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Novelty and emotion: pupillary and cortical responses  
during viewing of natural scenes

Vera Ferrari<sup>1</sup>, Andrea De Cesare<sup>2</sup>, Serena Mastria<sup>2</sup>, Luisa Lugli<sup>3</sup>, Giulia Baroni<sup>3</sup>,  
Roberto Nicoletti<sup>3</sup>, Maurizio Codispoti<sup>2</sup>

<sup>1</sup>Department of Neuroscience, University of Parma,

<sup>2</sup>Department of Psychology, University of Bologna

<sup>3</sup>Department of Philosophy and Communication, University of Bologna

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Address correspondence to:

Vera Ferrari, Ph.D.

Department of Neuroscience, University of Parma

Via Volturno, 39

43100 Parma, Italy.

E-mail: vera.ferrari@unipr.it

Abstract

Given the remarkable similarities in the antecedent conditions—stimulus motivational relevance and novelty (i.e., probability of occurrence)—that elicit amplitude modulation of the late positive potential (LPP) and the pupillary dilation response, the present study examines whether these two indexes of orienting response reflect common processes that are responsible for modulatory patterns in motivationally relevant contexts. In the present study, the LPP and the pupillary dilation response were co-registered in a free-picture viewing context in which stimulus novelty was manipulated through repeated presentation of the same picture exemplar. More specifically, pictures depicting both emotional and neutral contents could be novel, that is never seen before in the course of the study, or repeated 4-8 times in a row (i.e., massed repetition).

Results showed that, despite massed repetitions, the late positive potential amplitude continued to be highly modulated by picture content, whereas affective modulation of pupil dilation decreased with picture repetition.

These findings indicate that, although the LPP and pupil dilation are similarly affected by motivational relevance during the viewing of novel pictures, they differ when pictures are highly familiar, possibly reflecting different functional meanings in the context of the orienting response.

## Introduction

The processing of novel and significant stimuli is crucial to protect and sustain the life of the individual. On one hand, new stimuli pose novel opportunities that may result in beneficial outcomes; on the other, new stimuli may pose a threat. Novel or emotional events induce a variety of physiological changes, which Sokolov (1963) collectively called the orienting response (OR). A defining feature of the orienting response was that these physiological changes were elicited in the context of stimulus change, and that orienting habituated with stimulus repetition.

Motivationally relevant stimuli, such as emotional scenes, can elicit an orienting response, which is observable as a modulation of a broad range of physiological reactions (Bradley, 2000; Lang, Greenwald, Bradley, & Hamm, 1993). Some of these reactions, especially those involving autonomic activation, have been shown to habituate rapidly with stimulus repetition (i.e. heart rate, skin conductance; Bradley, Lang, & Cuthbert, 1993; Codispoti, Ferrari, & Bradley, 2006), indicating that some processes engaged in the initial orienting response to motivationally relevant stimuli are no longer necessary when stimuli are repeated or expected.

At the cortical level, the centro-parietal late positive potential (LPP) is reliably modulated by motivational relevance (Cacioppo, Crites, Gardner, & Berntson, 1994; Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000; Codispoti, Mazzetti, & Bradley, 2009; Radilova, 1982; Schupp, Flaisch, Stockburger, & Junghöfer, 2006). This affective modulation begins approximately 300–400 ms following picture onset and develops into a sustained positive slow wave throughout the picture presentation period (1-6 seconds; Cuthbert et al., 2000; Gable, Adams, & Proudfit, 2015). The largest positive amplitudes have been observed for highly arousing picture contents, either pleasant or unpleasant, suggesting that the late positive potential is more clearly

a brain response to stimulus motivational relevance, rather than to its valence (De Cesarei & Codispoti 2011; Weinberg & Hajcak et al, 2010; Schupp et al., 2004; 2006). Moreover, unlike autonomic orienting responses, this cortical index of emotional processing has been found to be only slightly affected by picture repetition (Codispoti, Ferrari, De Cesarei, & Cardinale, 2006; Codispoti, Ferrari, & Bradley, 2007; Ferrari, Bradley, Codispoti, & Lang, 2011), consistent with the idea that the orienting response cannot be considered a unitary process (Barry & O'Gorman, 1987; Bradley, 2009; Codispoti et al., 2006).

One index of the orienting response that has been only recently investigated in the context of emotional picture viewing is the pupil dilation response (Bradley, Miccoli, Escrig, & Lang, 2008). Like the late positive potential amplitude, pupil diameter is larger during viewing of emotionally arousing pictures, pleasant or unpleasant, compared to neutral scenes, and this modulatory effect begins with the initial light reflex and persists throughout the picture viewing interval (Bradley et al., 2008; Henderson, Bradley & Lang, 2014). Moreover, recent animal studies suggest that pupillometry may be used as a useful index to study cortical arousal, as higher cortex desynchronization and stronger responsiveness to external stimuli have been observed during pupil dilation, as opposed to constriction (Reimer et al., 2014; Vinck, Batista-Brito, Knoblich, & Cardin, 2015).

Given the remarkable similarities in the antecedent conditions (i.e. stimulus relevance) that elicit the amplitude modulation of the LPP and the pupil dilation, the current study examines whether these two indexes of orienting response reflect common neural mechanisms that are responsible for modulatory patterns in motivationally relevant contexts. In human research, only a few studies have co-registered cortical activity (i.e. P3 component of event-related potentials) and pupil

diameter within the same experiment, and found that they were highly sensitive to stimulus novelty, with both measures increasing in amplitude as the probability of occurrence of the eliciting stimulus decreased (Friedman, Hakerem, Sutton, & Fleiss, 1973; Murphy, Robertson, Balsters, & O'Connell, 2011; Verbaten, Roelofs, Sjouw, Slangen, 1986).

In the present study, the LPP and the pupillary dilation response were co-registered in a free-viewing context in which participants viewed blocks of novel pictures, emotional and neutral, alternated with blocks of massed repetitions, wherein the same picture exemplar was consecutively presented 4-8 times in a row.

The specific comparison between the affective modulation of the LPP and the pupillary dilation response as a function of repetition would be informative regarding the nature of the processes underlying their affective modulation: If modulatory differences in the LPP and in the pupil diameter as a function of affective picture content index the same processes, we expected similar effects of picture repetition. Therefore, based on previous findings (Bradley & Lang, 2015; Codispoti et al., 2006, 2007; Ferrari et al., 2011), we expected that the viewing of repeated emotional pictures would prompt enhanced LPP and pupil diameter, compared to the viewing of repeated neutral pictures.

On the other hand, the affective modulation of the pupillary dilation response has been shown to covary with skin conductance changes (Bradley, et al., 2008). Considering that skin conductance reactivity is clearly affected by picture repetition, showing a rapid habituation of the affective modulation following a few picture repetitions (Bradley, Lang & Cuthbert, 1993), we might expect an enhanced sensitivity to picture repetition of the pupillary response modulation compared to the late positive potential.

## Method

### Participants

A total of 27 participants (14 females) took part in the study for course credits. Age ranged from 18 to 32 ( $M = 23.11$ ,  $SD = 3.72$ ). All participants had normal or corrected to normal vision, and none of them reported current or past neurological or psychopathological problems. The participants had no previous experience with the materials used in this experiment. The experimental protocol conforms to the declaration of Helsinki, and was approved by the Ethical Committee of the University of Bologna. Because of failure of the eye tracker, we could not collect pupil data from 5 participants. For pupil analyses, final Ns are 22 participants, females = 12.

### Materials and Design

The stimuli were 176 pictures selected from the International Affective Picture System (Lang, Bradley, & Cuthbert, 2008) and other sources, representing an equal number of emotionally arousing (pleasant and unpleasant) and neutral pictures. Pleasant categories included erotic couples, romance, families/babies, puppies and sports; unpleasant categories included mutilations, human threat, animal threat, disgust and accident, and neutral categories included faces and people in urban or domestic contexts<sup>1</sup>. Each picture was adjusted to an average luminance and contrast value and resized to 160 x 120 pixels. Of these, 32 (half emotional, half neutral) pictures were repeated across trials whereas the remaining pictures were presented only once (i.e. novel). Repetitions were consecutive (massed), and the number of repetitions was variable across trials (from four to eight times in a row). Novel pictures were presented in series from three to six pictures. Throughout the study, there were 32 series of repeated pictures and 32 series of novel pictures, for a total of 320 trials; novel and repeated series alternated regularly. Using the same 176 pictures,



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six pseudorandomized presentation orders were constructed that varied the specific pictures presented in the repeated and novel sets across participants.

To find the appropriate luminance and timing, which allow the pupil to recover from the light reflex (Ellis, 1981), we collected pilot data from 8 participants who did not take part in the final experiment. In this pilot group, we varied the inter-trial interval (ITI) and the luminance of the stimuli and background. Room illumination, picture as well as background luminance were measured using a diode-type digital luxmeter. The mean room illumination was 11 lux. The background color that surrounded the picture, also used as a background screen in the ITI, was black. The grey patch used to replace the picture during the ITI had a luminance of 10 lux. Pictures had an average luminance of 14.52 lux. Based on these pilot data and these luminance values, the average ITI was set to 5 seconds.

Each trial consisted of the presentation of a grey blank patch (sized 160 x 120 pixels) at the center of a black screen for 2 sec. In place of the grey patch, a picture was displayed for 4 sec, and was again followed by a grey patch lasting 3 sec, plus or minus a random additional time between 0 and 250 ms (average ITI: 5 sec  $\pm$  250 ms).

### *Apparatus*

Participants were seated in front of a SMI RED 500 remote eye tracking system, positioned below a 22-in LCD monitor on which the pictures were presented and situated approximately 52 cm from the participant's head. The visual angle subtended by each image was 8.42 (horizontal) x 4.65 (vertical) degrees.

*Pupil recording and scoring:* Pupil size was acquired continuously, at a rate of 500 samples per second, from the left eye. Data were converted and analyzed using ILAB (Gitelman, 2002) and in-house developed Matlab routines. To identify missing or bad data, the following procedure was used. Pupil diameters with missing values, or with

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values above or below 2.5 standard deviations from the average pupil diameter in each trial, were excluded from data analysis. The data of trials in which large pupil changes ( $> .3$  mm) occurred, where an exceedingly small pupil size was reported ( $< 1$  mm), were excluded. Similarly, data segments of less than 5 samples, which were preceded and followed by excluded data, were excluded. If the pupil data contained more than 50% blinks or excluded data, the whole trial was discarded from data analysis. Otherwise, excluded data from the remaining trials were replaced by linear interpolation starting from 5 samples before and ending 5 samples after (van Orden, Jung, Makeig, 2000). On average, the pupil data of 307 (SD = 21, min = 241, max = 316 out of a total of 320) trials were included in the analysis. The baseline pupil size was defined as the average pupil size (mm) in the 500 ms interval preceding picture onset. In each trial, the baseline pupil size was linearly subtracted from the pupil diameter changes following stimulus onset. For statistical analyses, these pupil diameter changes were then averaged between 500 ms and 1000 ms from stimulus onset for the light reflex window, and between 1000 ms and 4000 ms for the late pupil diameter change.

*EEG Recording and Processing:* EEG was recorded at a sampling rate of 512 Hz from 256 active sites using an ActiveTwo Biosemi system. An additional sensor was placed below the participant's left eye, to allow for detection of blinks and eye movements. The EEG was referenced to an additional electrode located near Cz during recording. A hardware 5th order low-pass filter with a -3dB attenuation factor at 50 Hz was applied online. Off-line analysis was performed using Emegs (Peyk, De Cesarei, & Junghöfer, 2011). EEG data were initially filtered (0.1 Hz high-pass and 40 Hz low-pass), and eye movements were corrected by means of an automated regressive method (Schlögl et al., 2007). Trials and sensors containing artefactual data were

detected through a statistical procedure specifically developed for dense array EEG (statistical correction of artifacts in dense array studies, SCADS; Junghöfer, Elbert, Tucker, & Rockstroh, 2000). For each participant, trial and sensor, the distributions of EEG amplitude, first derivative, and amplitude variability were calculated. Then, trials exhibiting extreme values on any of these distributions were identified and marked for exclusion. If a high number of neighboring bad sensors was present on a single trial, then the whole trial was discarded; for the remaining trials, sensors containing artefactual data were replaced by interpolating the nearest good sensors. The percentage of good trials was 74.20% and 71.34% for emotional and neutral novel pictures, and 87.29% and 71.60% for emotional and neutral novel pictures. Finally, data were off-line re-referenced to the average of all sensors, and a baseline correction based on the 200 ms prior to stimulus onset was performed. ERP statistical analysis was conducted on the mean amplitudes in a window 500-1000 ms for the late positive potential, and in the window 1000-4000 ms for the slow wave, in the centro-parietal region. Sensor cluster was selected on the basis of the difference between emotional and neutral pictures ( $> 1 \mu\text{v}$ ; see inset in Figure 1 for sensor cluster, grey dots).

#### Statistical analysis

A repeated measures ANOVA was conducted on pupil dilation and centro-parietal ERPs with picture Content (2 levels: Emotional and Neutral), Repetition (2 levels: Novel and Repeated) and Window (2 levels: 500-1000ms; 1000-4000ms from picture onset), as within-subjects factors. Huynh–Feldt correction was used when appropriate. The partial eta squared statistic ( $\eta^2_p$ ), indicating the proportion between the variance explained by one experimental factor and the total variance, was calculated and reported.

## Results

### *Centro-parietal event-related potentials<sup>2</sup>*

Figure 1 illustrates ERP waveforms averaged over centro-parietal sensors (inset on the top figure shows the sensor cluster) for emotional and neutral pictures during novel presentations (top) and massed repetitions (bottom). Significant main effect of picture Content,  $F(1,26)=75$ ,  $p<.0001$ ,  $\eta^2_p =.23$  indicated an overall larger ERP positive amplitude when subjects viewed emotional pictures, compared to neutral contents. The interaction picture Content x Window,  $F(1,26)=43.8$ ,  $p<.0001$ ,  $\eta^2_p =.63$ , primarily indicates that the enhanced positive amplitude for emotional pictures significantly decreased over the picture viewing interval (LPP vs slow wave,  $F(1,26)=15.5$ ,  $p<.005$ ,  $\eta^2_p =.4$ ), while no significant change was observed for neutral scenes. Affective modulation was significant in both time intervals, the LPP window,  $F(1,26)=83$ ,  $p<.0001$ ,  $\eta^2_p =.8$ , and the slow wave window,  $F(1,26)=47$ ,  $p<.0001$ ,  $\eta^2_p =.6$ .

The three-way interaction, picture Content x Repetition x Window,  $F(1,26)= 7.7$ ,  $p<.05$ ,  $\eta^2_p =.23$ , indicated that picture repetition prompted a small but significant reduction in the affective modulation of the late positive potential (Repetition x Content,  $F(1,26)=8.5$ ,  $p<.01$ ,  $\eta^2_p =.25$ ), whereas the amplitude difference between emotional and neutral pictures in the slow wave window was not significantly affected by stimulus repetition. More important, replicating many previous studies, viewing massed repetitions of emotional pictures still prompted significantly larger ERP positive amplitudes than when viewing neutral pictures  $F(1,26)=20$ ,  $p<.0001$ ,  $\eta^2_p =.4$ , and this was evident during the whole picture viewing interval,  $F(1,26)=26.4$ ,  $\eta^2_p =.5$  for the LPP,  $F(1,26)=11$ ,  $p<.005$ ,  $\eta^2_p =.3$  for the slow wave.

### *Pupil diameter*

### *Pupillary and cortical responses*

Figure 2 illustrates pupil diameter across the picture-viewing interval for emotional and neutral pictures during novel presentation (top) and massed (bottom) repetition. Here, we observed a pattern of initial pupil constriction (light reflex), which slowly returned towards baseline values. Beginning at about 500 ms after stimulus onset picture repetition prompted a relatively smaller pupil constriction (see inset of Figure 2), evident during the whole picture viewing interval (overall repetition effect,  $F(1,21)=9.1$ ,  $p<.01$ ,  $\eta^2_p=.3$ ). Moreover, the significant interaction Window x Repetition,  $F(1,21)=4.7$ ,  $p<.05$ ,  $\eta^2_p=.18$ , indicated that this repetition effect was even larger in the later (1000-4000ms),  $F(1,21)=9.7$ ,  $p<.01$ ,  $\eta^2_p=.32$ , compared to the earlier (500-1000ms),  $F(1,21)=5.5$ ,  $p<.05$ ,  $\eta^2_p=.21$ . In addition, the interaction of picture Content x Repetition,  $F(1, 21)=6$ ,  $p<.05$ ,  $\eta^2_p=.22$ ,  $\eta^2_p=.22$ , indicated that the affective modulation of pupil diameter--that is a smaller pupil constriction during viewing of novel emotional, compared to neutral, pictures,  $F(1,21)=7.4$ ,  $p<.05$ ,  $\eta^2_p=.26$  -- was significantly affected by repetition, such that the viewing of repeated pictures did not prompt any affective modulation of the pupil diameter across the whole picture viewing interval (see Figure 2).

### *Correlations between LPP and pupil responses*

In order to directly assess the relationship between the centro-parietal late positive potential (LPP) and the pupil diameter change, we examined the correlations between these measures. In a first analysis, Pearson's correlation coefficients were calculated between the LPP (500-1000 ms) and the pupil diameter change (500-1000 ms) in those participants who had both measures intact ( $n=22$ ). During viewing of novel pictures, either emotional or neutral, correlation coefficients between the LPP and the pupil responses were low ( $r=.14$ ,  $p=.55$ , and  $r=.27$ ,  $p=.22$ , respectively), so as they were during the viewing of repeated emotional and neutral pictures ( $r=-.09$ ,  $p=$

.70, and  $r = .31$ ,  $p = .16$ ). An additional analysis was carried out to compare the correlation coefficients between pupil diameter changes and LPP during the viewing of emotional and neutral pictures, separately in the novel and in the repeated condition (Raghunathan, Rosenthal, and Rubin, 1996). Neither for novel nor for repeated condition the correlation coefficients for emotional and neutral pictures differed from each other,  $z = -0.62$ ,  $p = .53$ ,  $z = -1.54$ ,  $p = .12$ , respectively. Finally, correlation coefficients of the affective modulation (emotional minus neutral) were not significant, neither for novel nor for repeated pictures ( $r = -.07$ ,  $p = .75$ ,  $r = -.03$ ,  $p = .91$ ), and did not differ from each other,  $z = -0.16$ ,  $p = .88$ .

### Discussion

In the present study, both the late positive potential (LPP) and the pupil diameter were concurrently assessed during free viewing of emotional and neutral scenes that were either novel, that is never seen before in the course of the study, or repeated with massed repetition, in which the same picture exemplar was consecutively repeated.

Despite massed repetition determined a reduction in LPP affective modulation, the late positive potential amplitude continued to be highly modulated by picture content, with a larger LPP amplitude during the viewing of emotional compared to neutral scenes, even after a series of massed repetitions (an average of 4-8). Pupil diameter varied as a function of emotional content in the novel condition, with larger pupil size (or smaller pupil constriction) for arousing pictures, compared to neutral scenes, consistent with previous findings (Bradley et al., 2008, Bradley & Lang, 2015; Henderson, et al., 2014). However, the present study showed that this pupillary affective modulation was highly dependent on stimulus novelty, as repeated pictures prompted a pupil response that did not vary as a function of picture motivational content. A recent study by Bradley and Lang (2015) observed that the affective

modulation of pupil diameter, although attenuated, was always prompted by the viewing of repeated emotional (i.e. erotica and violence) and neutral pictures. It should be noted, however, that compared to this previous study, in the present study the number of massed repetitions was higher and emotional pictures varied in contents and arousal (including only a few high arousing erotic and mutilation pictures), which might account for the enhanced habituation of the affective modulation of pupil dilation observed here.

The overall effect of stimulus repetition on pupil diameter, that is a smaller pupil constriction for repeated compared to novel pictures, is consistent with a number of previous findings (Bradley & Lang, 2015; Gardner, Mo, & Borrego, 1974; Heaver & Hutton, 2011; Naber, Frässle, Rutishauser, & Einhauser, 2013; Kafkas & Montaldi 2015; Papesh, Goldinger, & Hout, 2012; Vo et al., 2008), who observed smaller pupil constriction (or a relative larger pupil diameter) for old as compared to new stimuli. Interestingly, this modulation pattern was observed both in the context of a general constriction following the light reflex, as in the present study, (e.g., Bradley & Lang, 2015; Gardner et al., 1974; Naber et al., 2013), and in the context of pupil dilation (e.g., Kafkas & Montaldi, 2015; Heaver & Hutton, 2011; Papesh et al., 2012; Vo et al., 2008). In both contexts, a relative dilation was observed for old compared to new stimuli. This pattern indicates that, at least in some experimental contexts, pupil diameter changes do not reflect orienting to novel stimuli, consistent with the literature showing that pupil response may be also modulated by memory processes (Gardner et al., 1974; Kafkas & Montaldi, 2011; Heaver & Hutton, 2011).

These findings show that although the LPP and pupil size were similarly affected by motivational relevance during the viewing of novel pictures, they differed concerning the habituation pattern of affective modulation, with pupil size being more

sensitive to stimulus repetition. Indeed, a substantial body of work indicates that a range of physiological changes, which have been associated with the orienting response, do not covary, suggesting that different components reflect different facets of the orienting response (Bradley, 2009; Barry, & O'Gorman, 1987).

Among responses classically associated with the orienting response, several findings indicated that pupillary changes and skin conductance response share functional similarities, for instance in the fact that changes in both measures are elicited by the viewing of motivationally relevant pictures (Bradley et al., 2008). The magnitude of skin conductance response, which covaries with emotional arousal of the stimuli (Bradley, Codispoti, Sabatinelli, & Lang, 2001; Lang, Greenwald, Bradley, & Hamm, 1993) habituates rapidly with stimulus repetition and is reinstated only when new pictures are presented (Bradley et al., 1993; Codispoti et al., 2006). Here, affective modulation of pupil dilation has been shown to decrease with scene repetition. These findings are consistent with the hypothesis that both pupillary and skin conductance changes during affective picture viewing are mediated by increased sympathetic activity that is critical to prepare the organism for potential action. Motivationally relevant stimuli generally prompt heightened responses in these measures, but orienting decreases when it is clear that no adaptive action is necessary, as when the same stimulus is repeatedly presented (or distant; Codispoti & De Cesare, 2007).

Increase in palmar sweat gland activity and pupil dilation are classic autonomic components of the orienting response, along with a transient change in brain activity. Several similar cortical ERPs have been linked to the orienting response, including the P3 (P3b), the late positive complex, and the “orienting” wave, all showing an enhanced positive change over central-parietal sensors when



processing significant stimuli (Barry, 1979; 2009; Connor & Lang, 1969; Donchin et al., 1984; Roth, 1983; Weerts & Lang, 1973). The late positive potential (LPP) is a similar slow potential, and is the most reliable ERP component modulated by emotional arousal during the viewing of natural scenes (Cuthbert et al., 2000; Schupp et al., 2004; De Cesarei & Codispoti, 2011). Unlike pupil dilation and skin conductance changes, the LPP continues to be enhanced when viewing emotional, compared to neutral, pictures, even following multiple contiguous repetitions (Ferrari et al., 2011; Ferrari et al., 2015).

Moreover, the present study showed that also the positive slow wave (1000-4000ms from picture onset), which was modulated by picture content for the entire picture viewing interval, was unaffected by picture repetition. Previous studies indicated that the amplitude of the slow wave is sensitive to the emotional content of visual scenes (Cuthbert et al., 2000; Codispoti, De Cesarei, & Ferrari, 2012), and that this effect is not merely explained by a slow return to baseline for the stimuli which elicited the most pronounced P3 and LPP amplitudes (Gable et al., 2015). Here we show that this slow wave affective modulation is also resistant to habituation, as it is still evident after massed repetitions, suggesting that the affective modulation of the slow wave indexes motivational activation, rather than sustained attentional engagement which should decline with stimulus repetition.

Previous studies that have co-registered ERPs and pupil diameter within the same experiment found that both indexes were highly sensitive to stimulus novelty, manipulated through stimulus probability (Friedman et al., 1973; Nieuwenhuis, De Geus, & Aston-Jones, 2011; Steinhauer & Hakerem, 1992), which have led to the idea that both physiological responses index the same psychological process, and are controlled by a common neural mechanism (Nieuwenhuis et al., 2011; for a

dissociation see Laeng, Ørbo, Holmlund, & Miozzo, 2011). Specifically, it has been proposed that the simultaneous occurrence of the P3 and the pupillary dilation response reflects the co-activation of the locus coeruleus-norepinephrine system and the peripheral sympathetic nervous system by their common major afferent: the rostral ventrolateral medulla (Nieuwenhuis et al., 2011). Consistently, a recent study found that pupil diameter covaries with blood-oxygen level dependent (BOLD) activity in human locus coeruleus (Murphy, Robertson, Balsters, & O'Connell, 2014). In addition, the antecedent conditions for the P3 are similar to those reported for the locus coeruleus (LC) phasic response, and it has been suggested that the P3 reflects the LC-mediated phasic enhancement of neural responsivity in the cortex (Nieuwenhuis et al., 2011). The present study manipulated novelty through repeated presentation of the same scenes, and observed that the emotional modulation of the LPP and the pupillary dilation response were differentially affected by stimulus repetition; these results seem to suggest that these indexes might be controlled by partially different neural mechanisms. Similar findings were reported by a previous study on affective picture perception that co-registered ERPs and another autonomic component of the orienting reflex: skin conductance response (Codispoti et al., 2006). Moreover, in the present study, the correlations between LPP and pupil dilation response were absent, consistent with few previous studies that have co-registered P3 and phasic pupil dilation (Murphy et al., 2011).

The apparent inconsistency between the present study and previous research addressing the effect of novelty on ERPs and pupillary dilation response could be due to some critical methodological differences. Previous studies that co-registered P3 and the pupillary dilation response, and reported that both indexes were sensitive to novelty, employed only neutral stimuli and manipulated stimulus probability to

examine the effect of novelty (Nieuwenhuis et al., 2011; Steinhauer & Hakerem, 1992). In the present study, we operationalized novelty as stimulus repetition and presented both emotional and neutral stimuli. Several studies indicated that whereas locus coeruleus cells show rapid habituation following repetitive exposure to the same neutral stimulus (Bouret & Sara, 2005; Herve-Minvielle & Sara, 1995; Vankov, Herve-Minvielle, & Sara, 1995; Swick, Pineda, Schacher, & Foote, 1994; Foote, Aston-Jones, & Bloom, 1980), little habituation of these neurons is observed when a cue signals a motivationally relevant event (e.g., reward; Bouret & Sara, 2004). Moreover, although the P3 and the LPP are very similar potentials in terms of latency and topography (often overlapping), and are similarly affected by motivational significance (Nieuwenhuis et al., 2011; Schupp et al., 2006), they probably cannot be considered the same ERP component.

The orienting response towards novelty has been associated with the same motivational circuits that underlie the response to emotional stimuli (Bradley, 2009; Ferrari, Codispoti, Cardinale, & Bradley, 2008). The activation of several subcortical structures (e.g., the locus coeruleus and the amygdala) is enhanced in response to neutral novel stimuli, compared to repeated ones, suggesting that novelty is integral of their function (Schomaker & Meeters, 2015; Schwartz et al., 2003; Wright et al., 2003; Zald, 2003). Future studies should clarify the mechanisms underlying the relationships between cortical and autonomic components of the orienting response by exploring the different facets involved in the concept of motivational significance such as novelty and emotional arousal.

References

- Barry, R.J. (2009). Evoked activity and EEG phase resetting in the genesis of auditory Go/NoGo ERPs. *Biological Psychology*, *80*, 292-9. doi: 10.1016/j.biopsycho.2008.10.009
- Barry, R.J. (1979). A factor-analytic examination of the unitary OR concept. *Biological Psychology*, *8*, 161–178. doi: 10.1016/0301-0511(79)90045-0
- Barry, R. J., & O'Gorman, J. G. (1987). Stimulus omission and the orienting response: Latency differences suggest different mechanisms. *Biological Psychology*, *25*, 261-276. doi: 10.1016/0301-0511(87)90051-2.
- Bouret, S., & Sara, S.J. (2004). Reward expectation, orientation of attention and locus coeruleus-medial frontal cortex interplay during learning. *European Journal of Neuroscience*, *20*, 791-802. doi: 10.1111/j.1460-9568.2004.03526.x
- Bouret, S., & Sara, S.J. (2005). Network reset: a simplified overarching theory of locus coeruleus noradrenaline function. *Trends in Neurosciences*, *28*, 574-82. doi:10.1016/j.tins.2005.09.002.
- Bradley, M. M. (2000). Emotion and motivation. In J. T. Cacioppo, L. G. Tassinary, & G. Berntson (Eds.), *Handbook of psychophysiology* (pp. 602–642). New York: Cambridge University Press.
- Bradley, M. M., & Lang, P. J. (2015). Memory, emotion, and pupil diameter: Repetition of natural scenes. *Psychophysiology*. doi: 10.1111/psyp.12442.
- Bradley, M. M., Codispoti, M., Sabatinelli, D., & Lang, P. J. (2001). Emotion and motivation II: Sex differences in picture processing. *Emotion*, *1*, 300-319. doi: 10.1037/1528-3542.1.3.300.

- Bradley, M. M., Miccoli, L., Escrig, M. A., & Lang, P. J. (2008). The pupil as a measure of emotional arousal and autonomic activation. *Psychophysiology*, *45*, 602-607. doi: 10.1111/j.1469-8986.2008.00654.x
- Bradley, M.M. (2009). Natural selective attention: orienting and emotion. *Psychophysiology*, *46*, 1-11. doi: 10.1111/j.1469-8986.2008.00702.x
- Bradley, M.M., Lang, P.J., Cuthbert, B.N. (1993). Emotion, novelty, and the startle reflex: habituation in humans. *Behavioral Neuroscience*, *107*, 970–980.
- Cacioppo, J. T., Crites Jr., S. L., Gardner, W. L., & Berntson, G. G. (1994). Bioelectrical echoes from evaluative categorizations: I. A late positive brain potential that varies as a function of trait negativity and extremity. *Journal of Personality and Social Psychology*, *67*, 115-125.
- Codispoti, M., & De Cesarei, A. (2007). Arousal and Attention: Picture Size and Emotional Reactions. *Psychophysiology*, *44*, 680-686, doi: 10.1111/j.1469-8986.2007.00545.x
- Codispoti, M., Ferrari, V., De Cesarei, A., and Cardinale, R. (2006). Implicit and explicit categorization of natural scenes. *Progress Brain Research*. *156*, 53–65. doi:10.1016/S0079-6123(06)56003-0
- Codispoti, M., Mazzetti, M., & Bradley, M. M. (2009). Unmasking emotion: exposure duration and emotional engagement. *Psychophysiology*, *46*(4), 731–8. doi:10.1111/j.1469-8986.2009.00804.x
- Codispoti, M., De Cesarei, A., & Ferrari, V. (2012). The influence of color on emotional perception of natural scenes. *Psychophysiology*, *49*, 11-16. doi: 10.1111/j.1469-8986.2011.01284.x
- Codispoti, M., Ferrari, V., & Bradley, M. M. (2006). Repetitive picture processing: Autonomic and cortical correlates. *Brain Research*, *1068*, 213-220. doi:10.1016/j.brainres.2005.11.009

- Codispoti, M., Ferrari, V., & Bradley, M.M. (2007). Repetition and Event-related Potentials: Distinguishing Early and Late Processes in Affective Picture Perception. *Journal of Cognitive Neuroscience*, *19*, 577-586. doi:10.1162/jocn.2007.19.4.577
- Connor, W.H. & Lang, P.J. (1969). Cortical slow-wave and cardiac rate responses in stimulus orientation and reaction time conditions. *Journal of Experimental Psychology*, *82*, 310-320. doi: 10.1037/h0028181
- Cuthbert, B. N., Schupp, H. T., Bradley, M. M., Birbaumer, N., & Lang, P. J. (2000). Brain potentials in affective picture processing: Covariation with autonomic arousal and affective report. *Biological Psychology*, *52*, 95–111. doi:10.1016/S0301-0511(99)00044-7
- De Cesarei A., & Codispoti, M. (2011). Affective modulation of the LPP and alpha-ERD during picture viewing. *Psychophysiology*, *48*, 1397–1404. doi: 10.1111/j.1469-8986.2011.01204.x
- Donchin, E., Heffley, E., Hillyard, S.A., Loveless, N., Maltzman, I., Ohman, A., Rösler, F., Ruchkin, D., & Siddle, D. (1984). Cognition and event-related potentials. II. The orienting reflex and P300. *Annals of the New York Academy of Sciences*, *425*, 39-57. doi: 10.1111/j.1749-6632.1984.tb23522.x
- Ellis, C.J. (1981). The pupillary light reflex in normal subjects. *British Journal of Ophthalmology* *65*, 754-759 doi:10.1136/bjo.65.11.754
- Ferrari V., Bradley M.M., Codispoti M., & Lang, P.J. (2011). Repetitive exposures: Brain and reflex measures of emotion and attention. *Psychophysiology*, *48*, 515-522. doi: 10.1111/j.1469-8986.2010.01083.x
- Ferrari V., Codispoti M., Cardinale R., & Bradley M.M. (2008). Directed and Motivated Attention during Processing of Natural Scenes. *Journal of Cognitive Neuroscience*, *20*, 1753-1761. doi:10.1162/jocn.2008.20121

*Pupillary and cortical responses*

- Ferrari, V., Bradley, M. M., Codispoti, M., & Lang, P. J. (2015). Massed and distributed repetition of natural scenes: Brain potentials and oscillatory activity. *Psychophysiology*. doi: 10.1111/psyp.12424
- Ferrari, V., Bradley, M.M., Codispoti, M., & Lang, P.J. (2010). Detecting novelty and significance. *Journal of Cognitive Neuroscience*, 22, 404-411. doi:10.1162/jocn.2009.21244
- Foote, S.L., Aston-Jones, G., & Bloom, F.E. (1980). Impulse activity of locus coeruleus neurons in awake rats and monkeys is a function of sensory stimulation and arousal. *Proceedings of the National Academy of Sciences*, 77, 3033-7.
- Friedman, D., Hakerem, G., Sutton, S., & Fleiss, J.L. (1973). Effect of stimulus uncertainty on the pupillary dilation response and the vertex evoked potential. *Electroencephalography and Clinical Neurophysiology*, 34, 475-84. doi: 10.1016/0013-4694(73)90065-5
- Gable, P. A., Adams, D. L., & Proudfit, G. H. (2015). Transient tasks and enduring emotions: The impacts of affective content, task relevance, and picture duration on the sustained late positive potential. *Cognitive, Affective, & Behavioral Neuroscience*, 15, 45-54. doi: 10.3758/s13415-014-0313-8.
- Gardner, R.M., Mo, S. S., & Borrego, R. (1974). Inhibition of pupillary orienting reflex by novelty in conjunction with recognition memory. *Bulletin of the Psychonomic Society*, 3, 237-238. doi: 10.3758/BF03333458
- Gitelman, D.R. (2002). ILAB: A program for postexperimental eye movement analysis. *Behavior Research Methods, Instruments, and Computers*, 34, 605-612.
- Heaver, B., & Hutton, S. B. (2011). Keeping an eye on the truth? Pupil size changes associated with recognition memory. *Memory*, 19, 398-405. doi: 10.1080/09658211.2011.575788.

- Henderson, R. R., Bradley, M. M., & Lang, P. J. (2014). Modulation of the initial light reflex during affective picture viewing. *Psychophysiology*, *51*, 815-818. doi: 10.1111/psyp.12236
- Hervé-Minvielle, A., & Sara, S.J. (1995). Rapid habituation of auditory responses of locus coeruleus cells in anaesthetized and awake rats. *Neuroreport*, *6*, 1363-8.
- Johnston, V. S., Miller, D. R., & Burleson, M. H. (1986). Multiple P3s to emotional stimuli and their theoretical significance. *Psychophysiology*, *23*, 684-694. doi: 10.1111/j.1469-8986.1986.tb00694.x
- Junghöfer, M., Elbert, T., Tucker, D. M., & Rockstroh, B. (2000). Statistical control of artifacts in dense array EEG/MEG studies. *Psychophysiology*, *37*, 523-532. doi: 10.1111/1469-8986.3740523
- Kafkas, A. & Montaldi, D. (2015). The pupillary response discriminates between subjective and objective familiarity and novelty. *Psychophysiology*, *52*, 1305-1316. doi: 10.1111/psyp.12471
- Laeng, B., Ørbo, M., Holmlund, T., & Miozzo, M. (2011). Pupillary Stroop effects. *Cognitive Processing*, *12*, 13-21. doi: 10.1007/s10339-010-0370-z
- Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (2008). *International Affective Picture System (IAPS): Affective rating of measures and instruction manual*, (Technical Report No. A-6). Gainesville, FL: University of Florida
- Lang, P.J., Greenwald, M.K., Bradley, M.M., & Hamm, A.O. (1993). Looking at pictures: affective, facial, visceral, and behavioral reactions. *Psychophysiology*, *30*, 261-73. doi: 10.1111/j.1469-8986.1993.tb03352.x.
- Murphy, P.R., O'Connell, R.G., O'Sullivan, M., Robertson, I.H., & Balsters, J.H. (2014). Pupil diameter covaries with BOLD activity in human locus coeruleus. *Human Brain Mapping*, *35*, 4140-4154. doi: 10.1002/hbm.22466.



- Murphy, P.R., Robertson, I.H., Balsters, J.H., & O'Connell, R.G. (2011). Pupillometry and P3 index the locus coeruleus-noradrenergic arousal function in humans. *Psychophysiology*, 48, 1532–1543. doi: 10.1111/j.1469-8986.2011.01226.x
- Naber, M., Frässle, S., Rutishauser, U., & Einhäuser, W. (2013). Pupil size signals novelty and predicts later retrieval success for declarative memories of natural scenes. *Journal of Vision*, 8, 13:11. doi: 10.1167/13.2.11.
- Nieuwenhuis, S., De Geus, E.J., & Aston-Jones, G. (2011). The anatomical and functional relationship between the P3 and autonomic components of the orienting response. *Psychophysiology*, 48, 162-75. doi: 10.1111/j.1469-8986.2010.01057.x
- Papesh, M.H., Goldinger, S.D., & Hout, M.C. (2012). Memory strength and specificity revealed by pupillometry. *International Journal of Psychophysiology*, 83, 56–64. doi: 10.1016/j.ijpsycho.2011.10.002
- Peyk, P., De Cesarei, A., & Junghöfer, M. (2011), ElectroMagnetoEncephalography software: overview and integration with other EEG/MEG toolboxes. *Computational Intelligence and Neuroscience*, 861705. doi:10.1155/2011/861705
- Radilova, J. (1982). The late positive component of visual evoked response sensitive to emotional factors. *Actavitas Nervosa Superior (Praha)*, 3, 334–337.
- Raghunathan, T. E., Rosenthal, R, & Rubin, D. B. (1996). Comparing correlated but nonoverlapping correlations. *Psychological Methods*, Vol 1(2): 178-183. doi: 10.1037/1082-989X.1.2.178
- Reimer, J., Froudarakis, E., Cadwell, C.R., Yatsenko, D., Denfield, G.H., & Tolias, A.S. (2014). Pupil fluctuations track fast switching of cortical states during quiet wakefulness. *Neuron*, 22, 355-62. doi: 10.1016/j.neuron.2014.09.033

- Roth, W. T. (1983). A comparison of P300 and skin conductance response. In A. W. K.Gaillard & W.Ritter (Eds.), *Tutorials in event related potentials: Endogenous components* (pp. 177–199). Amsterdam: North-Holland.
- Schlögl, A., Keinrath, C., Zimmermann, D., Scherer, R., Leeb, R., & Pfurtscheller, G. (2007). A fully automated correction method of EOG artifacts in EEG recordings. *Clinical Neurophysiology, 118*, 98–104. doi:10.1016/j.clinph.2006.09.003
- Schomaker, J., & Meeter, M. (2015). Short- and long-lasting consequences of novelty, deviance and surprise on brain and cognition. *Neuroscience & Biobehavioral Reviews, 12*, 55, 268-279. doi: 10.1016/j.neubiorev.2015.05.002
- Schupp, H. T., Cuthbert, B. N. , Bradley, M. M. , Hillman, C. H. , Hamm, A. O. , & Lang, P. J. (2004). Brain processes in emotional perception: Motivated attention. *Cognition and Emotion, 18*, 593–611. doi:10.1080/02699930341000239
- Schupp, H. T., Junghöfer, M., Weike, A. I., & Hamm, A. O. (2004). The selective processing of briefly presented affective pictures: An ERP analysis. *Psychophysiology, 41*, 441-449. doi:10.1111/j.1469-8986.2004.00174.x
- Schupp, H. T., Stockburger, J., Codispoti, M., Junghöfer, M., Weike, A. I., & Hamm, A. O. (2007). Selective visual attention to emotion. *Journal of Neuroscience, 27*, 1082-1089. doi:10.1523/JNEUROSCI.3223-06.2007
- Schupp, H.T., Flaisch, T., Stockburger, J., & Junghöfer, M. (2006). Emotion and attention: event-related brain potential studies. *Progress Brain Research, 156*, 31-51. doi:10.1016/S0079-6123(06)56002-9
- Schwartz, C.E., Wright, C.I., Shin, L.M., Kagan, J., Whalen, P.J., McMullin, K.G., Rauch, S.L. (2003). Differential amygdalar response to novel versus newly familiar neutral faces: a functional MRI probe developed for studying inhibited temperament. *Biological Psychiatry, 15*, 854-62. doi:10.1016/S0006-3223(02)01906-6

- Sokolov, E. N. (1963). *Perception and the conditioned reflex*. New York: Macmillan.
- Steinhauer, S. R., & Hakerem, G. (1992). The pupillary response in cognitive psychophysiology and schizophrenia. *Annals of the New York Academy of Sciences*, 658, 182-204. doi: 10.1111/j.1749-6632.1992.tb22845.x
- Swick, D., Pineda, J.A., Schacher, S., & Foote, S.L. (1994). Locus coeruleus neuronal activity in awake monkeys: relationship to auditory P300-like potentials and spontaneous EEG. *Experimental Brain Research*, 101, 86-92. doi: 10.1007/BF00243219
- van Orden, K.F, Jung, T.P., Makeig, S. (2000). Combined eye activity measures accurately estimate changes in sustained visual task performance. *Biological Psychiatry*, 52, 221–240. doi:10.1016/S0301-0511(99)00043-5
- Vankov, A., Hervé-Minvielle, A., & Sara, S.J. (1995). Response to novelty and its rapid habituation in locus coeruleus neurons of the freely exploring rat. *European Journal of Neuroscience*, 7, 1180-7. doi: 10.1111/j.1460-9568.1995.tb01108.x
- Verbaten, M.N., Roelofs, J.W., Sjouw, W., Slangen, J.L., 1986. Habituation of early and late visual ERP components and the orienting reaction: the effect of stimulus information. *International Journal of Psychophysiology*, 3, 287–298.
- Vinck, M., Batista-Brito, R., Knoblich, U., & Cardin, J.A. (2015). Arousal and locomotion make distinct contributions to cortical activity patterns and visual encoding. *Neuron*, 6, 740-54. doi: 10.1016/j.neuron.2015.03.028
- Vo, M. L. H., Jacobs, A. M., Kuchinke, L., Hofmann, M., Conrad, M., Schacht, A., & Hutzler, F. (2008). The coupling of emotion and cognition in the eye: Introducing the pupil old/new effect. *Psychophysiology*, 45, 130–140. doi: 10.1111/j.1469-8986.2007.00606.x

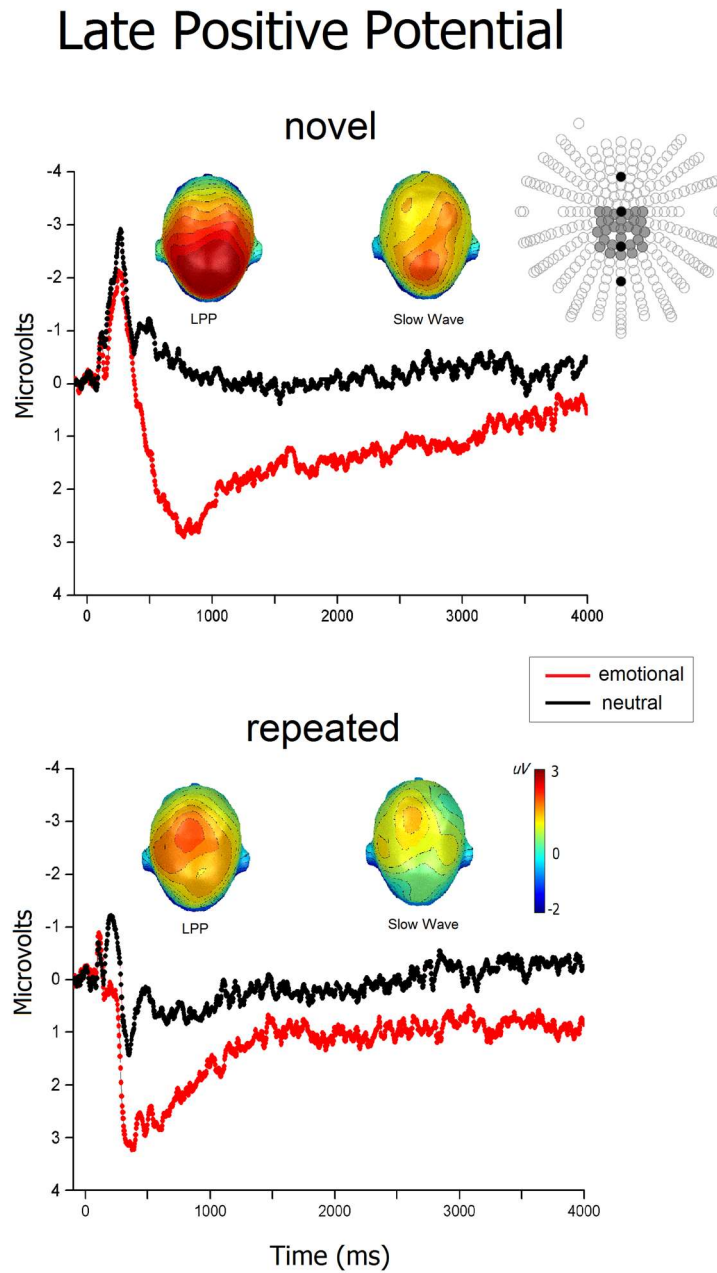
- Weerts, T.C., & Lang P.J. (1973). The effects of eye fixation and stimulus and response location on the contingent negative variation (CNV). *Biological Psychology, 1*, 1-19. doi:10.1016/0301-0511(73)90010-0
- Weinberg, A., & Hajcak, G. (2010). Beyond good and evil: Implications of examining electrocortical activity elicited by specific picture content. *Emotion, 10*, 767-782.
- Wright, C.I., Martis, B., Schwartz, C.E., Shin, L.M., Fischer, H.H., McMullin, K., & Rauch S.L. (2003). Novelty responses and differential effects of order in the amygdala, substantia innominata, and inferior temporal cortex. *Neuroimage, 18*, 660-9. doi:10.1016/S1053-8119(02)00037-X
- Zald, D.H. (2003). The human amygdala and the emotional evaluation of sensory stimuli. *Brain Research Reviews, 41*, 88-123. doi:10.1016/S0165-0173(02)00248-5

Footnotes

1. Picture set comprised both IAPS and non-IAPS pictures. More specifically, non-IAPS pictures were selected from public domain pictures available on the Internet, and from document scanning, with the purpose to reach a sufficient number of images in the neutral category (n=88). Examples of some IAPS images used in the study that closely resemble the neutral non-IAPS pictures are: 2110, 2390, 2396, 2595, 2036, 2339, 2102, 2191, 2357, 2372, 2374, 2377, 2384, 2400, 2411, 2488, 2489, 2521, 2635, 2745, 7493, 7550. Pleasantness (p) and arousal (a) mean (standard deviation) ratings for IAPS neutral pictures: p=5.4 (0.5); a=3.7 (0.4); pleasant pictures: p=7.2 (0.6); a=5.6 (1.1); unpleasant pictures: p=2.3 (0.7); a=6.3 (0.7).

2. An additional analysis focused on the modulation of ERPs in an early time interval (150– 350 ms) over occipito-temporal regions (i.e. posterior P2, or EPN, early posterior negativity; Schupp, Junghöfer, Weike, & Hamm, 2004; Schupp et al., 2007). A main effect of Repetition was observed,  $F(1, 26) = 32.32$ ,  $p < .001$ ,  $\eta^2 p = .55$ , with less positive ERPs in the repeated compared to the novel condition. Additionally, a main effect of Content was observed,  $F(1, 26) = 43.39$ ,  $p < .001$ ,  $\eta^2 p = .63$ , with less positive amplitudes for emotional as compared to neutral pictures. No interaction between Repetition and Content was observed in this analysis,  $F < 1$ .

Figures

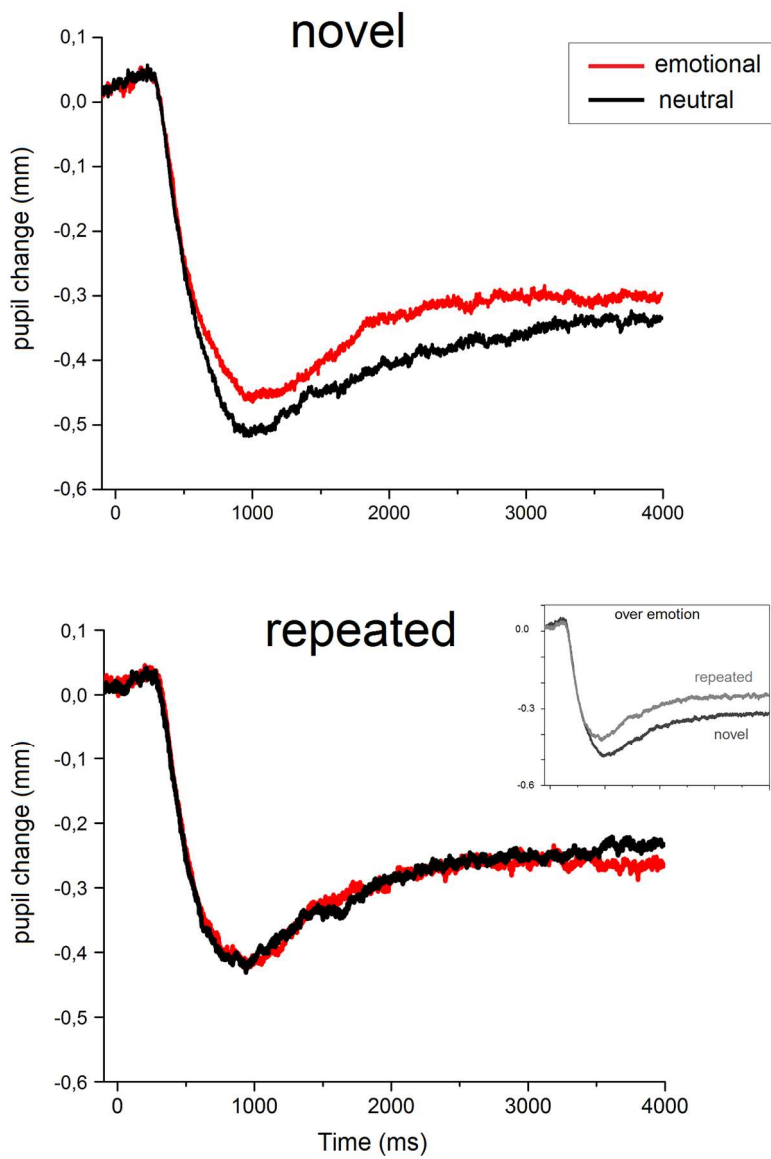


**Figure 1.** Grand average ERP waveforms of the centro-parietal scalp region (grey dots in inset) when viewing emotional (red line) and neutral (black line) pictures that were novel or repeated. For both novel and repeated pictures, scalp topography (top view) represents the difference between emotional and neutral pictures in the late positive potential window (500-1000 ms) and in the slow wave window (1000-4000 ms). Inset shows the centro-parietal sensor cluster (grey dots) used for statistical

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analysis in both the LPP and the slow wave window. Black dots represents four representative midline sensors, respectively, FZ, CZ, PZ, OZ.

## Pupil diameter



**Figure 2.** Average pupillary responses to the viewing of emotional (red line) and neutral (black line) pictures that were novel or repeated. Inset shows the overall pupil repetition effect—smaller pupil constriction for repeated compared to novel pictures.