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ScienceDirect
Biological Psychology 78 (2008) 150–158
BIOLOGICAL PSYCHOLOGY
www.elsevier.com/locate/biopsycho

Differential early ERPs to fearful versus neutral facial expressions: A response to the salience of the eyes?

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Received 4 April 2007; accepted 2 February 2008

Abstract

Several event-related potential (ERP) studies have demonstrated a negative shift in ERPs for fearful relative to neutral facial expressions ~170–300 ms post-stimulus over occipital-temporal scalp. In the present study, three experiments were conducted to examine the importance of the eye region for this ERP differentiation. ERPs and behavioral discrimination responses were measured to fearful and neutral expressions when only the eye region of the expression was visible (the eyes and eyebrows or the eyes alone) and when the eye region (the eyes and eyebrows or the eyes alone) was covered by dark glasses. The results showed a negative shift in ERPs for fearful relative to neutral expressions over lateral temporal sites, starting ~160–210 ms post-stimulus. The visibility of the eye region but not the eyes per se was critical for these ERP differences to occur. There were, however, indications that information in the eyes is also coded and used in the categorization of facial expressions.

Keywords: Event-related potential; Emotion; Face perception; Facial expression

Specialized neural mechanisms are thought to exist that serve recognition of stimuli relevant to our core motivation of minimizing danger and maximizing pleasure (Williams, 2006). These mechanisms act to ensure that biologically relevant stimuli are rapidly and automatically detected and prioritized in the competition for access to awareness. Consistent with this view, behavioral studies have shown that spatial attention is automatically shifted towards a fearful faces over a simultaneously presented neutral face (Holmes et al., 2005), and that fearful faces hold attention for a longer period of time than neutral faces (Georgiou et al., 2005).

Recordings of event-related potentials (ERPs) have further shown that threat-related (fearful/angry) facial expressions elicit different patterns of brain activity compared to positive/neutral facial expressions starting from components associated with early visual processing (P100, N170). Specifically, the P100 and the face-sensitive N170 components over occipital-temporal scalp regions have been shown to be of larger amplitude for fearful relative to neutral faces (Batty and Taylor, 2003; Leppänen et al., 2007b; Pourtois et al., 2005; Stekelenburg and de Gelder, 2004). Some studies have not confined the analyses to any specific ERP components but have instead quantified ERP differences to threat-related and neutral facial expression over a broader temporal window. These studies have shown a negative shift in ERP activity for fearful/angry relative to neutral expressions, starting at the latency of the N170 component or slightly later and lasting for 100 ms or more (Eimer et al., 2003; Eimer and Kiss, 2007; Leppänen et al., 2007a; Schupp et al., 2004; Sprengelmeyer and Jentzsch, 2006). The negative shift in ERPs to threat-related relative to neutral facial expressions may arise from enhanced processing of emotionally salient stimuli in perceptual representation areas (Schupp et al., 2004). A similar negative shift is observed for task-relevant target stimuli relative to task-irrelevant distractors in studies using non-emotional material such as colors or geometric shapes (Hillyard and Anllo-Vento, 1998). The onset of the negative shift can, therefore, be used as a marker of the time at which emotionally/motivationally relevant and neutral (or task-irrelevant) stimuli have been discriminated and are subjected to differential processing in cortical visual systems.

An important but little investigated question concerns the stimulus features that underlie the rapid discrimination of fearful and neutral facial expressions. One possibility is that fearful facial expressions are detected on the basis of some relatively simple facial features that are “diagnostic” for this category of facial expressions (cf. Smith et al., 2005).

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doi:10.1016/j.biopsycho.2008.02.002

Facial expressions of fear are characterized by several appearance changes in the face, of which the most important are wide open eyes, furrowed and raised eyebrows, and stretched mouth (Kohler et al., 2004). There are, however, indications that the salience of the eye region in fearful faces may provide the critical diagnostic information that allows fearful facial expressions to be rapidly distinguished from other facial expressions. Most notably, the impairment in the recognition of fearful facial expressions in patients with amygdala lesion appear to be attributable to a failure to utilize information in the eye region (Adolphs et al., 2005). The amygdala, which is thought to play a key role in rapid detection of threat, is activated not only by fearful faces but also by fearful eyes embedded in a neutral face (Morris et al., 2002) and by fearful eyes presented in isolation (Whalen et al., 2004). There is also evidence that early face-related ERPs (N170) are particularly responsive to the eyes (Bentin et al., 1996; but see Eimer, 1998). We are, however, not aware of any studies that would have examined whether the differential processing of fearful and neutral facial expressions in the early stages of cortical processing (i.e., at the level of the N170 component and beyond) is driven by the cues in the eye region.

Fig. 1. Examples of stimuli used in Experiments 1 and 2.

To directly test the hypothesis that the rapid discrimination of fearful and neutral faces is driven by the expressive cues in the eye region, three experiments were conducted to examine ERPs and behavioral reaction times (RTs) to fearful and neutral expressions using face stimuli in which (a) the whole face was visible; (b) only the eye region of the face was visible; and (c) the whole face except the eye region was visible. We hypothesized, first, that if discrimination of fearful and neutral faces is based on the expressive cues in the eye region, then the differential early ERPs to fearful and neutral expressions (i.e., a negative shift in ERPs to fearful expressions ~200–300 ms post-stimulus) should be observed, and fearful and neutral expressions should be behaviorally discriminated at above chance level even when the eye region of the faces is presented in isolation. Second, we hypothesized that covering the eyes impedes the recognition of fearful expressions, resulting in attenuated/abolished early ERP differentiation of fearful and neutral faces, and delayed and less accurate behavioral discrimination of fearful expressions.

1. Experiment 1

In Experiment 1, ERPs to fearful and neutral facial expressions were examined by using a paradigm in which facial expressions were presented as non-target stimuli and observers were not given any specific instructions to explicitly process (i.e., to categorize or label) the expressions. In some studies, ERP differentiation between emotional and neutral faces was observed only when participants were explicitly attending to the emotional content of the facial expressions (e.g., Krolak-Salmon et al., 2001). There are, however, also studies suggesting that reliable effects can also be obtained in a passive task (Batty and Taylor, 2003; Leppänen et al., 2007b).

1.1. Methods

Participants. The participants were 18 volunteers (11 females, age $M = 27$ years, range 18–50 years). Four additional participants were tested but excluded due to excessive artifact and poor signal-to-noise ratio.

Stimuli. The stimuli were color pictures of fearful and neutral facial expressions of two male and two female models from the MacBrain Face Stimulus Set[1] (Tottenham et al., 2002). Fearful and neutral facial expressions with eyes covered were created by drawing "dark glasses" on the original faces (see Fig. 1). This way only the eyes were covered and the stimuli still appeared relatively natural (as opposed, for example, if the eye region had been covered with a black rectangle). The faces subtended approximately $9^\circ \times 12^\circ$ when viewed from a distance of 77 cm. Fearful and neutral expressions with only the eye region of the face depicted were created for each original face. The isolated eyes ("letter box") subtended approximately $7^\circ \times 3^\circ$. Fearful and neutral expressions did not differ in the mean pixel luminance in any of the stimulus conditions ($ps > .25$). Stimulus presentation and timing were controlled by Neuroscan Stim software.

Procedure. Faces with eyes visible, faces with eyes covered, and isolated eyes were presented in three separate blocks, each consisting of a total of 128 face stimuli (64 per emotion category). The ordering of the blocks was balanced between participants. The stimuli were presented for 500 ms followed by a 2000-ms interstimulus interval (ISI). Fearful and neutral expressions were presented in random order. A passive task with no instruction given to the participants to recognize the expressions was used. However, to ensure that the participants attended to the screen throughout the testing session, participants

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[1] Development of the MacBrain Face Stimulus Set was overseen by Nim Tottenham and supported by the John D. and Catherine T. MacArthur Foundation Research Network on Early Experience and Brain Development.

were asked to respond by a button press to additional pictures of a car presented 12 times in each block. Subsequent analyses showed that participants detected 100% of the target stimuli, indicating that all participants were highly attentive to the task.

Acquisition and analysis of ERPs. Continuous EEG was recorded using an electrode cap (Electrocap) with 21 electrodes positioned according to the 10–20 system. The left mastoid served as an on-line reference. Vertical (VEOG) and horizontal (HEOG) electrooculogram was monitored bipolarly from the sites above and below the midpoint of the left eye and beside the outer canthus of each eye. Electrode impedances were below 5 kΩ. The EEG was band-pass filtered from 0.1 to 100 Hz, amplified with a gain of 5000, and stored on a computer disk at the sample rate of 500 Hz (Neuroscan/Synamps). The continuous EEG signal was digitally filtered off-line using a 30 Hz lowpass filter and segmented to 600-ms segments starting 100 ms prior to stimulus presentation. The segments were baseline-corrected against the mean voltage during the 100-ms prestimulus period. Segments with eye movements and blinks were excluded from further analysis by using ± 70 μV thresholds for the HEOG and VEOG channels. The remaining segments were visually scanned for other artifacts. Average waveforms for each individual participant within each experimental condition were calculated. Average waveforms were re-referenced to an average of all recording channels excluding HEOG and VEOG. A limitation of the present study is that the average reference is based on a relatively small number of electrodes (Dien, 1998). The average reference was, however, used because a recent study shows that the average reference provides the best reference for recording of the N170 and for detecting differences between stimulus categories at posterior electrode sites (Joyce and Rossion, 2005).

Statistical analyses of the ERP data were targeted to examine the effects of emotional expressions on the ERPs at occipital-temporal electrode sites. The first set of analyses examined facial expression effects on the P100 and N170 components. The peak amplitude of the P1 was measured by determining the maximum positive peak within a 80–120 ms time window and the peak amplitude of the N170 by determining the minimum amplitude within a 120–200 ms time window. The P100 was analyzed for medial (O1/O2) and the N170 for lateral (T5/T6) electrodes overlaying the left and right occipital-temporal regions. Second, we examined potential amplitude differences in ERPs to fearful and neutral facial expressions beyond the N170 component by quantifying the mean amplitude of the ERP activity for lateral temporal electrodes (T5 and T6) in two consecutive 50-ms time windows starting from the mean latency of the peak of the N170 component (160–210 and 210–260). The selected analysis period and electrodes covered the time interval and the spatial loci in which early posterior negativity for fearful/angry relative to neutral faces have been observed in previous studies (Eimer and Kiss, 2007; Schupp et al., 2004). The amplitude scores were analyzed by repeated measures analyses of variance (ANOVA) with Facial Expression (fearful, neutral), Stimulus Type (whole faces with eyes visible, whole faces with eyes covered, isolated eyes), and Hemisphere (left, right) as within-subject factors. Of particular interest in the present study were the main effects of Facial Expression and potential Facial Expression × Stimulus Type interactions. Although also of potential interest, the main effects of Stimulus Type were not considered in the present analyses because such effects would have been difficult to interpret given the obvious low level differences between different stimulus types.

1.2. Results

ERPs over lateral occipital-temporal scalp to fearful and neutral faces in each stimulus condition are shown in Fig. 2. All facial stimuli elicited a positive deflection (P100) at a mean latency of 101 ± 8 ms followed by a prominent negative deflection (N170) at a mean latency of 153 ± 7 ms.

The peak amplitude of the P100 component did not differ for fearful and neutral expressions. Consistent with previous studies (Bentin et al., 1996), the N170 was larger over the right (-9.2 μV) compared to the left (-6.7 μV) hemisphere, $F(1, 17) = 6.9$, $p < .02$, but there were no significant differences in the peak amplitude of the N170 between fearful and neutral expressions, $p > .10$. A slight negative shift was however, observed for fearful relative to neutral expressions in each stimulus condition starting at the peak of the N170 component and lasting for approximately 100 ms (Fig. 2). A 2 (Facial Expression) × 2 (Stimulus Type) × 2 (Hemisphere) ANOVA yielded a significant main effect of Facial Expression in the 160–210 ms interval, $F(1, 17) = 43.4$, $p < .001$, and no significant interaction between Facial Expression and Stimulus Type or Facial Expression and Hemisphere, $ps > .10$. A significant main effect of Facial Expression was also observed in the 210–260 ms time interval, $F(1, 17) = 7.1$, $p < .02$. Again, there were no significant interactions between Facial Expression and Stimulus Type or between Facial Expression and Hemisphere, $ps > .10$. Separate one-way ANOVAs on the amplitude scores (collapsed across hemispheres and time intervals) confirmed a significant main effect of Facial Expression within each of the three stimulus conditions, $Fs(1, 17) > 4.4$, $ps \leq .05$.

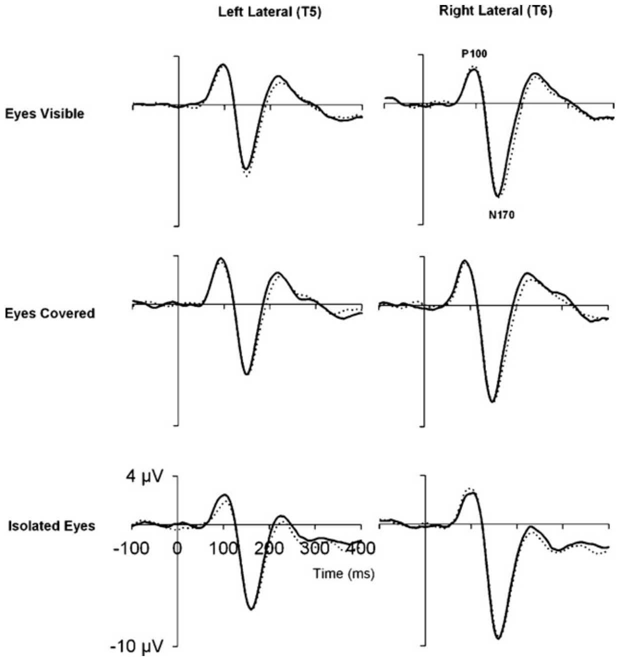


Fig. 2. Grand average ERP waveforms for fearful (dotted line) and neutral (solid line) facial expressions from electrodes overlying the left and right lateral temporal regions in Experiment 1.

1.3. Discussion

The results of Experiment 1 showed no differences in the peak amplitude of the P100 and N170 components between fearful and neutral facial expressions. However, the expected pattern of a negative shift of the ERP amplitude for fearful relative to neutral expressions over lateral temporal scalp was observed 160–260 ms post-stimulus (Eimer et al., 2003; Eimer and Kiss, 2007; Leppänen et al., 2007a; Schupp et al., 2004). Adding to the existing literature, the present results showed that the negative shift in ERPs for fearful expressions was observed not only when full faces were used as stimuli but also when the eye regions of the faces were presented in isolation. This finding gives support for the hypothesis that discrimination of fearful and neutral facial expressions can be achieved on the basis of the cues in the eye region alone (i.e., the eyes and/or the eyebrows). Importantly, however, the present results also showed that the visibility of the eyes per se is not critical since the ERP differentiation was found when the eyes of the stimulus faces were covered.

No differences in the peak amplitude of the N170 for fearful and neutral expressions were observed. The lack of differences in the N170 amplitude is consistent with several earlier studies (Krolak-Salmon et al., 2001; Schupp et al., 2004). However, other recent studies have shown expression-related effects on the amplitude of the N170 component (Batty and Taylor, 2003; Leppänen et al., 2007b; Stekelenburg and de Gelder, 2004). The critical factors underlying the discrepancies in findings across studies are presently not known. A review of the literature suggests that the studies showing differences and the studies showing no differences in the N170 amplitude do not differ systematically in the nature of the perceptual task (passive vs. active). It seems, however, that several of the studies showing differences in the N170 amplitude between fearful and neutral expressions used a high-density electrode array to measure ERPs and an average reference (Batty and Taylor, 2003; Leppänen et al., 2007b; Stekelenburg and de Gelder, 2004), suggesting that high-density recordings may allow for better characterization of early differences. However, in the absence of direct comparisons, this possibility remains speculative.

2. Experiment 2

Experiment 2 examined whether information in the eye region is important for behavioral categorization decisions between fearful and neutral facial expressions. First, we tested the hypothesis that fearful and neutral expressions are discriminated at above chance level on the basis of cues in the eye region alone. Second, we examined whether covering the eyes reduces the accuracy of discriminating fearful and neutral expressions and results in longer discrimination times.

An additional purpose of Experiment 2 was to further examine the somewhat unexpected finding that the ERP differences for fearful and neutral expressions were not attenuated when the eyes were covered. One potential explanation for this result is that the natural bias to attend to the eyes (e.g., Guo et al., 2003) was inhibited by a strategic allocation of attention to diagnostic features outside the eye region in the Eyes Covered condition (e.g., cues in the mouth region). The use of such a strategy was possibly facilitated by the fact that faces with eyes visible and faces with eyes covered were presented in separate blocks, giving observers advance knowledge about the visibility of the eyes. To test this possibility, in Experiment 2, we presented the stimuli in two different ways: in separate blocks (as in Experiment 1) and intermingled within one block. We predicted that the intermingled condition would be perceptually more demanding than the blocked condition because observers have no advance information about the cues that will be available on each trial. It is possible that, when the observer is not able to predict the location of the diagnostic information, the default tendency to attend to the eye region (Guo et al., 2003) is more apparent and, consequently, the visibility of the eyes would have a larger contribution on discrimination performance.

2.1. Methods

Participants. Twelve volunteers (10 females, age $M = 27$ years, range 19–46 years) participated in Experiment 2.

Stimuli and procedure. The stimuli were the same as those used in Experiment 1. Stimulus presentation, timing, and response registration were controlled by E-Prime program and E-Prime button box panel. As in Experiment 1, the stimuli were presented in the center of a computer screen with a 500-ms stimulus presentation time and 2000-ms ISI. Fearful and neutral expressions were presented in random order. Participants were asked to identify whether the expressions presented on the screen was fearful or non-fearful, and to press a correspondingly marked response key in the button box. The left/right position of the response key for fearful and non-fearful faces was counterbalanced between the participants. Both hands were used to respond so that the index finger of the left hand was used to press the left-hand key and the index finger of the right hand the right-hand response key. Prior to the actual testing, 12 practice runs were performed. Thereafter, two series of 96 test trials were run (16 trials/stimulus condition). In one series, full faces, full faces with eyes covered, and isolated eyes were presented in separate blocks separated by brief breaks (32 stimuli/block). In another series, the different stimulus types were presented in an intermingled fashion without breaks. The ordering of the two series was balanced between subjects.

Data analysis. Behavioral data were analyzed by calculating the percentages of correct responses and mean reaction times (RTs) for fearful and neutral faces in each stimulus condition. Mean RTs were calculated for correct responses after RTs ± 2 S.D. below/above each individual participant's mean RT were removed (an average of 3.6% of the trials).

2.2. Results

Percentages of correct responses and RTs to fearful and neutral facial expressions in each stimulus condition are shown in Table 1. A 2 (Facial Expression) × 3 (Stimulus Type) × 2 (Condition: blocked, intermingled) ANOVA on percentages of correct responses yielded a significant main effect of Stimulus Type, $F(2, 22) = 10.1$, $p < .002$, and a trend toward Stimulus Type × Facial Expression interaction, $F(2, 22) = 3.2$, $p = .06$. Separate analyses of fearful and neutral expressions showed that, for fearful expressions, the accuracy scores were highest in the Eyes Visible condition (97%, collapsed across presentation conditions). The accuracy scores were marginally lower in the Eyes Covered condition (92%), $p = .08$, and significantly lower in the Isolated Eyes condition (86%), $F(1, 11) = 12.0$, $p < .005$. For neutral faces, the accuracy scores in the Eyes Visible condition (97%) were slightly lower than those obtained in the Eyes Covered condition (98%), $F(1, 11) = 6.6$, $p < .03$. Again, the accuracy scores in the Isolated Eyes condition (90%) differed significantly from those in the Eyes Visible condition, $F(1, 11) = 9.7$, $p < .02$. There was no effect of Condition (blocked/intermingled) on the percentages of correct responses nor any interactions involving factor Condition.

Table 1
Mean percentages of correct responses and reaction times (RTs) for fearful and neutral facial expressions in different experimental conditions in Experiment 2

Stimulus type	Facial expression			
	Fearful		Neutral	
	%Correct	RT	%Correct	RT
Blocked presentation				
Eyes visible	96.4 (7.3)	583 (163)	95.3 (3.9)	580 (139)
Eyes covered	89.1 (15.1)	550 (139)	99.0 (2.4)	553 (91)
Isolated eyes	84.9 (15.2)	600 (100)	91.1 (10.5	625 (115)
Intermingled presentation				
Eyes visible	97.4 (5.6)	609 (176)	98.4 (2.8)	612 (141)
Eyes covered	94.8 (7.5)	645 (205)	97.9 (4.1)	621 (125)
Isolated eyes	87.0 (14.5)	670 (135)	89.1 (11.0)	695 (154)

Standard deviations are shown in parentheses.

A 2 × 3 × 2 ANOVA on RT data showed a significant main effect of Condition, $F(1, 11) = 6.9$, $p < .03$, reflecting slower overall RTs in the intermingled (642 ms) compared to the blocked (582 ms) condition. There was also a significant main effect of Stimulus Type, $F(2, 22) = 17.9$, $p < .001$, and a significant Stimulus Type × Condition interaction, $F(2, 22) = 5.6$, $p < .02$. The main effect of Facial Expression was not significant nor was there any interaction between Facial Expression and other factors, $ps > .10$. Separate analyses of RTs in the blocked and intermingled condition (collapsed across facial expressions) showed that in the blocked condition, RTs did not differ in the Eyes Visible (582 ms) and Eyes Covered (551 ms) conditions, but RTs in the Isolated Eyes condition were significantly slower than RTs in the Eyes Covered condition, $F(1, 11) = 25.6$, $p < .001$. In the intermingled condition, however, RTs were significantly faster in the Eyes Visible (611 ms) compared to the Eyes Covered (633 ms) condition, $F(1, 11) = 7.3$, $p < .03$, and Isolated Eyes (683 ms) condition, $F(1, 11) = 39.5$, $p < .001$. This pattern was seen for both fearful and neutral expressions (Table 1).

2.3. Discussion

The results of Experiment 2 showed that fearful and neutral expressions were categorized well above chance level (50%) even when just the eye region of the expressions was presented, supporting the hypothesis that the eye region provides sufficient information for classifying faces as fearful vs. non-fearful. The results also showed, however, that categorization decisions were less accurate and slower when the eye region alone was presented compared to stimuli showing the whole face. This indicates that information in the eyes alone is not used for categorization decisions and that the additional cues present in whole face expressions permit faster and more accurate categorization performance. The results of Experiment 2 further indicated that the speed of categorization decisions was not affected by the visibility of the eyes when faces with eyes visible and faces with eyes covered were presented in separate blocks. However, in the intermingled condition, a small but significant speed advantage for faces with eyes visible compared to faces with eyes covered was observed. This result is consistent with the hypothesis that the contribution of the eyes is more apparent when observers have no advance information about the visibility of the eyes and the task context does not encourage attention away from the eye region.

3. Experiment 3

Experiment 3 was conducted to replicate the findings of Experiments 1 and 2 and to further examine the importance of the eye region for the ERP and behavioral discrimination of fearful and non-fearful facial expressions. Experiment 3 also incorporated several amendments to the stimuli and experimental design. First and most importantly, the isolated eye stimuli in Experiments 1 and 2 included eyebrows in addition to the eyes, making it difficult to determine whether the findings in these experiments reflected a use of visual information in the eyes and eyebrows or in the eyes alone. To address this question, Experiment 3 included stimuli depicting the eyes and eyebrows and stimuli showing the eyes alone. Likewise, two types of faces with eyes covered were created, faces with the eyes and eyebrows covered and faces with the eyes alone covered (as in Experiments 1 and 2). Second, an intermingled rather than blocked presentation of different types of stimuli was used to increase the likelihood of detecting condition differences in ERPs and behavioral performance. For the same reason, an active emotion processing task in which observers were asked to categorize facial expressions as fearful and non-fearful was used. Third, the number of face models in the stimulus set was increased to extend the generalizability of the findings.

3.1. Methods

Participants. Twelve female volunteers participated in Experiment 3 (age $M = 20$ years, range 18–22 years).

Stimuli. The stimuli were color pictures of fearful and neutral facial expressions of five male and five female models from the MacBrain Face Stimulus Set (see footnote 1) (Tottenham et al., 2002). Two different sizes of "dark glasses" were drawn and superimposed on faces to create whole faces with eyes and eyebrows covered and whole faces with eyes alone covered. Also, two types of "letter box" images were created, one showing the eyes and eyebrows and the other showing the eyes only. Fig. 3 shows examples of the stimuli. The stimuli were framed by a black frame, which subtended approximately 5.7° × 6.4° when viewed from a distance of 77 cm. The stimuli were presented against light gray background and the size of the black frame was the same in each condition. Stimulus presentation and timing were controlled by Neuroscan Stim2 software.

Procedure. The stimuli were presented for 500 ms followed by a question mark, which remained on the screen until the participant's response. Participants were asked to identify whether the expressions presented on the screen was fearful or non-fearful, and to press a correspondingly marked response key when the question mark was presented on the screen. The left/right position of the response key for fearful and non-fearful faces was counterbalanced between the participants. Following the response, there was a 1000-ms interstimulus interval (ISI) before the next trial started. A total of 500 trials were run (50 trials per condition) with short breaks after every 100 trials. Fearful and neutral expressions and different stimulus types were presented in random order.

Acquisition and analysis of ERPs. Continuous EEG and HEOG/VEOG were recorded using the same recording settings as those used in Experiment 1. The nosetip served as an on-line reference. Off-line, the continuous EEG signal was corrected for blink artifact using a regression-based blink reduction algorithm (Semlitsch et al., 1986). Epochs with eye movements other than blinks and other visible artifacts were excluded on the basis of visual inspection and by using a threshold of ±50 μV for the HEOG channel ($M = 4.1\%$ of trials excluded, S.D. = 3.3%). Filtered, segmented, and baseline-corrected (see Experiment 1) epochs were averaged within each experimental condition. To avoid differences in trial counts between conditions, both correct and incorrect responses were included in the ERPs. Average waveforms were re-referenced to an average of all recording channels excluding HEOG and VEOG. Statistical analyses of the ERP data were focused on facial expression effects on the amplitude of the P100 (80–120 ms post-stimulus) and N170 (120–200 ms post-stimulus) components,

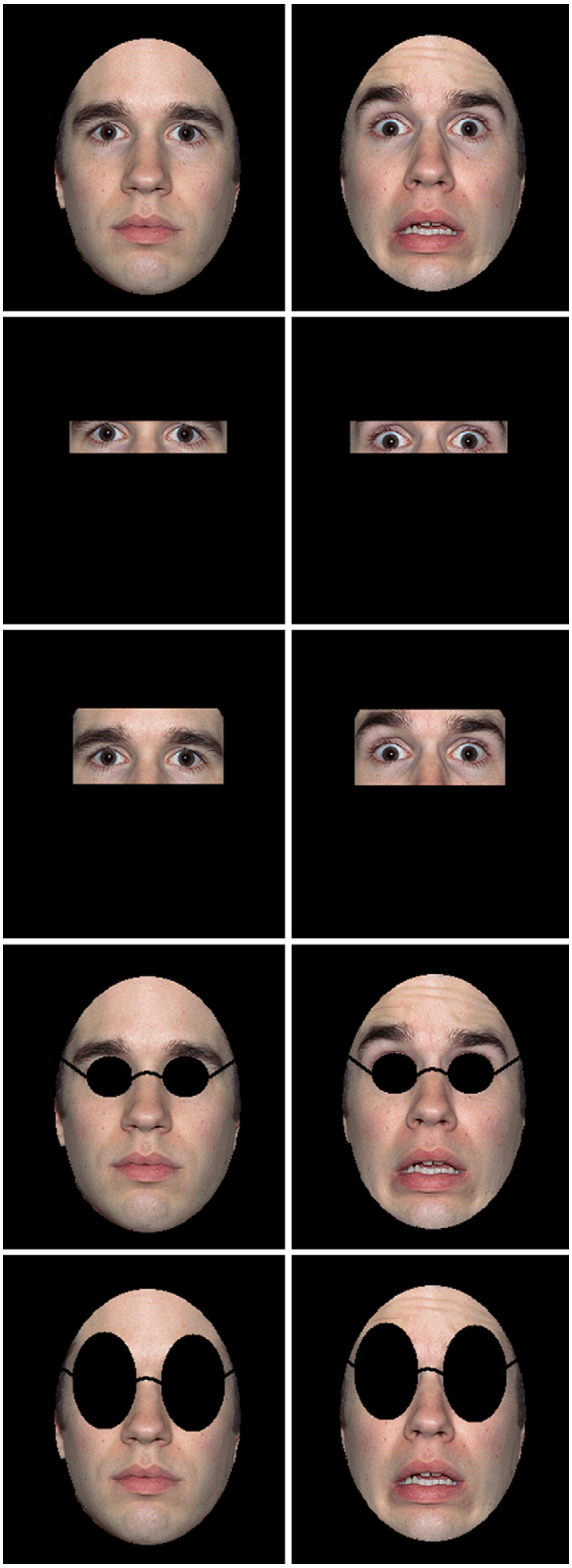


Fig. 3. Examples of stimuli used in Experiment 3.

Table 2
Mean percentages of correct responses for fearful and neutral facial expressions in different experimental conditions in Experiment 3

Stimulus type	Facial expression	
	Fearful	Neutral
Whole faces eyes visible	89.9 (7.9)	97.5 (5.9)
Isolated eyes and eyebrows	86.9 (13.2)	92.9 (9.8)
Isolated eyes	86.0 (16.3)	94.0 (7.7)
Eyes covered	71.3 (20.0)	98.5 (3.1)
Eyes and eyebrows covered	73.2 (19.3)	99.5 (1.3)

Standard deviations are shown in parentheses.

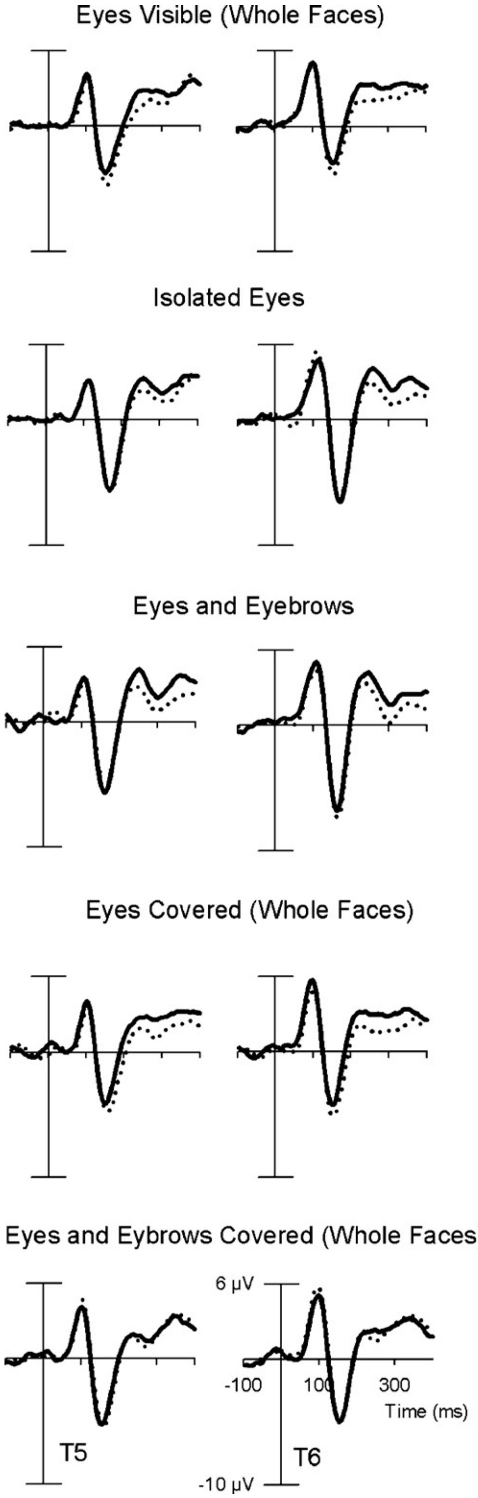
and on the amplitude differences in ERPs to fearful and neutral facial expressions in two consecutive 50-ms time windows starting from the mean latency of the peak of the N170 component (160–210 and 210–260 ms post-stimulus). The amplitude scores were analyzed by repeated measures analyses of variance (ANOVA) with Facial Expression (fearful, neutral), Stimulus Type (whole faces with eyes visible, whole faces with eyes covered, whole faces with eyes and eyebrows covered, isolated eyes, and isolated eyes and eyebrows), and Hemisphere (left, right) as within-subject factors. When necessary, the degrees of freedom were adjusted by Greenhouse-Geisser epsilon (for clarity, uncorrected d.fs. are reported).

3.2. Results

Behavioral results. Behavioral data of one subject was not included in the analysis due to a technical problem in data storing. For the remaining participants, the percentages of correct response for fearful and neutral faces in different stimulus conditions are shown in Table 2. A 2 (Facial Expression) × 5 (Stimulus Type) repeated-measures ANOVA yielded a significant main effect of Facial Expression, $F(1, 10) = 14.8$, $p < .005$, and Facial Expression × Stimulus Type interaction, $F(4, 40) = 7.2$, $p < .02$. Further analyses indicated that for fearful expressions, the percentages of correct responses were highest in the Whole Faces with Eyes Visible condition (90%). The percentages of correct responses were slightly lower in the Isolated Eyes and Eyebrows (87%) and Isolated Eyes (86%) conditions but the difference was not significant, $p > .10$. However, in the Whole Faces with Eye Covered condition (71%) and Whole Faces with Eyes and Eyebrows Covered condition (73%), the percentages of correct responses were significantly lower, *p*s $< .04$ (Bonferroni corrected). For neutral expression, no significant differences between stimulus types were found, *p*s $> .10$.

ERPs. Fig. 4 shows ERPs to fearful and neutral expressions over lateral occipitotemporal sites in each stimulus condition. An early positive deflection (P100) was observed for all stimuli, followed by the N170 component at the mean latency of 161 ± 9 ms. The peak amplitude of the P100 component did not differ for fearful and neutral facial expressions, but there was a significant main effect of Facial Expression, $F(1, 11) = 11.7$, $p < .01$, and a significant Facial Expression × Stimulus Type interaction, $F(4, 44) = 3.0$, $p < .05$, on the peak amplitude of the N170 component. The N170 was larger for fearful than neutral expressions in the whole face condition when the eyes were visible, ($M = -5.7$ μV for fearful and $M = -4.9$ μV for neutral expressions $F(1, 11) = 8.0$, $p < .02$) and when the eyes were covered ($M = -6.7$ μV for fearful and $M = -5.7$ μV for neutral expressions, $F(1, 11) = 13.3$, $p < .01$). The amplitude of the N170 did not differ for fearful and neutral expressions in the other three stimulus conditions, *p*s $> .10$.

Analysis of the ERP amplitudes beyond the N170 component revealed a significant main effect of Facial Expression, $F(4, 44) = 23.0$, $p < .01$, and a significant Facial Expression × Stimulus Type interaction in the 160- to 210-ms interval, $F(4, 44) = 3.1$, $p < .05$. A significant main effect of Facial Expression was also observed in the 210–260 ms time interval, $F(1, 11) = 18.2$, $p < .01$. The Facial Expression × Stimulus Type interaction was not significant in the 210- to 260-ms interval, $F(4, 44) = 1.4$. Given the significant Facial Expression × Stimulus Type interaction in the 160- to 210 interval and the predicted differences between stimulus conditions, ERPs to fearful and neutral facial expressions were analyzed separately within each stimulus condition. These analyses revealed that the negative shift in ERP activity for fearful relative to neutral facial expression was significant in the Whole Faces with Eyes Visible condition from 160 to 260 ms, $Fs(1, 11) > 10.2$, *p*s $< .01$, in the Eyes Covered condition from 160 to 260 ms, $Fs(1, 11) > 18.7$, *p*s $< .01$, in the Isolated Eyes and Eyebrows condition from 210 to 260 ms, $F(1, 11) = 4.8$, $p \leq .05$, and in the Isolated Eyes condition from 210 to 260 ms post-stimulus, $F(1, 11) = 8.2$, $p < .05$. In contrast, there was no evidence for a significant negative shift for


Eyes Visible (Whole Faces)

Isolated Eyes

Eyes and Eyebrows

Eyes Covered (Whole Faces)

Eyes and Eybrows Covered (Whole Faces)

Fig. 4. Grand average ERP waveforms for fearful (dotted line) and neutral (solid line) facial expressions from electrodes overlying the left and right lateral temporal regions in Experiment 3.

fearful relative to neutral expressions in the Eyes and Eyebrows Covered condition, all *p*s > .50.

3.3. Discussion

The results of Experiment 3 showed a negative shift in ERPs to fearful relative to neutral expressions over lateral temporal electrode sites starting approximately 160 ms after stimulus onset. Consistent with the results of Experiment 1, the negative shift was observed not only for the whole face expressions (eyes visible) but also when the eyes and eyebrows of the original faces were shown in isolation or when the eyes of the original faces were covered. The results of Experiment 3 further showed that information in the eyes per se was sufficient to elicit the ERP differentiation of fearful and neutral expressions as the negative shift was observed even when the eyes alone were presented. It is noteworthy, however, that the eyes were again not critical. The negative shift was observed when the eyes were covered. The visibility of the eye region (eyes and eyebrows), instead, appeared to be more critical as no differences in ERPs to fearful and neutral expressions were observed when the eyes and eyebrows were covered.

Two additional aspects of the ERP results of Experiment 3 are important to note. First, the results showed that the negative shift for fear became significant ~160 ms post-stimulus in the whole face conditions (irrespective of the visibility of the eyes) whereas differences were observed only at ~210 ms post-stimulus when the eyes and eyebrows or the eyes of the original faces were presented in isolation. This finding echoes the behavioral findings of Experiment 2 showing faster categorization of fearful and neutral expressions from the whole face expressions as compared to isolated eyes and eyebrows. It is plausible that the whole faces convey additional information that is used in the discrimination of fearful and neutral expressions (e.g., stretched mouth in fearful expressions).

A second additional aspect of the results of Experiment 3 concerns the slightly earlier onset of the ERP differentiation between fearful and neutral expressions than that observed in Experiment 1. In Experiment 1, the N170 component did not differentiate between fearful and neutral expressions. Experiment 3, in turn, showed larger N170 for fearful than neutral whole face expressions. This finding is consistent with some previous studies (Batty and Taylor, 2003; Leppänen et al., 2007b; Pourtois et al., 2005; Stekelenburg and de Gelder, 2004). It is possible that the earlier onset of the ERP differentiation in Experiment 3 is explained by the changes made to the experimental design and task (i.e., intermingled presentation of different stimulus types, active emotion categorization task, and a larger stimulus set).

The behavioral results of Experiment 3 showed that fearful and neutral facial expressions were categorized at a relatively high level of accuracy when the eyes were presented in isolation. Conversely, covering the eyes reduced categorization accuracy. These findings are consistent with the hypothesis that information in the eyes is coded and used in facial expression categorization. In Experiment 3, the accuracy of categorization of fearful and neutral expressions did not differ significantly in the Whole Faces with Eyes Visible condition and Isolated Eyes and Eyebrows condition. In Experiment 2, the corresponding difference was significant. There is no ready explanation for this discrepancy in the findings of the two experiments. It is of note, however, that in both experiments, the mean percentage of correct responses was lower in the Isolated Eyes and Eyebrows condition. The inclusion of a larger number of face models in Experiment 3 may partly explain why the difference did not become significant in this experiment (i.e., categorization performance was possibly not as much affected by idiosyncratic features of some models in the stimulus set).

4. General discussion

In line with previous ERP studies, the present results showed a negative shift in ERPs for fearful relative to neutral facial expressions over occipital-temporal scalp region (Eimer et al., 2003; Eimer and Kiss, 2007; Leppänen et al., 2007a; Schupp et al., 2004; Williams et al., 2006). This negative shift started at the latency of the N170 component (Experiment 3) or slightly later (Experiment 1). The posterior ERP negativity is thought to reflect selective processing of stimuli that have been tagged as motivationally significant (or task-relevant) and are subjected to enhanced processing in perceptual representation areas (Schupp et al., 2004). Although speculative, it is possible that the amygdala plays an important role in the early ERP differentiation between emotionally salient and neutral facial expressions. Scalp-recorded ERPs cannot reflect amygdala activity directly because of the closed-field organization of neurons in the amygdala and the deep position of the amygdala with respect to scalp surface (Eimer and Holmes, 2007). It is known, however, that the amygdala responds differentially to fearful and neutral facial expressions (Morris et al., 2002; Whalen et al., 2004). The amygdala also exerts a modulatory influence on face-sensitive areas in the occipital-temporal cortex (Amaral et al., 2003; Vuilleumier, 2005), including those areas in the occipitotemporal cortex that are thought to generate the early face-sensitive ERPs at scalp surface.

Fearful facial expressions are distinguished from most other facial expressions by salient changes in the eye region, including wide-open eyes and raised eyebrows (Kohler et al., 2004). The present results supported the hypothesis that these cues in the eye region are important for the early ERP differentiation between fearful and non-fearful facial expressions. Specifically, the ERP differentiation and relatively accurate behavioral discrimination of fearful and neutral expressions were observed not only when the whole face was presented but also when only the eye region of the faces was visible. Conversely, the ERP differentiation was abolished and behavioral discrimination accuracy reduced when the eyes and eyebrows were covered by dark glasses. These results point to the importance of the eye region in the processing of fearful expressions and they are consistent with a recent study which used a combination of a technique called ‘‘bubbles’’ and ERPs to show that the eye region provides the most useful diagnostic information for the recognition of fearful facial expressions, and that information in the eye region is coded earlier (i.e., it modulates ERPs earlier) than information in other facial features (Schyns et al., 2007).

We also examined the hypothesis that within the eye region, the eyes are especially important for the rapid ERP differentiation to occur. The size of the white sclera exposed above and on sides of the dark iris and pupil is larger in fearful than in most other facial expressions. Given that the amygdala responds differentially to fearful and happy eye whites (Whalen et al., 2004), it is possible that the eyes are also important in the rapid ERP discrimination between fearful and non-fearful facial expressions. Consistent with this view, the present results showed that fearful and neutral expressions were behaviorally discriminated at above chance level and ERP negativity for fearful relative to neutral expressions was observed when the eyes alone were presented. The eyes were not critical, however, because clear ERP differences were seen in response to whole face expressions when the eyes were covered. This finding suggests that, in addition to information in the eyes per se, the eye region contains other expressive cues that permit rapid ERP differentiation of fearful and neutral facial expressions. These cues may involve the shape of the eyebrows and spatial-relational information pertaining to the spatial relations and distance between the eyes and the eyebrows.

Although fearful and neutral facial expressions were discriminated when the eye region of the faces alone was visible, the present results also showed that the ERP differentiation occurred at a shorter latency when the whole face instead of the eye region alone was visible. Behavioral discrimination performance was also faster and more accurate when the whole face was visible. These findings suggest that, although the eye region provides sufficient information for classifying faces as fearful vs. non-fearful, whole face expressions provide additional diagnostic cues that permit faster and more accurate discrimination performance (e.g., cues in the mouth region). The fact that fearful and neutral expressions were discriminated at above chance level when the whole eye region was covered (Experiment 3) is also consistent with this idea.

In conclusion, the present results suggest that the eye region is critical for the rapid ERP differentiation between fearful and neutral facial expressions. The important cues in the eye region may relate to the shape of the eyebrows, spatial relations between the eyebrows, and information in the eyes (size of the white sclera above and on sides of the dark iris and pupil). The results also showed that, although information in the eye region provides sufficient diagnostic information for ERP and behavioral discrimination between fearful and neutral facial expressions, the discrimination is both faster and more accurate when the whole facial expressions is visible. Together these findings point to flexibility in the way neural systems underlying facial expression processing use the available facial information. That is, rather than responding to the cues in the eye region in a fixed fashion, the neural systems underlying categorization of facial expressions may respond and utilize information from multiple sources to ensure fast and accurate categorization performance.

Acknowledgements

This study was supported by grants from Academy of Finland (Projects # 115536 and 1111850) and Finnish Cultural Foundation.

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