Spontaneous blink rate as an index of attention and emotion during film clips viewing

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Abstract

Spontaneous blinking is a non-invasive indicator known to reflect dopaminergic influence over frontal cortex and attention allocation in perceptual tasks. 38 participants watched eighteen short film clips (2 min), designed to elicit specific affective states, and arranged in six different emotional categories, while their eye movements were recorded from the vertical electroculogram. The largest blink rate inhibition, reflecting greater attention allocation to the movie, was observed during the presentation of Erotic clips, excerpts on wilderness depicting beautiful landscapes (Scenery), as well as clips showing crying characters (Compassion). Instead, the minimum blink rate inhibition was found for Fear clips, which induced a defensive response with stimulus rejection. Blink rate across time evidenced how Compassion clips elicited early inhibition while Sadness clips induced a slower, later inhibition. Correlation analyses also revealed a negative correlation ($r < -0.40$) between total blink rate recorded during Erotic and Compassion clips and self-reported interest. Overall, the main variable explaining blink rate was emotional Valence. Results suggest that blink modulation is related with the motivational relevance and biological significance of the stimuli, tracking their differential recruitment of attentional resources. Furthermore, they provide a solid background for studying the emotion-attention patterns and their deficits also in clinical samples (e.g., neurological and psychiatric patients) using spontaneous blinking as a not-interfering psychophysiological measure.

Key Words: VEOG; Emotion; Film; Affect; Orienting response; Motivation;

1. Introduction

1.1.   Spontaneous blinking and attention

Spontaneous blinking refers to closure of eyelids that occurs automatically without any external stimulation (McMonnies, 2010). Its neurobiological basis is still partly unknown, although recent work suggested that the spinal trigeminal complex may play a role in its generation and regulation, through a complex relationship with the dopaminergic nigrostriatal nuclei (Kaminer, Powers, Horn, Hui, & Evinger, 2011). It is generally believed that main purpose of blinking is to systematically restore the thin tear film that protects the cornea (Al-Abdulmunem, 1999), but many evidences proved that spontaneous blink frequency exceeds the one needed for keeping the eye moist. Among the factors that influence blink rate other than maintaining eye homeostasis, many researchers suggested that eye blink rate may be tied with cognitive processing, especially with attentional engagement, vigilance and mental load (Bentivoglio et al., 1997; Martin & Carvalho, 2015; McIntire, McKinley, Goodyear, & McIntire, 2014). At rest, humans blink with a rate that varies between 10 and 25 times per minute, but the involvement in tasks that require active deployment of attentional resources (i.e. reading), makes blink frequency to drop to less than 5 blinks per minute (Bentivoglio et al., 1997). Moreover, involvement in these tasks determines changes in the pattern of eye blinks, which tend to occur at specific breakpoints in the task (i.e. the end of a phrase) and to be synchronized across individuals (Nakano & Kitazawa, 2010; Nakano, Yamamoto, Kitajo, Takahashi, & Kitazawa, 2009). The explanation for these evidences is that blink suppression is necessary to minimize the loss of information caused by the interruption of the visual information stream, thus in conditions that demand a great attentional investment spontaneous blinking will be largely inhibited. Bolstering the association between attention and blinking regulation, a recent work highlighted that, under naturalistic circumstances (i.e. video watching), blink events are associated with increased activity in the default mode network and a reciprocal decrease of activity in the cortical regions that constitute the dorsal attention network (Nakano, Kato, Morito, Itoi, & Kitazawa, 2013). This evidence clearly suggests that, during spontaneous blinking, brain areas involved in orienting the attention toward the environment are inhibited, an observation that supports the notion that spontaneous eye blink may index attentional engagement, especially during naturalistic perceptual conditions like free viewing of a movie. A recent study carried out in our lab has clearly confirmed blink rate as a measure of attention allocation, as it showed that blink rate decreased with increasing demand and difficulty in a task of sustained attention, the Mackworth clock task (Maffei & Angrilli, 2018). In addition, the relationship between dopaminergic activity and spontaneous blinking makes this index extremely interesting for the non- invasive investigation of the mediating role of this neurotransmitter in neurological and psychiatric disorders (Karson, Burns, LeWitt, Foster, & Newman, 1984; Karson, Dykman, & Paige, 1990), as well as in normal cognitive functioning. For instance, recent studies exploited BR to reveal that central dopaminergic levels are linked with increased cognitive flexibility, better inhibitory action control and reinforcement-driven learning (for a complete review see Jongkees & Colzato, 2016).

1.2.   Does emotion interact with attention in spontaneous blinking modulation?

A field that may largely benefit from the application of spontaneous blinking is the study of motivated attention, namely the interaction of emotional and attentional processes. Indeed, in cognitive

neuroscience research attention and emotion are very often conceived and studied as separate, non-interacting processes, although there is much evidence that in the natural environment, the motivational relevance of a stimulus exerts a strong influence over its cognitive processing (Bradley, 2009). The Orienting reflex (Sokolov, 1963) represents a complex psychophysiological phenomenon that characterizes this relationship. When facing a novel stimulus with an intrinsic motivational relevance, a series of physiological modifications occur, involving both the somatic and the central nervous system, that reflect heightened attention toward the stimulus itself (Bradley, 2009). In human research this response has been investigated mainly using a picture presentation paradigm, a task inducing a repeated brief attention-related phasic response (i.e., the most studied form of orienting response), corresponding to the brief presentation (5-6 sec. each) of a series of pictures with varying emotional content (pleasant vs. unpleasant) and varying arousal. Indeed, using the widely known emotional slide paradigm developed by Lang and co-workers (IAPS, International Affective Picture System (1999)), attentional effects of emotions were measured with a classical response time interfering task, in which participants had to respond as fast as possible to an acoustic tone while watching a series of emotional slides (Buodo, Sarlo, & Palomba, 2002). Interestingly, results showed that the most arousing pictures (erotic and blood-injuries) compared to the low arousal ones (sport, neutral and threat) were associated to the longest reaction times (i.e. greatest interference) and therefore to the greatest attention allocation. One critical issue concerns the fact that the reaction time task, for its interfering characteristic, may disrupt stimulus processing and may alter full emotional induction as the subject is presented with a dual task rather than the more ecological emotional task alone. Using simpler passive viewing task instead it is possible to highlight the physiological changes linked with the orienting response and the interaction between emotion and attention. Typical markers of this orienting response are cardiac deceleration and pupil dilation. These physiological modifications are associated with increased sensitivity of the organism toward salient and motivationally relevant stimuli in the environment (Lang & Bradley, 2013). Indeed, the larger the motivational relevance and the arousal of the picture, the larger these physiological changes are (Bradley, Miccoli, Escrig, & Lang, 2008; Lang & Bradley, 2013). Nevertheless, although the use of emotional slides has been widespread, it is somehow limited in the effectiveness of eliciting a strong or ecological emotional reaction, especially when compared with other techniques (Westermann, Stahl, & Hesse, 1996). Therefore, past paradigms do not allow to study how emotions influence attentional engagement in ecological situations that are close to real life. Movies represent an excellent alternative to pictures, since they allow to overcome these limitations, while still giving the experimenter the possibility to reach a good degree of control over the stimuli (Rottenberg, Ray, & Gross, 2007). In the literature, several film clip sets have been validated for emotion induction (Gabert-Quillen, Bartolini, Abravanel, & Sanislow, 2015; Gross & Levenson, 1995; Philippot, 1993; Schaefer, Nils, Sanchez, & Philippot, 2010), but a recent one developed in our lab (Maffei, Vencato, & Angrilli, 2015) provided several advantages specifically for psychophysiological research: similar duration across clips (about 2 min), homogeneous categories (e.g. fear clips do not show blood, typically inducing disgust), similar arousal across different emotional categories (unbalanced arousal is often a confounding variable explaining most of statistical variance across emotional categories). Here we propose the use of this new set of emotional movies (Maffei et al., 2015), in combination with the analysis of task-related spontaneous blinking (Maffei & Angrilli, 2018), to assess the emotional modulation of attentional engagement during movie presentation. Since blinking is a physiological mechanism that minimizes information loss, we predicted that the emotional effect on blink frequency would be reflected in inhibited blink rate to film contents able to capture attention of the viewer, due to their biological significance. Thus, we expected the most interesting clips, but also pleasant and high arousal stimuli to induce the greatest blink rate inhibition. Furthermore, taking into account the characteristics of the

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experimental setting used (i.e. simple passive viewing), we expected that, in response to the unpleasant (namely high arousal unpleasant, i.e. Fear) movies, blink rate would have been relatively higher, due to a mechanism of "stimulus rejection" (Hahn, 1973), that is, a reduced processing of sensory information in order to prepare a behavioral defensive response. Due to the dynamic nature of film clips, an evolution of emotional and attentional processes across the 2 min of clip presentation was expected: therefore, the time course of blink rate was studied by dividing the whole film into five intervals. An initial strong orienting of attention (blink rate inhibition) common to all clips was expected in the first interval while a category differentiation in the following intervals, especially in the last two, was foreseen in correspondence of the emotional climax reached in the last 20-30 sec of each clip. The aim of the study is to show how spontaneous blinking can be fruitfully used for tracking the effects of relevant affective information on the processing mechanisms of dynamic stimuli, and to suggest a new perspective about the suitability of this index for exploring affectively-laden attention allocation mechanisms in ecological settings.

2. Methods

2.1. Participants

The number of participants was selected based on an *a-priori* estimate computed with G*Power 3 software (Faul, Erdfelder, Lang, & Buchner, 2007), which suggested a sample size of 38 participants for detecting a small effect size (0.2) with a power of 0.8. Thirty-eight students (18 females, mean age 23.3 ± 3 years) from the University of Padova participated in the study in exchange of course credits. University students were selected based on a convenience sampling strategy. All the participants were healthy, had no history of neurological or psychiatric illnesses, and had normal or corrected-to-normal vision. They were asked to refrain from caffeine and nicotine consumption for at least 2 hours before the experimental session. All the procedures were conducted according to the principles of the Declaration of Helsinki and were approved by the local Ethics committee.

2.2. Stimuli

Eighteen film clips (duration 120 ±10 s) were selected from a validated database of emotional film clips (Maffei et al., 2015) for use in the present study. Movies were arranged in six emotional categories (3 clips per category): Erotic, Scenery, Compassion, Sadness, Fear and a Neutral category. These categories were selected in order to cover a broad range of positive and negative affective state, and to provide a fine grained distinction among emotional state that are often confounded. Specifically, we distinguished clips depicting characters crying after a loss (Compassion category) from clips featuring themes of loneliness and helplessness that did not include crying scenes (Sadness category). This distinction aimed at targeting two similar but distinct affective states. Compassion clips was expected to trigger in the viewer a reaction characterized by empathic concern and prosocial approach toward the characters, through an empathic mechanism of emotional resonance elicited by tears. Sadness clips, instead, aimed at eliciting in the viewer feelings of sadness together with isolation and angst characterized by a withdrawing motivational tendency[1]. We also introduced a new category, Scenery, comprising clips depicting wilderness and natural environments that in past have been used as neutral

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[1] The theoretical distinction between these two categories has been confirmed by validation data (Maffei et al., 2015; Maffei & Angrilli, *under review*) that show that Compassion clips elicit a sad mood characterized also by feeling touched, while Sadness clips elicit a sad mood together with feelings of anxiety and anguish.

stimuli, despite the compelling evidences that support the view of nature as a source of positive emotions.

The excerpts were edited using Adobe Premiere CS5 for producing a single continuous clip, interspersed with intervals necessary for subjective evaluations and 30 s baseline, with a 1280x720 resolution. In order to control for difference in sound pressure levels, the audio tracks of the clips were peak-normalized to the value of -8 dBFS (decibel relative to full scale) using the "Normalize All Peaks" function in Adobe Premier. The order of presentation of the excerpts in the final clip was pseudo-randomized in order to avoid that two excerpts belonging to the same category were consecutive. The stimuli were presented on a 27-inch monitor placed at 70 cm from eyes, and audio was delivered through stereo headphones.

2.3. Eye blink acquisition and preprocessing

Spontaneous eye blinks were identified through vertical electroculogram collected by means of two Ag/AgCl 10 mm cup electrodes placed above and below the right eye. Signal was amplified with a gain of 4000 and online filtered with a time constant of 10 seconds (cut-off frequency 0.016 Hz) and a low pass filter set at 80 Hz. Sampling rate was set to 250 Hz by means of a National Instruments A/D PCMCIA 12 bit board (DAQCard-AI-16E-4). Data acquisition and analyses were carried out with LabVIEW software (National Instruments) according to Angrilli (1995). In order to remove slow oscillations due to movement artifacts a high pass filter set at 0.5 Hz was applied offline. Eye blinks were defined as a peak of positive voltage change exceeding the threshold of 100 microvolts in a time window of 500 ms (Colzato, van den Wildenberg, van Wouwe, Pannebakker, & Hommel, 2009). In order to assess blink rate changes across time, each clip was divided in 5 blocks of 22 s and blink number was converted into blinks/min for each block. In addition, a baseline blink rate was computed for each clip from the 22 sec period preceding the onset of the clip. Finally, blink rates of clips belonging to the same category were averaged before statistical analysis.

2.4. Procedures

Upon arrival, each participant was given a description of the experimental procedure to fulfill the requirements of signed informed consent. In order to avoid self-monitoring and control of blinks, this description did not mention that blinking was the focus of the study. Then electrodes were attached, the light dimmed and the session started. After each clip, participants were asked through on-screen instructions to rate the valence and the arousal of the affective state induced by the clip, and how much interesting they found the clip. Valence was assessed on a 9 point Likert scale ranging from 1 (Extremely unpleasant) to 9 (Extremely pleasant), arousal was assessed on a 9 point Likert scale ranging from 1 (Calm) to 9 (Extremely aroused), and interest was assessed on a 5 point Likert scale ranging from 1 (Not interesting at all) to 5 (Extremely interesting). Since blink frequency increases in the evening (Barbato et al., 2000), data were not collected after 5 p.m.

2.5. Statistical analysis

Valence and arousal evaluations of film clips were analyzed using repeated-measures ANOVAs designed with one within-subjects factor (Film Category) and one between-subjects factor (Sex). Analysis of blink rate was performed with a repeated-measures ANOVA designed with two within-subjects factor (Film Category and Time) and one between-subjects factor (Sex). When appropriate,

Greenhouse-Geisser procedure was used and corrected probability values were reported. Significant effects were further explored using post-hoc pairwise comparisons with the Newman-Keuls procedure for multiple comparisons, using a significance level of $p < .05$.

In addition, Pearson's correlations were carried out between the blink rate for each category and the mean rating of interest reported by the participants for each film category, controlling the false discovery rate with the Benjamini & Hochberg procedure (1995).

Finally, in order to better characterize which affective dimension most affected blink modulation during movie presentation, a linear mixed-effect model was fitted to the blink rate data including self-reported Valence, Arousal, Interest and their interactions as predictors and a random intercept modeling the repeated measurements within each participant (Baayen, Davidson, & Bates, 2008). To improve parameters interpretability, predictors were mean-centered before inclusion in the model.

3. Results

Analysis of Arousal ratings (Fig. 1 left panel) showed a significant main effect of Sex ($F_{(5,36)} = 17.91$, $p = 0.00012$, $\eta^2_p = 0.33$) and of Film ($F_{(5,180)} = 62.56$, $p < 0.0001$, $\eta^2_p = 0.63$). Women reported an overall greater arousal than men (see Fig. 1 left panel). Post-hoc test of the Film effect showed that arousal ratings were the lowest for Neutral clips, while Erotic, Fear and Compassion categories were rated as the most arousing clips, with no significant differences among them. Analysis of Valence (Fig. 1 right panel) showed a significant main effect of Film category ($F_{(5,180)} = 34.33$, $p < 0.0001$, $\eta^2_p = 0.48$). Post-hoc comparisons revealed that Erotic and Scenery clips were rated as more pleasant compared to all the other categories ($p<0.05$), while Fear, Sadness and Compassion clips were rated as more unpleasant, with Fear judged as significantly more unpleasant than the other two ($p<0.05$).

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Insert Fig. 1 about here
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Analysis of blink rate (Fig. 2) revealed significant main effects of Time ($F_{(5,180)} = 13.35$, $p = 0.00013$, $\eta^2_p = 0.27$) and Film ($F_{(5,180)} = 4.9$, $p = 0.0019$, $\eta^2_p = 0.11$), and a significant Film x Time interaction ($F_{(25,900)} = 2.25$, $p = 0.0084$, $\eta^2_p = 0.05$) (see Fig. 2). Post-hoc analysis of the interaction revealed that Erotic and Compassion clips elicited a larger blink rate inhibition compared to the Fear and Sadness categories in the first interval ($p<0.05$). Fear clips were associated with higher blink rates than Erotic ones also in the fourth and fifth interval, than Compassion in the second interval, and than Scenery in the second and fourth intervals (all $ps<0.05$). In addition, analysis across intervals revealed that, compared with the baseline, blink rate was lower during film watching, starting from the first interval and continuing till the last interval, for all the categories. For the Sadness clip, blink rate in the third and fourth intervals was significantly lower ($p<0.05$) than in the first clip interval (0-22 s).

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Insert Fig. 2 about here
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Within-subjects Pearson's correlation (Fig. 3) revealed a relatively large negative correlation between total blink rate and self-reported interest for Erotic ($r_{(38)}$ = -0.45, $p_{fdr-corrected}$< .05) and Compassion ($r_{(38)}$ = -0.41, $p_{fdr-corrected}$< .05) clips: the subjects with the lowest blink rate (during the whole clip) in these two clips, exhibited the greatest self-reported interest (see Fig. 3). No significant correlation were found instead for Fear ($r_{(38)}$ = -0.22, n.s), Sadness ($r_{(38)}$ = -0.31, n.s), Neutral ($r_{(38)}$ = 0.02, n.s) and Scenery ($r_{(38)}$ = -0.3, n.s) categories.

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Insert Fig. 3 about here
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Mixed-model analysis revealed that Valence was significantly associated to blink modulation (β = -0.56, $t_{(188)}$ = -2.3, $p < 0.05$), showing that blink rate increases with negative valence and decreases with positive affect. No significant effects were found instead for the Arousal (β = -0.13, $t_{(188)}$ = -0.8, n.s.) and Interest (β = -0.54, $t_{(191)}$ = -1.29, n.s.) dimensions nor for the interactions among the predictors.

4. Discussion

The goal of the present paper was to use spontaneous blink rate as an index of the attentional engagement during the presentation of a set of 18 emotional film clips: we expected that different emotions elicited through short film clips would have shown different attention patterns depending on their valence and biological relevance. Stimuli with high biological relevance are those inducing high arousal and automatic, unconditioned responses as they represent primary emotional conditions hardwired evolutionarily in our brains (see for a review: Lang & Bradley, 2010; Pessoa & Adolph, 2010) and physiology. Thus, threat stimuli elicit fear-related defensive responses preparing the body for fight-flight options; erotic stimuli elicit reproductive, approach-oriented rewarding response; compassion scenes with crying characters induce a pro-social supportive approach. To test our main hypothesis we used blink rate measured by means of the vertical oculogram, which is a reliable index of attention allocation, and has been also suggested to reflect the dopaminergic influence on this process (Maffei & Angrilli, 2018). The innovative aspect of our study is twofold: first we chose an interesting psychophysiological index (blink rate) used so far in specific niche studies, that demonstrated to own an interesting potential for studying attention and emotions in non-invasive ecological settings. Second, this index was used to measure time-dependent evolution of attention in a new set of validated emotional film-clips specifically devised for psychophysiological research. The set of clips included several

important emotional categories well balanced and controlled for arousal level, some of which are biologically relevant according to the literature (e.g., Erotic and Fear, Lang & Bradley, 2010). The use of the blink rate on the new emotional set allowed us to investigate how basic and complex emotions modulate attention allocation across time, an issue rarely addressed in past research using emotional movies.

Subjective evaluation of the clips confirmed the expected differences among the six different emotional categories along the subjective dimensions of arousal and valence. Concerning emotional valence, Erotic and Scenery induced a comparable pleasant, high arousing, emotional state (Fig. 1), while Sadness and Compassion were rated both moderately unpleasant. Fear clips showed instead the greatest unpleasantness and arousal. Valence ratings did not reveal any differences between genders. Arousal evaluations showed a main effect in which women reported an overall greater arousal than men, this difference was particularly evident for unpleasant clips, especially fear. This is in line with current literature on both emotional slides and clips showing a greater sensitivity of women compared to men to emotional stimulation in general and especially to unpleasant contents (Bianchin & Angrilli, 2012; Bradley, Codispoti, Sabatinelli, & Lang, 2001; Maffei et al., 2015).

Concerning blink rate, results clearly showed how it was modulated by the affective content of the films suggesting that processing of the motivational values of the stimulus may play a crucial role in this mechanism. All film clips induced a strong significant inhibition of blink rate in the first 22 sec interval with respect to the baseline; the inhibition was sustained across all intervals of clip presentation. Therefore, independently of the category, all clips induced in participants a greater attention allocation due to the increased cognitive resources that visual processing of dynamic stimuli demanded. Within this overall trend, blink rate was modulated in a different way depending on both the emotional content and the time interval, but no gender differences were found for this index. In the first 22 sec interval, the unpleasant Fear and Sadness clips showed a relatively larger blink rate compared to Erotic and Compassion (Fig. 2). According to past literature on heart rate and emotion (Bianchin & Angrilli, 2012; Bradley, 2009) greater attention orienting was found when unpleasant stimuli were presented. Therefore, in principle, for Fear films a prevalence of attention orienting over defensive response could be hypothesized. But in most past studies using slides the sample of emotional stimuli included a mixture comprising mainly blood-mutilation rather than frightening stimuli (see e.g. Bianchin & Angrilli, 2012; Bradley, 2009). Thus, depending on the emotional category used, there is evidence that the defensive response prevails in fear and threat situations while the attention orienting response dominates during blood-mutilation presentation (Buodo et al., 2002; Sarlo et al., 2010). In the present experiment, Fear and Sadness clips induced a relative higher blink rate throughout the whole clip duration, and it was significantly higher than that observed during Erotic clips in four out five intervals. The observed withdrawal of attentional resources, indexed by reduced blink rate inhibition, is related with the motivated withdrawal emotional state induced by these two high arousal categories, characterized, especially in the first explorative interval, by anguish, anxiety and distress: in this condition it might be biologically relevant to extract only a few relevant and informative (for surviving) elements from the clip, in order to plan and organize a fast (Fear) or slow (Sadness) defense response. This condition is what in the literature has been defined as "stimulus rejection" (Hahn, 1973), typical of defense responses, which indicates a reduced processing of sensory stimuli. In a dangerous situation it is not adaptive to spend precious time by analyzing the details of the whole scene, as instead it occurs in the pleasant or neutral conditions. Interestingly, a similar result was reported by Palomba and colleagues (2000) who found that blink rate for a threatening movie was not different from a control neutral condition, while it was substantially inhibited during the viewing of a surgery clip: the difference

between the two negative clips showed the specificity of threat with respect to other unpleasant conditions. Similarly, in a past study based on startle reflex (Sarlo et al., 2010), fear stimuli induced a clear defense-related potentiated startle reflex which has been compared with another category of unpleasant stimuli (blood-mutilations), which instead elicited an attention-related startle inhibition This result is also consistent with a past study based on emotional slides in which Threat stimuli were associated with fast response times (i.e. less attention allocation to an incidental tone), close to those induced by Neutral stimuli (Buodo et al., 2002), thus reflecting a relatively reduced attention allocation on Fear stimuli.

Compassion clips are emotionally unpleasant but showed a different pattern with respect to the other two negative clips, as they induced, in the first and second interval, a greater blink rate inhibition compared to Fear and, in the first interval only, larger inhibition also compared to Sadness. The emotion induced by sad stimuli with crying characters (Compassion clips) is prosocial and, unlike other negative emotions, is biologically grounded to move the observer towards the suffering person for providing help and support (Vingerhoets, Cornelius, Van Heck, & Becht, 2000; Vingerhoets, van de Ven, & van der Velden, 2016), a disposition which can be classified within the approach motivation domain. Compassion stimuli, compared to Fear, are associated with significantly lower levels of distress, anxiety and jittery (Maffei et al., 2015) and thus do not elicit a defensive response. Furthermore, Compassion clips require the viewer, especially in the first scenes, to explore the situation and analyze the scene in detail in order to understand the context and the cause of the character's suffering (this holds especially for short clips in which initially the context is unknown with respect to whole films lasting 1.5 hr or more). This specificity may explain the initial attention-related blink rate inhibition observed in the first two intervals (0-22 sec and 23-44 sec) with respect to Fear, but not in the last two intervals.

The Sadness category has many features in common with Compassion stimuli, both induce sadness, but while Sadness movies feature themes of loneliness, isolation and hopelessness, Compassion clips show characters crying because of loss and mourning. Nevertheless, these two categories differ for some aspects: Sadness is expected to induce motivated withdrawal, Compassion motivated approach. Indeed, Sadness clips, in the first interval, revealed a blink rate overlapping with that induced by Fear movies, and significantly higher than that elicited by Erotic and Compassion, a result which indicates, in addition to the poor environmental details and context, a possible stimulus rejection and initial precautionary defense response. In the following intervals, second and third (23-44 sec and 45-66 sec), Sadness movies induced a strong and significant blink rate inhibition with respect to the first interval (0-22 sec). This result is consistent with an initial anxiety-related higher blink rate followed by an increased attention allocation developing with time. Therefore, blink rate was able to detect very different patterns between these two similar categories across time, a dynamic feature which can be explored and analyzed only using movies. With regard to positive film categories, Scenery movies elicited a contemplative, aesthetic and approach-motivated pleasant condition, and, compared to Fear clips, were associated with an attention- related blink rate inhibition, that developed more slowly than Erotic and Compassion clips. Our results showed that, compared to Fear, the greatest blink rate inhibition during these clips, occurred in the second and fourth intervals of movie presentation (23-44 sec and 67-88 sec). Erotic clips are instead biologically relevant stimuli that orient attention rapidly and intensely, thus these stimuli induced a quick intense attention-related blink rate inhibition compared to Fear, in the first interval and then in the fourth and fifth ones. This is in agreement with past research which used response times during slide presentation (Buodo et al., 2002): slower response times, indicating greater attention allocation, were found while subjects were looking at erotic compared with neutral slides. In general, stimuli which are interesting, complex and biologically relevant are expected to induce a "stimulus intake" condition

characterized by strong orientation of attention towards external cues (Hahn, 1973).

The time dependent patterns found in the present study may depend on the mean duration (2 min) established for this sample of movies, which may characterize early and late responses. Interestingly, at about 1 min from movie onset, in the central interval (45-66 sec), blink rate of all movies tended to overlap: this is the only interval in which no effects between-categories were found. Although this pattern requires further replication, it might indicate the presence of ultradian rhythms in attention engagement with a periodicity of 1 min: after strong attentional allocation captured by initial information of the clips, attention may drop for a few seconds to restart in the second min of presentation. Using longer emotional clips may reveal additional latency and periodicity of cognitive-emotional response patterns interacting with emotional category. Nevertheless, the attention-emotion effects observed in the first intervals analyzed in the present study are not expected to change using longer movies.

Of particular interest are the two significant negative correlations found between the blink rate computed during the whole 110 sec interval of clip presentation and the self-reported interest ratings collected from participants soon after each clip. This correlation was relatively high and was found for the Compassion and Erotic clips, two approach-motivated biological relevant emotional categories. Therefore, those participants who found more interesting the clips of these two categories had also the greater attention-related blink rate inhibition. This from one side strengthens the interpretation of blink rate as a measure of attention-interest for the stimuli through an index (self-reported interest) falling within the cognitive-behavioral domains. From the other side, the correlation found for these two categories, Compassion and Erotic, indicates for these a clear, coherent pattern across the whole clip duration, of the prevalence of a consistent attention-interest-driven processing of the clips. The Fear category, by inducing a larger blink rate marking a reduced processing of these stimuli, showed a dissociation between the induced moderate interest and the limited blink rate inhibition, thus indicating how this biologically relevant emotional category is not dominated by a consistent univocal attention-related process.

Concerning the role of dopamine in affective processing, the present results might be interpreted in line with the role played by dopamine in modulating the cortico-subcortical interactions that mediate avoidance behavior. Indeed, animal studies showed that dopaminergic influence on the amygdala is critical for the development of fear conditioning (Guarraci, Frohardt, & Kapp, 1999) and that inhibition of this influence leads to impaired defensive reactions, such as reduced startle reflex (Greba, Gifkins, & Kokkinidis, 2001). Moreover, investigation of patients affected by Parkinson's disease, characterized by reduced striatal dopamine (which leads also to abnormal blinking patterns), revealed that they are impaired at recognizing negative emotions, especially Fear, Sadness and Disgust (Kan, Kawamura, Hasegawa, Mochizuki, & Nakamura, 2002; Péron et al., 2010). Nevertheless, it should be acknowledged that recently the relationship between eye blink rate and dopamine has been questioned (Dang et al., 2017; Sescousse et al., 2018), so this interpretation might be considered as tentative.

It is interesting to observe that the pattern of blink rate inhibition found for affectively relevant material is quite similar to the pattern that describes the effect of emotional induction on heart rate dynamics. In response to stimuli with strong motivational relevance heart rate decreases, with a deceleration, the extent of which depends on significance of the stimulus (Lang, Greenwald, Bradley, & Hamm, 1993). This phenomenon is usually interpreted as the result of an orienting mechanism aimed at facilitating the sensory intake of the relevant information (Bradley, 2009; Lang et al., 1993). Here, blink rate inhibition

might reflect the same attentional mechanism, aiming at favoring stimulus processing by reducing the likelihood that the most relevant pieces of information are lost because of eye closure. On the other hand, the relatively greater blink rate observed for Fear category throughout the clips, may index a mechanism of increased arousal in response to potential threat mediated by subcortico-cortical pathways centered around amygdala. Indeed, amygdala is responsible for the modulation of arousal of the body when a potential threat is detected (Pessoa, 2010), and its activation is linked, among the others, to connections with the dopaminergic fronto-striatal pathway, whose activation is reflected in blink rate (Jongkees & Colzato, 2016). Thus, relative increase of blink rate to fearful movies may index the automatic activation of defensive schemes, similar to the mechanisms observed in other psychophysiological responses, like heart rate and electrodermal activity.

A further issue is the lack of a gender effect on blink rate. Although in the field of psychophysiology of emotions sex differences have been found, depending on the psychophysiological variable investigated, (see e.g. Bianchin & Angrilli, 2012; Chivers, Seto, Lalumière, Laan, & Grimbos, 2010) in the present research, gender differences have been found (as expected from past research) in the subjective evaluation domain only. The lack of a gender effect on blink rate may depend on the fact that this measure is more sensitive to a cognitive variable like attention, but it should be also acknowledged that the inter-individual variability in blink rate is larger than in other psychophysiological measures, so that it may mask possible smaller gender effects. Further research is necessary to address this issue.

Finally, multiple regression analysis, also helped to disambiguate which affective dimension exerted the greatest influence on blink modulation. Results showed a unique association between valence and eye-blink rate. The higher is the unpleasantness of the viewer the higher was the blink rate. This result is consistent with the observed blink modulation induced by film clips, and further supports the idea that assessing spontaneous blinking might be useful to track individual emotional state (Kret, 2015). Indeed, taken together these results suggest that blinking is reduced when processing positive material and experiencing a positive affect, while its increase might signal a negative emotional state, specifically related with a withdrawal motivation.

5. Limits and Future Directions

The present study has a series of limitations that should be considered when examining the results. Basic visual features (i.e. luminance, contrast, number of transitions) of the clips have not been explicitly controlled, so it is not possible to estimate their impact on the results. This is an unavoidable drawback of choosing ecological stimuli that are necessary for analyzing emotions in more natural and less artificial setting. One possible confounding variable which deserves to be investigated in future studies is represented by the change in luminance/brightness: it is possible that quick and strong changes in brightness, including scene transitions, may have elicited some blinks. We believe that the present clips did not include strong brightness changes and that attention grabbed by novel scenes after a transition can overtake the tendency to blink to the same event. Thus, a specific ad-hoc study on the correlation between spontaneous blink rate and number of transitions/brightness changes is advisable. However, we believe that averaging across different clips within the same category should have reduced most of listed confounds as the variability of clips in these psychophysical domains would represent random non-systematic noise. Furthermore, it is necessary to acknowledge that these confounding psychophysical variables may play an instrumental role in the capacity of a film clip to evoke a specific emotion. Future

investigation should definitely aim at characterizing the complex interrelationship between spontaneous blinking, emotions and stimulus features. Moreover, video clips compared to static pictures offer the possibility to dynamically characterize visual features of the scenes in term of complexity and amount of information and would be interesting to test the hypothesis that the increase in spontaneous blinking observed for Fear clips might be also moderated by a reduced complexity and informative content of these kind of scenes compared with other categories (i.e. Scenery). Another potential limitation of the present study is represented by the absence of a complete between-subjects stimuli randomization. Although we think that the possible order effect is limited by the randomized and interspersed distribution of the clips of different categories along the whole presentation, future studies should employ a complete randomized design in order to overcome this limitation. Finally, future studies should also aim at extending the variety of participants' sample, in order to test the generalizability of results drawn from university students to the general population. It can be expected that, since dopamine brain levels change across ages (Wahlstrom, Collins, White, & Luciana, 2010), but also emotional responding changes (e.g. in aged individuals the recall of positive events is prevailing, Nowlan, Wuthrich & Rapee, 2014), differences across extreme ages (adolescence and elderly) may reveal interesting age-related patterns in blink rate.

6. Conclusions

Blink rate proved to be a useful index of the effects of emotion on attentional engagement which, compared to other peripheral indexes, could serve as an optimal choice for ecological investigation of this effect. First, blink rate is among the many psychophysiological indices available, one of the easiest to collect and analyze. Second advantage of blink rate is represented by its looser physiological constraints (Shultz et al. 2011). Indeed, blinking is deeply tied to the visual system, making its inhibition a simple and effective psychophysiological mechanism evolved to grant the processing of information critical for organism survival. Finally, blink rate best satisfies the minimal invasiveness required by ecological investigation, but owns also a minimal demand characteristic, thus, it is also suited for research involving special populations for which high compliance is difficult to attain (e.g. children, medical patients). Indeed, one open field of the use of blink rate is its application to the study of emotional-cognitive deficits in psychiatric patients, individuals with personality disorders, and especially in neurological patients with attention or affective impairments, or with lesion in the dopaminergic circuits such as individuals affected by Parkinson disease, who typically exhibit pathological reduction of spontaneous blink as a consequence of *substantia nigra* degeneration (Karson et al., 1984).

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Film Category	Sex	Mean Valence	SD Valence	Mean Arousal	SD Arousal	Mean Interest	SD Interest
Erotic	F	6.00	1.66	6.86	1.09	2.26	0.84
Erotic	M	6.57	1.29	6.26	1.49	2.67	0.93
Scenery	F	6.75	1.77	5.16	2.00	3.04	1.16
Scenery	M	6.25	1.54	4.37	2.06	2.84	1.18
Neutral	F	5.33	1.50	3.35	1.93	2.7	1.22
Neutral	M	5.23	1.41	2.35	1.75	2.42	1.22
Sadness	F	4.47	1.83	5.74	1.79	2.68	1.18
Sadness	M	4.37	1.61	4.23	1.82	2.39	1.15
Compassion	F	4.63	2.40	6.96	1.38	3.09	1.14
Compassion	M	4.25	1.70	5.37	1.84	2.61	1.10
Fear	F	3.54	1.72	7.35	1.14	2.33	1.01
Fear	M	3.84	1.73	5.72	1.72	2.51	1.00

TABLE 1: summary statistics (Mean and SD) of self-reports of Arousal, Interest and Valence arranged by film category and gender.
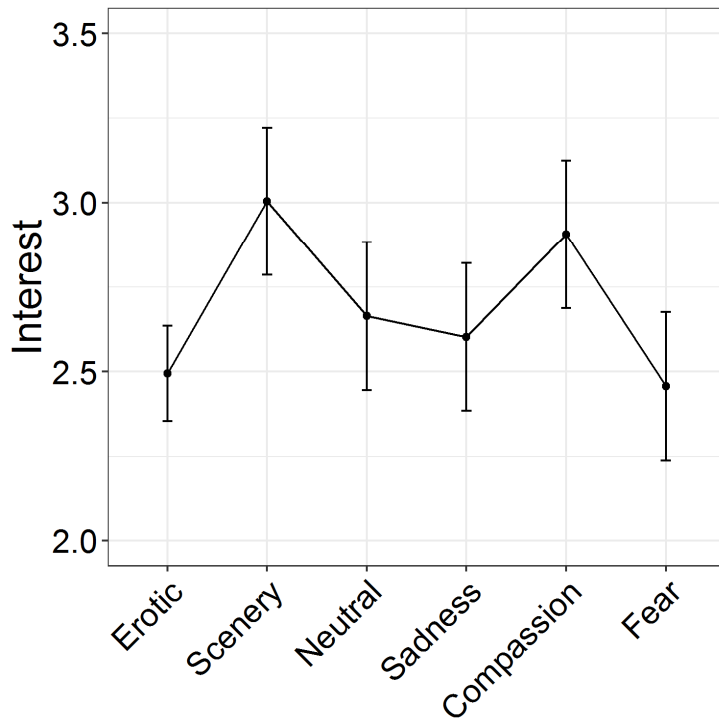

Figure legend: the main effect of Film category was significant ($F_{(5,180)}= 3.51$, $p < 0.005$, $\eta^2_p = 0.09$) and post-hoc analysis showed greater interest to Scenery compared with Erotic ($P< 0.03$) and Fear ($p<0.02$) clips. Bars indicate SE.
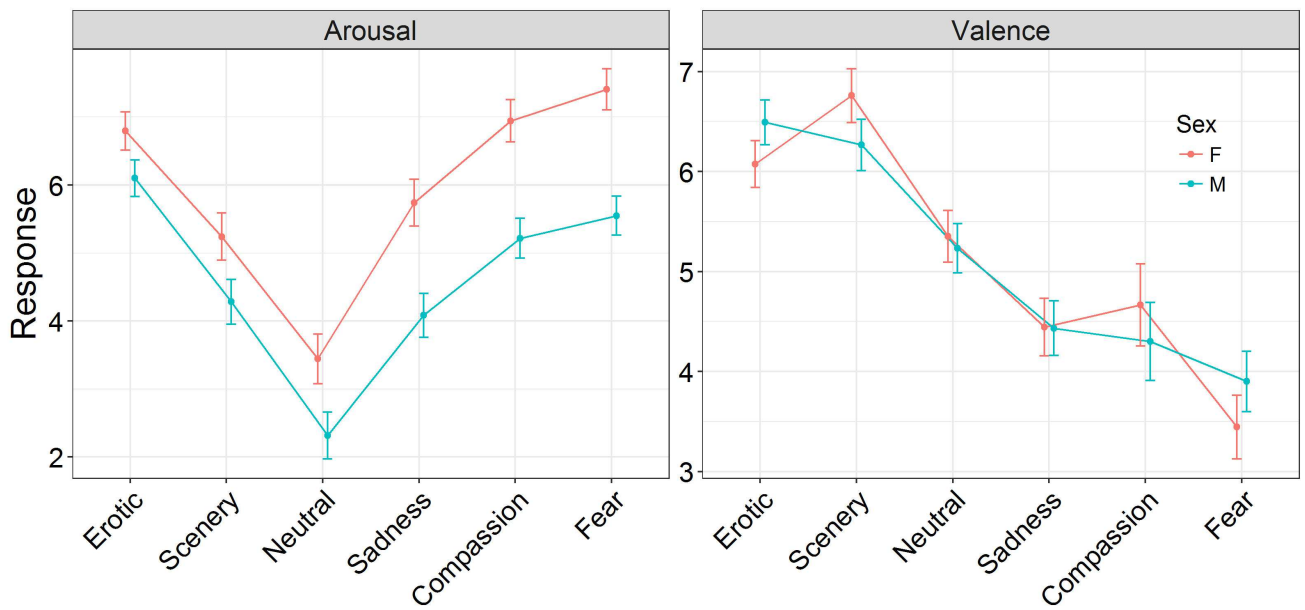
Figure 1. Effect of film category on self-report evaluation of valence and arousal, divided for gender. Bars indicate Standard Error (SE).

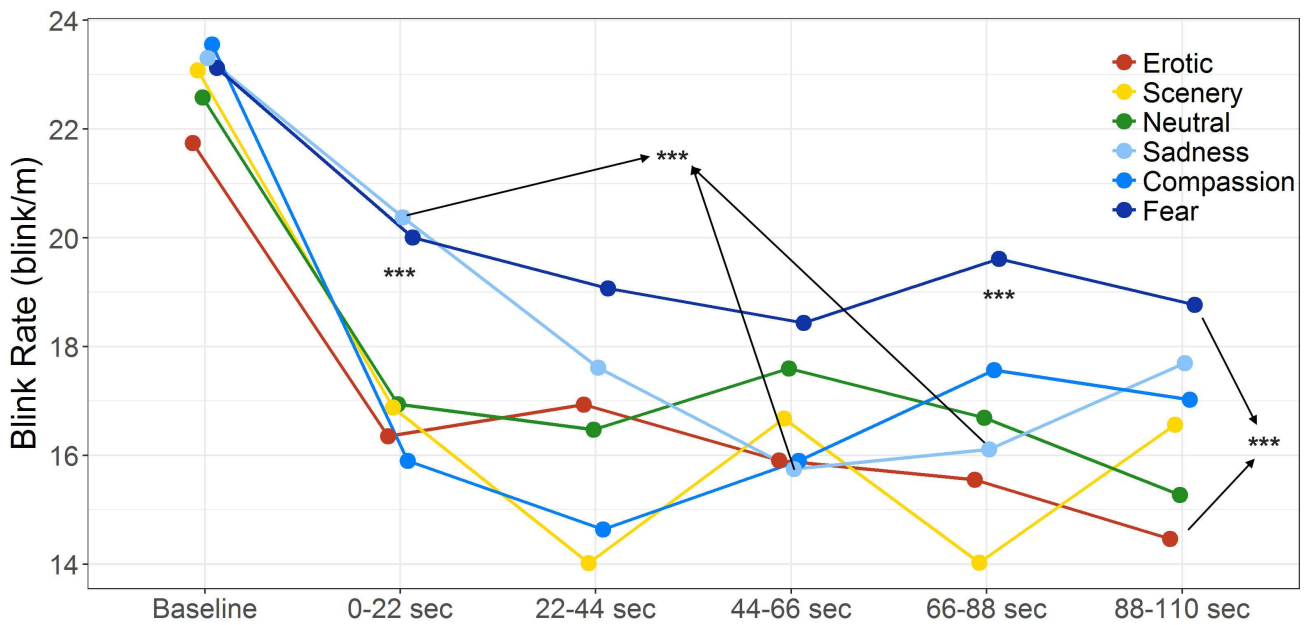Figure 2. Interaction effect of film category by time interval on spontaneous blink rate. *** indicates significant post-hoc effects with p< 0.05.
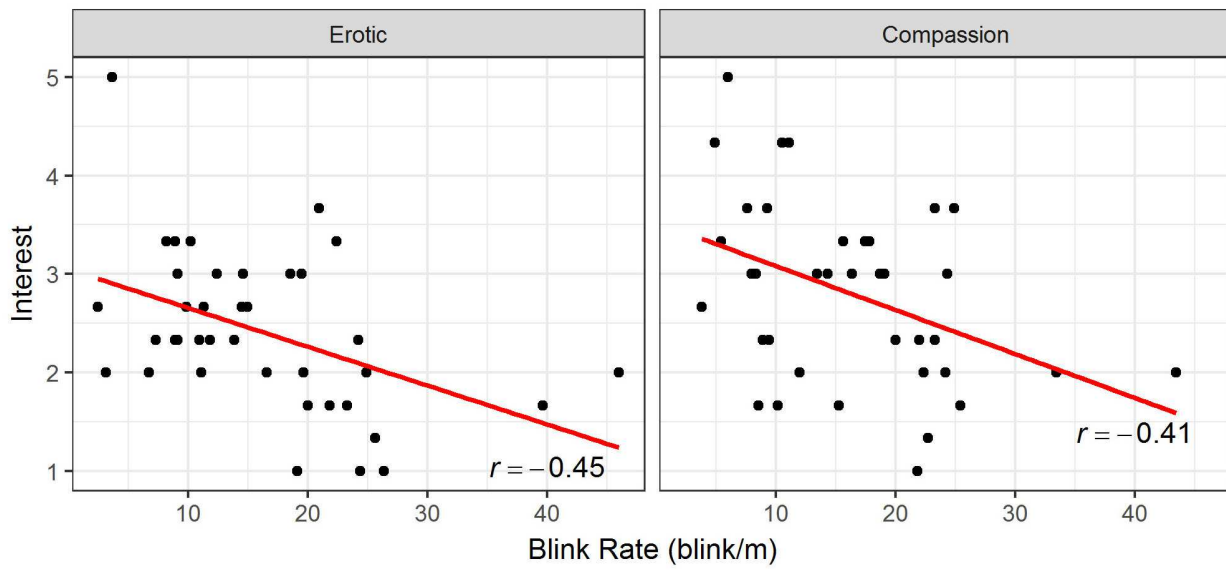
Figure 3. Pearson's correlation between self-reported interest and total blink rate during presentation of Erotic and Compassion clips