Transient pupil constrictions to faces are sensitive to orientation and species

C. A. Conway   School of Psychology, University of Aberdeen, UK

B. C. Jones   School of Psychology, University of Aberdeen, UK

L. M. DeBruine   School of Psychology, University of Aberdeen, UK

A. C. Little   School of Psychology, University of Stirling, UK

A. Sahraie   School of Psychology, University of Aberdeen, UK

Previous studies have reported transient pupil constrictions to basic visual attributes (e.g., color and movement) that are processed along the ventral and the dorsal pathways. Specific cortical areas are activated more for faces than most other types of stimuli, raising the possibility that stimulus-specific transient pupil constrictions might also occur for faces. Such pupil responses may be sensitive to stimulus orientation and species since these parameters have been found to affect electrophysiological and behavioral responses to faces. Here we show transient pupil constrictions to upright human faces that are greater than those to scrambled versions, inverted versions, or macaque monkey faces. Similar to findings from electrophysiological studies, the inversion effect occurred for human faces but not macaque faces. Collectively, our findings show that transient pupil constrictions to faces are sensitive to the same parameters that have been found to influence electrophysiological and behavioral measures of face processing (i.e., orientation and species) and thus reveal a novel, objective, and non-invasive method for studying face perception.

Introduction

The pupil light reflex is a change in pupil size to variation in ambient light that optimizes retinal illuminance for perception (Loewenfeld & Lowenstein, 1999). Studies have also demonstrated pupil responses to various low-level visual attributes that are processed along the ventral and dorsal pathways (Barbur, 1991; Barbur & Forsyth, 1986; Sahraie & Barbur, 1997). Small rapid transient constrictions of the pupil occur following the onset of visual attributes such as spatial structure (Barbur & Forsyth, 1986), coherent motion (Sahraie & Barbur, 1997), and color (Barbur, 1991). These pupil responses cannot be explained by fluctuations in accommodation or convergence and occur independently of the pupil light reflex (e.g., Barbur, Harlow, & Sahraie, 1992).

Sinusoidal gratings of equal and lower luminance than the background also elicit pupil constrictions (e.g., Barbur & Forsyth, 1986; Slooter & van Norren, 1980). The amplitudes of pupil constrictions to gratings and checkerboard patterns vary systematically with the spatial frequency and check-size, respectively (Barbur & Forsyth, 1986; Slooter & van Norren, 1980), and show frequency response curves that are similar to those of the viewer's contrast sensitivity (Barbur & Thomson, 1987). Thus, these pupil responses can be used as an objective measure of visual acuity (Slooter & van Norren, 1980). Pupil color responses are produced when interchanging isoluminant monochromatic lights are presented (Young & Alpern, 1980) or when presenting color defined patterns (Barbur, 1991). Both grating and color responses occur independently of retinal illuminance (e.g., Barbur et al., 1992). The onset of coherent motion embedded in a random dot pattern also elicits a rapid transient pupil constriction (Sahraie & Barbur, 1997). These pupil motion responses also vary systematically with changes in the direction and speed of the moving target (Sahraie & Barbur, 1997) and therefore cannot be explained by the pupil light reflex. Unlike the pupil light reflex, very little is known of the possible functions of pupil constrictions to spatial structure (e.g., gratings), coherent motion, and color. Nonetheless, these pupil responses have often been used as indices of differences in cortical processing (Barbur, 1995).

Evidence for cortical involvement in the generation of stimulus-specific pupil responses comes from findings that grating, color, and motion pupil responses are either absent or reduced when stimuli are presented to the blind

hemifield of patients with occipital brain lesions (Barbur, 1995). For example, the hemianope, GY, demonstrated no pupil response to red-green equiluminant gratings that were presented in the blind hemifield but demonstrated a reliable pupil response when stimuli were presented in the sighted hemifield (Weiskrantz, Cowey, & Le Mare, 1998). Furthermore, GY demonstrated reduced sensitivity to achromatic gratings at higher spatial frequencies presented in the blind hemifield than those in the intact field (Weiskrantz et al., 1998). GY also showed small but significant pupil motion responses within his blind hemifield (Sahraie & Barbur, 1997). Other evidence for cortical involvement in pupil responses comes from a study of patients with lesions to dorsal pretectal areas (i.e., Paurinaud's syndrome). These patients did not show pupil light reflexes, while grating and color responses were typically smaller and had longer latencies than those that were evident in control subjects (Wilhelm, Wilhelm, Moro, & Barbur, 2002).

The latency of pupil responses to visual stimuli (i.e., the time between the onset of a stimulus and the onset of the pupil response) may provide direct information about the level in the visual system at which particular stimulus attributes are processed (see Barbur, Wolf, & Lennie, 1998). For example, short response latencies may indicate subcortical processing, while longer latencies may reflect processing at "higher" cortical levels (Barbur et al., 1998).

While rapid transient pupil constrictions to simple stimulus attributes (e.g., color and spatial structure) are well established, relatively few studies have tested for pupil constrictions to more complex visual patterns that are processed later in the visual pathway. Faces are an example of a complex and salient category of stimulus that viewers are adept at processing (Haxby et al., 2001; Kanwisher, 2000; Tarr & Gauthier, 2000). fMRI studies have revealed specific cortical areas (e.g., the fusiform "face" area) that are activated more for face stimuli than for most other types of stimuli (e.g., Haxby et al., 2001; Ishai, Ungerleider, Martin, Schouten, & Haxby, 1999). Similarly, single-cell recording studies have revealed neurons in the monkey temporal cortex that respond more to faces than other stimuli (e.g., Perrett, Rolls, & Caan, 1982). That specific cortical areas are activated more by face stimuli than by most other types of stimuli suggests that stimulus transient pupil constrictions might also occur following the onset of face stimuli. As neuroimaging and single-cell recording studies have shown that cortical areas activated by face processing are further along the visual pathway than those responding to low level attributes (Haxby et al., 2001; Kanwisher, 2000; Perrett et al., 1982; Tarr & Gauthier, 2000), the latency of pupil responses to faces may also be expected to be similar to, or longer than, those for basic visual attributes (260–340 ms; Barbur, 1991).

Two recent studies have investigated pupil responses to face stimuli (Harrison, Singer, Rotshtein, Dolan, & Critchley, 2006; Porter, Hood, & Troscianko, 2006). Both studies demonstrated dilatory pupil responses to faces following an initial constriction. Crucially, however, it was not clear from these studies whether the initial pupil constriction to the onset of face stimuli occurs independently of the pupil light reflex or whether the pupil constriction observed is unique to face stimuli. Consequently, we conducted 3 experiments that compared the magnitude of rapid transient pupil constrictions to face stimuli and carefully matched control stimuli (scrambled versions of the face images). Comparing pupil responses to non-scrambled and scrambled versions of face stimuli may reveal rapid transient pupil constrictions that are associated with face processing and that cannot be explained by pupil light reflexes since total light levels will be identical for scrambled and non-scrambled versions.

We also investigated whether visual parameters that have been found to affect behavioral and electrophysiological responses to faces also affect rapid transient pupil constrictions to faces. Findings from behavioral studies of face perception show that face processing is sensitive to stimulus orientation (i.e., upright faces are processed more efficiently than inverted faces, Maurer, Grand, & Mondloch, 2002; Yin, 1969) and also species (e.g., human faces are processed more efficiently than other-species faces, Mondloch, Maurer, & Ahola, 2006). Furthermore, electrophysiological studies of face perception have found that the event related potential component, the N170, has shorter latencies for upright human faces than for inverted human faces or monkey faces (de Haan, Pascalis, & Johnson, 2002). Indeed, while an inversion effect was evident for human faces, there was no inversion effect for monkey faces (de Haan et al., 2002). Single-cell recording studies have also shown that macaque inferotemporal neural responses are sensitive to face species (Kiani, Esteky, & Tanaka, 2005). Although findings for neural sensitivity to face orientation in the fusiform face area are somewhat mixed (for a review, see Rhodes et al., 2004), recent neurobiological evidence (Yovel & Kanwisher, 2005) and orientation-contingent face aftereffects (Rhodes et al., 2004) suggest that the fusiform face area may well be sensitive to the orientation of faces. Furthermore, single-cell recording studies have revealed neural sensitivity to face orientation in the monkey temporal cortex and have found that upright faces are represented more extensively than faces in other orientations (Perrett, Oram, & Ashbridge, 1998). More extensive representation of upright faces than faces in other orientations may occur because upright is the orientation in which faces are typically seen (Perrett, Oram, et al., 1998). Consequently, other familiar face categories (e.g., own-species) might also be represented more extensively than relatively unfamiliar face categories (e.g., other-species). In light of these findings for neural sensitivity to orientation and species, we compared pupil responses to upright and inverted human faces (Experiment 1), upright and inverted macaque faces (Experiment 2), and human and macaque faces (Experiment 3). Since inversion disrupts the processing of familiar face categories more than unfamiliar face

categories (Sangrigoli & de Schonen, 2004) and electro-physiological studies have reported inversion effects for human faces but not for monkey faces (de Haan et al., 2002), we anticipated that pupil constrictions to inverted human faces would be smaller than those to upright human faces, but that inversion would have a relatively small effect on pupil constrictions to macaque faces.

Following previous studies of human (Perrett, May, & Yoshikawa, 1994; Perrett, Lee, et al., 1998) and macaque (Waitt et al., 2003) face perception, we used prototype (i.e., average) human and macaque face images in our experiments to ensure that our face stimuli were highly representative of human and macaque face categories (see also Little, DeBruine, Jones, & Waitt, 2008).

Experiment 1

In Experiment 1, we compared the magnitude and the latency of pupil constrictions following the onset of 4 different versions of a prototype human face: (1) an upright face, (2) an inverted version of this face, (3) a scrambled version of the upright face, and (4) a scrambled version of the inverted face. Since the human face processing system is sensitive to the orientation of faces (e.g., de Haan et al., 2002; Maurer et al., 2002; Perrett, Oram, et al., 1998; Rhodes et al., 2004; Yin, 1969), we predicted a greater pupil constriction to the upright prototype face than to the inverted or scrambled versions.

Method

Participants

Twelve participants took part in Experiment 1 (1 male and 11 females). All participants were aged between 20 and 30 years (mean age = 25.33 years, SD = 3.28 years) and had normal or corrected to normal visual acuity.

Stimuli

The stimuli presented were gray scale images of a human prototype (i.e., average) face, in upright and inverted orientations, and scrambled versions of these stimuli (see Figure 1). The images subtended 8.4° × 11.8° at a viewing distance of 76 cm and were presented on a gray background.

The original human prototype face stimulus was manufactured using computer graphic techniques for creating prototype face images that are reported in Perrett, Lee, et al., 1998. To manufacture this prototype, first 179 landmark points were placed on 20 digital images of White women. The average face shape was then calculated for the sample by calculating the mean x and y coordinates for each landmark point. Each original image
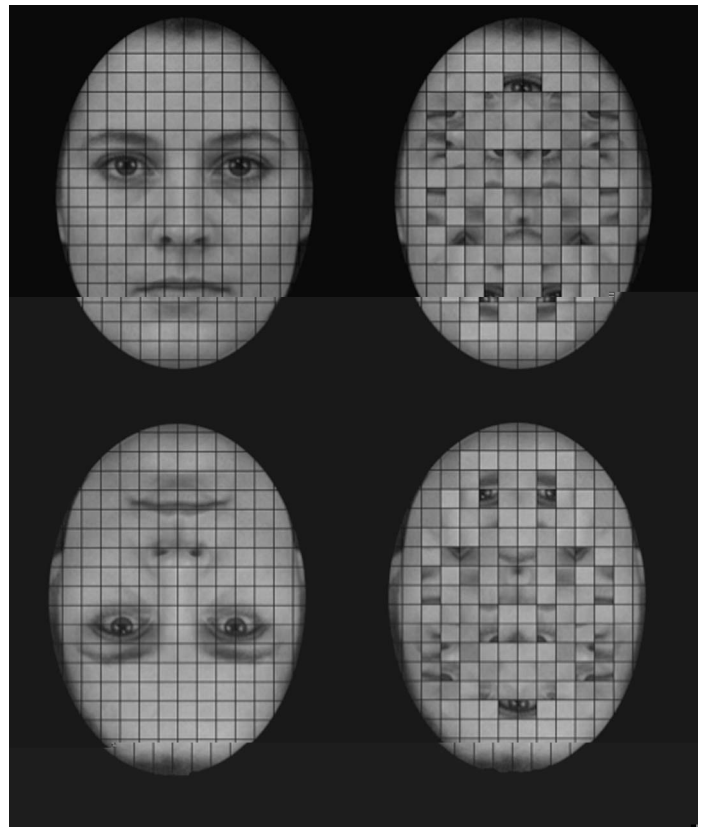

Figure 1. Upright and inverted human face stimuli and their scrambled counterparts used in Experiment 1. Grids were superimposed on all versions to control for possible effects of pupil grating responses. Scrambled versions were manufactured to retain high symmetry typical of faces.

was warped into this average shape, and the average RGB color values for each pixel were applied to the average face shape. Finally, representative texture was calculated using a wavelet-based algorithm, which adjusts RGB values accordingly to give the face representative texture details (for technical details, see Tiddeman, Burt, & Perrett, 2001). This method for manufacturing average faces has been used to manufacture face stimuli in many previous studies of face perception (e.g., Perrett et al., 1994; Perrett, Lee, et al., 1998).

Next, scrambled versions of the prototype face were manufactured. These scrambled versions were manufactured to retain the high symmetry typical of face images, so as to control for possible effects of processing symmetric vs. asymmetric stimuli on pupil responses. The scrambled versions of the upright prototype were constructed by dividing the face image into blocks of 20 × 20 pixels and randomizing the position of the blocks in the left hand side of the image. The blocks on the right hand side of the image were positioned to correspond to the scrambled positions of the blocks on the left, such that the image maintained symmetry. A total of 20 scrambled images were constructed using this

method. Scrambling faces by blocks of pixels imposes a lattice structure on the images that is not present in non-scrambled faces. To control for possible effects of this lattice structure on pupil responses (e.g., pupil grating responses, e.g., Barbur & Forsyth, 1986; Slooter & van Norren, 1980), we superimposed a grid pattern marking the boundaries of the pixel blocks in the scrambled faces on both the non-scrambled and scrambled versions of the prototype face (see Figure 1).

A new scrambled version of the prototype face was manufactured as a target stimulus in order to equate familiarity with the image between non-scrambled and scrambled conditions. Inverted versions of the prototype face and this scrambled face were manufactured by rotating the upright images through 180 degrees.

Procedure

On each trial, participants viewed a sequence of images consisting of a scrambled image (1000 ms), the target stimulus (1000 ms), and another scrambled image (2000 ms). There were 320 trials in total, consisting of 80 trials each of "upright face", "inverted face", "upright scramble," and "inverted scramble" conditions. An upright scrambled face image was used as the target stimulus for all trials in the upright scramble condition, and an inverted version of this scrambled face was used as the target stimulus for all trials in the inverted scrambled condition. This scrambled image had been randomly selected from the full set of scrambled images described in the Stimuli section. Using the same scrambled image as the target stimulus for all trials in the upright scrambled and inverted scrambled conditions controls for possible effects of differences in familiarity with the face and scrambled images that might otherwise be generated across trials during the experiment (i.e., participants saw the same "face identity" as the target stimulus for all trials in the upright and inverted face conditions but also saw the same "scramble identity" as the target stimulus for all trials in the upright and inverted scrambled conditions). The scrambled images that were presented immediately before and immediately after the target stimulus were randomly selected for each trial.

There were 4 blocks of 80 trials for each participant, each containing 20 trials from each of the 4 conditions. Trial order within each block was randomized. A central fixation cross remained on the screen throughout each trial. Onset and offset of stimulus presentation were signaled by an auditory beep, and participants were instructed to look at the center of the fixation point and to remain fixated throughout each trial. Participants took breaks between blocks and were also encouraged to take short regular breaks as needed between trials to avoid fatigue. The duration of inter-trial intervals was randomly determined to avoid pupil responses becoming synchronized with biological rhythms (e.g., heart rate, breathing). Stimuli were presented on a computer monitor enclosed within a cubical at a viewing distance of 76 cm. Participants were seated with their head supported by a chin/headrest to minimize head movement. Prior to the start of each block and following any breaks, participants were adapted to the ambient light in the testing room for five minutes.

An ASL5000 pupillometer with headrest-mounted optics was used to monitor eye movements and to measure pupil responses monocularly (right eye). Participant fixation was monitored throughout the trials by the experimenter, who observed a magnified image of the eye on a viewing monitor. The pupil diameter was extracted from a circle automatically fitted to the image of the pupil and recorded at a frequency of 60 Hz. The start and end of recording of pupil responses were automatically synchronized with the screen refresh video signal of the stimulus monitor. The extracted parameter data were transmitted from the pupillometer to a second PC via an RS232 link.

Initial processing of data

The data were first processed offline using *P-trace* software (developed at the Vision Research Laboratories, University of Aberdeen; see Sahraie, Weiskrantz, Trevethan, Cruce, & Murray, 2002), which allowed examination of each individual pupil trace and the subsequent removal of those containing artefacts (such as blinks and other breaks in fixation), sorting of the pseudorandom order of segments, and averaging and extracting pupil diameter change for each stimulus condition. Blinks and other breaks in fixation cause visible "breaks" in the pupil trace for that individual trial, meaning that trials containing blinks and other breaks in fixation can easily be identified by visually inspecting the pupil trace for each trial. Following this process, less than 10% of trials were discarded on average [mean number of valid trials per condition for each participant = 73.46 (maximum possible = 80), SD = 4.87, minimum = 61.3, maximum = 78.5]. Repeated measures ANOVA [dependent variable: number of valid (i.e., retained) trials; within-subjects factors: *orientation* (upright, inverted), *stimulus type* (non-scramble, scramble)] revealed no significant effects [all $F(1, 11) < 1.12$, all $p > .30$], indicating that there was no significant bias in the number of discarded trials among conditions.

For each participant, the average pupil response trace for each condition was calculated from the remaining measured pupil response traces. In order to determine the presence of a stimulus-specific pupil response in each condition, differences in pupil diameter between two 100 ms intervals were calculated from each participants' average trace for each condition. These intervals were at the onset and offset of the pupil response and were determined by visual inspection. Two different researchers independently carried out this visual inspection. For each participant, the average change in pupil diameter (i.e., the average pupil constriction between the onset and the offset

of the response) was calculated for each stimulus condition for each independent measurer. The response latency was also measured and refers to the time interval between the stimulus onset and the onset of the change in diameter. Since intercorrelations between values produced by each measurer were high (mean r for amplitude = 0.99; mean r for latency = 0.91), we averaged responses from each independent measurer. It was these latter (i.e., averaged) scores that were used in subsequent

analyses. Average pupil responses for each condition are shown in Figure 2a.

Results

Pupil diameter

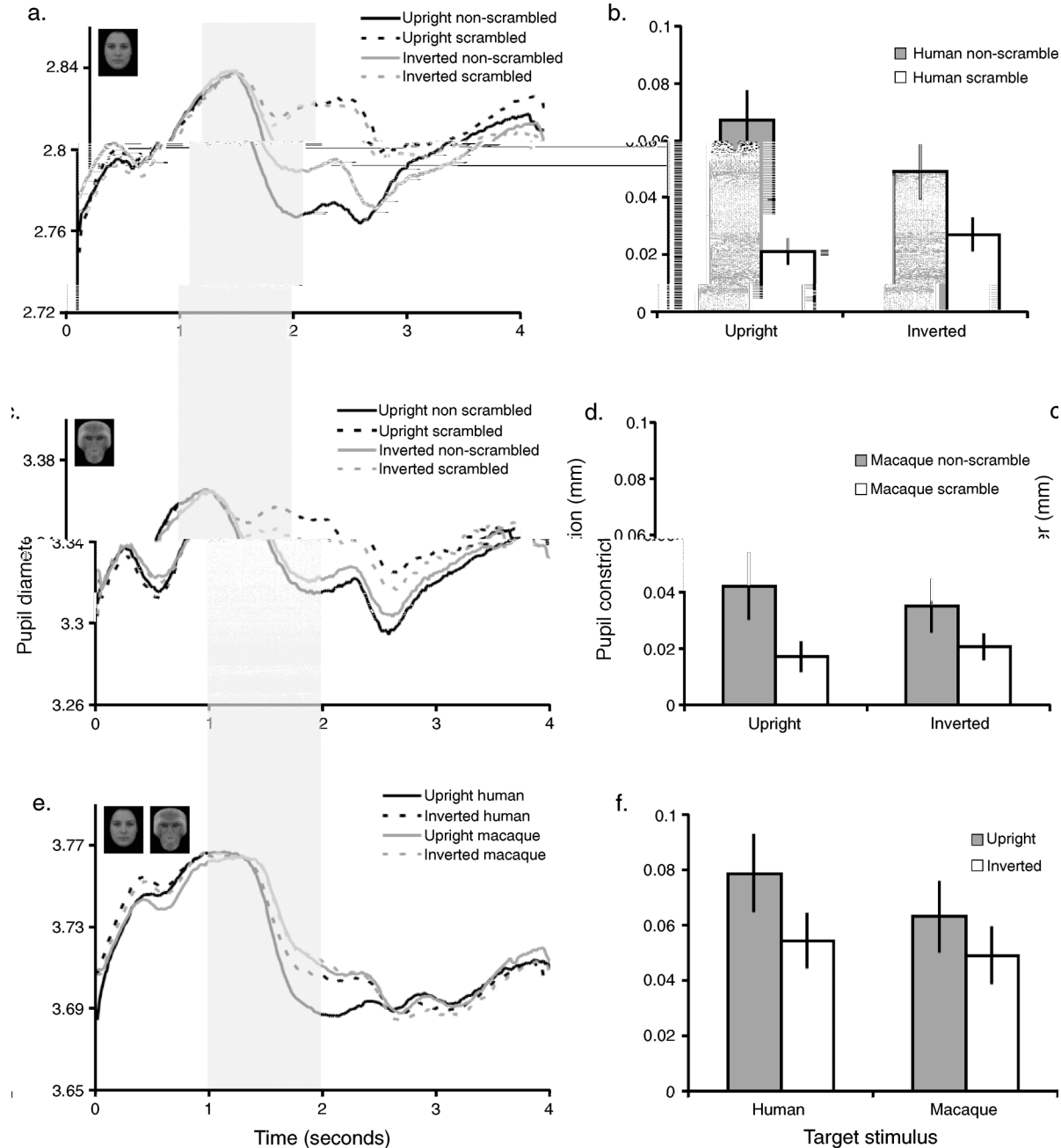A 2 × 2 repeated measures ANOVA with within-subjects factors of *orientation* (upright, inverted) and

Figure 2. Average pupil responses for each condition over time (panels a, c, and e) and average pupil constriction for each condition (panels b, d, and f). Bars in panels b, d, and f show means and *SEM*s. The shaded sections of panels a, c, and e correspond to the presence of the target stimulus for each condition. Panels a and b show results for Experiment 1 (human faces), panels c and d show results for Experiment 2 (macaque faces), and panels e and f show results for Experiment 3 (human vs. macaque faces). Collectively, these findings show that pupil constrictions when viewing upright human faces are greater than when viewing other types of stimuli.

stimulus type (non-scramble, scramble) revealed a significant main effect of *stimulus type* [$F(1, 11) = 21.46$, $p = .001$, $\eta_p^2 = .661$] and a main effect of *orientation* that approached significance [$F(1, 11) = 4.16$, $p = .066$, $\eta_p^2 = .274$]. These main effects were qualified by the predicted interaction between *orientation* and *stimulus type* [$F(1, 11) = 11.66$, $p = .006$, $\eta_p^2 = .515$, see Figure 2b].

Paired samples *t*-tests demonstrated that pupil constrictions to the non-scrambled faces were significantly greater than those to their scrambled versions for both the upright face [$t(11) = 4.71$, $p = .001$] and the inverted face [$t(11) = 3.57$, $p = .004$]. Pupil constrictions to non-scrambled upright faces were also significantly greater than those to non-scrambled inverted faces [$t(11) = 3.28$, $p = .007$]. By contrast, pupil responses to the upright and inverted scrambled faces did not differ significantly [$t(11) = -1.55$, $p = .149$].

Pupil latency

The response latencies for the upright ($M = 358$ ms, $SD = 71$ ms), upright scrambled ($M = 374$ ms, $SD = 63$ ms), inverted ($M = 377$ ms, $SD = 104$ ms), and inverted scrambled ($M = 374$ ms, $SD = 85$ ms) faces were analyzed with a 2×2 repeated measures ANOVA with within-subjects factors of *orientation* (upright, inverted) and *stimulus type* (non-scramble, scramble). This analysis revealed no significant effects [all $F(1, 11) < 1.83$, all $p > .203$, $\eta_p^2 < .143$].

Experiment 2

The results from Experiment 1 show that pupil constrictions to non-scrambled face images are greater than to scrambled versions and that pupil constrictions to upright non-scrambled faces are greater than to inverted non-scrambled faces. Since scrambled and non-scrambled faces and upright and inverted faces do not differ in total light levels, these findings cannot be explained by the pupil light reflex. Indeed, there was no significant difference between pupil responses to upright scrambled faces and inverted scrambled faces. Collectively, these findings demonstrate a small rapid transient pupil constriction that appears to be more sensitive to upright faces (i.e., that is greater than that to inverted or scrambled faces). In Experiment 2, we repeated Experiment 1 substituting a female macaque prototype face for the female human prototype face. As human faces are processed more efficiently than monkey faces (Mondloch et al., 2006) and electrophysiological studies have reported that the N170 is also sensitive to species (de Haan et al., 2002), we predicted that the pupil response to the macaque prototype would be smaller than that observed for the human

prototype in Experiment 1. Since inversion may disrupt processing of familiar face categories to a greater extent than it disrupts processing of relatively unfamiliar face categories (Sangrigoli & de Schonen, 2004) and de Haan et al. (2002) reported no effect of inversion on the N170 for monkey faces, we did not predict that inversion would affect pupil responses to the macaque prototype.

Method
Participants

Ten participants took part in this study (1 male and 9 females). All participants were aged between 20 and 30 years (mean age = 25.1 years, $SD = 3.57$ years) and had normal or corrected to normal visual acuity.

Stimuli

The stimuli presented were gray scale images of a prototype female macaque face in upright and inverted orientations and scrambled versions of these images (see Figure 3). The images subtended $10.8° \times 13.1°$ at a
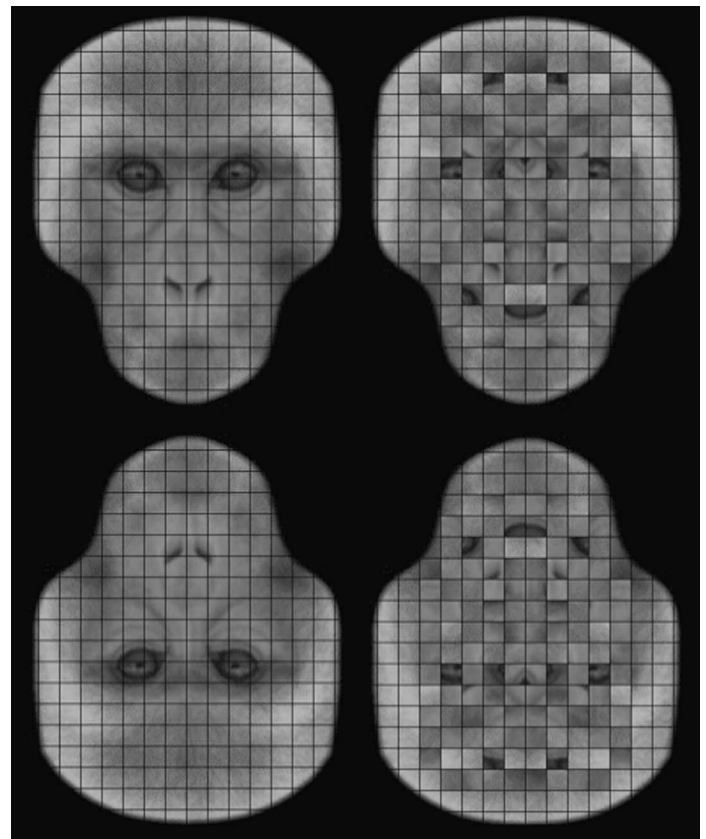

Figure 3. Upright and inverted macaque face stimuli and their scrambled counterparts used in Experiment 2. Grids were superimposed on all versions to control for possible effects of pupil grating responses. Scrambled versions were manufactured to retain high symmetry typical of faces.

viewing distance of 76 cm and were presented on a gray background. Upright and inverted macaque faces and scrambled versions were constructed using methods that were identical to those used to manufacture human face stimuli in Experiment 1.

Procedure and initial processing of data

The procedure for Experiment 2 was identical to that in Experiment 1.

Data were processed in the same way as in Experiment 1 (i.e., for each participant, we calculated the average change in pupil diameter for each stimulus condition and the latency for this change). As in Experiment 1, inter-measurer reliability was high (mean r for amplitude = 0.91; mean r for latency = 0.75). Less than 10% of trials were discarded on average [mean number of valid trials per condition for each participant = 75.83 (maximum possible = 80), SD = 2.42, minimum = 72.0, maximum = 78.3]. Repeated measures ANOVA [dependent variable: number of valid (i.e., retained) trials; within-subjects factors: *orientation* (upright, inverted), *stimulus type* (non-scramble, scramble)] revealed no significant effects [all $F(1, 9) < 1.47$, all $p > .25$], indicating that there was no significant bias in the number of discarded trials among conditions. Average pupil responses for each condition are shown in Figure 2c.

Results

Pupil diameter

A 2×2 repeated measures ANOVA with within-subjects factors of *orientation* (upright, inverted) and *stimulus type* (non-scramble, scramble) revealed a significant main effect of *stimulus type* [$F(1, 9) = 7.01$, $p = .027$, $\eta_p^2 = .438$], whereby pupil constrictions were greater to non-scrambled images than to scrambled versions. There were no other significant effects (all $F < 1.55$, all $p > .245$, $\eta_p^2 < .147$; see Figure 2d).

Pupil latencies

The response latencies for the upright ($M = 329$ ms, $SD = 52$ ms), upright scrambled ($M = 325$ ms, $SD = 76$ ms), inverted ($M = 338$ ms, $SD = 64$ ms), and inverted scrambled ($M = 318$ ms, $SD = 51$ ms) faces were analyzed with a 2×2 repeated measures ANOVA with within-subjects factors of *orientation* (upright, inverted) and *stimulus type* (non-scramble, scramble). This analysis revealed no significant effects [all $F(1, 9) < .636$, all $p > .446$, $\eta_p^2 < .066$].

Experiment 3

In Experiment 1, we found that pupil constrictions to upright non-scrambled human faces were greater than those to scrambled or inverted versions. By contrast, while pupil constrictions to non-scrambled macaque faces were greater than to scrambled versions in Experiment 2, the magnitude of this difference was not affected by inversion. The absence of an inversion effect on pupil responses to macaque faces is consistent with findings from behavioral studies of face processing that have shown inversion disrupts processing of familiar face categories more than unfamiliar face categories (Sangrigoli & de Schonen, 2004) and also with findings of inversion effects for N170s to human faces but not to monkey faces (de Haan et al., 2002). Nonetheless, it is also possible that the lack of any difference between pupil responses to the upright and inverted macaque faces is due to there being different groups of participants in Experiments 1 and 2. Consequently, in Experiment 3 we compared the magnitude of pupil constrictions to upright and inverted versions of human and macaque faces in a single group of participants.

Method

Participants

Twenty participants took part in this study (4 males and 16 females). All participants were aged between 19 and 25 years (mean age = 20.40 years, SD = 1.67 years) and had normal or corrected to normal visual acuity.

Stimuli

The upright and inverted human and macaque prototype faces were the same as those used in our earlier experiments.

Procedure

As in Experiments 1 and 2, participants completed 320 trials, each consisting of a scrambled face (1000 ms), target stimulus (1000 ms), and scrambled face (2000 ms). By contrast with Experiments 1 and 2, however, no *scrambled* faces were presented as the target stimulus (i.e., target stimuli were upright and inverted human or macaque prototype faces). For each participant, there were 2 blocks of trials: one block where target faces were either upright or inverted human faces and the other block where target faces were either upright or inverted macaque faces. The order in which these blocks were presented was fully counterbalanced. Each block contained 80 presentations of each target stimulus (i.e., 160 trials in total).

Initial processing of data

The data were processed in the same way as Experiments 1 and 2. The average change in pupil diameter for each stimulus condition and the latency of this change were calculated for each individual participant. As in Experiments 1 and 2, inter-measurer reliability was high (mean r for amplitude = 0.98; mean r for latency = 0.78). In Experiment 3, less than 14% of trials were discarded on average (mean number of valid trials per condition for each participant = 74.93 (maximum possible = 80), SD = 3.34, minimum = 69.0, maximum = 79.0). Repeated measures ANOVA [dependent variable: number of valid (i.e., retained) trials; within-subjects factors: *orientation* (upright, inverted), *stimulus type* (non-scramble, scramble)] revealed no significant effects [all $F(1, 19) < 2.0$, all $p > .17$], indicating that there was no significant bias in the number of discarded trials among conditions. Average pupil responses for each condition are shown in Figure 2e.

Results

Pupil diameter

A 2×2 repeated measures ANOVA with within-subjects factors of *species* (human, macaque) and *orientation* (upright, inverted) revealed a significant main effect of *species* [$F(1, 19) = 4.62$, $p = .045$, $\eta_p^2 = .196$] and a marginally significant main effect of *orientation* [$F(1, 19) = 4.33$, $p = .051$, $\eta_p^2 = .186$]. These main effects were qualified by a significant interaction between *species* and *orientation* [$F(1, 19) = 46.32$, $p = .021$, $\eta_p^2 = .250$; see Figure 2f].

Paired samples t-tests comparing responses to upright and inverted human faces demonstrated a significant difference in pupil constriction between the two conditions [$t(19) = 2.80$, $p = .011$]. By contrast, there was no significant difference in pupil constrictions to upright and inverted macaque faces [$t(19) = .87$, $p = .395$].

Our analyses show that pupil constrictions were greater to upright human faces than to inverted human faces and that inversion did not affect pupil responses to macaque faces. Collectively, these findings show that pupil responses to human faces are greater than those to macaque faces when controlling for the pupil light reflex (i.e., the difference between responses to upright and inverted human faces was greater than the difference between responses to upright and inverted macaque faces).

Pupil latencies

The response latencies for the upright human ($M = 383$ ms, $SD = 70$ ms), upright macaque ($M = 450$ ms, $SD = 72$ ms), inverted human ($M = 413$ ms, $SD = 65$ ms), and inverted macaque ($M = 442$ ms, $SD = 52$ ms) faces were analyzed with a 2×2 repeated measures ANOVA with within-subjects factors of *species* (human, macaque) and *orientation*

(upright, inverted). This analysis revealed a significant main effect of *species* [$F(1, 19) = 14.64$, $p = .001$, $\eta_p^2 = .435$] and no significant main effect of *orientation* [$F(1, 19) = 1.43$, $p = .245$, $\eta_p^2 = .070$]. There was also a significant interaction between *species* and *orientation* [$F(1, 19) = 6.63$, $p = .019$, $\eta_p^2 = .259$]. This interaction reflected pupil responses tending to occur earlier for upright than inverted conditions when viewing human faces [$t(19) = -2.03$, $p = .056$], but not when viewing macaque faces [$t(19) = 1.09$, $p = .291$]. This interaction should be treated cautiously, however, given Experiments 1 and 2 revealed no significant differences among conditions for response latencies.

Discussion

In Experiment 1, we observed a greater pupil constriction in response to both the upright and inverted human face stimuli than to their matched control stimuli (i.e., scrambled versions of the images). Importantly, comparing pupil constrictions to non-scrambled and scrambled images controls for the pupil light reflex. Additionally, we observed a greater pupil constriction in response to viewing an upright human face than an inverted human face, indicating that the pupil response to faces is sensitive to face orientation (see also Experiment 3). Experiment 2 demonstrated that pupil constrictions to upright and inverted macaque faces were also greater than pupil constrictions to their scrambled counterparts. However, unlike human faces, there was no significant difference between pupil constrictions in response to upright and inverted macaque faces, suggesting that pupil responses to macaque faces are *not* sensitive to face orientation (see also Experiment 3). Finally, in Experiment 3, we compared responses to upright and inverted human and macaque prototype faces in a larger sample of participants. We observed a greater pupil constriction in response to an upright human face than to the other stimulus conditions. These latter findings demonstrate that pupil responses to faces are sensitive to face species. Collectively, these findings demonstrate that transient pupil constrictions to upright human faces are greater than to scrambled versions, inverted non-scrambled versions and macaque faces. While rapid transient pupil constrictions have been widely reported for basic stimulus attributes (e.g., gratings, motion, color), here we show a pupil response that seems to be more sensitive to upright human faces than to scrambled human faces, inverted human faces, or macaque faces.

Our experiments show that transient pupil constrictions can occur for complex visual patterns. Importantly, the greater pupil responses to upright faces than to our other stimuli cannot be explained by total light flux change (i.e., pupil light reflexes), pupil responses to the block pattern

in scrambled faces (i.e., pupil grating responses), greater symmetry in faces than scrambled faces, or repeated presentation of target stimuli (i.e., the same scrambles were used as the target on every trial). In our experiments, each trial consisted of a scrambled image, followed by the target stimulus, followed by another scrambled image. This sequence allowed the change in pupil response to be measured to scrambled and non-scrambled versions of either the human or macaque faces. Since these scrambled and non-scrambled versions have the same global outline, the different pupil responses to human and macaque faces that we observed cannot be explained by differences in the outline of human and macaque faces. Additionally, using human and macaque prototypes as stimuli ensures that our face stimuli are optimally representative of their respective categories.

Our findings show that pupil constrictions to faces are sensitive to two of the parameters that have been found to affect behavioral and electrophysiological responses to faces (i.e., orientation and species). Since inversion is thought to disrupt configural processing of faces (for a review see Maurer et al., 2002) and configural processing is more pronounced for human faces than monkey faces (Mondloch et al., 2006), it is possible that greater pupil responses to upright human faces than inverted versions or macaque faces may be a consequence of greater configural processing of upright human faces. However, because greater configural processing occurs for familiar face categories than for unfamiliar face categories (Mondloch et al., 2006), pupil responses in our experiments could reflect either configural processing or be associated with the processing of highly familiar categories of objects more generally. While these explanations of our findings emphasize the possible role of configural processing and/or stimulus familiarity, an alternative interpretation is that upright human faces are a particularly meaningful object category that triggers a greater autonomic nervous response than other stimuli. While we note that these explanations are not necessarily mutually exclusive, further research is needed to investigate these explanations and other possibilities (e.g., that the pupil responses are associated with suppression of scanning patterns when viewing faces). Additionally, while we show a pupil response that is greater when viewing upright human faces than scrambled versions (i.e., versions in which some featural information is preserved), this finding does not preclude the possibility that pupil responses to isolated face features might also occur. Indeed, previous studies have demonstrated that certain face features (i.e., eyes) elicit robust N170 event-related potentials (Bentin, Allison, Puce, Perez, & McCarthy, 1996).

The mean latency of the pupil responses observed to upright faces was around 360 ms. Previously established pupil responses to spatial structure (e.g., pupil gratings response) typically occur between 260 and 340 ms (Barbur, 1991). Since face processing is thought to occur further along the ventral pathway than the neural mechanisms that analyze these basic aspects of spatial structure, the longer latencies may indicate a "higher" level of processing. The latency of the pupil responses to faces and scrambled faces that we observed appear to occur after perceptual effects (e.g., configural processing) are likely to have been completed, suggesting that these pupil responses may reflect post-perceptual processes. Such a conclusion should be treated cautiously, however, since little is known about the manner in which the time courses of pupil responses relate to actual time courses of perceptual and post-perceptual processes. It is possible, and even likely, that there is a substantial lag between perceptual processes occurring and the occurrence of pupil constrictions, as has already been established for pupil responses to coherent motion (see, e.g., Barbur et al., 1998).

The findings from our three experiments demonstrate a transient pupil constriction that is greater for upright human faces than inverted versions, scrambled versions, or macaque faces. This pupil response may be modulated by the same neural mechanisms that underpin the specificity of behavioral and electrophysiological responses for upright human faces compared to other types of face stimuli (de Haan et al. 2002; Maurer et al., 2002; Mondloch et al., 2006; Perrett, Oram, et al., 1998). While previous studies have demonstrated a late onset dilatory pupil response to face stimuli (Harrison et al., 2006; Porter et al., 2006), our findings demonstrate an early transient constriction component of the pupil response to a face stimulus that is relatively specific to upright human faces. Collectively, these findings are the first that we know of to demonstrate a rapid transient pupil constriction to a complex visual stimulus and may reveal a novel, objective, and non-invasive method for studying face perception.

Acknowledgments

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