valence had a significant effect on the CMRs inducing the highest amplitude responses during unpleasant picture viewing. As the long-latency component of the CMR is most likely cortically controlled [15] and occurs reflexively, our results show that emotional valence plays a role in precognitive control of motor activity. Our finding that this cortically mediated reflexive response was augmented by the viewing of unpleasant pictures is in accordance with the findings of the TMS studies of Coelho et al. [13] and Koganemaru et al. [14], which reported augmentation of cortically elicited motor responses during the unpleasant state.

The motivation priming hypothesis of Lang et al. [4] implies that if the reflex is non-defensive, as in the case of spinal reflexes, it is not expected to be affected by emotional state or modulated merely by emotional arousal, [8]. The supraspinal CMR is regarded a fairly non-defensive reflex consisting of two excitatory components of which the one with a latency of approximately 60 to 80 ms is considered to be a reflex response that is modulated cortically via the supraspinal pathway (e.g. [15, 16]. This response was elicited by electrical stimulation that activates the cutaneous Aα nerve fibers and skin mechanoreceptors but not the nociceptors, i.e. does not cause pain [19], and thus it is not experienced as unpleasant. TMS pulses are not similarly regarded as perceptually threatening environmental stimuli that should be avoided. Nevertheless, the motor outputs of both supraspinal CMRs and TMS-elicited motor-evoked potentials have been augmented in the unpleasant emotional state (e.g. [13, 14]). The reflex loop of the CMR is not clearly known yet, it is possible that also the non-motor frontal cortical area, which directly projects to the spinal motoneuron [20] might modulate the muscle responses, unfortunately our experiment cannot shed light on the possible role of these areas.

Also, emotional stimulation may induce changes in the autonomic nervous system and galvanic skin response may decrease skin resistance to a small degree. However, our stimulation technique included use of conductive paste in flexible steel ring electrodes. This should minimize the changes in resistance if GSR would appear. In addition it is unlikely that if GSR would appear it could produce so short term changes what would be needed to have an effect within the experimental run. However, these results, the augmentation of CMR response during unpleasant picture viewing compared to pleasant and neutral picture viewing, suggests that negatively valenced state rather than arousal primes the cortical motor control of reflexes for fast responding. The increase in corticospinal excitability has been suggested by van Loon et al. [12], Hajcak et al. [10] and Coombes et al. [11] to be sensitive to arousal. van Loon et al. [12] found facilitation of action readiness in a choice task during unpleasant and pleasant picture viewing and Hajcak et al. [10] and Coombes et al. [11] showed augmentation of muscle activity (the abductor pollicus brevis muscle) during viewing both pleasant and unpleasant pictures. However, Coelho et al. [13] and Koganemaru et al. [14] have shown contradictory results that support the view favouring valence-specific modulation: these studies found augmentation of muscle activity and improved motor behaviour during unpleasant picture viewing, respectively. Our results support the view that emotional valence, or at least the unpleasant state, modulates the related reflexive responses. It may be that even if both the pleasant and unpleasant emotional states increase motor cortex excitability and motor preparedness, the action itself is facilitated only in the unpleasant state. It is, quite simply, more crucial to act to avoid harmful things than to approach pleasant ones. To conclude, these results indicate that cortically mediated non-defensive reflexes are affected by emotional valence in a similar way as defensive reflexes.

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[1] The IAPS (Lang et al. 2001) pictures were:
Pleasant: 1440, 1460, 1600, 1603, 1610, 1620, 1750, 1920, 2040, 2050, 2080, 2091, 2208, 2250, 2331, 4650, 4660, 4680, 5200, 7270, 7280, 7330, 7350, 8030, 8080, 8120, 7200, 7230, 8200, 8510

Neutral: 5500, 5510, 7000, 7010, 7020, 7030, 7050, 7060, 7080, 7090, 7100, 7110, 7150, 7170, 7500, 5900, 7004, 7184, 7211, 7217, 7233, 7130, 7180, 2514, 5740, 5750,7002, 7009, 7031, 7705

Unpleasant: 2120, 3000, 3010, 3030, 3120, 3130, 3140, 3150, 6200, 6230, 9040, 9050, 1120, 1274, 1280, 1300, 2095, 2683, 3530, 6370, 9490, 2710, 2800, 6415, 6555, 9001, 2053, 2141, 7380, 9041

Figure 1.
Schematic illustration of the CMR procedure.

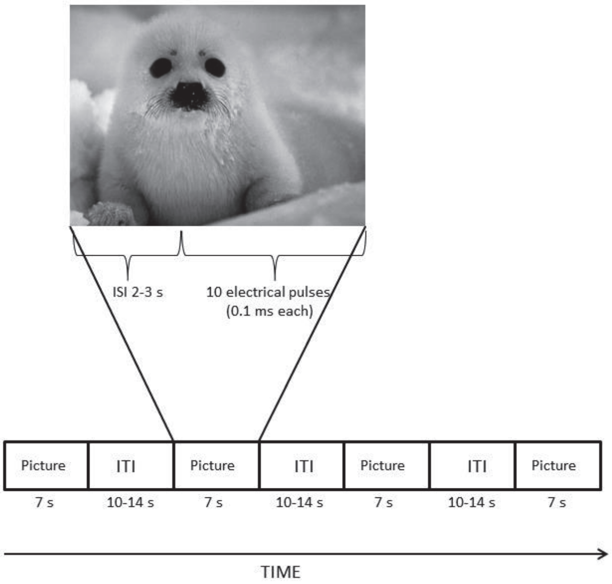

Figure 2.
Violin plot shows the comparison and distributions of CMR amplitudes during viewing of pleasant (pl), neutral (neu) and unpleasant (unpl) pictures (white half-dots: median; black box: 50th percentiles; tail ends: minimum and maximum; *p < 0.05, 2-tailed).

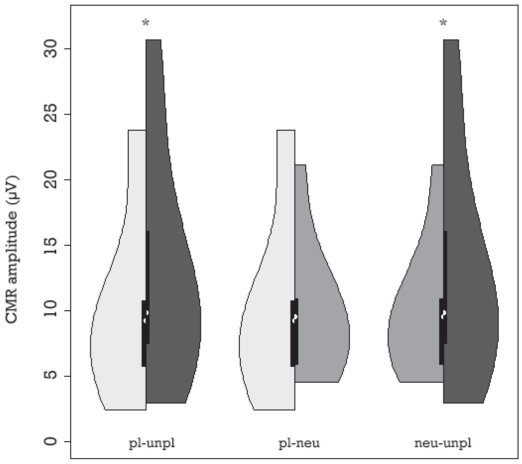

Figure 3.
Grand mean CMR waveform of all 10 participants. E1, I1 and E2 refer to early excitatory (short-latency) component, inhibitory component and late excitatory (long-latency) component of CMR, respectively.