Pupil dynamics during bistable motion perception

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Pupil size not only varies to changes in illumination but is also modulated by several cognitive factors, making it a potentially versatile physiological marker of cortical states. We recorded pupil dynamics while subjects continuously reported their bistable perception of ambiguous moving stimuli, plaids, and partially occluded rotating diamonds. We observed small (about 5% of surface change on average) but reliable pupil dilation around (-300 ms to 1.5 s) the button presses indicating the changes of percepts. We found that 70% of pupil dilation could be accounted for by the motor response. The remaining perceptual component was similar for spontaneously occurring transitions and transitions triggered by physical stimulus manipulations. Moreover, the amplitude of pupil modulation in the spontaneous condition was unrelated to the duration of each perceptual state. It is therefore unlikely that the mechanisms of endogenous perceptual bistability reflect in the pupil. In addition, we measured a clear constriction of the pupil after blinks (about 8% of surface change on average). As pupil changes have the potential to entail retino-cortical activity, their monitoring in studies of visual processing could prove worthwhile.

Introduction

One function of the pupil is to regulate the flux of light entering the eye in response to changes in illumination. This pupil light reflex (PLR) involves a non-linear closed-loop feedback system. The predominant input to the PLR originates in melanopsin-containing, intrinsically photosensitive, retinal ganglion cells (ipRGC; Guler et al., 2008). The reflex loop then involves the pretectal olivary nucleus (PON; Clarke, Zhang, & Gamlin, 2003), the Edinger–Westphal nucleus (EW), and the ciliary ganglion, belonging to the parasympathetic pathway, which drives the sphincter muscle that constricts the iris (Loewenfeld, 1999). Pupil size depends also on the antagonistic activity of the dilator muscle, which is driven by a sympathetic circuit originating in the hypothalamus (Loewenfeld, 1999; for a synthetic diagram of main connections, see Szabadi & Bradshaw, 1996, reproduced in Merritt, Schnyders, Patel, Basner, & O'Neill, 2004). This pathway is not concerned with the PLR, and its function is far less specific. Regulation of pupil size is complicated by

multiple modulations and interactions between these two pathways. The PLR parasympathetic pathway can be modulated at the level of the PON, which receives multiple cortical and subcortical inputs (Gamlin, 2006), possibly at the origin of a non-(directly) retinal component of the PLR (Barbur, 2004). The EW nucleus is thought to receive multiple inhibitory influences, in particular from the locus coeruleus (LC) and the cortex (Breen, Burde, & Loewy, 1983; Loewenfeld, 1999, Merritt et al., 2004).

Pupil dilation in response to many situations may therefore be mainly explained by inhibition of the parasympathetic sphincter pathway at the level of the EW and excitation of the dilator sympathetic pathway, through the hypothalamus, which receives projections from the cortex, the frontal cortex in particular. According to Loewenfeld (1999, p. 319), "Any sensory stimulation (with the exception of light), can elicit pupillary dilation; and spontaneous thoughts and emotions have the same effect as sensory stimuli." Well-studied examples of pupil dilations in response to non-sensory processing comprise cognitive load (Beatty & Wagoner, 1978; Kahneman & Beatty, 1966), attention (Beatty, 1977), stimulus probability

(Reinhard & Lachnit, 2002), and emotional factors (Partala & Surakka, 2003). In addition, changes of pupil size have been observed for specific visual computations supposed to involve the visual cortex, like the transition from incoherent to coherent motion (Sahraie & Barbur, 1997), color perception (Barbur, Weiskrantz, & Harlow, 1999; Wilhelm, Wilhelm, Moro, & Barbur, 2002), or the processing of faces (Conway, Jones, DeBruine, Little, & Sahraie, 2008), raising the possibility that pupil dynamics reflects some aspects of cortical processing.

We further investigate the relationship between cognitive states and pupil dynamics by recording pupil size during long-lasting observation of visual moving bistable stimuli. We were interested to determine whether changes in perception entail concomitant changes in pupil size. Perceptual bistability is an interesting tool in this regard as an unchanging stimulus yields different interpretations correlated to different brain states (reviews in Blake & Logothetis, 2002; Long & Toppino, 2004), such that any change in pupil size cannot be accounted for by a change in luminance distribution. Comparisons with conditions where perceptual bistability is induced by smooth physical stimulus changes that do not in themselves entail pupil modulation further allow assessing whether changes in pupil size are specific to endogenous or exogenous modification in perception, so as to shed light on the mechanisms of bistable perception.

Recent studies indicate that pupil transiently dilates when subjects change their perception of ambiguous stimuli (Einhäuser, Stout, Koch, & Carter, 2008; Lamirel, Hupé, & Lorenceau, 2008). Whether these changes relate to the cause or the effect of perceptual transitions or are simply an epiphenomenon remains an open issue that we address in the present study. Since pupil responds to many non-specific factors, we ask whether this modulation of pupil size is related to the cortical reorganization responsible for the percept change, to the change of the percept itself, to attentional, predictive or decisional factors, or simply to the motor command needed to report a perceptual switch. In order to dissect the origin of the observed pupil dilation, additional experiments involved sound induced and endogenously generated button presses, perceptual transitions induced by smooth and abrupt physical stimulus changes without a motor response, and the passive viewing of a static stimulus whose contrast distribution varied over time. Our analyses reveal that pupil dilation is related to both motor response and perceptual transitions. Einhäuser et al. (2008) observed that pupil dilation around the time of perceptual switches (starting before a button press) was correlated to the relative duration of the following percept, suggesting that a structure involved in controlling pupil dilation, the locus coeruleus, plays a role in the perceptual reorganization itself, owing to its widely distributed, ascending projections to the neocortex (Aston-Jones & Cohen, 2005). Here, we performed a similar study, but we do not observe such a correlation. An unexpected result, however, is the observation of a long-lasting pupil constriction after blinks. Thus, we observe marked pupil changes during the bistable perception of ambiguous percepts, due to button presses, percept changes, and blinks.

Methods

Stimuli and procedure

Two experiments were run independently in Toulouse by JMH and in Paris by CL and JL. The Toulouse experiments used moving plaids (Hupé & Rubin, 2003), the Paris experiments used rotating diamonds with varying luminance distribution along their line segments (Lorenceau & Shiffrar, 1992).

Experiment 1: Plaids

Stimuli

See http://cerco.ups-tlse.fr/~hupe/plaid_demo/ for examples of plaid stimuli. Plaids were displayed on a 19-in. monitor (26.25-cm vertical viewable screen size) at a frame rate of 75 Hz. The screen resolution was 1024 × 768 pixels. Two rectangular-wave gratings were presented through a 6-degree radius circular aperture on a computer screen 57 cm away. The gratings comprised thin dark stripes (−31.7% contrast over the mean luminance, duty cycle = 0.3, spatial frequency = 0.3 cycle/deg) on a lighter background (36% contrast) and appeared as figures moving over the background. The intersecting regions were darker than the gratings (−47.9% contrast, in the middle of the transparency range). Mean luminance was 33 cd/m^2. The gratings were drifting at 1.5 deg/s in directions 105 or 125 degrees apart. Plaids could move in the four cardinal directions. As a secondary goal of this study was to test observers' capability to bias their bistable percept at will, an "intention" instruction was printed on the screen ("neutral", "coherent", "transparent") before each trial. A red fixation point over a 1-degree radius circular gray mask was added in the middle of the circular aperture and subjects were instructed to fixate this point throughout stimulus presentation. The stimulus can be perceived either as a single plaid moving in a given direction ("coherent" percept) or as two gratings sliding in opposite directions on top of each other ("transparent" percept; Hupé & Rubin, 2003; Wallach, 1935; Wuerger, Shapley, & Rubin, 1996; we shall further name this percept the "incoherent" percept, for semantic compatibility with the diamond study, even though the transparent interpretation requires as much global processing as the coherent one: Rubin & Hupé, 2005).

Procedure

Fourteen observers (8 males and 6 females) gave informed consent to participate in the experiment (average

age: 27) with normal or corrected-to-normal eyesight. They were comfortably seated in front of the screen, with their chin and forehead resting on a chin rest (University of Houston College of Optometry). They were instructed to report their conscious perception of each stimulus during 1-min periods by pressing the right mouse button continuously for transparent percepts and the left button for coherent percepts. They were asked to release both buttons if they were unsure of their percept (this happened on average 3% of the time). Twenty-four stimuli (8 different stimuli by 3 instructions) were presented in a randomized order. Observers were given a few practice trials. Seven subjects had run previously other plaid experiments. On average, observers reported the coherent percept for 54% of the time (range [36% 68%]). The effect of intention will be described in another paper. The analyses presented below used all available data, but similar conclusions apply if restricting the analysis to the "neutral" trials (not shown; figures and analyses were, however, sent to the reviewers).

Eye position (difference between the pupil and corneal reflection centers) and pupil diameter were recorded binocularly at 120 Hz (10 subjects) or 240 Hz by using an ISCAN ETL-200 system (Burlington, MA, USA). The cameras were attached to the chin rest just above the eyes and looked at the eyes through semi-transparent mirrors.

Experiment 2: Diamonds

Stimuli

The "hidden" moving diamond used in this study consisted in a set of four disconnected segments translating sinusoidally along a vertical axis (Lorenceau & Alais, 2001). The phase relationship between segment motions was chosen such that their perceptual integration resulted in seeing a diamond shape translating along a circular trajectory of 0.5° radius at 1 Hz. When segregated, the segments were seen as two independent pairs oscillating up and down. The diamond subtended a visual angle of about 6 degrees at a viewing distance of 50 cm. A small red fixation disk was present at all times at the center of the screen. Background luminance was 15 cd/m². The stimulus was displayed on a 22-in. monitor (30.48-cm vertical viewable screen size, screen resolution of 1024 × 768 pixels) at a frame rate of 85 Hz. Seven different conditions were tested in long-lasting run of 4 min each. These different conditions, detailed below, were designed to disentangle the possible contributions of perception, attention, prediction, decision, and motor commands to pupil changes.

Condition 1: Active report of spontaneous perceptual switches of an ambiguous rotating diamond

In this condition, a physically unchanging version of the "broken" diamond was used. The stimulus was ambiguous and could either be perceived as a single diamond translating clockwise along a circular trajectory ("coherent" percept) or as four independent segments oscillating up and
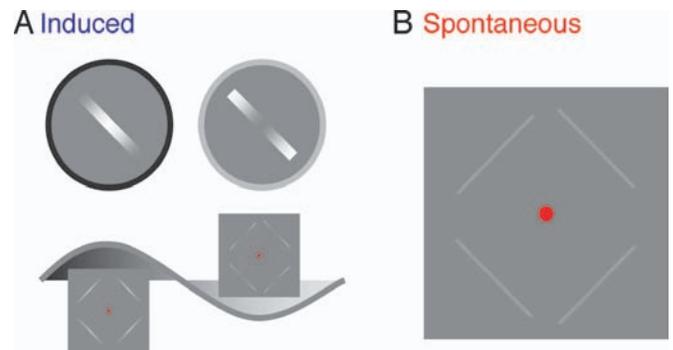

Figure 1. Diamond display. (A) Induced bistability was controlled through smooth modulation of the contrast gradient along the line segments (8-s period). Low contrast line ends yield a coherent percept (black shaded area) while high contrast line ends yield an incoherent percept (gray shaded area). Mean contrast was constant over time. (B) A fixed contrast gradient derived from preliminary testing was used in the spontaneous condition.

down ("incoherent" percept). As contrast gradients along the line segments can favor either the coherent or the incoherent percept (see below, Condition 2; Lorenceau & Shiffrar, 1992), we set the contrast gradient of the stimulus so as to yield well-balanced perceptual alternations (Figure 1).

Condition 2: Active report of induced perceptual switches

The stimulus was the same as in Condition 1, but the contrast gradient along each line segment was smoothly varied to periodically induce a perception of either the coherent or the incoherent percept (Lorenceau et al., 2008; Lorenceau & Shiffrar, 1992). Segments with low contrast line ends and a high contrast center favor the perception of a global shape rotation while segments with high contrast line ends and a low contrast center entails the perception of independent up and down translating segments, despite average luminance being the same in both cases (Figure 1). Smooth periodic transitions from one contrast gradient to the other induces alternations between coherent and incoherent perceptual states with an 8-s cycle, thus leading to a 4-s theoretical average duration of perceptual episodes. Preliminary experiments were performed prior to the main experiments to determine the contrast gradient corresponding to well-balanced coherent/incoherent episodes. This gradient was used in Condition 1 and as the midpoint gradient in Condition 2.

Condition 3: Active report of a sound stimulus

A brief sound (1000 Hz, 100 ms) to which observers must respond as in the remaining conditions (by alternating their finger presses) was produced every 4 s while a static version of the diamond of Condition 1 was displayed on the screen throughout the 4-min run to maintain the same mean luminance.

Condition 4: Constant stimulation

Only a static version of the diamond of Condition 1 was displayed on the screen.

Condition 5: Induced perceptual switches with no report

The stimulus was the same as in Condition 2, but observers were looking at the stimulus without reporting their perception.

Condition 6: Static diamond with dynamic contrast gradient

The stimulus was the same as in the previous condition but remained static. The only change was the distribution of the contrast along each line segment, with the same dynamics as before. This control condition aimed at verifying that no pupil changes correlated to the contrast changes would occur.

Condition 7: Abrupt changes with no report

The contrast gradient along the line segments changed abruptly at random intervals of 1, 2, 3, or 4 s from a diamond strongly biased to promote the coherent percept to a diamond strongly biased toward the incoherent percept, so as to grossly mimic unpredictable perceptual switches as those of Condition 1. Based on previous experiments (Lorenceau & Shiffrar, 1992), such abrupt changes reliably drive perceptual alternations between coherent and incoherent percepts. These abrupt time-stamped changes were used to trigger changes in perception for analyzing purposes.

Procedure

Ten observers with normal or corrected-to-normal eyesight (8 males and 2 females; average age: 28) gave their informed consent to participate in the experiments. They were seated in front of the screen using a chin rest with a bite bar. Each subject ran each condition once. The order of appearance of the different conditions was counterbalanced across subjects using a Latin square. After an initial fixation-only period lasting 4 s the stimulus was presented for 240 s. A fixation-only period of 2 s ended the trial. Subjects were instructed to fixate the central fixation disk for the entire course of all trials.

In Conditions 1 and 2, subjects were instructed to report their visual percept—coherent or incoherent—by continuously pressing one of two mouse buttons. The assignment of buttons to coherent and incoherent perceptual states was balanced across subjects. Observers were given a few practice trials. In Condition 1, they reported the coherent percept on average for 38.5% of the time (range [12% 65%]). In Condition 2, we verified that the gradient corresponding to the average transition point between both percepts was the midpoint gradient found in the preliminary experiments and that perceptual alternations followed luminance variations. However, we observed a hysteresis effect, which is expected given the underlying cooperative processes involved in motion binding (Lorenceau,

Gimenez-Sastre, & Lalanne, 2003). Intra- and inter-subject variability of response times to stimulus changes was also observed (see Results section), as expected with stimuli supporting several interpretations.

In Condition 3, subjects were instructed to continuously press a mouse button and to change button every time they heard the sound. In Condition 4, subjects had to continuously press a mouse button and to change it at will. In both cases, there was no change at all of the visual display.

In Conditions 5 to 7, subjects were instructed to pay attention to their perceptual state but without reporting their percept (no button press), while still looking at the center of the diamond as in all other conditions.

Eye position (difference between the pupil and corneal reflection centers) and pupil diameter were recorded monocularly (right eye for all subjects) at 240 Hz using an ISCAN ETL-400 system (Burlington, MA, USA). The camera looked at the right eye through a semi-transparent mirror.

Data analysis

Pretreatment

Oculomotor signal was pretreated using an interactive interface written in Matlab. Each trace was inspected and processed in three steps. First, blinks were removed by relying on pupil size. By default, a blink was identified when pupil signal was below 60% of the median size for at least 50 ms (we systematically verified that this threshold was below actual pupil size). Blinks separated by less than 100 ms were aggregated to a single blink. Each detected blink was verified by inspecting the signal on both eyes (Experiment 1) as well as eye position around each blink (since partial occlusion of the pupil by the lid when the eyes are closing is recorded as eye position moving down fast just before signal loss). Blinks could thus be removed or added manually, which was necessary only in a few cases. Next, eye position was scaled, using a 9-point calibration procedure recorded just before the experiments. Finally, artifacts were removed (loss of signals not due to blinks, aberrant eye positions). The signal did not need being filtered.

Exclusion criteria

After a first-pass analysis, we observed variations of pupil diameter after blinks (see Figure 9) and around button presses (see Figure 2). In order to measure both effects properly, we selected blinks and button presses that were isolated enough from other events (blink or button press). Pupil response was significantly modulated for about 1.5 s after a blink or a button press. For button presses, modulation started about 500 ms before the press. An event was therefore kept for the analysis only if no other event happened within 1.5 before the beginning of
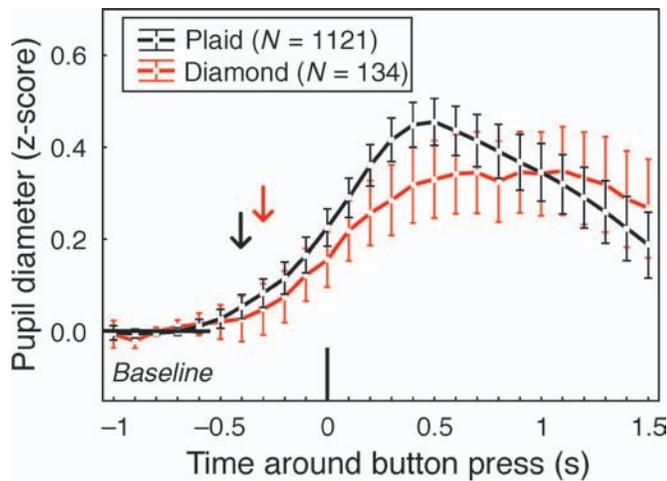
Figure 2. Pupil response around perceptual switches of ambiguous plaids and diamonds (mean ± SEM). Plaids: Data from 14 subjects, N = 1121 (10 outliers removed. The total number of transitions was 3390). Diamonds: Data from 10 subjects, N = 134 (9 outliers removed. The total number of transitions was 332). Subjects indicated percept changes by releasing and pressing mouse buttons. Since pupil response to blinks last on average 1.5 s (see Figure 9), button presses were excluded if a blink occurred within 2.5 s before (i.e., 1.5 s before baseline) or 1.5 s after the button press. In order to avoid between events contamination, button presses were excluded if following another press by less than 2.5 s or preceding another press by less than 2 s. Average pupil diameter was computed for the period [−1 −0.6] s before each button press and subtracted. For plaids, pupil diameter started to increase significantly above zero 400 ms before button press (black curve, $F(1, 1093) = 6.6$, $p = 0.021$, $\eta_p^2 = 0.29$). The increase was significant for 13 out of the 14 subjects (individual data shown in Figure 8). For diamonds, it started 300 ms before button press (red curve, $F(1, 114) = 5.3$, $p = 0.026$, $\eta_p^2 = 0.09$). The increase was significant for 8 out of the 10 subjects (individual data shown in Figure 5: red curves).

baseline and up to 1.5 after the event (2 s if the next event was a button press). Only about a third of the events could be included in the analyses. Refer to figure legends for exact numbers. Such strict exclusion criteria were necessary to ensure that the analyses and conclusions drawn from pupil dynamics would not be contaminated by irrelevant factors.

Statistical analysis

A baseline was computed before each event (start and end values of the baseline period are indicated in the figure legends) and subtracted to the signal to get rid of slow pupil variations. Time zero was the time of event. Since blinks last some time, time before each blink was relative to the beginning of the blink, time after each blink relative to the end of the blink. Median blink duration was 100 ms (range of 21–567 ms) in Experiment 1 and 137 ms

(range of 21–1446 ms; "long" blinks correspond in fact to several blinks) in Experiment 2. Pupil size was converted to z-scores in order to collapse data across subjects. Peri-event time histograms were computed at the original sampling rate and then downsampled to 10 Hz for display and statistics purposes (note that we verified that sampling at 60 Hz gave exactly the same result; 10 Hz corresponds better to pupil dynamics). Time "zero" in the graphs corresponds to the signal averaged from 50 ms before up to 50 ms after the event. ANOVAs were performed in Statistica 8 (Statsoft) independently at each time around the event. Since these dependent variables are temporally correlated, no correction for multiple comparisons was applied. Some procedures do exist to balance temporal correlation and multiple comparisons (see, e.g., Hupé et al., 2001), at least for two-sample comparisons. Here, however, we indicated p-values only to help the reader have an idea of the average effect robustness. We also indicate effect sizes (partial Eta Squared, η_p^2, varies between 0 and 1). Data from individual subjects are presented when necessary. Some of the effects were in any case very strong (e.g., Figures 2 and 9), others borderline significant (e.g., Figures 6 and 7). Results are discussed accordingly. The variable "subject" was included in all statistical analyses and treated as a random factor. Residuals were inspected for every analysis and dependent variable. Residuals were always normally distributed and variances reasonably homogenous. Events were excluded if the absolute standardized residual value was larger than expected for a normal distribution given the sample size. The Gaussian distribution predicts that 1% of values should be either larger than 2.96 or smaller than −2.96, and 1 over 1000 either larger than 3.65 or smaller than −3.65. We set the threshold value for outliers as the value for which the (two-sided) Gaussian probability multiplied by sample size was less than 0.5. This means that for a sample size of 1000, our outlier threshold was 3.84. Here, residual values were therefore considered as outliers if their absolute value was larger than about 3.9 (Experiment 1, Experiment 2: analyses of Figures 4 and 6), about 3.6 (Experiment 2: analysis of Figure 7), or about 3.2 (Experiment 2, Condition 1) for at least one of the dependent variables.

Raw and relative modulations

We also performed analyses on raw data (no z-score transformation) to have an idea of the amplitude of pupil modulations relative to pupil diameter (% change). This analysis does not take into account the different noise levels measured in different subjects, contrary to the z-score analysis that we favored. In addition, the amplitude of the pupil light reflex was measured for all subjects in Experiment 2 and four subjects in Experiment 1, by modulating the screen luminance from minimum to maximum. For the 10 other subjects of Experiment 1, this amplitude was estimated by the difference between

maximum and minimum pupil sizes (we verified that both measures were tightly correlated and close to each other). This allowed us to compare the size of pupil modulations in our experiments to the maximum range of pupil size variations.

Vergence

A possible confound in considering pupil size is its link with eye vergence. To determine the extent to which vergence eye movements could account for changes in pupil size, we measured vergence eye movements in Experiment 1 using binocular recordings. For most subjects, vergence mirrored pupil dilation. Vergence modulation was about half the size of pupil modulation when both measures were computed as *z*-scores, be the cause of pupil modulation a button press, a blink, or a luminance change. Average vergence was about 0.05 degrees after button presses. We conclude that vergence modulations in our data were a measure artifact due to the fact that for most subjects pupil centers diverge when pupil dilates (anatomical constraint, Camellin, Gambino, & Casaro, 2005; Wyatt, 1995; Yang, Thompson, & Burn, 2002). No other vergence eye movements could be observed in our data. Eye position was also stable around button presses, stimulus changes, and blinks.

Correlation between pupil diameter and percept duration

We measured the correlation between pupil size and percept duration in Experiment 1, as in Einhäuser et al. (2008) by computing a "normalized measure of 'post-switch' duration relative to the corresponding 'preswitch' duration": Relative duration = (post − pre) / (post + pre). We selected only the switches that were not contaminated by other events, as described previously. The first and last switches of each trial were also excluded, since relative duration for these switches cannot be computed. Traces around switches were downsampled to 10 Hz (but the results were identical at 60 Hz). For each subject, we computed the correlation coefficients between pupil size (*z*-score, no baseline removal) and relative duration for each time point between 1 s before and 1.5 s after the switch. The same number of data was used to compute each correlation coefficient (mean number of values by subject = 70, range = [21 121]). We also computed a correlation coefficient over all values. We performed the same analysis for the data of Experiment 2 (for the condition of spontaneous perceptual switches of ambiguous rotating diamonds), but much less data was available (10 subjects, mean number of values by subject = 13, range = [3 24]). We also performed the analyses using absolute postswitch duration (log values) instead of relative duration. In order to compare directly our results to those of Einhäuser et al. (2008), we also performed the
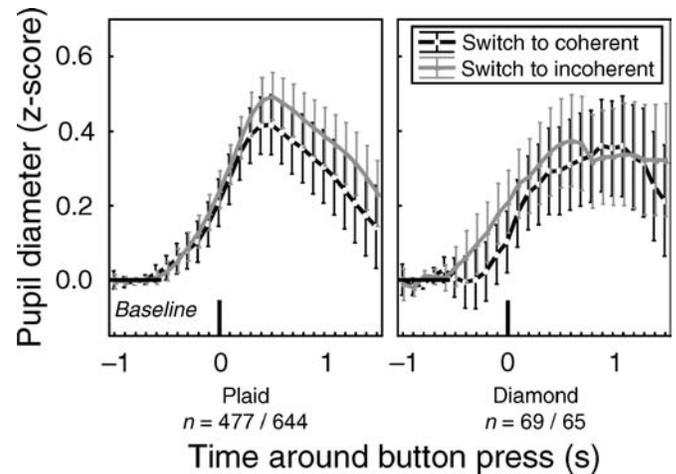

Figure 3. Percept type has no or little effect on the amplitude or timing of pupil responses (mean ± *SEM*). Same data set as in Figure 2. For spontaneous diamonds, we observed a tendency for the pupil response to start earlier for switches to incoherent percepts. However, amplitude difference between both traces did not reach significance: The stronger difference occurred at the time of button press ($F(1, 114) = 3.02$, $p = 0.098$, $\eta_p^2 = 0.14$). Inspection of data of individual subjects confirmed that this latency difference was not reliable in our data set.

correlation analyses without exclusion criteria. In addition, we considered several possible biases. We performed the analyses without *z*-transformation (pupil size was then measured in millimeters), or by selecting only trials for which competing percepts were well balanced (close to 50%), or by selecting only trials with slow alternations (with no other exclusion criteria). All these analyses produced converging results (they are not detailed here, but the figures and statistical results were sent to the reviewers).

Results

Pupil response to perceptual switches

The pupil response to perceptual switches is presented in Figure 2 for both experiments. Switches were indicated by subjects by releasing the mouse button indicating the previous percept and pressing the other mouse button to indicate the new percept. The time reference is the button press corresponding to the new percept. Pupil dilation started before the time of button press (average increase is visible as early as 500 ms and significant 400 ms before the button press for plaids). Pupil response was similar for both types of perceptual transition and for both stimuli (Figure 3). This decorrelation between the "sign" of a transition—from coherent to incoherent, or the reverse-and pupil changes indicates that pupil dilation is not

related to a change in—perceived—contrast or luminance. Maximum effect size (η_p^2) was 0.67 for both plaids and diamonds, 100 to 200 ms after button press for diamonds, 200 to 400 ms for plaids. Pupil response was, on average, 2.2% of mean pupil diameter for plaids (range for individual subjects: 0.1% to 6.6%) and 2.4% for diamonds (range of 1.1% to 4.8%). Average modulation corresponds to 5.2% (range of 0.2% to 15.6%) of pupil maximal dilation (see Methods section) for plaids and 4.9% for diamonds (range of 1.8% to 10.9%).

Components of the pupil response

Since pupil dynamics is rather slow (the latency of the PLR is about 300 ms, with an absolute minimum of 180 ms due to the mechanical properties of the iris muscle: Loewenfeld, 1999), pupil dilation in Figures 2 and 3 was caused by an event that happened as early as 700 ms before the button press. This event might have been related to the neural mechanism of bistability leading to percept switching (like the reorganization of the neural networks corresponding to each interpretation), the perceptual change itself (and its awareness), with a possible component of surprise, the decision to press the button, the motor preparation and command to press the button, or a combination of these processes. Attentional components may also modulate several stages of the processes. For example, a spontaneous switch of perception may transiently increase attention, or subjects may pay more attention to their percepts when they have to report it.

To try disentangling these different processes and evaluate their respective contributions to pupil dilation, we ran six additional conditions, intermingled with the main "spontaneously changing diamond" condition in the same observers (see Methods section, Experiment 2).

First, we tested whether pupil dilation reflected specifically the neural mechanism of perceptual bistability (independently of the percept switching itself) by comparing the pupil response to spontaneous and induced transitions. We designed a "diamond" condition where perceptual transitions were induced by stimulus manipulations. Specifically, we smoothly varied the contrast gradient along each segment (period = 8 s; one switch every 4 s), alternating between gradients that cause incoherent and coherent percepts, while keeping the same mean luminance. The left panel of Figure 4 shows that pupil dilation is very similar for spontaneous and induced transitions, at least for the onset and the peak of dilation. Pupil dilation was on average more sustained for spontaneous transitions, but the difference between the two curves did not reach significance. Inspection of individual data (Figure 5, blue and red curves) reveals that this lack of significance is unlikely to be due to a lack of power, since several subjects with clear pupil responses for both conditions did not exhibit at all this effect (see, e.g., subjects 2, 7, and 8). It is therefore unlikely that the *neural*
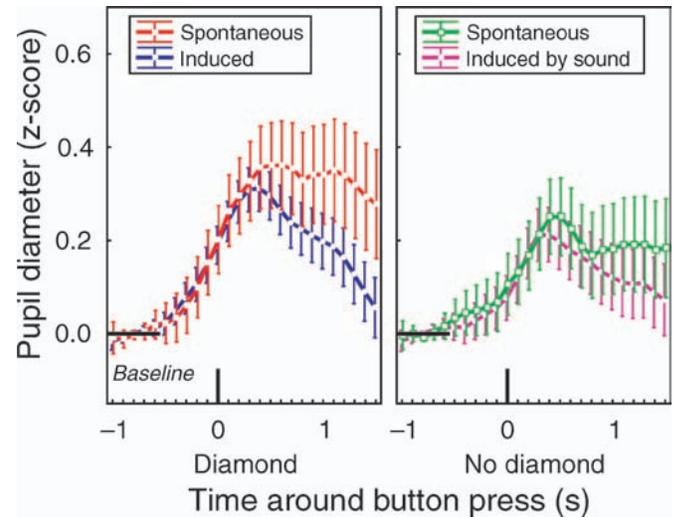

Figure 4. Pupil response for different tasks. The same 9 subjects ran all four experiments (subject 5 had too many blinks in the "green" condition and was therefore excluded in this graph). Red lines: Condition 1, diamonds were ambiguous and subjects experienced spontaneous changes of percept (same data set as in Figures 2 and 3). Blue lines: Condition 2, perceptual transitions were induced by stimulus manipulations. Purple lines: Condition 3, subjects were asked to switch mouse button each time there was a sound. Green lines: Condition 4, subjects were asked to press the mouse buttons randomly. In those two conditions, subjects were required to keep fixating a small disk at the center of a static occluded diamond (static version of the stimulus of Condition 1). Total number of events used for the computation of each PSTH was, respectively, 138, 320, 164, and 303 (20 outliers were removed). Pupil response was stronger in the presence of rotating diamonds from 100 ms before until 500 ms after button press (comparison between the curves of the left and those of the right panel; maximum effect of stimulus 100 ms after button press, $F(1,889) = 15.7$, $p = 0.003$, $\eta_p^2 = 0.63$). A trend for more sustained pupil dilation is visible for both "spontaneous" conditions, compared to the "induced" conditions, but the difference was not significant (comparison of the curves across both panels; maximum effect 1.5 s after button press, $F(1, 889) = 1.77$, $p = 0.22$, $\eta_p^2 = 0.18$).

mechanisms of perceptual bistability have a specific influence on pupil size. These two conditions differed also by both the timing and the possible prediction of percept switches. Spontaneous transitions happened at random times, with no warning, while induced perceptual transitions followed the periodic smooth changes of the stimulus and were therefore highly predictable. The lack of significant difference between the pupil responses in both conditions indicates that surprise and prediction have little effect on pupil dilation, at least for these experimental conditions.

We then tested whether neural processes related to decision and motor preparation were overall responsible for the changes in pupil size, with no specific influence of
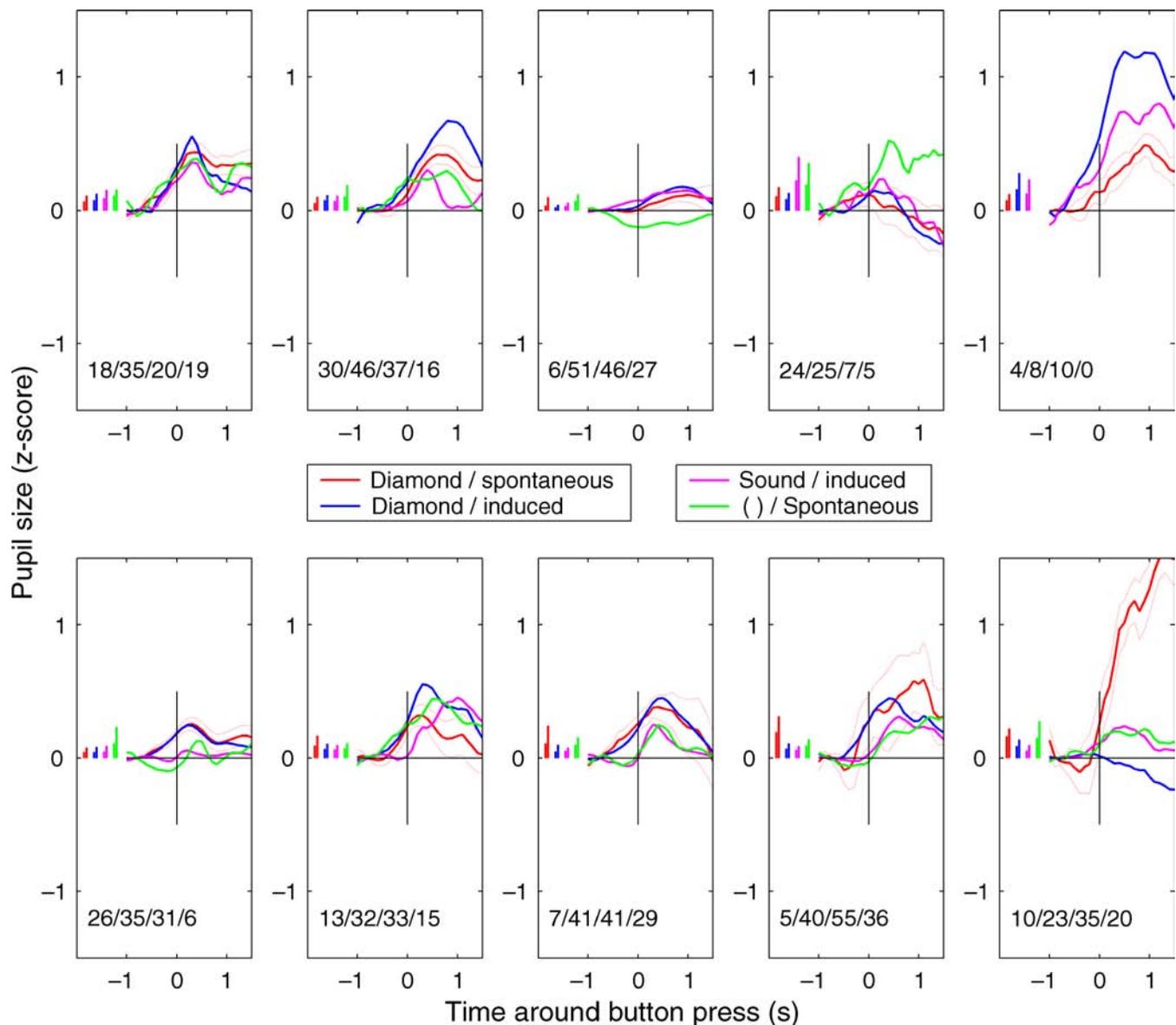
Figure 5. Data of individual subjects for perceptual reports. Same color conventions as in Figure 4. Baseline was [−1 −0.6] s before button press. *SEM* is plotted only for spontaneous perceptual switches of the diamond stimulus (pink lines around the red lines). Small vertical bars on the left of each panel indicate the average and maximum standard errors of the mean over time, for each condition and subject. The number of traces used to compute each PSTH is indicated within each panel (no outlier removal at this stage).

perceptual transitions. To that aim, we added two conditions with no change in visual perception. In the first one, we asked observers to switch button press after a brief sound produced every 4 s during 4 min. This timing was similar to the perceptual transitions induced by physical modulations of the contrast gradient in the diamond experiment. Since we were concerned that such exogenous drive would not resemble the endogenous inspection of one's perceptual state required with bistable stimuli, we also ran a second control condition where observers had to change button press at will, in the absence of any driving sensory stimulus (i.e., whenever they felt ready for it; endogenous drive). The right panel

of Figure 4 shows that pupil dilated around button presses, with a time course similar to that observed with bistable stimuli. However, the overall amplitude of pupil dilation was significantly less ($p = 0.003$, see legend of Figure 4) than that observed during the changing perception of diamonds, providing indirect evidence for a genuine contribution of perceptual alternations to pupil dynamics. On average, the decision and motor components contributed to 70% of the maximal pupil response (measured in both conditions 400 ms after button press). Inspection of individual data (Figure 5) makes it reasonably convincing that, despite large individual variability, pupil dilation was larger for the diamond conditions. The
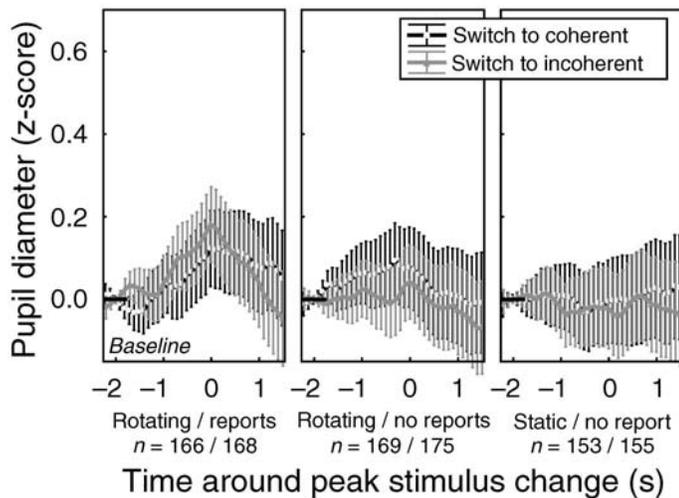
Figure 6. Pupil response after smooth changes of the diamond stimulus. The "rotating/reports" condition (left panel) is the same condition as the "diamond/induced" condition in Figure 4. The PSTH is computed over the same data but is now taking as time reference the stimulus change instead of the button press. Time zero corresponds to the time when the stimulus should induce the maximum of either coherence or incoherence. Stimulus cycle was 8 s, so a maximum was reached every 4 s. Traces were excluded if a blink occurred within 3.7 s before (i.e., 1.5 s before baseline) or 1.5 s after time zero. Average pupil diameter was computed for the period [−2.2 −1.8] s and subtracted. (Middle panel) Same stimulus, but subjects did not report their percepts. (Right panel) Static stimulus, no button press. The dynamics of local luminance changes were the same as in the other two conditions. The same 9 subjects as before (subject 5 had too many blinks in the static/ no report condition). Nine outlier values were removed. There was no effect of percept type. Luminance changes *per se* did not trigger any pupil response (right panel). There was a small pupil response due to the change of percept (middle panel, maximum response at time zero, average over both percepts, z-score = 0.07, $F(1,326) = 3.6$, $p = 0.08$, $\eta_p^2 = 0.25$). The effect of task was significant between −100 ms and 500 ms (maximum at 100 ms, $F(2, 932) = 5.25$, $p = 0.015$, $\eta_p^2 = 0.35$).

latency of pupil response was also shorter (by about 200 ms) when button presses followed perceptual switches, a further argument in favor of a perceptual contribution to pupil dilation. However, the latency difference could have been due to the amplitude difference (Figure 5, compare, e.g., subject 1 with subject 6 or 8). Comparing the pupil response to endogenously and exogenously driven button presses (the two curves of the right panel of Figure 4) revealed that pupil diameter decreased, on average, more slowly after an "endogenous" button press than after an "exogenous" button press. This effect was, however, not significant and probably due to random inter-subject variations (Figure 5). Several subjects with clear pupil responses for both conditions did not

exhibit at all this effect (compare the green and magenta curves for subjects 8, 9, and 10).

To further disentangle the specific contributions of perceptual and motor components to pupil dilation, we ran two additional conditions during which observers passively looked at the diamond stimulus for 4 min (no button press). We used the same smooth variations of contrast gradient along segments as before. In one condition (Condition 5), the segments were also moving in the same way as in Condition 2 (blue curve of Figure 4), in the other condition (Condition 6) the segments were stationary, in order to verify that variations of local luminance had no effect on pupil diameter by themselves. Pupil responses were now aligned to the reversals of contrast gradients (since there was no button press), that is the times of "higher terminator luminance/lower center luminance" and "lower luminance terminator/higher center luminance," which cause, respectively, incoherent and coherent percepts when the segments are moving. Figure 6 shows the results, together with the data of Figure 4 (blue curve) reprocessed in order to align the pupil response to the stimulus changes, instead of the button presses. Changing the contrast gradient of the stationary segments did not trigger any variation of pupil diameter (right panel). With a moving display inducing perceptual changes, there was a small, almost significant, pupil dilation (middle panel). This small variation is unlikely
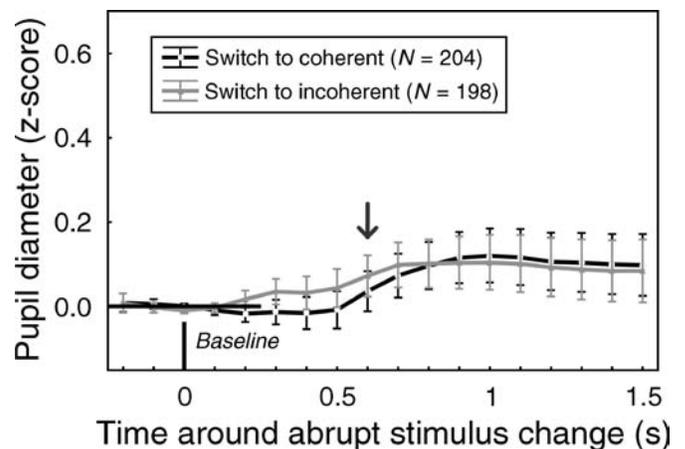

Figure 7. Pupil response after abrupt changes of the diamond stimulus. Each stimulus change was supposed to trigger a perceptual flip. Subjects did not indicate their percepts. The same 10 subjects as previously ($N = 402$, 8 outliers excluded). Traces were excluded if a blink occurred within 1.7 s before (i.e., 1.5 s before baseline) or 1.5 after the stimulus change. Average pupil diameter was computed for the period [−0.2 0.2] s and subtracted. There was no effect of the type of stimulus manipulation: Whether it should induce a switch to coherence or incoherence, there was a small increase of pupil diameter. On average, this increase was just significant 600 ms after the stimulus change (black arrow) and after (maximum significance at 700 ms, $F(1,382) = 5.83$, $p = 0.038$, $\eta_p^2 = 0.38$).

to be due to stimulus changes per se (considering the possibility that local luminance changes were more powerful for moving stimuli than for static ones), because pupil variations were not in opposite directions for both types of stimulus variations. Moreover, the time course of pupil dilation was similar to the one observed in the original condition, when button presses followed percept switches (left panel). When measuring the latency of those button presses relative to peak stimulus changes (time zero), we observed intra- and inter-subject variations. Induced perceptual switch was indicated by subjects 18% of the time at time −1 s, 50% at time zero, and 80% at time 1 s. This variation is larger than what we should expect from the motor command itself. This means that when smooth luminance variations are used, perceptual flips occur at slightly different times within and across
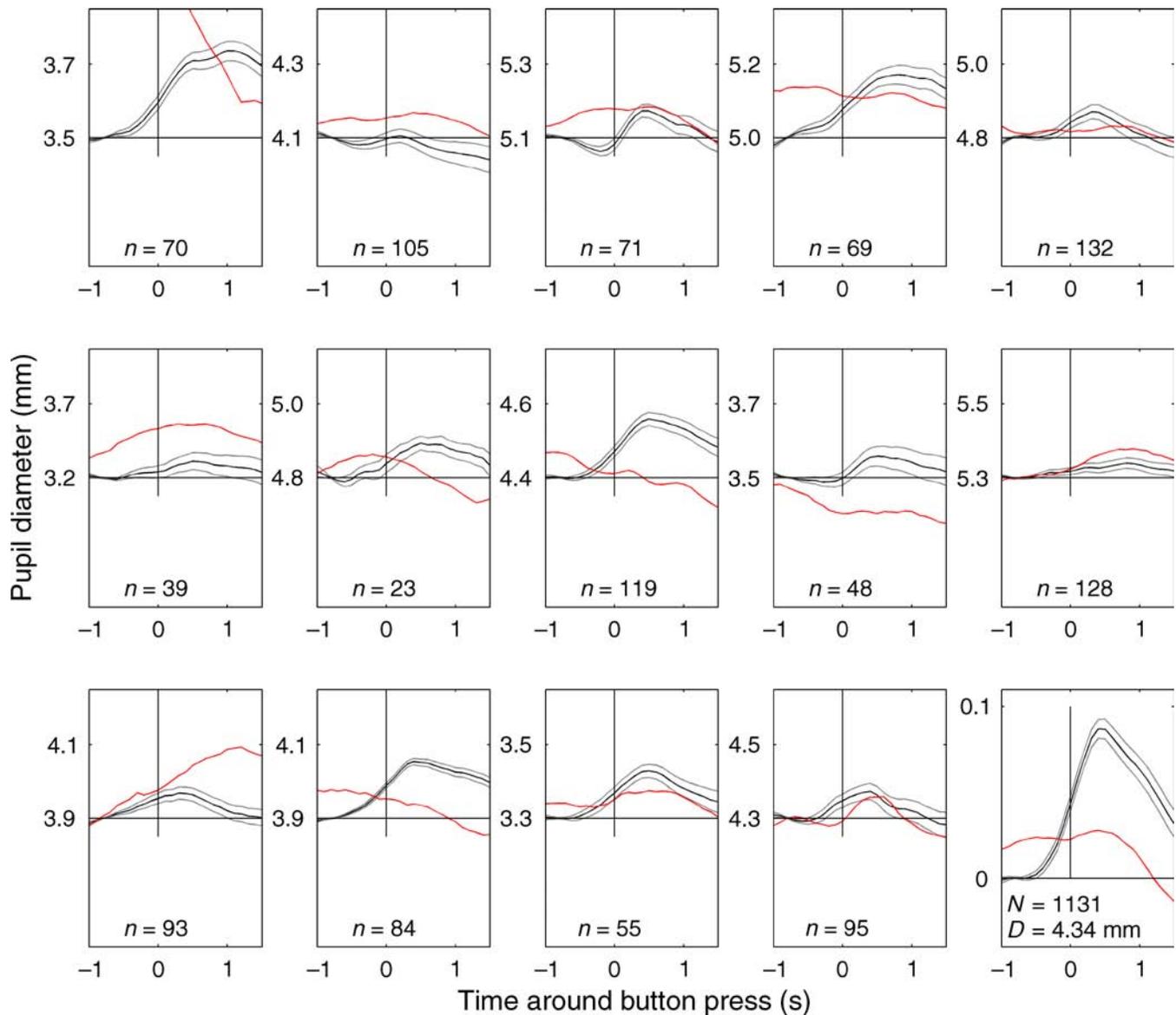

Figure 8. Pupil response and correlation analysis in the plaid experiment. Data for each subject and average (bottom right panel). Black curves: Pupil diameter as in Figure 2, but now measured in millimeters instead of *z*-scores, and with no outlier removed (mean ± *SEM*). Only switches that were not contaminated by blinks or other switches were included, as usual. Red curves: For each subject and time point, we measured the correlation coefficient between percept relative duration and pupil size (measured in millimeters). The *Y*-axis has the same scale as for pupil size but then indicates *r*-values plotted between −0.35 and 0.35 (horizontal lines denote zero correlation). In the bottom right panel, the correlation was computed over the data from the 14 subjects all together. *Y*-axis indicates *r*-values for the red curve and change of pupil diameter for the black curve (*D* indicates the average pupil diameter before button press). Correlations were always computed over less data than indicated in the figure panels, because relative duration could not be computed for the first and last transitions of each trial (total $N = 982$). *R*-values were not significantly different from zero and not modulated over time (max *r*-value 700 ms before button press, $r = 0.02$, $p = 0.61$, $\eta_P^2 = 0.0002$).

observers, maybe because of a hysteresis effect. When considering the effect of the unflagged perceptual flip on pupil dilation, averaging in reference to the stimulus changes may thus have "blurred" the effect.

In a last condition (condition 7), we abruptly and randomly changed the contrast distribution along the segments every 1, 2, 3, or 4 s, in order to trigger perceptual transitions with less time jitter than with smooth contrast variations. Subjects were still asked to passively look at the moving stimuli. Pupil dilation was modest but significant (Figure 7), with no difference between the "signs" of the contrast gradient.

Correlations between pupil diameter and percept duration

Overall, the results reveal the presence of two main components of the pupil response to perceptual switches indicated by button presses. The major component (about 70%) is correlated to the button press itself and may include attention, decision, and motor preparation and execution. The second, smaller, component is correlated to perceptual alternations, also possibly including an attentional component. Both components are present in the absence of any change in retinal illuminance and are therefore independent of the PLR. Perceptual changes can be induced either by exogenous stimulus manipulations or by endogenous perceptual reorganizations characteristic of bistable stimuli. The average pupil dilation is similar in both cases, but a possible relationship between pupil size and the mechanisms of bistable perception may have been subtler than a global effect on the amplitude of pupil dilatation. Einhäuser et al. (2008) suggested that pupil

dilation around the time of perceptual switches (and before button press) was predictive of the relative duration of the following percept. We tested this hypothesis for the bistable perception of ambiguous plaids (Experiment 1) and found that pupil size was not correlated to the relative duration of perceptual episodes in our data set (Figure 8). *R*-values of individual subjects were very small for most subjects. When computing the correlation over all data (bottom right panel), *r*-values were all very small and never reached significance, whether we computed pupil size in millimeters (as shown in the figure) or as *z*-score (max *r*-value 700 ms before button press, $r = 0.05$, $p = 0.11$, $\eta_p^2 = 0.003$).

We verified that this lack of effect was not due to our restrictive selection criteria. First of all, we computed a histogram of the percept durations selected to compute the correlations (not shown). We observed that even with short periods excluded, duration variability was large enough to have enough power for detecting potential correlations. Moreover, selected percepts followed the typical lognormal distribution. In addition, we varied our selection criteria, in order to balance possible biases and power, as indicated in the Methods section. Correlation only reached significance (while keeping a very small effect size) when applying exactly the same method as the one used by Einhäuser and colleagues, by using *z*-scores and with no exclusion criteria (max *r*-value 300 ms before button press, $r = 0.1$, $N = 2046$, $p = 3 \times 10^{-6}$, $\eta_p^2 = 0.01$). The same analysis but on raw pupil diameter, measured in millimeters, produced non-significant results (max *r*-value 500 ms before button press, $r = 0.02$, $N = 2046$, $p = 0.28$, $\eta_p^2 = 0.0006$). We also computed the correlations using the postswitch duration instead of the relative percept durations (not shown). We had to transform durations to their
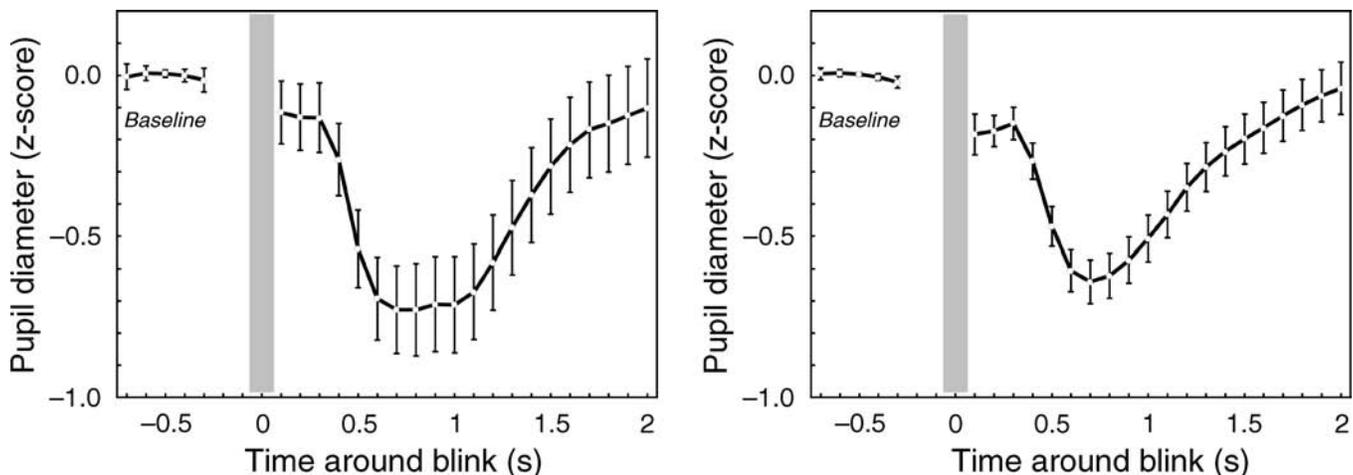

Figure 9. (Left) Pupil response around blinks in the plaid experiment (mean ± *SEM*). Data from 14 subjects, *N* = 417. The total number of blinks was 2267. Average pupil diameter was computed for the period [−0.7 −0.3] s before the beginning of each blink and subtracted to each trace. Blinks were excluded from the analysis if another blink or a button press occurred within 2.2 s before (i.e., 1.5 s before baseline) the beginning of the blink or 1.5 s after its end. Pupil diameter was significantly (*p* < 0.03) below zero between 400 ms and 1.5 s after the end of the blink. (Right) Pupil response around blinks in the diamond experiment. Data from 10 subjects, *N* = 492. The total number of blinks was 1980. Pupil diameter was significantly (*p* < 0.03) below zero between 400 ms and 1.5 s after the end of the blink.

log in order to compute sensible statistics. The results were similar (*r*-value 700 ms before button press, $r = 0.04$, $p = 0.20$, $\eta_p^2 = 0.02$; correlation computed over *z*-scores, with our exclusion criteria).

Pupil response to blinks

The time course of pupil size around blinks is displayed in Figure 9. There is a clear miosis after blinks in both the plaid (maximum effect 700 ms after blinking, $\eta_p^2 = 0.64$) and diamond experiments (maximum effect 600 ms after blinking, $\eta_p^2 = 0.68$). This constriction was significant for 12 out of the 14 subjects in the plaid experiment and 9 out of the 10 subjects in the diamond experiment. Miosis started about 350 ms after the end of a blink and lasted on average for more than 1 s. It was sometimes clearly visible on individual traces. Since blinks interrupt the visual input, reopening the eye might be equivalent to a flash of light. The time course of miosis after a blink is similar to the time course of the PLR, as measured in our experiments (see also Loewenfeld, 1999). Blink-induced miosis is, however, not exactly equivalent to the PLR, since there was no indication of pupil dilation during the blink, which could have been expected if a blink was equivalent to switching off the light: Pupil size was, on average, even slightly smaller just after a blink, and there was no positive correlation between blink duration and pupil size just after the blink (not shown). Second, there was also no correlation between blink duration and miosis peak amplitude (not shown).

Discussion

We recorded pupil size while subjects continuously reported their bistable percept of ambiguous moving stimuli. We observed reliable pupil dilation around the button presses indicating the percepts. Pupil dilation started 300 to 400 ms before a button press and lasted after it. In an attempt to dissect the origin of this pupil dilation, we ran several control experiments where observers either responded to—exogenous or endogenous—perceptual transitions, passively viewed the same stimuli, performed motor responses to a sound or at will. In addition, we verified that changing the contrast gradient along segments in the "diamond" experiment did not entail pupil changes by itself. From these control experiments, we could infer that both perceptual changes and motor responses contributed to pupil dilation. However, the correlation found between changes in pupil diameter and perceptual transitions does not necessarily mean that bistability is governed by non-visual—e.g., subcortical or frontal—structures or indicate that a non-visual generator of bistability is involved, because the magnitude of pupil

dilation was similar for spontaneous perceptual alternations and those triggered by physical manipulations of the stimulus. We also observed a clear constriction of the pupil after blinks in our data set, followed by a slow return to baseline.

Components of the pupil response

Pupil response to motor report of perceptual changes was, on average, 2.3% of pupil diameter, corresponding to 5% of pupil maximal dilation (Figure 2). Pupil changes around button presses were clearly visible on some but not all individual traces. Pupil dilation was also present when subjects had only to press a mouse button, either after a sound or at will. The amplitude of pupil dilation was, however, smaller than when button presses followed a perceptual change. By comparing both responses, we could estimate that the motor response *per se* (preparation and motor command) accounted for about 70% of the pupil dilation due to the reports of perceptual switches. The difference was significant (Figure 4) and reasonably consistent between subjects (Figure 5). Could this difference reflect a different attentional load in the test and control conditions rather than a perceptual influence on pupil size? The task may indeed have been slightly more demanding in the test condition with ambiguous stimuli, when subjects had to pay attention to unpredictable changes of their perceptual states. Control conditions were easy indeed. In one of them, subjects only had to press the mouse button at will. In another one, they had to switch button after each brief sound, occurring every 4 s. However, the task was also easy when percept changes were induced by smooth and periodic manipulations of the diamond stimulus. However, pupil dilation was not significantly smaller in that condition compared to the spontaneous condition (Figure 4), especially when considering the onset and peak response. It is therefore unlikely that difficulty or attentional load alone can account for the observed amplitude difference of pupil dilation.

More direct evidence of a perceptual component to pupil responses was obtained in the conditions where subjects passively looked at the stimulus. Percept changes *per se*, induced by physical changes of the stimulus, caused a small dilation of the pupil. We observed this response in two independent conditions, where perceptual changes were induced either by smooth and periodic or abrupt and random changes of the stimulus. Although the corresponding pupil responses were weak when considered independently, combining both data sets of Figures 6 (middle panel) and 7, aligning the peak responses (so time zero of the smoothed changes was aligned to time 1 s of the abrupt changes), maximum dilation was 0.1 (*z*-score) and $p = 0.005$ ($F(1, 695) = 10.8$, $\eta_p^2 = 0.43$). In addition, pupil response was similar for switches to coherency and switches to incoherency.

An important complementary result was the lack of pupil dilation for the static condition (right panel of Figure 6), which ascertains that the local contrast change used to induce perceptual switches did not trigger any pupil response by itself. Thus, the absence of pupil modulation indicates that pupil does not reflect *any* perceptual change but rather shows some degree of specificity.

Altogether, these results corroborate previous reports that changes in pupil size are coupled to perceptual changes and can signal visual cortical processes (see below). Pupil dilation is, however, small and cannot, at least in our hands, be measured on a trial-by-trial basis to infer a perceptual state without an objective report from the observer. Thus although pupil dynamics is a physiological signal related to perception, using it to read-out perceptual states appears limited.

Perceptual bistability and pupil response

Since pupil dilation follows specific and not any perceptual changes, an exciting possibility was that pupil dilation reflected the dynamics of neural events leading to percept changes in the absence of any stimulus modification. In other words, endogenous perceptual reorganization leading to bistable perception could reflect in the pupil. Unfortunately, this was not the case, since pupil dilation was similar for spontaneous changes of a constant ambiguous stimulus and for perceptual transitions induced by physical modifications of the stimulus (Figure 4, left). Our observation corroborates the result of Einhäuser et al. (2008), who had run a "replay" condition for two ambiguous displays (plaid and structure from motion), where induced transitions followed the time course of spontaneous percept switches recorded in a previous condition. They found that pupil modulation was very similar for the spontaneous and induced switches (see their Figure 2C).

However, there could have been more information in the pupil signal than its average amplitude. Einhäuser et al. (2008) had the original idea to test whether pupil size was correlated to the duration of perceptual episodes. They found the relative amount of dilation before switching being a weak but significant predictor of the duration of the subsequent percept. Noticing that pupil dilation is correlated to levels of norepinephrine (NE) released from the locus coeruleus (LC; Aston-Jones & Cohen, 2005), they suggested that NE could play a critical role in perceptual bistability. However, although we performed similar analyses using a larger data set, we were not able to replicate this finding. We already indicated in a commentary one possible reason that may explain the discrepant results between both studies (Hupé, Lamirel, & Lorenceau, 2008). In light of the data presented here (as well as the many additional analyses not shown here—see Methods section), we emphasize now what we think are the major controversial issues.

1. First of all, the correlation measured by Einhäuser et al. (2008) was very weak (max $r = 0.13$, so $r^2 = 0.017$; value obtained by pooling the data of six subjects obtained in four different experiments). Such a weak result was therefore in demand of confirmation. With 14 subjects, our correlation values never reached above 0.1 ($r^2 = 0.01$), even when applying possibly biased methods (see below).

2. Einhäuser et al. (2008) plotted *p*-values over time and not *r*-values, without indicating the number of values used to compute the correlation at each time point. They obtained a peak of significance before the button press, very suggestive of an interesting (even though weak) phenomenon. However, this peak may reflect only the greater number of values used for these time points. Indeed, to avoid using the same data point multiple times, they truncated each trace at the midpoints between switches. However, they used all their data points to compute the correlations. Since percept durations are variable, following a lognormal distribution (many short periods and a few very long periods), there were more data points around the time of switch compared to a few seconds before or after the switch. Applying exactly the same procedure on our data set, we did observe a peak of *p*-value around switch time, but this was only due to the greater number of values; *r*-values were positive and rather constant (around 0.1; not shown). In our analyses, we were careful to use the same number of values for each time point.

3. Measures of duration and pupil size are in fact not independent, mainly because of the slow dynamics of pupil dilation that encompass short perceptual episodes (Hupé, Lamirel et al., 2008). For percepts shorter than 3 s, switch-related pupil dilation could last until the next switch. In our data set, given the overall distribution of percept durations, the next percept lasted on average longer, which may be the cause of the positive correlation between pupil size and relative duration. Indeed, we observed that the correlation could be significant only when including short percepts (note however that the correlation was weak and even not significant if using raw data instead of *z*-scores). In their reply to our letter, Einhäuser and colleagues indicated that they did not observe that percepts tended to be longer after short percepts (<3 s). In fact, such a relation is expected only when one percept is more likely than the other, but the opposite relationship holds for variable switch rates. On a whole set of data, both relationships may exist. Einhäuser and colleagues did not try to exclude short percepts.

In their reply to our letter, Einhäuser et al. argue that if their correlation was an artifact due to short percept durations, they should have observed it also in their

"replay" condition. However, this is true only in a purely deterministic model, that is if each percept transition triggered a pupil response, or if the correlation was strong. In fact, pupil dilation is highly variable and the effect is robust only when averaging many trials. Their observation that pupil dilation is on average similar in the rivalry and replay condition of their Experiment 2 (see their Figure 2) does not mean that each transition contributed equally to the mean response in both conditions.

Pupil response to motor and to perceptual processes

One major but intriguing finding of the present study is the observation of a reliable pupil dilation correlated to a motor response, when, for example, observers had to press a key at will (Figure 4, green curve). This result corroborates a result by Simpson (1969) who observed enhanced pupil dilation for subjects who had to press a key to fulfill a pitch discrimination task, as well as pupil dilation for sound-induced keypresses irrelevant to the task. Since many experiments in cognitive neuroscience include a motor response, pupil dilation that may in turn entail a visual response (see below) needs to be stressed, at least for methodological if not theoretical purposes. It has long been known that pupil dilation is related to arousal. "In alert individuals, excitatory impulses arise from the cerebral cortex and travel through the reticular activating system and hypothalamus to cause pupil dilation" (Merritt et al., 2004). Loewenfeld (1999) notes that the reticular system in the brainstem receives connections from afferent sensory fibers as well as efferent motor fibers, accounting for pupillary dilation observed to about any sensory stimulation. The locus coeruleus (LC) is probably a key structure in this network, since it is involved in the regulation of sleep and arousal, exhibits an activity well correlated with decision processes and motor responses (Aston-Jones & Cohen, 2005), and projects to the region of the Edinger–Westphal nucleus (EW) driving the pupil (at least in cat: Breen et al., 1983). The LC could therefore be involved in pupil dilation related to manual motor responses.

The enhanced pupil response during reports of bistable perceptual changes as compared to a simple motor response further raises the possibility that cortical processing also influences pupil size (see above, "Components of the pupil response"), if one considers that bistable perception specifically involves cortical mechanisms. Hupé, Joffo, and Pressnitzer (2008) have defended, with others, the idea that perceptual bistability is generated at the level where the competing percepts are encoded, that is, the visual cortex for plaids or ambiguous diamonds (Fang, Kersten, & Murray, 2008; Lorenceau et al., 2008). In our experiments, the stimulus remained identical over time, providing a constant input to retinal ganglion cells

and subcortical structures. The observation that the PON, for example, receives significant cortical (e.g., V1, V4, IT, LIP, FEF) input, admittedly in addition to ventral thalamic (e.g., LGN), and midbrain (e.g., superior colliculus) input (Gamlin, 2006) provides evidence that an influence on pupil size originating in the visual cortex is possible. One issue is to uncover the nature of this potential influence, namely whether it induces a dilation, as observed here for bistable stimuli, or a constriction of the pupil as observed in other studies detailed below.

Pupil constriction is characteristically observed for the pupil light reflex (PLR), which is due to an increase of light. However, many stimulus changes made at constant illumination also trigger pupil constrictions and could involve cortical processing (see Barbur, 2004, for a review). The PLR originates in melanopsin-containing, intrinsically photosensitive, retinal ganglion cells (ipRGC), a special class of RGC that do not project to the LGN but to the PON (Guler et al., 2008). Melanopsin-based signals are slow and code the average illumination of the retina, so fast pupil responses to changes of visual stimuli are triggered by rods and cones whose signals converge to ipRGCs before being sent to the PON. Therefore, any stimulus change that trigger a retinal response, and not only a luminance change, may trigger the PLR. The strongest evidence in favor of a cortical component to the PLR is in fact based on lesion studies. Sahraie and Barbur (1997) observed pupil constriction when incoherent moving random dots were physically switched to a coherent motion flow, and they observed a reduction of this constriction for patient GY when the stimuli were presented in his blind hemifield. Barbur et al. (1999) observed that the PLR to isoluminant green (but curiously, not red) stimuli was abolished in the blind hemifield of two patients with cortical lesions. Wilhelm et al. (2002) observed that patients with Parinaud's syndrome, who had no pupil response to light changes, still displayed pupil constriction to gratings and isoluminant red stimuli. Since the PON of these patients was probably destroyed, this result argues strongly in favor of a pupil pathway independent of the ipRGC–PON pathway. They proposed that pupil constriction for these stimuli was due to cortical modulation of central inhibition at the level of the EW nucleus. Conway et al. (2008) reached a similar conclusion after observing a pupil constriction stronger for upright than for inverted images of faces. This effect was present only for human faces, not monkey faces, so they took it as the likely signature of a cortical process within the infero-temporal "face" area.

It is possible that neuronal activity within the visual cortex entails either dilation or constriction, depending on the mechanisms specifically involved. However, it is also possible that either the constrictions described above or the dilation observed in our study does not originate within the visual cortex. In favor of the first hypothesis, Loewenfeld suggested that reduced pupillary light reflex

in patients with cortical lesions are accounted for by retrograde trans-synaptic degeneration following a supra-geniculate lesion, eventually causing infrageniculate fiber loss (Loewenfeld, 1999, p. 220). Accordingly, she noted that deficits of the PLR always needed some time after the lesion in order to develop. Similarly, the effects of GY cortical lesion on pupil responses may indeed be due to neuronal degeneration within subcortical structures. Weiskrantz, Cowey, and Le Mare (1998) observed similar, specific, changes like decrease of spatial frequency sensitivity of the PLR for GY and after V1 lesions in monkeys. In the latter case, "the left dorsal lateral geniculate nucleus was uniformly and totally degenerated throughout". As noted above, the PON receives inputs from many structures, including the LGN. The PON may, however, not be invoked to explain the pupil responses observed in Parinaud patients (Wilhelm et al., 2002), but this does not exclude the possible involvement of other subcortical mechanisms (rather than cortical ones), possibly related to those of the near reflex, which is preserved. Concerning the study by Conway et al. (2008), they did not consider that faces undoubtedly have an emotional component, possibly involving early subcortical activity (Morris, Öhman, & Dolan, 1998). In addition, the stronger constriction for upright faces could be equally understood as a dilation component added to the constriction for inverted faces. This dilation component could be due to a greater (involuntary) effort to try to recognize the inverted face. The use of a constant visual input, as in our study, allowed us to better (if not totally) control for subcortical modifications. On the other hand, the fact that we observed dilation and not constriction leaves open the issue of whether the perceptual processes within the visual cortex are the origin of the observed pupil dilation or whether it is mediated by modulation of (possibly supra-modal) attentional processes or mental workload. More-over, several fMRI studies report a frontal activity during bistable perception (e.g., Sterzer, Russ, Preibisch, & Kleinschmidt, 2002). The existing projections from the frontal cortex to the hypothalamus driving the dilator of the pupil further suggests that frontal cortex activity may reflect in the pupil. In light of all these considerations, the questions of whether neuronal activity within the visual cortex reflects or not in the pupil and whether it may result in dilation or constriction of the pupil (or both) are still at stake.

Pupil response to blinks

We were surprised to observe a large and systematic miosis after blinks. We had to take these modulations into account in order to exclude postblink periods for our analyses of pupil size (see Methods section). We are not aware that such precautions were taken in other studies, or that such changes were even systematically observed. Fukuda, Stern, Brown, and Russo (2005) even observed pupil dilation after blinks. However, Loewenfeld (1999, p. 402) observed that blinks were "followed by small pupillary contractions and redilations", whose time course was similar to the one we observed. Such modulations were not observed when blinks happened in darkness, suggesting that this response is caused by the dark periods of lid closure and is therefore similar to the "darkness reflex", where retina gains sensitivity during short inter-ruptions of the light. With dim light conditions or short dark pauses (less than 500 ms), dilation of the pupil during the short dark period could be absent while the contrac-tion–redilation response was still present (Lowenstein & Loewenfeld, 1969, cited by Loewenfeld, 1999, p. 292), which may explain why we did not observe any dilation of the pupil just at the end of the blink. For the darkness reflex, however, the amplitude of the response depends on the duration of the dark period (Stark, 1962, cited by Loewenfeld, 1999, p. 293). We did not observe such a relationship with blinks, probably because blink durations did not vary enough. Most blinks lasted less than 500 ms, a duration where retinal sensitivity measured for the amplitude of the darkness reflex is poorly modulated by dark duration (*ibid.*). It is surprising that Fukuda et al. (2005) did not observe any pupil constriction after blinks. Maybe the "darkness reflex" could be inhibited under certain experimental conditions. It should be reminded that the pupil is controlled through an antagonistic influence of the sympathetic and parasympathetic path-ways and that several modes of pupil control have been considered as inhibition and excitation may interplay in a complex non-linear way (Yamaji, Hirata, & Usui, 2000). Note that these antagonistic activities may strongly depend upon the background and stimulus luminance, contrast or color, stimulus duration and characteristics, such that the outcome response may largely vary with these parameters.

Consequences for imaging studies

The observation of long-lasting changes in pupil diameter (around 1.5 s in the present study), be they due to perceptual changes, button presses, or blinks, suggest that neural activity (see below) and visual processing (e.g., contrast sensitivity) may be affected by these changes. Although the changes in pupil diameter reported herein are small (about 2% of pupil diameter), they correspond to non-negligible changes in pupil surface, thus changing the flux of light entering the eyes, as more clearly demonstrated when considering pupil size mea-sured in millimeters. The average increase of pupil diameter was 0.14 mm (range of 0.01 to 0.33 mm), corresponding to an average increased surface of 1.2 mm^2 (range of 0.06 to 4.6 mm^2), or 5.4% (0.5% to 13.5%). The average decrease of pupil diameter after blinks was 0.19 mm (range of 0 to 0.43 mm), corresponding to an

average decreased surface of 1.3 mm^2 (range of 0 to 3.5 mm^2), or 8.2% (0% to 15.1%).

All these values are somehow larger than the 0.02-mm average pupil dilation measured by Fukuda et al. (2005), which they relate to higher order processes, namely the "readiness to acquire new visual information", or the about 0.03 mm observed by Conway et al. (2008) for inverted vs. upright faces (see their Figure 2).

Accordingly, a change in pupil diameter unrelated to a change in a visual parameter, as those reported herein, entails a change in retinal illumination. Given the present findings, an intriguing possibility is that pupil dilation related to a cognitive or motor response elicits changing neuronal responses in the retina. This could be the case for a number of reasons: changing pupil size induces variations in the photon flux that could modulate the photoreceptors activity, and possibly the activity of the corresponding ganglion cells; opening or closing the pupil changes the optical characteristics of the eye, such as the depth field, which could also change the spatial frequency related activity, by recruiting ganglion cells tuned to different spatial frequencies (Campbell & Gubish, 1966; Strang, Atchison, & Woods, 1999). The neuronal responses elicited by these changes could in turn propagate through the thalamo-cortical pathways and modulate the neuronal responses in striate and extrastriate areas. If true, these would have two main consequences. One is that such a pupil-related cortical activity might modulate visual sensitivity in psychophysical detection tasks. The second is that changes of pupil size provide a possible source of visual noise that could be taken into account when analyzing fMRI, EEG, or MEG data, thus possibly improving the signal-to-noise ratio. Although this may appear speculative at first sight, Siegle, Steinhauer, Stenger, Konecky, and Carter (2003) used pupil variations as a regressor in their analysis of fMRI data, which did improve the sensitivity of their analyses.

We also speculate that the change of pupil diameter has a stronger effect on the ganglion cells situated at the periphery of the retina, since they would be rapidly covered or uncovered by the iris. For these neurons, changes of pupil diameter correspond to ON/OFF responses. These responses are not inhibited in any way at the level of the retina, since they can trigger the powerful "edge-light cycles" of pupil oscillations when the retina is illuminated only at the edge of the iris (Loewenfeld, 1999). Modulations of pupil diameter may therefore trigger neuronal responses in the parts of the visual cortex specifically coding the periphery of the visual field. Since blinks are followed by a biphasic pupil response, measured neural responses to blinks might be due in part to the change of pupil size, and not only to the brief shutting off of the visual input (which is usually not consciously detected, a phenomenon described as "blink suppression"; see, e.g., Bristow, Frith, & Rees, 2005. The following references dealt with the mechanisms of either blink suppression or blinking control. We cite them here

within a different context). Blinks occurring in light conditions trigger activity in the occipital cortex, including V1, as measured with fMRI (Bodis-Wollner, Bucher, & Seelos, 1999; Bristow et al., 2005; Kato & Miyauchi, 2003; Yoon, Chung, Song, & Park, 2005), EEG (Berg & Davies, 1988), and MEG (Bardouille, Picton, & Ross, 2006). Interestingly, activity is stronger in (or even limited to) the anterior calcarine sulcus, where V1 codes the periphery of the visual field (Stenbacka & Vanni, 2007a, 2007b), even though subjects only had to maintain their gaze over a fixation point (e.g., Bardouille et al., 2006; Bristow et al., 2005; Yoon et al., 2005). We observed similar blink-related BOLD activity in the retinotopically defined periphery of V1, although stimuli were presented only centrally (J.-M. Hupé & M. Dojat, unpublished observations). We are not aware that this specific activity in the periphery of V1 was either stressed out or explained (except by Simo Vanni, several years ago already: personal communication; see also Stenbacka & Vanni, 2007b). The observed 200- to 300-ms latency of these responses (Bardouille et al., 2006; Berg & Davies, 1988) is compatible (though a bit too early) with the time course of blink-induced pupil responses. A question at stake is therefore whether peripheral V1 activity related to blinks, when measured in light conditions, is only due to the mechanisms of blink suppression, or whether it is also related to blink-induced changes of pupil size.

Conclusions

The present work shows that pupil dilation accompanies changes in perception with unchanging bistable stimuli. The several experiments ran to identify the origin of pupil dilation suggests that it reflects different components related to both changes in perception and motor commands. The presence of a "true" perceptual component supports the hypothesis of an influence of activity within the visual cortex on pupil size. Although pupil dilation may not allow the decoding of perceptual states on a trial-by-trial basis, it could influence activity in the retino-geniculo-cortical pathways. Taking pupil activity into account may thus improve the signal-to-noise ratio in psychophysical or imaging research. Is there a functional interpretation of the pupil response to perceptual transitions? Einhäuser et al. (2008) suggest that pupil dilation reflects a consolidation of a perceptual state. Additional studies addressing more directly the individual long-term perceptual dynamics and its correlation to the pupillary responses seem necessary before such a statement can be made.

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