Pupil size tracks perceptual content and surprise

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Abstract

Changes in pupil size at constant light levels reflect the activity of neuromodulatory brainstem centers that control global brain state. These endogenously driven pupil dynamics can be synchronized with cognitive acts. For example, the pupil dilates during the spontaneous switches of perception of a constant sensory input in bistable perceptual illusions. It is unknown whether this pupil dilation only indicates the occurrence of perceptual switches, or also their content. Here, we measured pupil diameter in human subjects reporting the subjective disappearance and re-appearance of a physically constant visual target surrounded by a moving pattern ('motion-induced blindness' illusion). We show that the pupil dilates during the perceptual switches in the illusion and a stimulus-evoked 'replay' of that illusion. Critically, the switch-related pupil dilation encodes perceptual content, with larger amplitude for disappearance than re-appearance. This difference in pupil response amplitude enables prediction of the type of report (disappearance vs. re-appearance) on individual switches (receiver-operating characteristic: 61%). The amplitude difference is independent of the relative durations of target-visible and target-invisible intervals and subjects' overt behavioral report of the perceptual switches. Further, we show that pupil dilation during the replay also scales with the level of surprise about the timing of switches, but there is no evidence for an interaction between the effects of surprise and perceptual content on the pupil response. Taken together, our results suggest that pupil-linked brain systems track both the content of, and surprise about, perceptual events.

Introduction

It has long been known that the diameter of one's pupil changes during cognitive acts (Hess & Polt, 1964; Kahneman & Beatty, 1966). Specifically, the pupil dilates when subjects engage in cognitive tasks, independent of the level of retinal illumination (Einhauser et al., 2010; Preuschoff et al., 2011; Fiedler & Glockner, 2012; Wierda et al., 2012; Zylberberg et al., 2012; Shalom et al., 2013; de Gee et al., 2014). These pupil responses are associated with changes in the gain of neural interactions in the cerebral cortex (Eldar et al., 2013; Reimer et al., 2014). Indeed, mounting evidence suggests that the same brainstem centers that control fast changes in cortical state also control non-luminance-mediated pupil dynamics – in particular the noradrenergic system (Aston-Jones & Cohen, 2005; Murphy et al., 2014). Other brainstem systems such as the cholinergic system (Sarter et al., 2009; Lee & Dan, 2012; Yu, 2012) and the superior colliculus (Wang et al., 2012) involved in attentional control may also contribute to task-related pupil dynamics. Task-related pupil responses, therefore, provide a window into fast fluctuations in global brain state during cognitive processing.

In particular, the pupil dilates during illusory perceptual switches in so-called multi-stable perceptual phenomena, in which a constant sensory input induces illusory switches in perception (Einhauser et al., 2008; Hupe et al., 2009; Frassle et al., 2014). Similar dilation occurs during the stimulus-evoked replay of the perceptual switches (Einhauser et al., 2008; Hupe et al., 2009; Frassle et al., 2014). While the button press typically used for reporting the perceptual switches can account for part of the pupil dilation, significant dilation remains in the absence of any overt motor response (Hupe et al., 2009).

Here, we asked whether the switch-related pupil dilations only track the occurrence of perceptual switches, or if they also contain information about the content of perception. In some illusions, perception alternates between two asymmetric states. For example, in 'motion-induced blindness' (MIB), a salient visual target surrounded by a moving mask spontaneously disappears from perception, and then reappears after some time (Bonneh et al., 2001, 2014; Bonneh & Donner, 2011). This subjective target disappearance and

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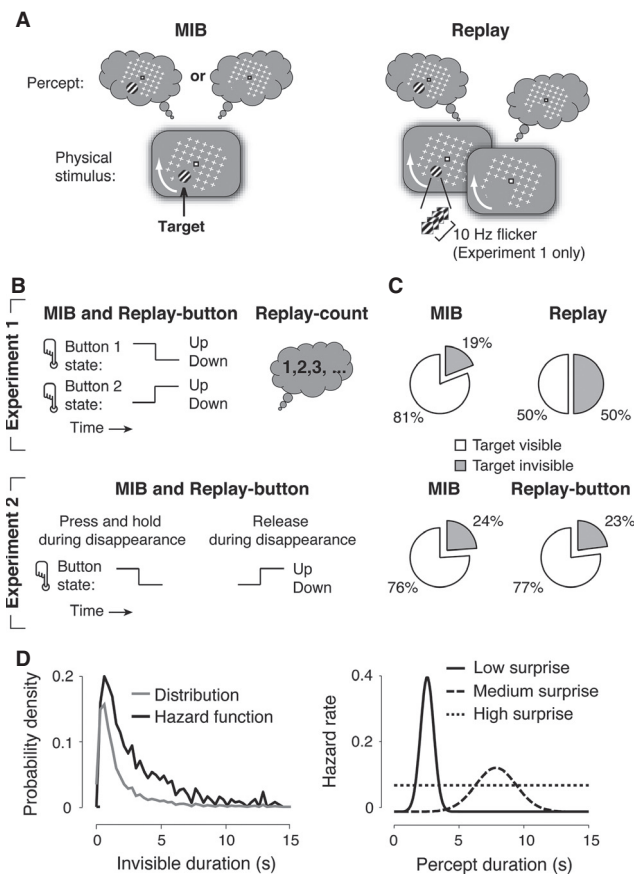E-mail: niels.kloosterman@gmail.com

FIG. 1. Stimuli and behavioral tasks. (A) Schematic snapshots of stimuli and alternating perception. Bottom left – motion-induced blindness (MIB) stimulus. A salient target stimulus (Gabor patch) was surrounded by a moving mask pattern (white), which appeared as a rotating grid. Top left, fluctuating perception of the target due to MIB. Right, corresponding stimulus and alternating perception during Replay. Replay stimuli were identical in Experiments 1 and 2, except that the target flickered at 10 Hz in Experiment 1. (B) Behavioral response regimes. Top, Experiment 1 – during MIB and Replay-button, subjects switched button press of two buttons. During Replay-count, subjects covertly counted the disappearances during the 3-min run, and reported the total after the end of the run in a 4AFC question. Bottom, Experiment 2 – subjects either pressed and held or released to report target disappearance (complementary for re-appearance). (C) Corresponding fraction of total target visible and invisible percept durations in the MIB and Replay conditions. Target onset and offset durations in Replay in Experiment 1 (top right) were sampled from the same distributions. (D) Left, distributions and corresponding hazard function of MIB durations in Experiment 1. Right, hazard functions used for generating percept durations during Replay in Experiment 1. The intervals between events were drawn from the probability distributions (not shown) corresponding to the three hazard functions shown here. Because subjects' expectation of event timing follows the hazard rate, events occurring around the mean interval in both conditions will elicit different levels of surprise.

re-appearance might be associated with different levels of cognitive engagement, which, in turn, might be reflected in the activity of pupil-linked brainstem systems.

We monitored pupil diameter of human subjects reporting their perceptual switches during MIB (Fig. 1A, left) and a physical replay of that illusion (Fig. 1A, right). We tested whether the subjective difference between target disappearance and re-appearance might be reflected in a difference of the associated pupil dilation. Because surprise about behaviorally relevant events has been shown to drive pupil dilation (Preuschoff *et al.*, 2011; Nassar *et al.*, 2012; Naber *et al.*, 2013) and the timing of perceptual switches in bistable illusions is typically unpredictable and hence surprising, we also investigated whether pupil dilation amplitude during replay tracked surprise about switch timing and if this surprise effect might interact with the effect of perceptual content.

Materials and methods

General stimuli and procedure

We measured pupil diameter during the MIB illusion (Fig. 1A, left) as well as during a number of control conditions collectively referred to as Replay, in which the target was intermittently removed from the screen for some period of time (Fig. 1A, right, and see below for details). The target was a full contrast black-and-white Gabor patch (diameter: 2°) located at 5° eccentricity in one of the four visual field quadrants selected individually for each subject to yield the maximum percentage of target invisible time. Because the mean luminance of the Gabor patch was equal to the luminance of the gray background, overall stimulus luminance remained constant during the target onsets and offsets in the Replay conditions. The target was surrounded by a rotating mask (17° × 17° grid of white crosses), separated from the target by a gray 'protection zone' subtending about 2° around the target. The fixation mark at the center of the screen was a square with red outline and white inside (0.8° width/length). Subjects were asked to monitor and report the disappearance and re-appearance of the target via various motor response regimes (Fig. 1B, and see below for details). Stimuli were presented and responses recorded by means of the Presentation software (NeuroBehavioral Systems, Albany, CA, USA). The diameter of the left eye's pupil was sampled at 1000 Hz with an average spatial resolution of 15–30 min arc, using an EyeLink 1000 697 Desktop Mount (SR Research, Osgoode, ON, Canada).

In this paper, we report data from two experiments. The main experiment was designed to assess the modulation of the pupil response by: (i) perceptual content; (ii) surprise about switch timing; and (iii) motor response. It also entailed magneto-encephalography recordings, which will be reported in a separate paper. The analyses of the main experiment showed a robust modulation of pupil response by perceptual content, which has not previously been shown. To replicate this effect in an independent sample, we also analysed data from a previously reported, within-subjects, placebo-controlled, pharmacological study of the perceptual dynamics in MIB (van Loon *et al.*, 2013). In this study, subjects were administered either a placebo or 1.5 mg of the γ-aminobutyric acid (GABA)-A receptor agonist lorazepam in each of two sessions (double-blind; session order counterbalanced across sessions). Because we found no differences between pupil responses in the two pharmacological conditions during MIB (Fig. S1), we collapsed across these conditions in the pupil results reported in this paper.

Experiment 1 (main experiment)

Subjects

Twenty-two subjects participated in the experiment (10 female, age range 20–54 years). Three subjects were excluded: one subject due to failure to complete all the sessions; one due to bad quality of the eye-tracking data; and one due to reaction times consistently being longer than 1 s during one of the Replay conditions. Thus, 19 subjects (10 female, age range 20–54 years) were included in the analysis. All subjects had normal or corrected-to-normal vision, and gave written informed consent. The experiment was approved by the

ethics committee of the Universitätsklinikum Hamburg Eppendorf in accordance with the Declaration of Helsinki.

Stimulus

The Gabor target contained two spatial cycles. During the Replay conditions, the different parts of the Gabor modulated at opposite phase at a temporal frequency of 10 Hz. The resulting counter-phase flicker rendered the target more salient and thus minimized the number of illusory target disappearances during Replay. The mask rotated at a speed of 160°/s. Stimuli were back-projected on a transparent screen using a Sanyo PLC-XP51 projector with a resolution of 1024 × 768 pixels at 60 Hz. Subjects were seated 58 cm from the screen in a dimly lit room.

Task

During the MIB and half of the Replay runs (henceforth called Replay-button condition), subjects were asked to press and hold a button with their right index finger during target disappearance and another button with their right middle finger during target re-appearance (Fig. 1B, top panel, left). In the other half of the Replay runs (henceforth called Replay-count condition), subjects were instructed to silently count the number of target offsets and report the total in a four-alternative forced-choice question displayed on the screen after the end of the run (Fig. 1B, top panel, right). There were always one correct and three incorrect numbers; the incorrect numbers were generated by randomly subtracting or adding 1, 2 or 3 from the actual number of disappearances, under the constraint that the four alternatives were all different from each other. Replay-button or Replay-count conditions were randomly selected before each run, under the constraint that each would occur equally often. The corresponding instructions were displayed on the screen and communicated verbally by the experimenter before each run. Each run had a duration of 3 min.

To test for an effect of surprise on the pupil dilation amplitudes, the predictability of the timing of perceptual events (stimulus disappearance or re-appearance) was manipulated during three different Replay conditions. To this end, the intervals between physical target offsets and onsets were randomly sampled from three different distributions, corresponding to narrow, intermediate and broad hazard functions (Fig. 1D). The hazard function describes the probability that an event will occur at a particular point in time, given that it has not occurred yet (Luce, 1986), and is computed as

$$\lambda(t) = \frac{f(t)}{1 - f(t)}, \tag{1}$$

where $f(t)$ is the value of distribution f at time t, $\lambda(t)$ is the value of the hazard function at t, and $f(t)$ is the area under the curve of f until t. Varying the hazard function changes the overall level of surprise elicited by stimulus changes, which can be formalized as the negative logarithm of the probability of an event (Friston, 2010). Thus, most events in the narrow, intermediate and broad hazard function conditions (i.e. those that occur around the center of mass of the corresponding distribution) are associated with low-, medium- and high-surprise, respectively. For simplicity, we here refer to these conditions overall as 'low-surprise', 'medium-surprise' and 'high-surprise' conditions, respectively (Fig. 1D). Splitting up individual events by the level of the associated surprise (which varies from event to event within each condition) might enable a more sensitive analysis; because we found significant modulation of the pupil dilation amplitude by the surprise condition (see Results), the analysis reported here seemed sufficiently sensitive.

Procedure

The experiment consisted of two sessions of approximately 2 h duration each, and subjects completed a total of 44 × 3-min runs (MIB: 6; Replay: 38). Subjects completed 16 low-, 16 medium- and 6 high-surprise Replay runs. Different numbers of runs per hazard function were used to obtain a similar number of trials for each of the three conditions (the high-surprise condition yielded more trials per unit time). Subjects performed the MIB and high-surprise Replay conditions in one session, and the medium- and low-surprise Replay conditions in the other session. The two types of runs in each session were presented within two separate blocks to allow subjects to learn the event distributions of each condition as much as possible. The order of blocks within a session and the order of sessions were counterbalanced across subjects.

Experiment 2 (replication)

Subjects

Nineteen subjects (12 female, age range 21–39 years) participated in the experiment. All subjects had normal or corrected-to-normal vision, and gave written informed consent. Four subjects were excluded due to excessive blinking. The experiment was approved by the ethics committee of the Department of Psychology at the University of Amsterdam in accordance with the Declaration of Helsinki.

Stimulus, task and procedure

The Gabor target contained four spatial cycles. The mask rotated at a speed of 120°/s. Stimuli were displayed on a 32-bit LaCie Electron Blue 4 CRT monitor with a resolution of 1024 × 768 pixels at a refresh rate of 85 Hz. Subjects were seated in a silent and dark room, with their head positioned on a chin rest, 50 cm in front of the computer screen. Subjects reported perceptual switches by pressing or releasing the space bar on a keyboard, using their preferred hand. The mapping between perceptual switch and motor response was flipped between two blocks of six runs of about 15 min each – button press for indicating target disappearance (release for re-appearance) in one block, and button release for disappearance (press for re-appearance) in the other block (Fig. 1B, bottom panel). Block order was counterbalanced across subjects. During the Replay-button condition, the target was physically removed from the screen in the same temporal sequence as it had previously disappeared during one of several previous MIB runs completed by that same subject. The design ensured that each Replay-button run would be preceded by at least two MIB runs. Replay-button was otherwise identical to MIB. Each run lasted 2 min.

Data analysis

Periods of blinks were detected using the manufacturer's standard algorithms with default settings. All the remaining data analyses were performed using custom-made MATLAB (The Mathworks, Natick, MA, USA) software and the Fieldtrip toolbox (Oostenveld et al., 2011).

Pre-processing

Blinks were removed by linear interpolation of values measured just before and after each identified blink (interpolation time window – from 0.1 s before until 0.1 s after blink). Fixation errors were defined as gaze positions outside of a permissible window of 4.5° from fixation mark. Pupil responses during cognitive events are

sluggish and confined to a frequency range below 4 Hz (Hoeks & Levelt, 1993; Loewenfeld & Lowenstein, 1993). Consequently, signal fluctuations above 4 Hz reflect mainly measurement noise. To remove the high-frequency noise, we low-pass-filtered the interpolated pupil time series from each block of runs using a third order Butterworth filter with a cutoff of 4 Hz. We obtained qualitatively identical results without filtering (data not shown), but we found that removing high-frequency noise increased the reliability of our single-trial pupil amplitude estimates, thus quantitatively boosting single-trial decoding of perceptual report type from the pupil response (see 'Statistical tests of modulations of the pupil response' below).

Event-related analysis of pupil responses

Epochs for event-related analysis of pupil diameter changes around perceptual switches were extracted from the low-pass-filtered pupil time series. In the following, we refer to the extracted epochs as 'trials', noting that the actual stimulus presentation was continuous (except for the target offsets and onsets in the Replay conditions). For MIB, trials were always aligned to subjects' behavioral report of target disappearance and re-appearance (button presses, releases or button switches). For Replay-count, trials were always aligned to the physical stimulus change. For Replay-button, we performed two separate analyses – one in which trials were aligned to the physical stimulus change; and one in which trials were aligned to behavioral report.

In all report-locked analyses (MIB, Replay-button), the following constraints were used for trial extraction: (i) the maximum trial duration ranged from −1 to 1.5 s relative to report (response-locked analysis) or −0.5 to 2 s relative to physical target on- and offsets (stimulus-locked analysis); (ii) when another report occurred within this interval, the trial was terminated 0.5 s from this report; and (iii) when two reports succeeded one another within 0.5 s, no trial was defined. For the analysis of Replay-button, we included only those reports that were preceded by a physical change of the target stimulus within 0.2–1 s, thus discarding reports following illusory target disappearances or button press errors and re-appearances. In Experiment 1, 8% of disappearance trials and 9% of re-appearance reports were discarded for Replay-button due to this constraint. When analysed separately, the pupil time courses of these discarded trials were similar as during the main analysis (data not shown).

In the stimulus-locked analysis of all the Replay conditions, trials were aligned to physical target on- and offsets. Trials in which either blinks or fixation errors occurred for more than 10% of the time occurred during the interval from 1 s before until 1 s after the trigger event were excluded from further analysis. After trial extraction, we down-sampled the data to 50 Hz to boost the sensitivity of our (sample-by-sample) statistical comparisons of pupil time courses (see below).

We used established procedures (Hupe *et al.*, 2009; de Gee *et al.*, 2014) to normalize the raw pupil diameter time courses. We subtracted the baseline pupil diameter value at the start of each trial from each sample of the time course and divided the time course by the mean pupil diameter across the experiment (i.e. all samples and trials pooled across two experimental sessions from a given subject). This normalization procedure transformed the pupil diameter time courses into units of percent modulation.

As baseline intervals, we used the following intervals for the report-locked and stimulus-locked analyses, respectively: from 1 (or later) to 0.6 s before report and from 0.5 (or later) to 0.1 s before stimulus change. The difference of 0.5 s between the intervals for the two analyses corresponds to the median reaction time measured during the Replay-button conditions. As a consequence, the baseline interval for both analyses contained predominantly pupil measurements preceding the (illusory or stimulus-evoked) perceptual switch. The above-described trial extraction procedure caused the start of the baseline intervals to be later (i.e. closer to the trigger event) for some trials.

Quantification of pupil response amplitudes

Because the pupil response is sluggish, any transient neural input will be smeared out in time (Hoeks & Levelt, 1993). Hence, the quantification of the response amplitude should take into account the entire pupil response time course rather than only the peak. We used a linear projection (de Gee *et al.*, 2014) procedure to collapse each single-trial time course into a single measure of response amplitude, according to

$$a_i = r_i \frac{\bar{r}}{\|\bar{r}\|^2}, \qquad (2)$$

where a_i is the scalar amplitude estimate for trial i, r_i, is a row vector containing the pupil response time course of that trial, and $\bar{r}$ is a column vector containing the average pupil response time course across all trials of all conditions for a given subject. The term $\frac{\bar{r}}{\|\bar{r}\|^2}$ normalized the mean response vector to be of unit length. In words, we computed the inner product between each trial's pupil response and a 'template', which was each individual's normalized mean response. This procedure is well established for the quantification of functional magnetic resonance imaging response amplitudes (Ress *et al.*, 2000). It yields robust estimates of single-trial amplitudes. By using a separate template per subject, it also accounts for the individual differences in the pupil impulse response function in a data-driven fashion (de Gee *et al.*, 2014).

We used two different time windows, for linear projection in the report-locked and stimulus-locked analysis, again shifted by 0.5 s to account for the median reaction time from the Replay-button conditions. In the report-locked analysis, we used the time window 0–1.5 s from report. For the stimulus-locked analysis, we used the time window 0.5–2 s from the stimulus change. These time windows consistently included the peak of all individual pupil dilation responses.

If a given trial was shorter than the above time windows (due to the trial extraction), then $\bar{r}$ was clipped to the length of the trial. Because the resulting amplitude values calculated according to Eqn 2 scale with the number of samples per trial (due to the addition of the products of individual samples), we divided each trial's amplitude value by the number of samples. A control analysis in which we computed the projection over the interval from 0 to 1 s (response-locked) or 0.5 to 1.5 s (stimulus-locked) included only trials of complete length. This analysis yielded similar results as the analysis including all the trials (Fig. S6).

Removing the effect of baseline pupil diameter

There is a known negative correlation between the baseline pupil diameter and the amplitude of phasic pupil responses (Gilzenrat *et al.*, 2010; Murphy *et al.*, 2011; de Gee *et al.*, 2014), which we also observed in the present data (Fig. S2). Because of this statistical dependence, differences in pupil response amplitudes between conditions (e.g. disappearance and re-appearance) could be 'inherited from' differences already existing during the baseline interval, even after subtraction of the baseline diameter when computing the response (de Gee *et al.*, 2014). We addressed this concern in a con-

trol analysis (see Fig. 2, bottom right bar graphs in each panel), in which we used linear regression to remove the effect of baseline pupil diameter on the single-trial amplitudes a_i before testing for differences between disappearance and re-appearance.

Statistical tests of modulations of the pupil response

We used two-tailed non-parametric permutation tests to test for differences between the pupil response time courses and amplitudes (Efron & Tibshirani, 1998). These tests were performed across subjects. For each test, we randomly permuted the labels of the observations (e.g. the condition labels of pupil response amplitudes), and recalculated the difference between the two group means (1000 permutations). The P-value associated with the original difference between the means was given by the fraction of shuffles in which the original difference was exceeded by the difference between the means obtained for the permuted data. For the pupil modulation time courses, we performed permutation tests at each time point with cluster-based multiple comparison correction across time points, as implemented in the Fieldtrip toolbox (Maris & Oostenveld, 2007). For testing significance of the correlations, we computed the correlation after each permutation of the labels of the observations, and obtained the P-value by comparing the correlations found after each permutation to the observed correlation, as explained above.

We used receiver-operating characteristic (ROC) analysis (Green & Swets, 1966) to compare the distributions of pupil modulation values from individual trials between the two types of perceptual reports (disappearance and re-appearance). The ROC index ranges between 0 and 1, and quantifies the probability with which one can predict the report type based on the pupil response measured during individual trials. An index of 0.5 implies chance level prediction. The index was first computed for the interval 0–1.5 s after report within each individual subject, averaged across subjects, and tested

for significant deviation from chance (i.e. 0.5) with a two-tailed permutation test across subjects (1000 permutations).

For testing whether the modulation amplitudes differed significantly across the three surprise conditions, we used a one-way ANOVA across subjects. To quantify the overall surprise effect in each subject with a scalar value (collapsing across Replay-count and Replay-button), we averaged the following two amplitude differences: high-surprise – medium-surprise and medium-surprise – low-surprise. We then correlated (across subjects) the magnitude of this overall surprise effect to the magnitude of the perceptual modulation (i.e. difference between disappearance and re-appearance amplitudes, pooled across MIB and Replay-button/Replay-count).

For testing whether reaction times and pupil amplitudes differed between disappearance and re-appearance across surprise conditions, we used a two-way ANOVA across subjects.

Eye position control analysis

The measurement of pupil diameter in video-based eye trackers depends on eye position. Although we found that subjects fixated well on the fixation mark at the center of the screen throughout all experiments (Fig. 2), we wondered whether the observed differences in pupil modulation could be explained by differences in eye position between target disappearance and re-appearance trials. We computed three-dimensional histograms of the subject's gaze behavior in Experiment 1 across the complete stimulus screen consisting of 1024×768 pixels. We normalized each subject's eye position data by dividing the number of observations per x,y bin by the total number of x,y observations. Then, we subtracted the re-appearance from the disappearance maps for both MIB and Replay, and tested these maps across subjects against zero with a permutation test using a cluster-based procedure (Oostenveld et al., 2011) to correct for multiple comparisons (Fig. 2). We found no significant differences
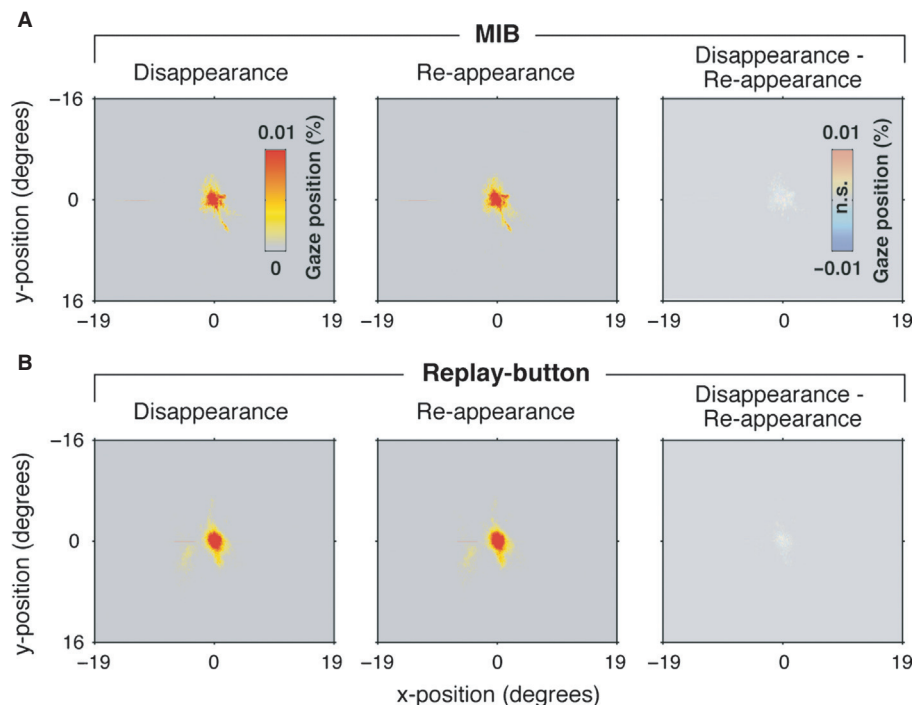

FIG. 2. No difference in eye position during target disappearance and re-appearance. Gaze fixation histograms averaged across subjects, indicating the proportion of fixation time spent at each position on the screen around perceptual reports during motion-induced blindness (MIB) and Replay-button in Experiment 1. (A) Left, MIB disappearance; middle, re-appearance; right, disappearance – re-appearance. Transparency level highlights clusters of significant modulation ($P < 0.05$, two-sided permutation test, cluster-corrected for multiple comparisons). (B) Corresponding histograms for Replay-button.
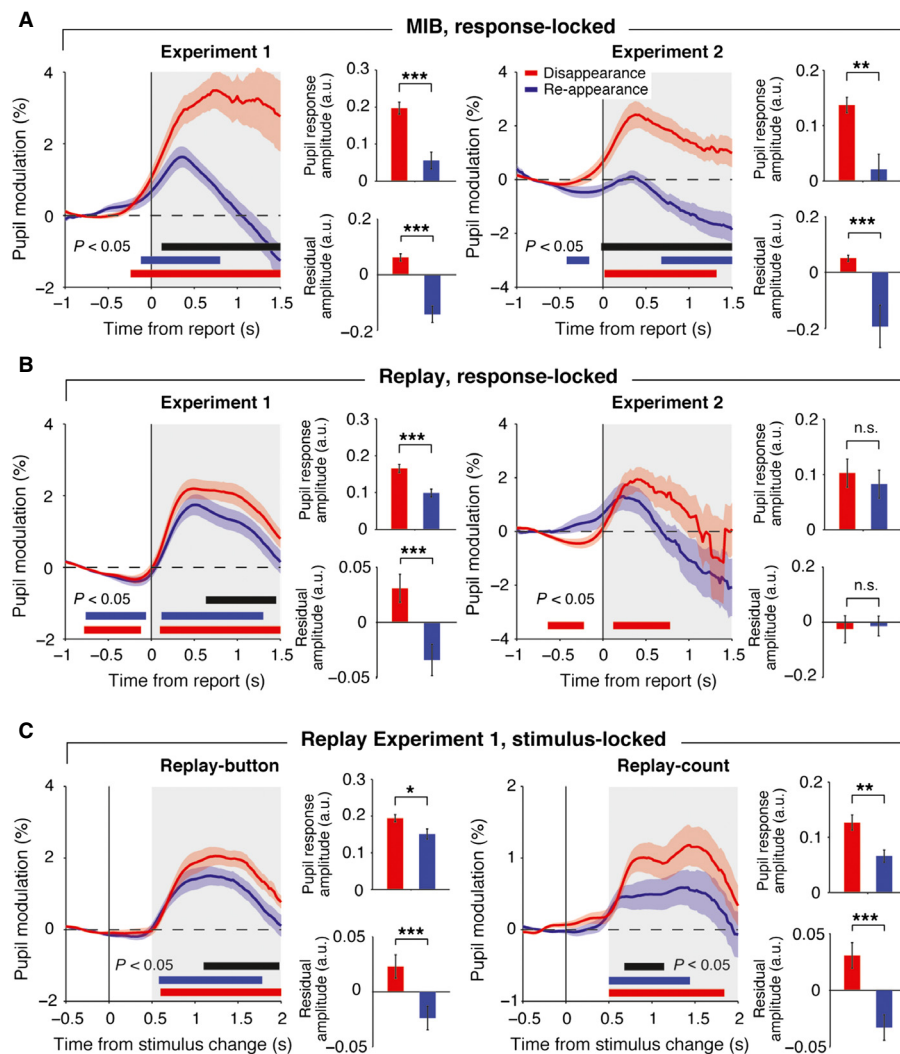
FIG. 3. Pupil dilation reflects content of perceptual report. Time courses of pupil diameter modulation around target disappearance and re-appearance. In each panel, the bar graphs on the right show scalar pupil response amplitudes in the time window indicated by the gray area underlying the time courses. Top, total pupil response amplitude. Bottom, residual response amplitudes after removing the effect of baseline pupil diameter. Colored bars indicate clusters of significant modulation; black bar indicates significant differences between colored traces ($P < 0.05$, cluster-corrected, Experiment 1: $N = 19$; Experiment 2: $N = 15$). Shaded areas time courses, SEM across subjects. Gray shaded area, interval used for estimation of response amplitude. Bar graphs – baseline pupil diameter, averaged over subjects. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n.s., not significant. (A) MIB Experiments 1 and 2, time-locked to behavioral report. (B) Replay Experiments 1 and 2, time-locked to behavioral report. (C) Replay Experiment 1, separately for active and count conditions, time-locked to stimulus change.

(all $P > 0.05$, cluster-corrected for multiple comparisons). To assess whether subjects fixated equally well during target disappearances and re-appearances, we quantified the similarity between the eye position distribution maps of the two types of perceptual switches. The spatial correlations between these maps were high for both MIB and Replay ($r = 0.73$ and $r = 0.82$, respectively), and significantly different from zero ($P < 0.001$, permutation test). Taken together, these analyses rule out the concern that the difference between pupil responses for target disappearance and re-appearance reported in the Results might be due to differences in eye position.

Results

Pupil dilation response reflects content of perceptual switch events

We linked pupil dynamics to perceptual switches during the MIB illusion and a physical replay of that illusion (Fig. 1). We used a

Gabor patch as the target to ensure that the overall stimulus luminance remained constant around targets offsets and onsets during the Replay condition. This was intended to minimize, as much as possible, the effect of pupillary response due to subjective (Laeng & Endestad, 2012) or physical stimulus changes during the perceptual switches. The pupil dilated from the time of subjects' perceptual report to about 1 s after report (Fig. 3). This was the case during the MIB illusion (i.e. in the absence of any physical change in the stimulus; Fig. 3A) and during Replay (Fig. 3B). The similar responses during the MIB illusion and Replay indicate that the pupil dilation during MIB did not reflect the endogenous neural events causing the spontaneous perceptual switches (then it should have occurred only during the illusion), but was a consequence of these switches. This is consistent with pupil dilation responses observed across a range of bistable perceptual phenomena other than MIB (Einhauser *et al.*, 2008; Hupe *et al.*, 2009; Naber *et al.*, 2011).

Critically, we found that the switch-related pupil dilation was significantly larger for disappearance than re-appearance (Fig. 3). We

henceforth refer to this amplitude difference between disappearance and re-appearance as the perceptual content effect. This effect was evident in the main experiment (Experiment 1; left panels of Fig. 3A and B) conducted in a group of 19 subjects as well as in an independent replication (Experiment 2) conducted in another group of 15 subjects. For both the pupil response time courses as well as the response amplitudes (see bar graphs), the perceptual content effect was statistically highly significant for MIB in both experiments (Fig. 3A), and it was statistically highly significant for Replay in Experiment 1 (Fig. 3B). In Experiment 1, subjects needed on average 60 ms longer to report target offsets than onsets, and the perceptual content effect was significant both when time-locked to the perceptual report (Fig. 3B, left panel) and to the physical stimulus change (Fig. 3C, left panel). Importantly, the occurrence of the perceptual content effect in the pupil response during the MIB condition implies that this effect is due to the subjective interpretation of the stimulus, rather than the physical stimulus itself.

We also assessed the impact of the temporal context of the disappearance and re-appearance events on the perceptual content effect. During the MIB illusion, subjects spent, on average, a smaller proportion of time in the target invisible than the target visible percept (Fig. 1C), as is commonly observed in this illusion when only a single target is presented (Bonneh *et al.*, 2001, 2014; Donner *et al.*, 2008; Bonneh & Donner, 2011). This asymmetry might have rendered target disappearances more salient. We used two approaches to test whether this might explain the larger pupil dilation during disappearance. First, we selectively analysed those subjects who spontaneously produced balanced percept durations, or a dominance of the duration of the target invisible percept. All three subjects satisfying this criterion exhibited larger pupil dilation around target disappearance than re-appearance; this effect was statistically significant within two of the subjects (Fig. S3). Second, in the Replay of Experiment 1, we matched (by design) the mean durations of the two percepts (Fig. 1C, top panel). Nonetheless, we observed the same perceptual effect in the pupil response (Fig. 3B, left panel, and C). Taken together, these observations suggest that the perceptual content effect is also unlikely to be caused by the asymmetry between the mean percept durations. We turn to the effect of the distribution of the intervals between events on the pupil response below ('Surprise about event timing also affects pupil response').

Perceptual content effect is not explained by baseline pupil diameter

Our analyses focused on the transient pupil responses around the perceptual switches, by subtracting the pupil diameter at the start of each trial from the response time courses (see Materials and methods). In a control analysis, we calculated the overall pupil diameter modulations around perceptual switches, thereby preserving potential differences between disappearance and re-appearance already in the 'baseline' pupil diameter (here defined as pupil diameter before the trigger event). Indeed, we found larger baseline pupil diameter values before target re-appearance than target disappearance during MIB (Fig. S4). This difference might be due to the above-described asymmetry between percept durations (on average, there was more time for the pupil diameter to decrease before disappearance than before re-appearance events). This indicates that the approach used in all the main figures was more suitable for isolating the event-related pupil responses. Nonetheless, we wondered whether the difference in baseline in combination with the correlation between baseline diameter and subsequent pupil response amplitude (Fig. S2)

might account for the perceptual content effect. In a control analysis, we removed (via linear regression) the component of the response amplitudes explained by the baseline diameter. The residual amplitudes exhibited the same perceptual content effect, with highly significantly larger amplitudes for disappearance than re-appearance (Fig. 3; see bottom right bar graphs in each panel).

Pupil response reflects perceptual content at single-trial level

The analyses presented so far have shown that the subjects' mean pupil responses differed significantly between target disappearance and re-appearance. How reliable are the pupil responses as a predictor of the content of individual perceptual switches? We applied ROC analysis (see Materials and methods) to the distributions of single-trial pupil response amplitudes sorted by the subjects' disappearance vs. re-appearance reports. The group mean ROC index for the post-report pupil dilation for the MIB condition was 0.61 ($N = 34$ across both experiments), which was significantly different from the 0.5 chance level ($P < 0.001$, two-sided permutation test). Thus, not only did the average pupil response amplitudes reflect the content of perceptual reports, but the single-trial pupil responses also enabled prediction of the content of individual reports.

Perceptual content effect is independent of motor report

Hupe *et al.* (2009) showed that a significant proportion of the switch-related pupil response in a bistable perceptual illusion could be accounted for by the button press used to report the switch. In the Replay-count condition of the main Experiment 1, we asked subjects to silently count the number of target disappearances. In line with Hupe *et al.* (2009), the overall pupil response in this condition was smaller than in the standard Replay-button condition (Fig. 4). But, critically, the perceptual content effect in the pupil was evident across a range of different report regimens. In Experiment 1, pupil responses were significantly larger for disappearance than re-appearance during Replay-count (Fig. 3C, right panel). In Experiment 2, we asked subjects to indicate target disappearance by means of button press in one half of the experiment and by means of button release in the other half (the converse for re-appearance; Fig. 1B, bottom). Pupil responses were similar across both button press regimes (Fig. S5) and, consequently, the perceptual content effect was significant after collapsing across these two response regimens (Fig. 3A and B, right panels). In sum, the perceptual modulation of pupil diameter was consistent across a range of different mappings between perceptual switch and motor response, and even in the complete absence of a motor response.

Surprise about timing of stimulus-evoked perceptual events also affects pupil response

The timing of perceptual switches in bistable illusions and their replay is typically unpredictable. Therefore, the perceptual switches may elicit surprise. Other forms of surprise have been shown to engage pupil-linked brainstem systems (Preuschoff *et al.*, 2011; Nassar *et al.*, 2012; Naber *et al.*, 2013). We wondered if the pupil dilation around the perceptual switches also reflected temporal surprise. It is difficult to experimentally control temporal surprise during MIB due to the spontaneous nature of the perceptual switches. Therefore, we focused this analysis on the Replay conditions in Experiment 1, in which we systematically manipulated the predictability of the

target onsets and offsets (Fig. 1D; see Materials and methods for details). This manipulation affected subjects' behavior – reaction times to the perceptual events occurring under these so-called low-, medium- and high-surprise conditions increased as a function of surprise level (490, 590 and 630 ms, respectively).

The pupil responses during target disappearances reflected surprise about event timing during Replay, in particular during the Replay-count condition (Fig. 5). Response amplitudes were largest for the high-surprise condition, and decreased in the medium- and low-surprise conditions during Replay-count (Fig. 5A: disappearance: $F_{2,56} = 4.6$; $P < 0.05$; re-appearance: $F_{2,56} = 7.58$; $P < 0.01$, one-way ANOVA). During Replay-button, response amplitudes were also larger in high- than low-surprise, but similar for high- and medium-surprise.

Importantly, the effects of surprise level and perceptual content were independent of each other during the Replay conditions, both for reaction times and pupil responses. Both the main effects of surprise level and type of perceptual switch on reaction time were significant (two-way ANOVA: surprise level, $F_{2,113} = 27.2$, $P < 0.001$;

perceptual switch type, $F_{1,113} = 14.3$, $P < 0.001$), but there was no significant interaction ($F_{2,113} = 0.06$, $P = 0.94$). These main effects were also significant for the pupil responses (surprise level, $F_{2,113} = 14.9$, $P < 0.001$; perceptual switch type, $F_{1,113} = 5.4$, $P = 0.02$), again with no significant interaction ($F_{2,113} = 0.06$, $P = 0.94$). Consistent with this finding, the overall effect of surprise (quantified as described in Materials and methods) on the pupil in a given subject did not predict the strength of the perceptual content effect (quantified as the amplitude difference between disappearance and re-appearance; $r = 0.13$, $P = 0.59$, permutation test, $N = 19$). In sum, despite clear effects of both effects of surprise and perceptual content on pupil and subjects' response behavior during Replay, we found no evidence for a relationship between these effects.

Discussion

A number of recent studies have linked modulations of pupil to surprise about behaviorally relevant events (Preuschoff *et al.*, 2011; Nassar *et al.*, 2012; Naber *et al.*, 2013), the content of perceptual
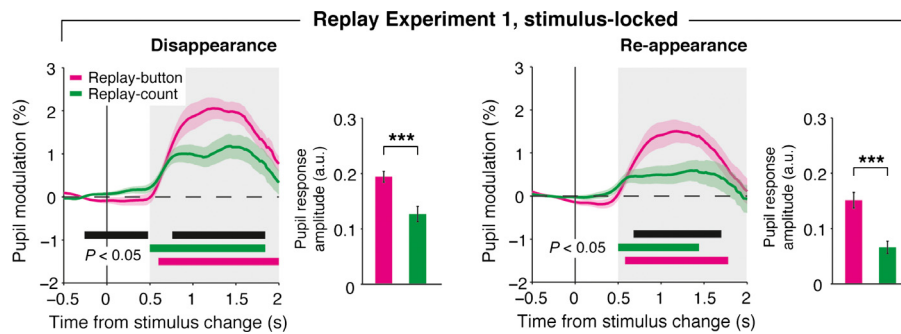
FIG. 4. Pupil dilation is larger for button pressing than for counting. Pupil diameter time courses around target disappearance (left) and re-appearance (right) during Replay-button vs. Replay-count. Conventions as in Fig. 3. ***$P < 0.001$.
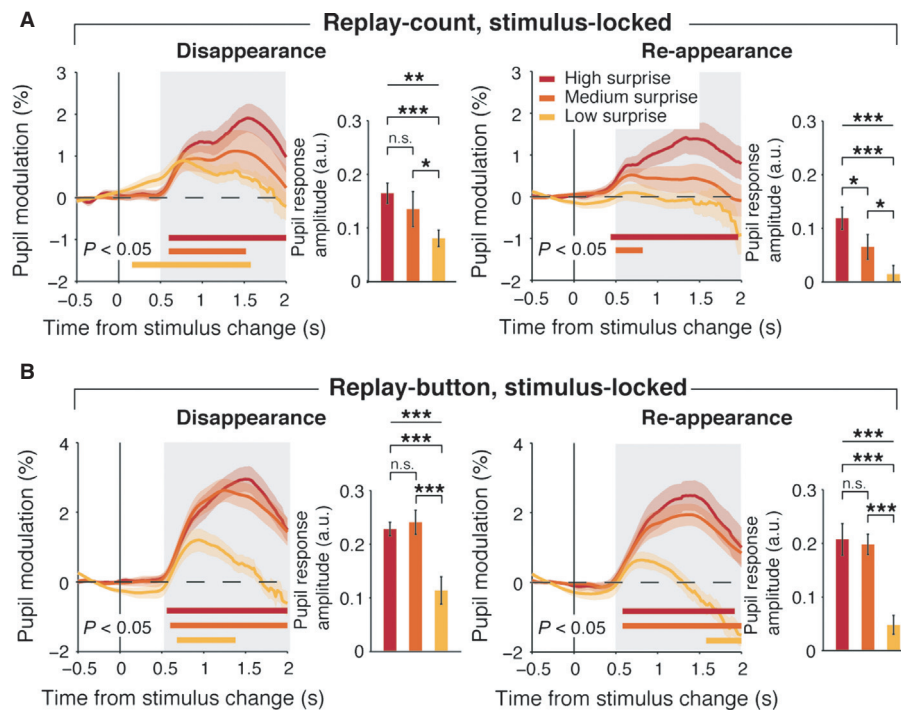
FIG. 5. Pupil dilation reflects surprise about timing of perceptual switches. Pupil diameter time courses around target offset and onset during low-, medium- and high-surprise Replay conditions. Conventions as in Fig. 3. (A) Replay-count. (B) Replay-button. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n.s., not significant.

decisions (Shalom *et al.*, 2013; de Gee *et al.*, 2014), and to the spontaneous perceptual switches in bistable illusions as well as the corresponding stimulus-evoked perceptual switches in replays of the illusions (Einhauser *et al.*, 2008; Hupe *et al.*, 2009; Naber *et al.*, 2011). Here, we show that pupil dilations following alternations in a visual stimulus can reflect the content of the perceptual switches as well as the level of subjects' surprise about the timing of the switches, both independent of motor response. Further, the effects of perceptual content and surprise on the pupil dilation during Replay seem to be distinct from one another.

The similar pupil dilations during the MIB illusion and its replay indicate that the pupil dilation during MIB does not reflect the endogenous neural processes that initiate the spontaneous perceptual switch in the MIB illusion (Donner *et al.*, 2008, 2013) – in this case, the effect should be specific for MIB (Donner *et al.*, 2008). Rather, the switch-related pupil dilation during both conditions reflects a process that is triggered by the switch, regardless of whether this switch is endogenously generated or evoked by a physical stimulus change. This result is in line with previous studies of pupil dynamics in bistable perception (Einhauser *et al.*, 2008; Hupe *et al.*, 2009). Further, while the dilation is small during passive viewing of a replay of an illusion (Hupe *et al.*, 2009), we here show robust dilation in the absence of immediate motor report, provided that subjects count the switches. The concomitant pupil dilation might reflect the updating of working memory. All the present results indicate that a substantial component (~ 65% of the report-locked pupil response during disappearance in Replay-button) of the pupil dilation measured during perceptual switches reflects elementary decision-making – the decision to report the switch by button press, or the decision to update (or not, in case of re-appearance) working memory.

The most notable aspect of the current results is that the pupil dilation does not only indicate the occurrence of perceptual switches, but also reliably differentiates between target disappearance and re-appearance (even at the level of individual reports). Some pupillometry studies of other types of perceptual decision-making have reported modulations of pupil responses by perceptual content (Shalom *et al.*, 2013; de Gee *et al.*, 2014; Laeng & Sulutvedt, 2014). Pupillometry studies of binocular rivalry have shown that the pupil tracks the luminance of the image seen by the currently dominant eye (Fahle *et al.*, 2011; Naber *et al.*, 2011), and older work indicates that a light flash in the dominant eye yields a larger pupil response than a flash in the suppressed eye (Bárány & Halldén, 1948; Lowe & Ogle, 1966). However, a perceptual content effect in pupil dilation during bistable perception has not been shown previously without manipulating luminance of the bistable stimulus (Einhauser *et al.*, 2008; Hupe *et al.*, 2009).

Although objective luminance was constant in our experiments, it is possible that the stronger pupil dilation around target disappearance than re-appearance may be due to concomitant changes in the perceived visual properties (brightness or contrast) of the disappearing and re-appearing target. Indeed, the pupil also responds to subjective changes in perceived brightness of large, centrally presented, black-and-white stimuli during constant physical luminance (Laeng & Endestad, 2012). However, we think that this possibility is unlikely to account for our findings for three reasons. First, the subjective pupil brightness response in Laeng & Endestad's study scales with the strength of the Kanisza illusion, which is determined by the stimulus configuration. In contrast, a uniformly gray patch does not appear to be brighter than the Gabor target, so it seems unlikely that a perceived brightness increase underlies the stronger pupil

dilation around target disappearance. Second, our gray, peripherally presented target stimulus was very small compared with the large and salient stimuli used by Laeng & Endestad. It seems unlikely that any subjective change in luminance around disappearance of the small target would have a large effect on pupil dilation. Third, the perceptual content effect seems also unlikely due to changes in perceived contrast, because stronger contrast of visual targets induces, at least under certain stimulus and task conditions, larger pupil dilations (Wang & Munoz, 2014). This effect would result in a stronger dilation around target re-appearance, in contrast with our results. Future work should test the effects of visual contrast and perceived brightness under the stimulus and task conditions used here.

We propose that the perceptual content effect in the pupil response reflects a difference in engagement that depends on the behavioral context. The effect might reflect a difference in the subjective saliency assigned to the disappearance and re-appearance events or, relatedly, a (stronger or more reliable) shift in covert attention towards the target triggered by the sudden target disappearance. Indeed, shifts in attention modulate the pupil response under certain conditions (Mathot *et al.*, 2013), and microstimulation of an important node in the attentional control network, the superior colliculus, induces transient pupil responses (Wang *et al.*, 2012). While neither physical stimulus properties nor the temporal context of the switch events seem sufficient to account for the perceptual content effect, one relevant aspect might be that only one of the two perceptual interpretations in MIB is illusory (target absent) whereas the other is veridical (target present). Future work should compare different types of perceptual illusions to determine the factors governing the subjective salience of the perceptual switch events.

Our findings indicate that the phasic activation of pupil-linked brainstem systems can be driven by purely subjective perceptual changes in a content-specific fashion. This conclusion has important implications for neurophysiological and neuroimaging studies of perception. Phasic brainstem activation is rapidly followed by changes in cortical state (Parikh *et al.*, 2007; Pinto *et al.*, 2013). Thus, our current and previous (Einhauser *et al.*, 2008; Hupe *et al.*, 2009; Naber *et al.*, 2011) pupillometry results may, at least in part, account for retinotopically global modulations of population activity that have been observed in early visual cortex during perceptual switches in various illusions (Donner *et al.*, 2008, 2013; de-Wit *et al.*, 2012; Kloosterman *et al.*, 2015; N. Rubin, personal communication). In particular, in MIB, this global activity modulation exhibits similar characteristics as the pupil diameter modulation reported here, occurring during both the illusion and its physical replay, and differentiating between target disappearance and re-appearance. An important difference, however, is that the activity modulation during MIB differs in sign between target disappearance and re-appearance (Donner *et al.*, 2008; Kloosterman *et al.*, 2015), as opposed to the scaling of the (generally positive) pupil response amplitude by perceptual content observed here.

In conclusion, our findings point to an intriguing feedback interaction between subjective perception and global brain state. Pupil-linked brainstem systems are phasically activated by changes in subjective perception. We here show that the amplitude of this phasic activation can depend on the contents of the perceptual changes. Because this phasic activation, in turn, causes widespread changes in cortical state, our finding implies that subsequent perception and cognition is affected in a way that depends on the contents of preceding perceptual changes.

Supporting Information

Additional supporting information can be found in the online version of this article:

Fig. S1. Pupil dilation during MIB is independent of drug manipulation.

Fig. S2. Correlation between baseline pupil diameter and subsequent pupil response.

Fig. S3. Pupil dilation reflects content of perceptual report also in subjects for whom target-visible durations are not longer than target-invisible durations.

Fig. S4. Baseline pupil diameter and overall modulation around perceptual Switches.

Fig. S5. Pupil dilation is independent of motor response regime.

Fig. S6. Pupil response amplitudes for disappearance and re-appearance trials of equal duration.

Conflict of interests

The authors declare no conflict of interest.

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Abbreviations

MIB, motion-induced blindness; ROC, receiver-operating characteristic.

References

Aston-Jones, G. & Cohen, J.D. (2005) An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annu. Rev. Neurosci.*, **28**, 403–450.

Bárány, E.H. & Halldén, U. (1948) Phasic inhibition of the light reflex of the pupil during retinal rivalry. *J. Neurophysiol.*, **11**, 25–30.

Bonneh, Y.S. & Donner, T.H. (2011) Motion induced blindness. *Scholarpedia*, **6**, 3321.

Bonneh, Y.S., Cooperman, A. & Sagi, D. (2001) Motion-induced blindness in normal observers. *Nature*, **411**, 798–801.

Bonneh, Y.S., Donner, T.H., Cooperman, A., Heeger, D.J. & Sagi, D. (2014) Motion-induced blindness and troxler fading: common and different mechanisms. *PLoS One*, **9**, e92894.

Donner, T.H., Sagi, D., Bonneh, Y.S. & Heeger, D.J. (2008) Opposite neural signatures of motion-induced blindness in human dorsal and ventral visual cortex. *J. Neurosci.*, **28**, 10298–10310.

Donner, T.H., Sagi, D., Bonneh, Y.S. & Heeger, D.J. (2013) Retinotopic patterns of correlated fluctuations in visual cortex reflect the dynamics of spontaneous perceptual suppression. *J. Neurosci.*, **33**, 2188–2198.

Efron, B. & Tibshirani, R. (1998) *An Introduction to the Bootstrap*. Chapman & Hall/CRC, Boca Raton.

Einhauser, W., Stout, J., Koch, C. & Carter, O. (2008) Pupil dilation reflects perceptual selection and predicts subsequent stability in perceptual rivalry. *Proc. Natl. Acad. Sci. USA*, **105**, 1704–1709.

Einhauser, W., Koch, C. & Carter, O.L. (2010) Pupil dilation betrays the timing of decisions. *Front. Hum. Neurosci.*, **4**, 18.

Eldar, E., Cohen, J.D. & Niv, Y. (2013) The effects of neural gain on attention and learning. *Nat. Neurosci.*, **16**, 1146–1153.

Fahle, M.W., Stemmler, T. & Spang, K.M. (2011) How much of the "unconscious" is just pre–threshold? *Front. Hum. Neurosci.*, **5**, 120.

Fiedler, S. & Glockner, A. (2012) The dynamics of decision making in risky choice: an eye-tracking analysis. *Front. Psychol.*, **3**, 335.

Frassle, S., Sommer, J., Jansen, A., Naber, M. & Einhauser, W. (2014) Binocular rivalry: frontal activity relates to introspection and action but not to perception. *J. Neurosci.*, **34**, 1738–1747.

Friston, K. (2010) The free-energy principle: a unified brain theory? *Nat. Rev. Neurosci.*, **11**, 127–138.

de Gee, J.W., Knapen, T. & Donner, T.H. (2014) Decision-related pupil dilation reflects upcoming choice and individual bias. *Proc. Natl. Acad. Sci. USA*, **111**, E618–E625.

Gilzenrat, M.S., Nieuwenhuis, S., Jepma, M. & Cohen, J.D. (2010) Pupil diameter tracks changes in control state predicted by the adaptive gain theory of locus coeruleus function. *Cogn. Affect. Behav. Ne.*, **10**, 252–269.

Green, D.M. & Swets, J.A. (1966) *Signal detection theory and psychophysics*. Wiley, New York.

Hess, E.H. & Polt, J.M. (1964) Pupil size in relation to mental activity during simple problem-solving. *Science*, **143**, 1190–1192.

Hoeks, B. & Levelt, W.J.M. (1993) Pupillary dilation as a measure of attention: a quantitative systems analysis. *Behav. Res. Meth. Ins. C.*, **25**, 16–26.

Hupe, J.M., Lamirel, C. & Lorenceau, J. (2009) Pupil dynamics during bistable motion perception. *J. Vision*, **9**, 10.

Kahneman, D. & Beatty, J. (1966) Pupil diameter and load on memory. *Science*, **154**, 1583–1585.

Kloosterman, N.A., Meindertsma, T., Hillebrand, A., van Dijk, B.W., Lamme, V.A.F. & Donner, T.H. (2015) Top-down modulation in human visual cortex predicts the stability of a perceptual illusion. *J. Neurophysiol.*, **113**, 1063–1076.

Laeng, B. & Endestad, T. (2012) Bright illusions reduce the eye's pupil. *Proc. Natl. Acad. Sci. USA*, **109**, 2162–2167.

Laeng, B. & Sulutvedt, U. (2014) The eye pupil adjusts to imaginary light. *Psychol. Sci.*, **25**, 188–197.

Lee, S.H. & Dan, Y. (2012) Neuromodulation of brain states. *Neuron*, **76**, 209–222.

Loewenfeld, I.E. & Lowenstein, O. (1993) *The pupil: anatomy, physiology, and clinical applications*. Iowa State University Press, Ames.

van Loon, A.M., Knapen, T., Scholte, H.S., St John-Saaltink, E., Donner, T.H. & Lamme, V.A. (2013) GABA shapes the dynamics of bistable perception. *Curr. Biol.*, **23**, 823–827.

Lowe, S.W. & Ogle, K.N. (1966) Dynamics of the pupil during binocular rivalry. *Arch. Ophthalmol.-Chic.*, **75**, 395–403.

Luce, R.D. (1986) *Response time: their role in inferring elementary mental organization*. Oxford University Press, New York.

Maris, E. & Oostenveld, R. (2007) Nonparametric statistical testing of EEG- and MEG-data. *J. Neurosci. Meth.*, **164**, 177–190.

Mathot, S., van der Linden, L., Grainger, J. & Vitu, F. (2013) The pupillary light response reveals the focus of covert visual attention. *PLoS One*, **8**, e78168.

Murphy, P.R., Robertson, I.H., Balsters, J.H. & O'Connell R, G. (2011) Pupillometry and P3 index the locus coeruleus-noradrenergic arousal function in humans. *Psychophysiology*, **48**, 1532–1543.

Murphy, P.R., O'Connell, R.G., O'Sullivan, M., Robertson, I.H. & Balsters, J.H. (2014) Pupil diameter covaries with BOLD activity in human locus coeruleus. *Hum. Brain Mapp.*, **35**, 4140–4154.

Naber, M., Frassle, S. & Einhauser, W. (2011) Perceptual rivalry: reflexes reveal the gradual nature of visual awareness. *PLoS One*, **6**, e20910.

Naber, M., Frassle, S., Rutishauser, U. & Einhauser, W. (2013) Pupil size signals novelty and predicts later retrieval success for declarative memories of natural scenes. *J. Vision*, **13**, 11.

Nassar, M.R., Rumsey, K.M., Wilson, R.C., Parikh, K., Heasly, B. & Gold, J.I. (2012) Rational regulation of learning dynamics by pupil-linked arousal systems. *Nat. Neurosci.*, **15**, 1040–1046.

Oostenveld, R., Fries, P., Maris, E. & Schoffelen, J.M. (2011) FieldTrip: open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Comput. Intell. Neurosci.*, **2011**, 156869.

Parikh, V., Kozak, R., Martinez, V. & Sarter, M. (2007) Prefrontal acetylcholine release controls cue detection on multiple timescales. *Neuron*, **56**, 141–154.

Pinto, L., Goard, M.J., Estandian, D., Xu, M., Kwan, A.C., Lee, S.-H., Harrison, T.C., Feng, G. & Dan, Y. (2013) Fast modulation of visual perception by basal forebrain cholinergic neurons. *Nat. Neurosci.*, **16**, 1857–1863.

Preuschoff, K., 't Hart, B.M. & Einhauser, W. (2011) Pupil dilation signals surprise: evidence for noradrenaline's role in decision making. *Front. Neurosci.*, **5**, 115.

Reimer, J., Froudarakis, E., Cadwell, C.R., Yatsenko, D., Denfield, G.H. & Tolias, A.S. (2014) Pupil fluctuations track fast switching of cortical states during quiet wakefulness. *Neuron*, **84**, 355–362.

Ress, D., Backus, B.T. & Heeger, D.J. (2000) Activity in primary visual cortex predicts performance in a visual detection task. *Nat. Neurosci.*, **3**, 940–945.

Sarter, M., Parikh, V. & Howe, W.M. (2009) Phasic acetylcholine release and the volume transmission hypothesis: time to move on. *Nat. Rev. Neurosci.*, **10**, 383–390.

Shalom, D.E., de Sousa Serro, M.G., Giaconia, M., Martinez, L.M., Rieznik, A. & Sigman, M. (2013) Choosing in freedom or forced to choose? Introspective blindness to psychological forcing in stage-magic *PLoS One*, **8**, e58254.

Wang, C.A. & Munoz, D.P. (2014) Modulation of stimulus contrast on the human pupil orienting response. *Eur. J. Neurosci.*, **40**, 2822–2832.

Wang, C.A., Boehnke, S.E., White, B.J. & Munoz, D.P. (2012) Microstimulation of the monkey superior colliculus induces pupil dilation without evoking saccades. *J. Neurosci.*, **32**, 3629–3636.

Wierda, S.M., van Rijn, H., Taatgen, N.A. & Martens, S. (2012) Pupil dilation deconvolution reveals the dynamics of attention at high temporal resolution. *Proc. Natl. Acad. Sci. USA*, **109**, 8456–8460.

de-Wit, L.H., Kubilius, J., Wagemans, J. & de Beeck, H.P.O. (2012) Bistable Gestalts reduce activity in the whole of V1, not just the retinotopically predicted parts. *J. Vision*, **12**, 12.

Yu, A.J. (2012) Change is in the eye of the beholder. *Nat. Neurosci.*, **15**, 933–935.

Zylberberg, A., Oliva, M. & Sigman, M. (2012) Pupil dilation: a fingerprint of temporal selection during the "attentional blink". *Front. Psychol.*, **3**, 316.