Who is afraid of the invisible snake? Subjective visual awareness modulates posterior brain activity for evolutionarily threatening stimuli

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ABSTRACT

Snakes were probably one of the earliest predators of primates, and snake images produce specific behavioral and electrophysiological reactions in humans. Pictures of snakes evoke enhanced activity over the occipital cortex, indexed by the "early posterior negativity" (EPN), as compared with pictures of other dangerous or non-dangerous animals. The present study investigated the possibility that the response to snake images is independent from visual awareness. The observers watched images of threatening and non-threatening animals presented in random order during rapid serial visual presentation. Four different masking conditions were used to manipulate awareness of the images. Electrophysiological results showed that the EPN was larger for snake images than for the other images employed in the unmasked condition. However, the difference disappeared when awareness of the stimuli decreased. Behavioral results on the effects of awareness did not show any advantage for snake images.

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1. Introduction

Snakes were one of the earliest predators of primates, and snake images appear to automatically draw more visual attention than other dangerous animals. Snakes have been living together with primates and their ancestors since prehistoric eras. According to the snake detection hypothesis (Isbell, 2006, 2009), the predatory pressure of these reptiles on primates may have caused evolutionarily driven changes in the primate visual system, which allows quick detection of these predators, thus increasing the chances of survival. The literature on the topic confirms that humans detect snake images faster than other animal stimuli (see Öhman, Flykt, & Esteves, 2001; Öhman & Mineka, 2001). From an evolutionary perspective, a fear module specifically activated by stimuli of a crucial importance for survival would be highly adaptive.

The amygdala may be the key structure dedicated to processing threatening stimuli (Mineka & Öhman, 2002). In macaques, pulvinar neurons respond faster and stronger to snake stimuli compared to other threatening or neutral images (Van Le et al., 2013). Pulvinar neurons may be part of a fast visual pathway that connects the retina and the superior colliculus to the amygdala. This pathway may enable rapid automatic visual detection of fear-related stimuli (Morris, Öhman, & Dolan, 1999; Tamietto & de Gelder, 2010).

Recently, several studies have provided electrophysiological support for the snake detection hypothesis in humans (He, Kubo, & Kawai, 2014; Van Le et al., 2013; Van Strien, Eijlers, Franken, & Huijding, 2014; Van Strien, Franken, & Huijding, 2014; Van Strien, Christiaans, Franken & Huijding, 2016). In four studies, Van Strien and colleagues examined the snake detection hypothesis in humans using event-related potentials (ERPs). The first study (Van Strien, Franken, & Huijding, 2014) showed an ERP component peaking at 225–300 ms after stimulus onset over the occipital scalp area (Early Posterior Negativity, EPN). The EPN amplitude was larger for snake pictures, intermediate for spider pictures and significantly lower for bird pictures. In a second study, Van Strien, Eijlers et al. (2014) and Van Strien, Franken et al. (2014) demonstrated that the EPN effect is not evoked by reptiles in general and that it is not directly related to the feeling of disgust or fear. Pictures of snakes elicited larger EPN compared to pictures of slugs and other dangerous and non-dangerous reptiles. The third study compared the ERPs produced by snake pictures with those produced by worm

pictures and demonstrated that the curvilinear shape of the snakes is not directly responsible for the enhanced EPN (Van Strien et al., 2016). Finally, a study using gray-scaled images showed no difference between spiders and other animal stimuli except snakes (He et al., 2014), contradicting previous findings (Van Strien, Eijlers et al., 2014; Van Strien, Franken et al., 2014). Furthermore, the results of He et al. (2014) showed a smaller snake advantage, as compared with the other studies.

Altogether, the results suggest that ancestral priorities may modulate early capture of visual attention. EPN has been suggested to reflect the early selective visual processing of emotional information that is not altered by habituation to the stimuli (Schupp, Flaisch, Stockburger, & Junghofer, 2006). Furthermore, EPN is modulated by motivational systems of approach and avoidance and it is sensitive to evolutionarily crucial stimuli (Schupp, Junghofer, Weike, & Hamm, 2003). A difference in the modulation of the EPN by snakes and spiders (He et al., 2014; Van Strien, Eijlers et al., 2014; Van Strien, Franken et al., 2014) is in line with the idea that snake detection is to a large degree driven by bottom-up flow of information, while the detection of other dangerous stimuli (e.g. spiders) depends on top-down processes (Soares et al., 2014; Kawai & Koda, 2016).

Van Strien et al. (e.g. Van Strien, Eijlers et al., 2014; Van Strien, Franken et al., 2014) reported that the EPN effect is modulated by ancestral priorities rather than consciously reported fear or arousal. However, these studies refer to consciousness as emotional awareness (conscious feelings) or conscious top-down processing (cognitive control) and not to subjective visual perception. On the basis of the argument that the snake detection advantage is automatic, evolutionary in origin and based on ancestral priorities, we found it reasonable to test whether or not this effect could be produced by unconscious visual processing.

There are currently no published studies that have investigated whether or not unaware images (e.g. of snakes) can produce the enhanced EPN deflection. However, previous studies have shown that masked snake images produce physiological effects (electrodermal responses) in phobic participants even under backward masking conditions that make the stimuli less visible (Öhman & Soares, 1993, 1994). Snake images seem to be more likely to be detected compared to other animals in challenging attentional conditions (Soares, Lindström, Esteves & Öhman, 2014). Furthermore, LoBue (2014) found an attentional bias toward dangerous stimuli due to perceptual, cognitive and emotional effects. Especially related to the detection of snakes, curvilinear shapes were easier to recognize compared to straight lines. Threatening words such as "snake" also produced faster responses when compared to non-threatening words.

On the other hand, even if the EPN represents an evolutionarily-driven electrophysiological reaction, the EPN itself might not be independent from subjective visual perception of the stimuli. For example, the EPN (225–300 ms) is a mid-latency evoked potential (Foxe & Simpson, 2002) and unconscious processing of emotional stimuli has been indexed in earlier components than EPN, over occito-temporal areas (see e.g. Pegna, Landis, & Khateb, 2008; Smith, 2012). However, the debate about the possibility of unconscious processing of emotional images is still ongoing (see the recent meta-analysis by Hedger, Gray, Garner, & Adams, 2016).

Furthermore, the EPN temporally and spatially overlaps with a well-known electrophysiological marker of subjective visual awareness called the Visual Awareness Negativity (VAN) (see Koivisto & Revonsuo, 2010; Koivisto et al., 2008; Railo, Koivisto & Revonsuo, 2011; Koivisto & Grassini, 2016; Koivisto, Salminen-Vaparanta, Grassini, & Revonsuo, 2016), which suggests that the EPN might reflect aware perception.

Finally, there are some methodological problems with the studies done so far. The previous EPN studies, with the exception of the study of He et al. (2014), face the limitation that the images were not equalized for contrast and luminance, making it possible that low level visual features associated with snake images may explain the effects. However, He et al. (2014) used gray scaled images, while colors might be important for the detection of threatening stimuli.

In the present study we strove to improve the current knowledge on the topic by studying (1) whether or not previous ERP results can be replicated using colored images, equalized for contrast level and luminance, and (2) whether or not conscious perception of the stimuli is crucial for the snake images to evoke the EPN effect. Previous studies describe the EPN effect of snake images as evolutionarily driven and "automatic" (see Van Strien, Eijlers et al., 2014; Van Strien, Franken et al., 2014), implicitly suggesting that such an effect might not require subjective visual awareness of the stimulus. The present research attempts to address this problem in a comprehensive way.

2. Methods

2.1. Participants

Participants were 27 university students (4 men, 23 women) with normal or corrected-to-normal vision. All participants were right-handed (Oldfield, 1971). Their age ranged from 19 to 29 years, with a mean age of 23.41 years (SD = 2.49). They participated in order to obtain course credits in introductory psychology at the University of Turku. The study was conducted with the understanding and written consent of each participant, in accordance with the Declaration of Helsinki, and was accepted by the Ethics Committee of The Hospital District of Southwest Finland.

2.2. Questionnaires

Before the experimental session, the participants were asked to rate their fear of snakes, spiders, birds and butterflies on questionnaires that were adaptations of the Spider Phobia Questionnaire (SPQ; Klorman, Weerts, Hastings, Melamed, & Lang, 1974; Muris & Merckelbach, 1996; Muris, Merckelbach, Ollendick, King, & Bogie, 2002), translated in Finnish. Each questionnaire contained 15 statement regarding fear of the animals displayed in the images that were employed in the study. For every statement, the participant had to rate 'yes' or 'no', and the score on each animal questionnaire could range from 0 (no fear) to 15 (very high fear). Furthermore, for every animal category, the participants were asked to indicate their familiarity with the animal by asking them how often they had seen the animal. This was done in order to examine possible effects of habituation. The score ranged from 0 (very rarely) to 10 (very often).

Finally, after the experiment, the participants were asked to rate from 0 to 10 the arousal provoked by every image presented during the experimental session.

2.3. Stimuli

Stimuli were presented using E-prime 2.0 software on a 19-in. CRT monitor with 1024 × 768 pixel resolution and 85 Hz screen refresh rate (1 refresh ≈ 12 ms). The stimuli were snake, spider, bird or butterfly images of the size of 600 × 450 pixels (Fig. 1), 9.8 × 7.4 cm from 1.5 m. 30 images per animal type were selected from free-to-use stock images on the internet. Snake, spider and bird images were employed in previous studies (see e.g. Van Strien, Eijlers et al., 2014; Van Strien, Franken et al., 2014), and were included in the present study to facilitate comparison with earlier studies. Butterfly images were included as non-threatening stimuli that feature colorful patterns that may resemble those of snakes. Using butterfly images we controlled for the possibility that

Fig. 1. Examples of images used as stimuli. From top left: snakes and spiders (threatening stimuli), butterflies and birds (unthreatening stimuli). These images were equalized for contrast and luminance using histogram equalization.

the color pattern alone would have been somehow responsible for the EPN. The luminance histogram of the images were equalized in Matlab (The MathWorks, Natick, MA) using the SHINE toolbox (Willenbockel et al., 2010). We did not equate the spatial frequency content of the stimuli, because arguably this information is crucial for the fast categorization of the stimulus (e.g. Tamietto & De Gelder, 2010). After luminance equalization, 20 images per category were selected, which allowed us to exclude those images that were spoiled in colors after the equalization procedure. Of these 20 images per category, 4 per category were selected to serve as stimuli in the assessment of awareness procedures, while the remaining 16 were included in the experimental task. Three different stimuli, created by mixing random parts of the experimental stimuli, were used as backward masks.

2.4. Assessment of awareness

Every experimental session started and ended with a block of trials assessing awareness. The assessment of awareness tasks (Fig. 2) investigated the subjective awareness of the stimuli under the 4 different levels of masking conditions which were employed in the present study. In masked conditions, the stimuli were presented for about 12 (1 frame), 24 (2 frames), 36 (3 frames) or 48 ms (4 frames). These stimuli were followed by a backward mask for about 282, 271, 259 or 247 ms. Hence, the combined duration of the stimulus and mask was always 25 frames.

In the assessment of awareness blocks, the participants were asked to make a forced-choice decision concerning which animal was presented (a snake, a spider, a butterfly, or a bird), pressing one out of four buttons on a response pad. They were then asked to rate their subjective awareness of the stimulus according to a modified perceptual awareness scale (PAS) (Ramsøy & Overgaard, 2004): 0 = I saw nothing, 1 = I saw something – full guess about the stimulus type, 2 = I saw weakly – good guess about the stimulus type, 3 = I saw the stimulus clearly.

In total, 136 stimuli were presented in the two blocks: 32 per animal category, plus 8 catch trials (2 frames of gray screen without an image and 23 frames of mask).

2.5. Experimental procedure

The EEG experiment consisted of 6 blocks (Fig. 3). In each block, the participants were asked to watch the continuous RSVP (rapid serial visual presentation) of 300 pictures of snakes, 300 pictures of spiders, 300 pictures of birds and 300 pictures of butterflies, presented randomly. Of these 300 pictures for each category, 60 were presented for about 300 ms (unmasked condition), and 60 in each four masking conditions.

In order to keep participants' attention on the stimuli, a word describing one of the animals (snake, spider, bird or butterfly) was shown randomly on the screen during the block. Participants were instructed to report the congruence or the incongruence of the word with the last image they saw on the screen using two buttons on the response pad. The last image before the word was always an unmasked image, and in total the words appeared 64 times in each block. The participants had 1 s to press the button. The results in this task confirmed that the participants were focusing on the RSVP (81% correct, SD = 10). After the response, the RSVP continued. ERPs in response to the images which were followed by words were not analyzed.

Each participant performed the task for 6 experimental blocks, for a total of 360 stimuli per condition (4 animal categories × 5 stimuli presentation durations). Following this, the participants completed a computerized questionnaire regarding arousal ratings of all images on a 0 to 10-point scale.

2.6. EEG

EEG was recorded with Ag/AgCl sintered ring electrodes connected to a recording cap (EASYCAP GmbH, Germany) using the international 10/20 system sites Fp1, Fp2, F3, F4, F7, F8, Fz, P3, P4, Pz, C3, C4, Cz, T3, T4, T5, T6, O1, O2. A reference electrode was placed on the nose and a ground electrode in front of electrode Fz. Vertical eye movements and blinks were recorded using an electrode below the left eye, and an electrode about 1.5 cm from the corner of the left eye was used for monitoring horizontal eye movements. EEG was amplified (SynAmps) using a band pass of 0.05 to 100 Hz, with a sampling rate of 500 Hz. An online 50 Hz notch filter was used. The impedance was kept below 5 kΩ in all the electrodes. EEG data were analyzed offline with Brain Vision Analyzer (Brain Products GmbH, Germany). Baseline was corrected to the activity from −100 to 0 ms before the onset of the stimulus. Trials with artifacts (> 50 μV and < 50 μV) in any of the electrodes were rejected offline (17% of the trials) and eye movements were corrected with the Gratton & Coles algorithm (Gratton, Coles, & Donchin, 1983). Data was filtered using 0.1 Hz high pass and 40 Hz low pass filters. ERP waveforms were averaged separately for trials in each condition, so that a total of 20 ERP waveforms were obtained (5 different stimulus durations × 4 animal categories). The mean amplitudes were calculated in the time window accounted for the EPN in previous studies (225–300 ms from the stimulus onset; see e.g. Van Strien et al., 2016).

2.7. Statistical analyses

For fear, familiarity, and arousal ratings, repeated-measures ANOVAs were used with stimulus category (snake, spider, butterfly,
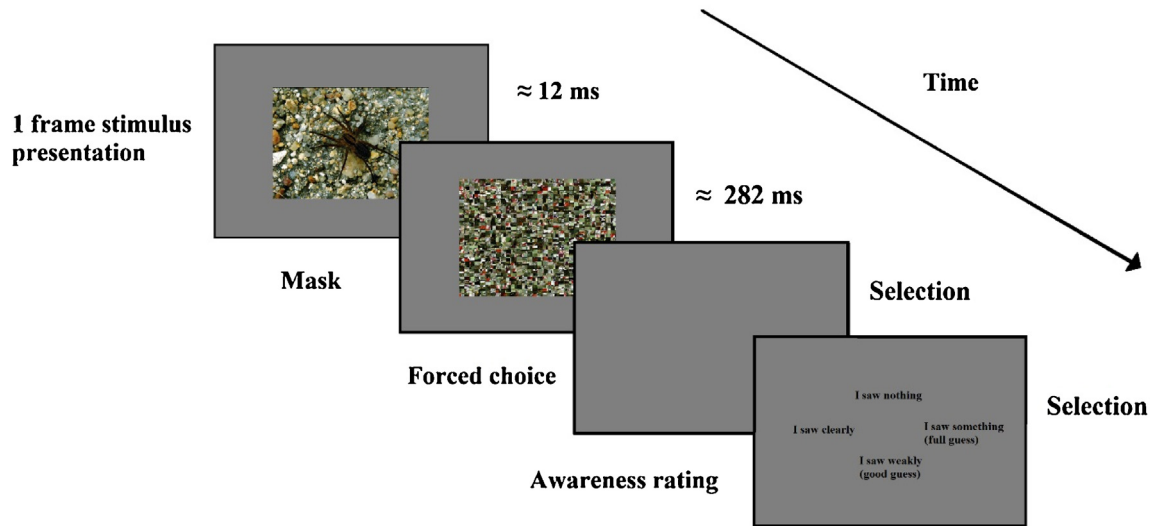
Fig. 2. An example of one trial in the assessment of awareness task. A stimulus representing one of the four animal categories was shown for a short duration, followed by a backward mask. Then the participant first made a forced-choice decision concerning the category of the animal that was shown, followed by the rating of subjective awareness.

bird) as factor. Repeated-measures ANOVAs were also conducted in order to test for differences in subjective awareness in the different stimulus categories. Accuracy was calculated separately for each subject for reported unaware stimuli and the computed values were compared using ANOVAs with stimulus category as factor (4). Signal detection theory (Tanner & Swets, 1954) was employed to understand possible perceptual differences between stimulus categories, for each stimulus category and each masking condition. D′ scores were calculated to assess the perceptual quality in each masking condition for each stimulus type, taking into consideration both aware and unaware reported trials. The computed values were compared using repeated-measures ANOVAS with stimulus category (4) and masking condition as factors (4). One sample *t*-tests were used to compare the d' scores to the chance level score 0.

For the EPN component, a repeated measures ANOVA was conducted, with stimulus category (4: snake, spider, butterfly, bird), presentation condition (5: unmasked and 1 frame, 2 frames, 3 frames, 4 frames masked) and occipital electrode hemisphere (2: O1, O2) as factors. This was done because according to previous studies these electrode sites reveal the modulation by evolutionarily stimuli (i.e., EPN) (Van Strien, Franken, & Huijding, 2014). In all the ANOVAs, when the sphericity assumption was violated, Greenhouse-Geisser correction was applied for *p*-values.

Six participants were excluded from the ERP analyses as they had less than 20% accepted trials at least in one of the conditions after artifact rejection.

To investigate a possible relationship between the reported fear, familiarity and arousal ratings with the EEG data, we calculated the
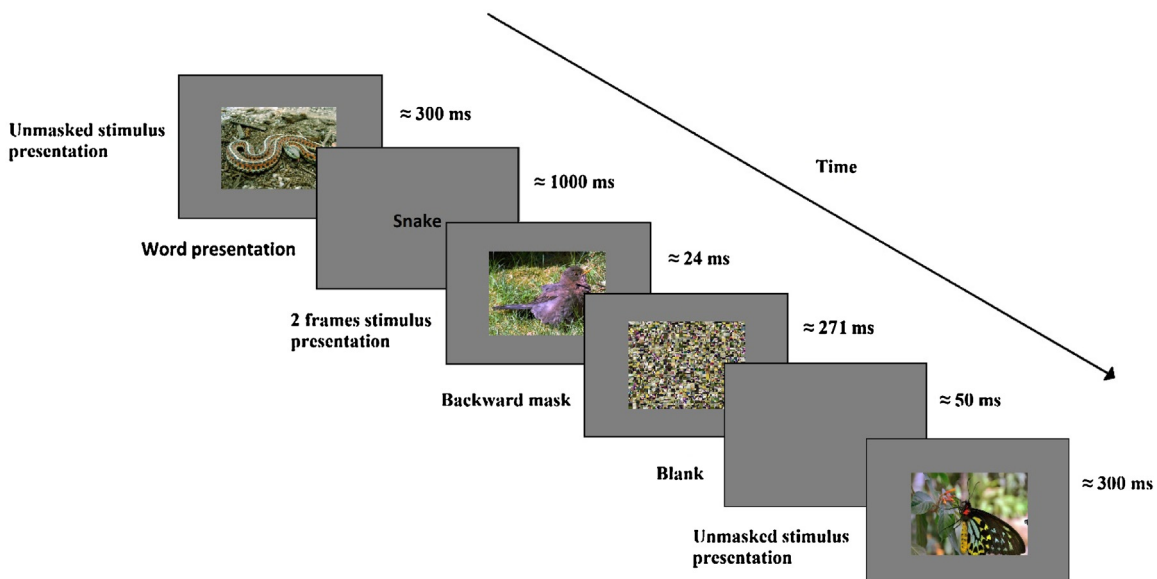
Fig. 3. Example of a part of the rapid serial visual presentation sequence. An animal stimulus was shown, either followed or not followed by a backward mask. During random intervals, a word was presented on the screen and it was either congruent or incongruent with the animal image presented previously. The subject had to press one of two buttons to report whether the word was congruent or incongruent with the previous image.

Table 1
Mean awareness rating (scale 0–3; SD in parenthesis) for each animal category as a function of presentation duration.

	Presentation duration (frames)				
Animal	1	2	3	4	MEAN
Snakes	0.55 (0.42)	1.37 (0.43)	1.98 (0.47)	2.23 (0.42)	1.53 (0.75)
Spiders	0.41 (0.37)	1.02 (0.43)	1.63 (0.55)	1.97 (0.48)	1.26 (0.69)
Butterflies	0.44 (0.33)	1.64 (0.54)	2.36 (0.56)	2.64 (0.47)	1.77 (0.98)
Birds	0.51 (0.35)	1.89 (0.56)	2.49 (0.50)	2.76 (0.38)	1.91 (1)
MEAN	0.48 (0.06)	1.48 (0.37)	2.12 (0.39)	2.40 (0.37)	

Table 2
The percentage (%) of trials in which the participants were aware of the images (ratings "seeing clearly" or "seeing something") for each animal category in each masking condition.

	Presentation duration (frames)				
Animal	1	2	3	4	MEAN
Snakes	20.2	54.7	79.1	81.7	58.9
Spiders	5.9	42.6	70.1	100	54.7
Butterflies	9.4	66.5	85.4	92.7	63.5
Birds	13.4	76.2	90.1	94.6	68.6
MEAN	12.3	60.0	81.2	92.3	

correlations between the questionnaire scores and the mean ERP amplitudes in the EPN time window to unmasked stimuli in each of the four different categories of stimuli. For the correlation analyses, the amplitudes registered in the two occipital electrodes O1 and O2 were averaged. In addition, we took the variability between the participants in the overall level of amplitudes into account by computing the correlations between the questionnaire scores and ERPs also for the amplitude differences between categories by using the bird category (which showed the weakest EPN) as the baseline, that is, for the snake minus bird, spider minus bird, and butterfly minus bird differences in amplitudes.

3. Results

3.1. Fear measures

Mean score in the fear questionnaire (scale 1 to 15) was 6.93 (SD = 2.79) for snakes, 4.26 (SD = 3.50) for spiders, 1.44 (SD = 1.89) for birds, and 0.19 (SD = 0.40) for butterflies. A significant category effect was revealed by the ANOVA, F(3,78) = 46.67, $p < 0.001$, $\eta_p^2 = 0.64$. Bonferroni corrected comparisons showed statistically significant differences between all possible pairwise comparisons (p-values < 0.011), with the snakes being the most feared, followed by spiders, birds and butterflies.

3.2. Familiarity measures

Mean score in the familiarity questionnaire (scale 1 to 10) was 6.41 (SD = 2.97) for snakes, 7.04 (SD = 2.55) for spiders, 9.15 (SD = 1.68) for birds, and 8.19 (SD = 1.92) for butterflies. A significant category effect resulted from the ANOVA, $F(3,78) = 20.15$, $p < 0.001$, $\eta_p^2 = 0.44$. Bonferroni corrected pairwise comparisons showed that participants were more familiar with butterflies and birds than with spiders and snakes (p-values < 0.014), but there was no difference in familiarity between spiders and snakes (p = 0.278).

3.3. Arousal measures

The arousal scores given at the end of the experiment for each image were averaged over the image category, obtaining scores ranging from a minimum of 1 to a maximum of 10. The mean score for the arousal measure was 6.16 (SD = 2.44) for snakes, 6.41 (SD = 2.15) for spiders, 1.98 (SD = 1.10) for birds and 2.00 (SD = 1.23) for butterflies. ANOVA showed a statistically significant difference between the categories, $F(3,78) = 82.88$, $p < 0.001$, $\eta_p^2 = 0.76$. Bonferroni corrected pairwise comparisons did not show statistically significant differences between snake and spider images (p = 1), nor between butterfly and bird images (p = 1), but both snakes and spiders differed from butterflies and birds (p-values < 0.001)

3.4. Awareness ratings

Awareness rating scores were calculated using both correct and incorrect answers, and are reported in Table 1. The ANOVA with

masking condition (4) × stimulus type (4) as factors showed a statistically significant interaction effect, F(9,234) = 19.22, $p < 0.001$, $\eta_p^2 = 0.43$, a main effect for masking condition F(3,78) = 312.49, $p < 0.001$, $\eta_p^2 = 0.92$, as well as for stimulus type, F(3,78) = 71.33, $p < 0.001$, $\eta_p^2 = 0.73$. Pairwise comparisons were conducted by applying Bonferroni corrections. All the masking conditions differed from each other for awareness ratings (p-values < 0.001) and all the stimulus types differ from each other (p-values < 0.002).

In the 1 frame condition, the effect for stimulus type was statistically significant, F(3,78) = 4.79, $p < 0.039$, $\eta_p^2 = 0.16$, with spider and butterfly images scoring lower than snakes (p-values < 0.033), and there was a statistical tendency for spiders to score less than birds (p = 0.056). Stimulus types differed also in the 2 frames, F(3,78) = 38.19, $p < 0.001$, $\eta_p^2 = 0.60$. In this condition also, the spider images were the least likely to reach conscious perception (p-values < 0.001). Snake images were more difficult to consciously perceive than bird (p < 0.001) and butterfly images (p = 0.27), while butterflies were rated as more difficult to perceive compared to birds (p = 0.20).

Pictures differed also in the 3 frames condition, F(3,78) = 39.93, $p < 0.001$, $\eta_p^2 = 0.61$. All the pairwise comparisons were statistically significant (p-values < 0.011), except butterfly vs. bird images (p = 0.122). Spiders were the least likely to be consciously perceived, followed by snakes.

Finally, there was a statistically significant main effect in the 4 frames masking condition, F(3,78) = 81.13, $p < 0.001$, $\eta_p^2 = 0.76$. In the Bonferroni corrected pairwise comparisons, all the comparisons were statistically significant (p-values < 0.019). Spiders were again the least likely to be consciously perceived, followed by snakes, butterflies and birds. The percentage of aware trials (ratings "seeing clearly" or "seeing something") for each condition are presented in Table 2.

3.5. Sensitivity index (d')

The d' scores were computed by defining correct forced-choice responses to each specific animal category as hits, and incorrect responses (the frequency that the specific animal was selected when some other stimulus category was presented) as false alarms.

The d' was first calculated only for trials where the subject reported not being able to recognize the animal in the images (awareness rating 0 and 1, Table 3). These two ratings were included

Table 3
Mean d's (standard deviations) in unaware trials (column "Unaware") and in each masking condition averaged across aware and unaware trials.

	Presentation conditions				
Animal	Unaware	1 frame	2 frames	3 frames	4 frames
Snakes	0.51 (0.34)	0.21 (0.42)	1.74 (0.62)	2.59 (0.65)	2.88 (0.58)
Spiders	0.29 (0.58)	0.03 (0.50)	0.73 (0.66)	1.49 (0.87)	1.94 (0.83)
Butterflies	0.30 (0.30)	0.11 (0.35)	1.89 (1.07)	2.60 (0.89)	3.07 (0.71)
Birds	0.36 (0.47)	0.20 (0.45)	1.68 (0.89)	2.78 (0.72)	3.17 (0.55)
MEAN	0.37 (0.10)	0.14 (0.87)	1.51 (0.52)	2.37 (0.61)	2.77 (0.56)

300 ms unmasked
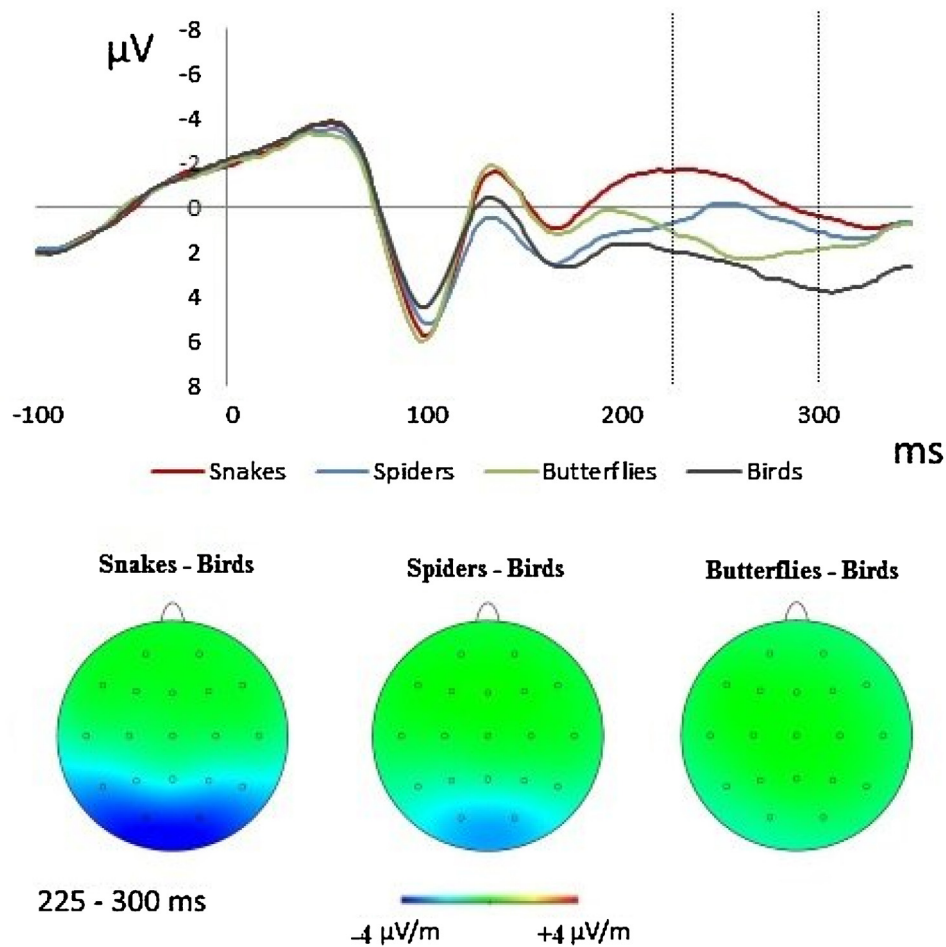
Fig. 4. On the top: Event-related potentials in the 300 ms unmasked condition for snakes (red line), spiders (blue line), butterflies (green line) and birds (black line) stimuli recorded at the occipital electrodes (average of O1 and O2). The vertical dotted lines show the EPN time window. On the bottom: Scalp distribution of brain activity in 225–300 ms from the stimulus onset. Bird images were used as baseline. The difference between snakes and birds and between spiders and birds was characterized by a negativity localized on the occipital area. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

as both refer to subjective unawareness of the category of the stimulus presented: in case of rating 0 it meant a total unawareness about the presence of the stimulus, and in rating 1 the participants had a somewhat aware perception of the stimulus, but it was a "full guess" about its category. Repeated-measures ANOVA on d' in unaware trials using stimulus type as factor (4) did not show any statistically significant difference ($p = 0.115$) between the categories.

Secondly, d' was calculated for each specific masking condition across aware and unaware trials (Table 3). The stimulus type (4) x masking condition (4) ANOVA showed a statistically significant interaction effect, $F(9,234) = 8.72$, $p < 0.001$, $\eta_p^2 = 0.25$. Statistically significant effects were found also for stimulus type, $F(3,78) = 53.19$, $p < 0.001$, $\eta_p^2 = 0.67$, as well as for masking conditions $F(3,78) = 259.25$, $\eta_p^2 = 0.91$, $p < 0.001$. Bonferroni corrected comparisons for stimulus type showed that only spider images differ from the other images types ($p < 0.001$), being more difficult to discriminate than the other images in masking conditions 3 frames, 2 frames and 1 frame (all p-values < 0.001).

Discrimination sensitivity (d') was compared to the chance level (value of 0) using one sample t-tests in unaware trials and in the 1 frame presentation. All the stimulus categories differed from the chance level in unaware trials (p-values < 0.016). In the 1 frame duration condition, only snakes and birds differed from the chance level (p-values < 0.029).

3.6. EPN (225–300 time window)

For the EPN amplitude, the interaction of stimulus category (4) × masking condition (5), $F(12,240) = 14.63$, $p < 0.001$, $\eta_p^2 = 0.42$), as well as the main effect for stimulus category, $F(3,60) = 28.39$, $p < 0.001$, $\eta_p^2 = 0.59$, and the main effect of masking condition, $F(4,80) = 25.73$, $p < 0.001$, $\eta_p^2 = 0.56$ were statistically significant. Other effects did not reach statistical significance. The source of the animal × masking condition interaction was studied by conducting separate ANOVAs for each presentation condition.
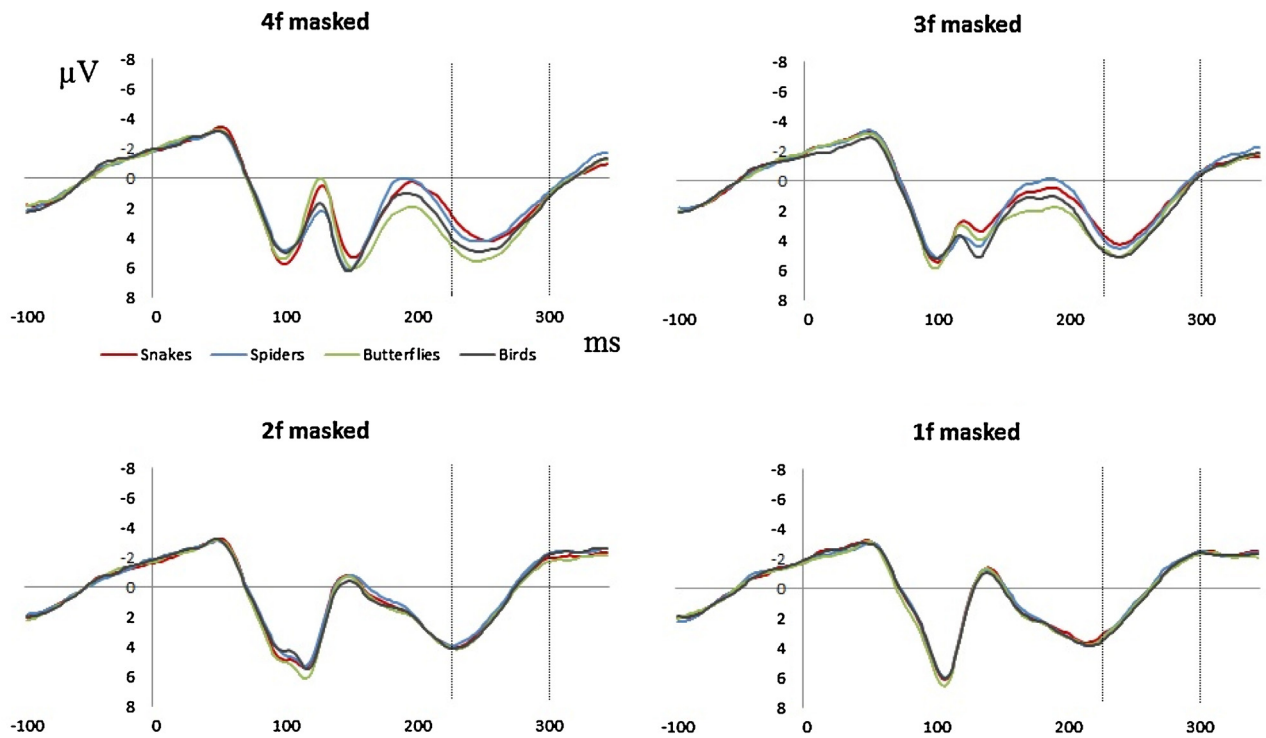
Fig. 5. Event-related potentials in 4, 3, 2 and 1 frame conditions with backward masking for snakes (red line), spiders (blue line), butterflies (green line) and birds (black line) measured at the occipital electrodes (average of O1 and O2). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.6.1. Unmasked presentation

In the unmasked condition the main effect for stimulus category was statistically significant, $F(3,60) = 41.08$, $p < 0.001$, $\eta_p^2 = 0.67$. Bonferroni corrected comparisons between stimulus categories revealed statistically significant differences between all animal categories (p-values < 0.037).The snake images produced the lowest average amplitude, followed by spiders, butterflies and birds (see Fig. 4).

3.6.2. Masked presentation

EPNs in masked conditions are presented in Fig. 5. Differences between animal types decreased as stimulus visibility decreased. In the 4 frames masked condition, the main effect for stimulus category was statistically significant, $F(3,60) = 10.02$, $p < 0.001$, $\eta_p^2 = 0.33$. Bonferroni corrected pairwise comparisons between stimulus categories revealed a statistically significant difference between snakes and butterflies ($p = 0.001$), but not between snakes and spiders ($p = 1$) and snakes and birds (although there was a tendency toward statistical significance, $p = 0.081$). Spiders differed from butterflies ($p = 0.003$), but not from birds ($p = 0.093$) and snakes. Amplitudes evoked by butterflies and birds did not differ from each other, but here also the difference was approaching significance ($p = 0.093$). In the 3 frames presentation plus masking condition, the main effect for stimulus category was statistically significant, $F(3,60) = 4.092$, $p < 0.010$, $\eta_p^2 = 0.170$. Bonferroni corrected comparisons among stimulus categories revealed a statistically significant difference only between snakes and birds ($p < 0.001$), and a tendency between snakes and butterflies ($p = 0.090$).

The main effect for stimulus category was not significant for the 2 frames and the 1 frame conditions.

3.7. Correlation analyses

Next we analyzed the correlations of the ERPs' average amplitude in the 225–300 time window (occipital electrodes) with the mean scores of the fear, familiarity, and arousal questionnaires. The correlations between amplitudes of the four stimulus categories in the unmasked condition and the fear scores of each specific animal category were not statistically significant. Also, the same analyses between the amplitudes of the stimulus categories and the questionnaire scores regarding animal familiarity and the arousal scores did not show any significant correlations. Also when these correlations were analyzed between the questionnaire scores and amplitude differences (snake − bird, spider − bird, butterfly − bird), no statistically significant correlations were found.

4. Discussion

The aim of this study was to explore whether or not snake pictures evoke enhanced early posterior negative potentials (EPNs) when the animal images are natural-looking, but equalized for luminance and contrast, and to study whether detection of snakes and the electrophysiological effects of snake perception are modulated by visual awareness of the stimuli.

Because snakes may be evolutionarily more fear-relevant compared to other animals (e.g., spiders, butterflies and birds), we expected larger EPN amplitudes in response to snake pictures and smaller amplitudes in EPN for the other stimuli (He et al., 2014; Van Le et al., 2013; Van Strien, Franken, & Huijding, 2014; Van Strien et al., 2016). Consistent with previous findings, we found that when participants were aware of the images, snakes provoked an enhanced EPN as compared with other animals. However, in contrast to the hypothesis that snake images might provoke

physiological reactions even when they remain unaware (Öhman & Soares, 1993, 1994), the differences in EPN between stimulus categories vanished as stimulus visibility decreased. This result suggests that awareness is necessary to elicit the selective ERP response to snake images. One should note, however, that our results do not rule out the possibility that phobic observers (Öhman, & Soares, 1994) might have developed mechanisms that make them more sensitive to fear-relevant stimuli than the non-phobic participants we studied. However, the previous studies (Öhman, & Soares, 1993, 1994) explored the possible importance of snake stimuli using electrodermal responses. It might be that such physiological methodology may be able to reveal effects that ERPs are not sensitive to. On the other hand, the present findings are in line with a wide body of literature that challenges the idea that unaware emotional stimuli may produce behaviorally observable effects (see meta-analysis of Hedger et al., 2016).

Previous findings in non-phobic observers indicating enhanced EPN for snake images, compared with images of other animals, were replicated with images containing identical luminance and contrast. This finding supports the snake detection theory (Isbell, 2006, 2009) which stresses the importance of snakes as an evolutionarily threatening stimulus. Furthermore, our results showed, in accordance with previous research (e.g. Van Strien et al., 2016), that spider images were also associated with enhanced EPN in comparison to images of unthreatening butterflies and birds. However, spider images differed from the snake images, with snake images producing larger EPN than spider images. These results for spiders partly contrasts with the findings of He et al. (2014) who did not find any difference between spider and nonthreatening images. The difference between the results of the studies may be due to the color stimuli used in the present study, as He et al. (2014) used gray-shaded images. Colors might play an important role in perception of dangerous stimuli, and spider stimuli might lose their effects if colors are removed.

In the present study, snakes were rated to elicit more fear than spiders, and this might have influenced the EPN amplitude. However, we did not find any correlation between fear and EPN, and also the arousal values did not differ between snakes and spiders, suggesting that perception of danger was not crucial for the difference in EPN. An earlier study (Van Strien, Eijlers et al., 2014; Van Strien, Franken et al., 2014) found no difference in reported fear between snakes and spiders, also suggesting that the EPN differences between snakes and spiders cannot be due to fear perception. Furthermore, even images of animals perceived as very dangerous (e.g., crocodile) did not evoke an EPN comparable with that produced by snake images (Van Strien, Franken & Huijding, 2014). The lack of association between fear measures and ERPs might be due to environmental factors. We tested a Finnish sample living in an urban environment: therefore our participants have not encountered snakes often in the real world. Familiarity scores were lower for snakes and spiders, and differed from the scores of birds and butterflies, opening the question of whether or not enhanced amplitude in EPN for spiders and snakes may be partly due to the novelty of the stimuli. However, there was no difference in familiarity between snakes and spiders, thus the EPN difference between these two stimuli types could not be explained by this factor. In addition, no correlations were found between EPN and familiarity.

Our main aim was to examine whether visual awareness modulates the EPN for snakes and whether there is a perceptual advantage favoring snakes. In order to manipulate awareness, the stimulus images were presented for different durations, followed by backward masks. This manipulation was effective: the ability to discriminate animal images (as indexed by d') and the rated awareness improved gradually as the presentation duration increased (Table 2). However, the snake images did not show any advantage in their ability to break into awareness in any of the masking conditions. Neither was there any advantage for snakes in the participants' ability to visually discriminate between different animals, either consciously or without consciousness. In fact, snake images were less likely to be consciously perceived than birds in all masking conditions except in the most difficult one. The spiders were the least likely to be consciously perceived in all masking conditions and the most difficult to discriminate from other animals in all masking conditions except in the easiest 4 frame condition. A lower general visibility for spiders compared to snakes may be the background factor for the ERP difference between snake and spider images in the 300 ms unmasked presentation. However, we did not collect awareness ratings or discrimination scores for the 300 ms presentation of unmasked stimuli, and therefore it remains open whether or not spiders have a discrimination or awareness disadvantage against snake images also when they are presented for 300 ms.

Electrophysiological differences between the animal categories strongly depended on awareness of the images. In the 4 frames masked condition, snake and spider images produced larger EPN as compared to butterflies. At the 3 frames presentation, the EPN evoked by the snake images differed from those evoked by bird images. In the masked conditions of 2 frames and 1 frame (with the 1 frame being the most effective at the group level), the differences between the animal categories in EPN disappeared completely. Importantly, the results showed that the EPN differences between the animal categories begun to gradually decrease already at the 4 frames stimulus presentation in which most of the subjects were well able to consciously perceive and discriminate the animal categories.

The present study found that awareness level is modulating the EPN response to dangerous evolutionary stimuli. The results do not challenge the general idea that snakes, and possibly spiders, capture attention more effectively than non-threatening animals, or a possible existence of a bottom-up attentional process selective for evolutionarily threatening stimuli. However, our findings suggest that the animal must be first consciously perceived, and only after that can the threatening stimulus be attended to preferentially. A recent masking study on emotional processing also suggested that emotions are elicited only after the stimulus is consciously recognized (Lähteenmäki, Hyönä, Koivisto, & Nummenmaa, 2015; Nummenmaa, Hyönä, & Calvo, 2010). In line with this idea, the time window in which the EPN typically occurs overlaps, or shortly follows, the time window (about 150–300 ms) in which aware stimuli typically show enhanced negativity as compared with unaware stimuli (for reviews, see Koivisto & Revonsuo, 2010). Thus, although snakes and spiders may not be processed unconsciously more efficiently than other animals, they may be more efficiently perceived in some challenging but conscious conditions (e.g., Soares et al., 2014). In line with this account, a recent priming study (Koivisto & Rientamo, 2016) suggests that discrimination between different animal species, which requires relatively fine-grained vision, depends on conscious vision, whereas unconscious visual processes are able only to discriminate coarsely between animal and non-animal images. Moreover, although fast behavioral reactions are typically assumed to be triggered by unconscious processes, recent evidence suggests that conscious vision can also guide fast motor behavior (Railo, Revonsuo, & Koivisto, 2015). Thus, it is possible that conscious perception may have had a significant role during evolution in dealing with dangerous animals.

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