The effect of covert visual attention on pupil size during perceptual fading

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Abstract

Pupil size is modulated by various cognitive factors such as attention, working memory, mental imagery, and subjective perception. Previous studies on this topic mainly focused on inducing or enhancing a subjective experience of brightness or darkness (for example by asking participants to attend to a bright or dark stimulus), and then showing that this affects pupil size. Surprisingly, the inverse has never been done; that is, it is still unknown what happens when a subjective experience of brightness or darkness is eliminated or strongly reduced even though bright or dark stimuli are physically present. Here, we aim to answer this question by using perceptual fading, a phenomenon where a visual stimulus gradually fades from visual awareness despite its continuous presentation. The study will contain two blocks: Fading and Non-Fading. In the Fading block, participants will be presented with black and white patches with a fuzzy outline that are presented at the same location throughout the block, thus inducing strong perceptual fading. In contrast, in the Non-Fading block, the patches will switch sides on each trial, thus preventing perceptual fading. Participants will covertly attend to one of the two patches, indicated by a cue, and report the offset of one of a set of circles that are displayed on top. We hypothesize that pupil size will be modulated by covert visual attention in the Non-Fading block, such that the pupil will be larger when attending to the dark as compared to the bright patch, but that this effect will not (or to a lesser extent) arise in the Fading block. This would imply that cognitive modulations of pupil size (gradually) disappear along with the subjective experience of brightness or darkness; in turn, this would suggest that cognitive modulations of pupil size reflect, at least in part, a high level of visual processing.

Keywords: pupil size, covert attention, adaptation, perceptual fading

Introduction

Pupil size is modulated by cognitive factors such as attention, working memory, mental imagery, and subjective perception (*attention*: e.g., Binda et al., 2014; Binda & Murray, 2015; Bombeke et al., 2016; Mathôt et al., 2013; Naber & Nakayama, 2013; Unsworth & Robison, 2017; Vilotijević & Mathôt, 2023a, 2023b; *working memory*: e.g., Hustá et al., 2019; Keene et al., 2022; Koevoet et al., 2023; Wilschut & Mathôt, 2022*; mental imagery*: e.g., Laeng & Sulutvedt, 2014; *subjective perception*: e.g., Binda et al., 2013; for reviews see Koevoet et al., 2023; Mathôt, 2020; Vilotijević & Mathot, 2023c). However, even though the conditions under which pupil size is modulated by cognition have been well characterized, much is still unclear about the physiological mechanisms through which this happens.

Previous studies have consistently shown that pupil size is modulated by covert visual attention (Binda et al., 2014; Mathôt et al., 2013; Naber et al., 2013; Unsworth & Robison, 2017). For instance, Mathôt et al. (2013) conducted a study in which participants were presented with a display that was vertically divided into bright and dark halves. Participants were cued towards either the bright or the dark side by an informative cue that predicted the location of an upcoming target. Results showed modulation of pupil size by covert visual attention: when participants covertly focused on the bright side, the pupil constricted more as compared to when participants covertly focused on the dark side, despite constant visual input and gaze fixation.

Similarly, Binda and colleagues (2013) showed that subjective perception modulates pupil size. In their study, participants were presented with naturalistic images of the sun and the moon that were carefully matched in luminance. The participants' task was to classify whether these images represented the sun or the moon. Results showed modulation of pupil size by subjective interpretation: when participants identified an image as the sun, their pupils constricted more as compared to when they identified an image as the moon, despite the images being matched in luminance.

Although studies such as those reviewed above have clearly shown that pupil size is modulated by cognitive factors, very little is known about the underlying physiological and cognitive mechanisms. Therefore, we recently conducted a study to better understand the underlying mechanisms (Vilotijević & Mathôt, 2023a); specifically, we focused on the question of whether modulation of pupil size by covert visual attention is mediated only by the so-called image-forming pathway, which is associated with rods and cones (Schmidt & Kofuji, 2008), or whether this is also mediated by the non-image-forming pathway, which is associated with intrinsically photosensitive retinal ganglion cells (ipRGCs) (non-human studies: Berson, 2003; human studies: Zele et al., 2011; Provencio et al., 2000; Barrionuevo et al., 2023; reviews: Schmidt & Kofuji, 2008; McDougal & Gamlin, 2015). To test this, we manipulated the color (blue/ red) of a to-be-attended stimulus in a spatial cueing paradigm, leveraging the fact that ipRGCs are predominantly responsive to blue light and thus cause the most prominent sustained constriction in response to blue light. Participants covertly attended to either dim or bright stimuli that were either red or blue for a prolonged period (15s). We replicated the classic effect of covert visual attention on pupil size as discussed above, finding that the pupil constricted more when covertly attending to bright as compared to dim stimuli (with the same color). However, we did not find any difference in pupil size when covertly attending to blue as compared to red stimuli (with the same luminosity), whereas we did observe this difference when participants directly looked at the same blue or red stimuli (a classic marker of the influence of ipRGCs on pupil size). This implies that the effect of covert visual attention (and perhaps cognitive factors more generally) on pupil size is mainly mediated by the image-forming rod-and-cone pathway, and not by the non-image-forming ipRGC pathway. This finding goes some way towards understanding the physiological and cognitive mechanisms behind the influence of cognitive factors on pupil size.

However, the image-forming pathway itself is complex and hierarchical and it is still unclear which level of processing is reflected in cognitive effects on pupil size. To date, studies have mainly focused on inducing a subjective experience of brightness or darkness, and showing that this affects pupil size (Binda et al., 2014; Binda & Murray, 2015; Bombeke et al., 2016; Mathôt et al., 2013; Naber & Nakayama, 2013; Unsworth & Robison, 2017; Vilotijević & Mathôt, 2023). Surprisingly, the inverse has never been done; that is, it is still unknown what happens when a subjective experience of brightness or darkness is eliminated or strongly reduced even though bright or dark stimuli are physically present. Do cognitive modulations of pupil size disappear along with the subjective experience of brightness or darkness? This is the question that we aim to test in the present study.

To do so, we make use of perceptual fading, a phenomenon where a fuzzy visual stimulus gradually disappears from perception or becomes less noticeable, despite the visual stimulus remaining physically present (Clarke, 1961; Kanai & Kamitani, 2003; Troxler, 1804). Perceptual fading is commonly explained through adaptation, but it is a distinct phenomenon (Bachy & Zaidi, 2014). Adaptation, in the sense that we use the term here, refers to the fact that photoreceptors and other neurons involved in the early stages of visual processing quickly reduce their firing rate after prolonged exposure to a stimulus (Fain et al., 2001; Kohn, 2007; Laughlin, 1989; McDougal & Gamlin, 2010; Stockman & Brainard, 2010; Webster, 2015). The extent and precise time course of adaptation depends on many factors, but it is in general a fast process that occurs within seconds (Kohn, 2007; Webster, 2015). However, adaptation does not always translate to a change in perception, because the visual system relies on many cues to keep perception stable despite changes in sensory input, including adaptation state. As a case in point, a bright circle with sharp edges is not susceptible to perceptual fading, presumably because the edges move in out of the receptive fields of photoreceptors, thus preventing adaptation of the edge, which serves as a cue that the circle is "still there" and in turn prevents perceptual fading of the circle as a whole: the visual system does not need much to understand that stimuli do not simply disappear. And even a bright circle with fuzzy edges fades from perception much more slowly than you would expect based on the time course of adaptation, presumably because the visual system relies on a heuristic of stability that keeps the perception of the circle intact for some time. Conversely, complete perceptual fading also does not require complete adaptation (Hsieh & Colas, 2012). Again as a case in point, a fuzzy bright circle fades—given enough time—from perception almost entirely, even though under conditions of normal display viewing, photoreceptors never become entirely silent (Krauskopf, 1963; Ramachandran et al., 1993). Taken together, and admitting that the exact relationship between adaptation and perceptual fading is not fully understood, perceptual fading appears to be a high-level phenomenon that is related to low-level adaption but is not identical to it, and that reflects visual awareness (Clarke & Belcher, 1962; Hsieh & Colas, 2012; Kanai & Kamitani, 2003; Kotulak & Schor, 1986; Ramachandran et al., 1993; Sheth & Shimojo, 2004). As such, it is an important question whether attending to a bright versus a dark stimulus affects pupil size, even when due to perceptual fading the stimulus is no longer perceived.

In the present study, we aim to induce near-complete perceptual fading and thus diminish the subjective experience of brightness while still keeping the stimuli physically present on the screen. To this end, the study will comprise two blocks: Fading and Non-Fading. In the Fading block, participants will be exposed to a visual display containing fuzzy black and white patches that are continuously shown on the same side of a gray screen. Both patches will be overlaid with randomly dispersed circles. Participants will receive a cue pointing to either the left or the right, and their task is to covertly attend to the indicated side and detect the offset of a single circle. In the Non-Fading block, the task is the same, except that the white and black patches switch sides on each trial, thus preventing perceptual fading.

Finally, we hypothesize that pupil size will be modulated by covert visual attention to bright/ dark in the Non-Fading block, but not (or to a lesser extent) in the Fading block. This would imply that cognitive modulations of pupil size (gradually) disappear along with the subjective experience of brightness or darkness, which would in turn suggest that cognitive modulations of pupil size depend at least in part on a high level of visual processing.

Sampling plan

Data collection will be done in the eye-tracking laboratory within the Department of

Experimental Psychology at the University of Groningen. On the basis of a checklist developed by the Ethical committee (EC-BSS) at the University of Groningen, the study was exempt from full ethical review (PSY-2324-S-0103).

Participants

To estimate the sample size, we conducted a power analysis using the `simR` package (Green & MacLeod, 2016). Specifically, we used pilot data (*N* = 2), collected on a version of the experiment that is very similar though not identical to the one that we will conduct here, and selected all the trials from the Non-fading block and trials from the Fading block where participants reported high levels of adaptation (ratings of 2 and 3) to test the interaction effect between Covertly attended brightness and Block type on Pupil size (Hypothesis 3). The model included Mean pupil size as a dependent variable, Covertly attended brightness, Block type, and their interaction as fixed effects, and random effects for participants. The power analysis revealed that a sample of 30 participants would be required in order for the interaction effect to be detected with a power of 100% and an alpha level of .02. Therefore, our target sample size will be 30 participants (psychology students at the University of Groningen) for the experiment. Normal or corrected-to-normal vision (no glasses or lenses) will be a prerequisite for participation in the experiment. Participants will be awarded course credits for their participation. Participants will provide informed consent before the start of the experiment.

Design of the study

The experiment and the analysis script are available here.

Apparatus and stimuli

The experiment is programmed in OpenSesame (Mathôt et al., 2012) using PyGaze for eye tracking (Dalmaijer et al., 2014). Stimuli will be presented on a 27-inch PROLITE G2773HBS-GB1 (EOL) monitor (1,920 × 1,080 pixels resolution; refresh rate: 60 Hz; maximum output: 300 cd/m^2 typical; gamma-calibrated) and an Eyelink 1000 (sampling frequency of 1000 Hz; SR Research Ltd., Mississauga, Ontario, Canada), will be used for eye tracking. The experiments will be conducted in a dimly lit room (lab illuminance: 39 lux)

Procedure

Prior to the start of each phase, after making sure that the participant is well seated at about 60 cm away from the computer, an eye-tracking calibration-validation procedure is run. A chin-rest is used to keep the participant's head in a stable position. In addition to the calibration-validation procedure, a 1-point eye-tracker recalibration ("drift-correction") is performed before each trial.

Participants are exposed to a display that features white ($SD_{grating}$: 5.77°; 99.60 cd/m2) and black ($SD_{grating}$: 5.77°; 0.14 cd/m^2) patches, one on each side, superimposed on a gray background (23.94 cd/m^2). On top of both patches, 10 randomly dispersed unfilled circles (r = 0.15°) with maroon outline are presented. Each trial begins with a presentation of a gray fixation dot at the center of the display for .5s, that is followed by a cue presentation lasting for 1s. The cue is a gray arrow, pointing either left or right, and indicating the side to which participants should covertly attend. Next, on 50% of trials, one randomly chosen circle (the target) disappears. The target disappears from the cued side in 80% of cases, and its offset occurs at a random moment in between 3 and 5s. Participants' task is to keep fixating at the center of the screen throughout the experiment and only covertly attend to the cued side, detect the offset of the target, and report it by pressing a 'space' key as fast as possible and to withhold a response on no-target-offset trials. Finally, participants are prompted to report the extent of fading they experienced at the moment that the question appeared (i.e. a retrospective judgment at the end of the trial). They report this by using arrow keys corresponding to a four-point Likert scale (left (0) = *not at all*; down (1) = *just a bit*; up (2) = *almost fully*; right (3) = *completely*).

The experiment consists of two blocks: Fading and Non-Fading. The Fading block contains trials where white and black patches are always presented on either the left or right side respectively (counterbalanced across participants). This means that the display is constant throughout the block, and the only thing that changes from trial to trial are the circles that are superimposed on the patches. On the other hand, the Non-Fading block contains trials where white and black patches switch sides from trial to trial (e.g. the white gabor is on the left on one trial, but on the right on the next trial).

To ensure that the accuracy is maintained at approximately 75% across all conditions, we will implement a Single-Interval Adjustment-Matrix (SIAM) procedure (Kaernbach, 1990). We will staircase the opacity of the circles based on the participant's response: after a hit, the opacity of the offsetting circle is decreased for 1% (thus increasing task difficulty); after a miss, the opacity of the offsetting circle is increased for 3% (thus decreasing task difficulty); after a false alarm, the opacity of the offsetting circle is increased for 4% (thus increasing task difficulty); after a correct rejection, no adjustments are made (thus not changing task difficulty).

The order of blocks is counterbalanced across participants. Before each block, participants complete a practice round (20 trials). Each block consists of 120 experimental trials.

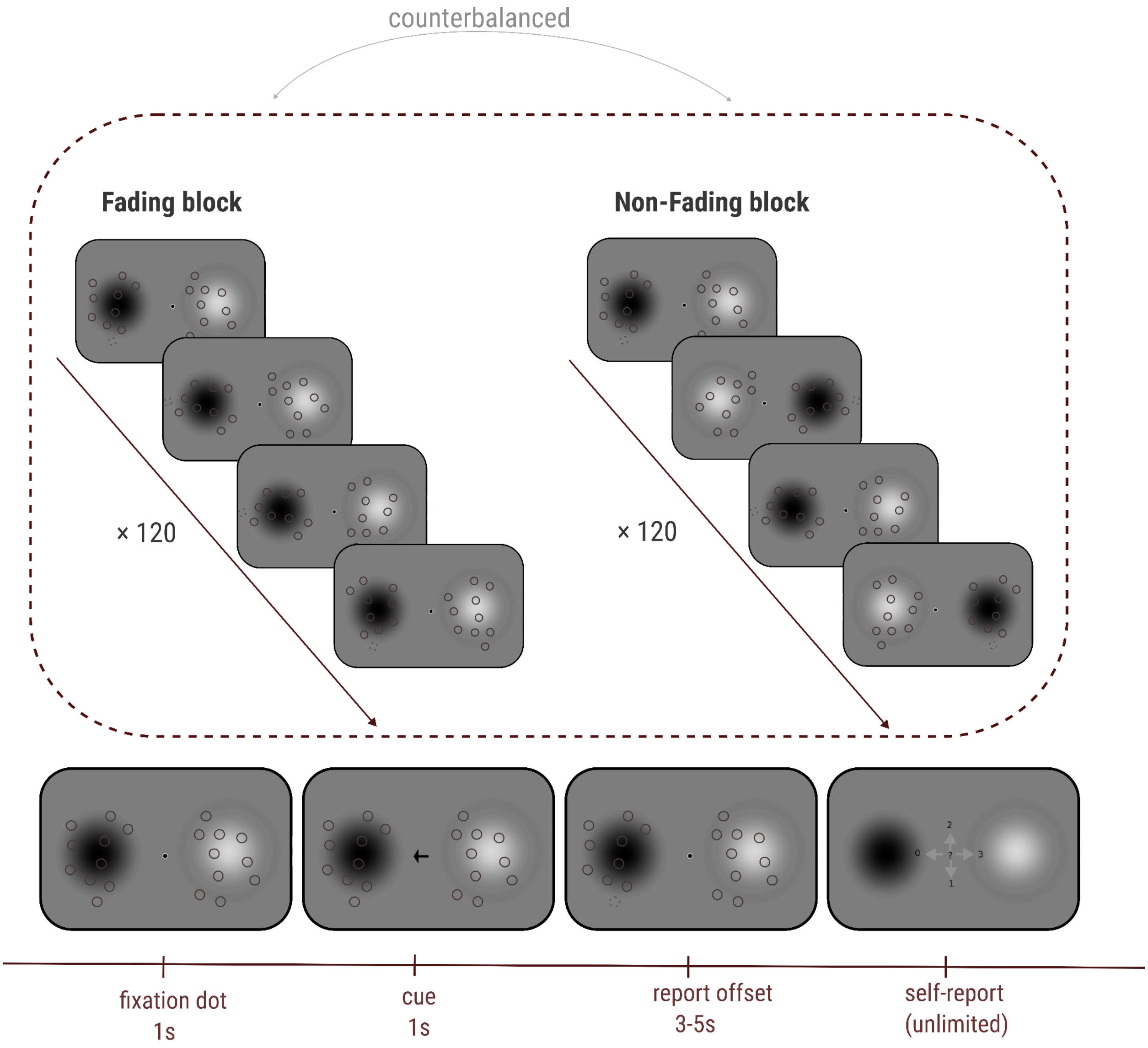

Figure 1. An example of a trial sequence in the experiment for both Fading and Non-Fading blocks. Participants are exposed to a display that features white and black patches, one on each side, superimposed on a gray background. On top of the patches, 10 randomly dispersed unfilled circles are presented on both sides. Each trial begins with a presentation of a fixation dot for 1s, that is followed by a cue lasting for 1s, indicating the side to which participants should covertly attend. Next, on 50% of trials, one randomly chosen circle (the target) disappears. Participants' task is to covertly attend to the cued side, detect the offset of the target, and report it (or withhold

a response on no-target-offset trials). Finally, participants are prompted to report the extent of fading they experienced using a four-point Likert scale (left (0) = *not at all*; down (1) = *just a bit*; up (2) = *almost fully*; right (3) = *completely*). The Fading block consists of trials where white and black patches are always presented to either left or right side respectively. On the other hand, the Non-Fading block consists of trials where white and black patches switch sides from trial to trial.

Hypotheses and prospective interpretation

We will test the following hypotheses (specific statistical tests are described in the next section):

Hypothesis 1: Covert visual attention to bright/ dark modulates pupil size in the Non-Fading block. We predict the pupil size to be modulated by the brightness of covertly attended stimuli in the Non-Fading block. Specifically, we predict to observe smaller pupils when covertly attending to the bright stimulus compared to the dark stimulus in the Non-Fading block. This finding is already well-established and would replicate previous studies on the effect of covert attention on the PLR (Binda et al., 2014; Binda & Murray, 2015; Bombeke et al., 2016; Mathôt et al., 2013; Vilotijević & Mathôt, 2023). This serves as a sanity check in our study (Figure 2).

Hypothesis 2: Pupil size is smaller in the Non-Fading as compared to the Fading block. We predict a pattern of the typical pupil response to visual change in the Non-Fading block, as a result of the switches that occur from trial to trial (Ellis, 1981; Hong et al., 2001; Mathôt, 2018). This response includes a rapid constriction immediately after the display onset, followed by a subsequent pupil escape, and eventually a response modulated by covert attention following the cue onset. We predict that this pattern will lead to smaller pupil sizes during the Non-Fading block compared to the Fading block (Figure 2). This is not a hypothesis of interest, but also serves as a sanity check because it is a strong prediction based on the general properties of the pupil response to visual change.

Hypothesis 3.1: Covert visual attention to bright/ dark does not modulate pupil size in the Fading block during full perceptual fading. This hypothesis holds that pupil size is *not* modulated by the brightness of covertly attended stimuli in the Fading block in the subset of trials where participants report maximal perceptual fading (adaptation self-report levels: 2 and 3). Specifically, this hypothesis predicts no differences in pupil responses when covertly attending to the bright stimulus and the dark stimulus in the Fading block. This finding would imply that pupil size is not modulated by covert attention once the subjective experience of brightness or darkness has disappeared. In broader terms, this would suggest that cognitive modulations of pupil size solely reflect a high level of visual processing (Figure 2).

Hypothesis 3.2: Covert visual attention to bright/ dark does modulate pupil size in the Fading block during full perceptual fading, but the effect is smaller than in the Non-Fading block. This hypothesis holds that pupil size is modulated by the brightness of covertly attended stimuli also in the Fading block in the subset of trials where participants report maximal perceptual fading (adaptation self-report levels: 2 and 3), but that the effect will be reduced as compared to the effect in the Non-Fading block. This finding would imply that pupil size is always modulated by covert attention, but that the effect decreases as subjective experience of brightness or darkness decreases. In broader terms, this would suggest that cognitive modulations of pupil size reflect a mixture of high-level and lower-level visual processing.

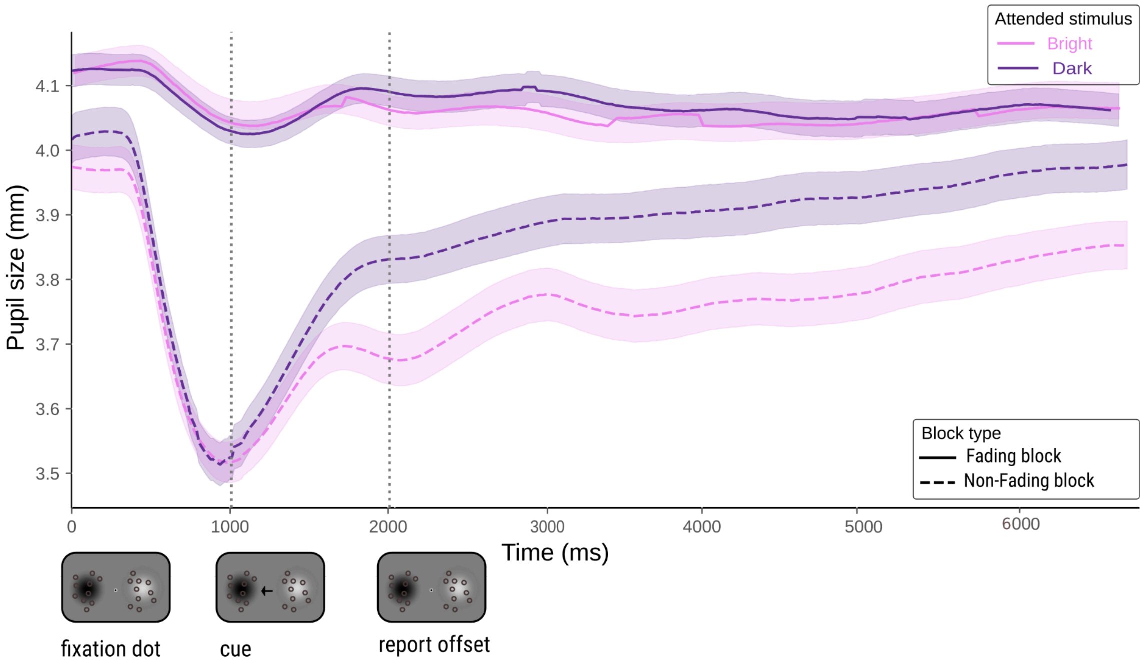

Figure 2. Predicted results. The interaction between Block type and Covertly attended brightness (bright/ dark) on Pupil size. This pattern confirms hypothesis 3.1. The plot is generated based on the pilot data (*N* = 2).

Hypothesis 4: The effect of covert visual attention on pupil size decreases over time in the Fading block but not in the Non-Fading block. We predict that the strength of perceptual fading will increase over the course of the Fading block, in turn resulting in decreased modulation of pupil size by covert attention. This would imply that the effect of covert attention gradually decreases along with the gradually decreasing experience of brightness/ darkness (Figure 3a). In contrast, we predict that the modulation of pupil size by covert attention will remain the same over the course of the Non-Fading block, because here the experience of brightness/ darkness will stay intact (Figure 3b).

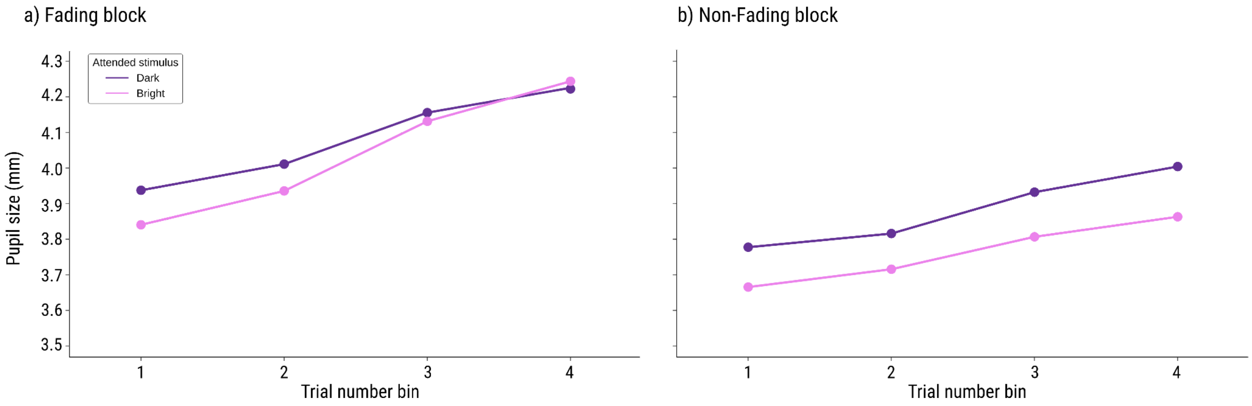

Figure 3. Predicted results. The three-way interaction between Block type, Trial number, and Covertly attended brightness (bright/ dark) on Pupil size. This pattern confirms hypothesis 4. a) A two-way interaction between Trial number and Covertly attended brightness in the Fading block. b) No two-way interaction between Trial number and Covertly attended brightness in the Non-Fading block. *Note*: the plots are generated based on hypothetical data.

Hypothesis 5: The effect of covert visual attention on pupil size decreases with increasing self-reported perceptual fading in the Fading block. We predict an interaction between Fading Self-Report and Covertly Attended Brightness on Pupil Size (Figure 4) in the Fading block, reflecting that the effect of covert attention on pupil size is inversely related to the subjective level of perceptual fading.

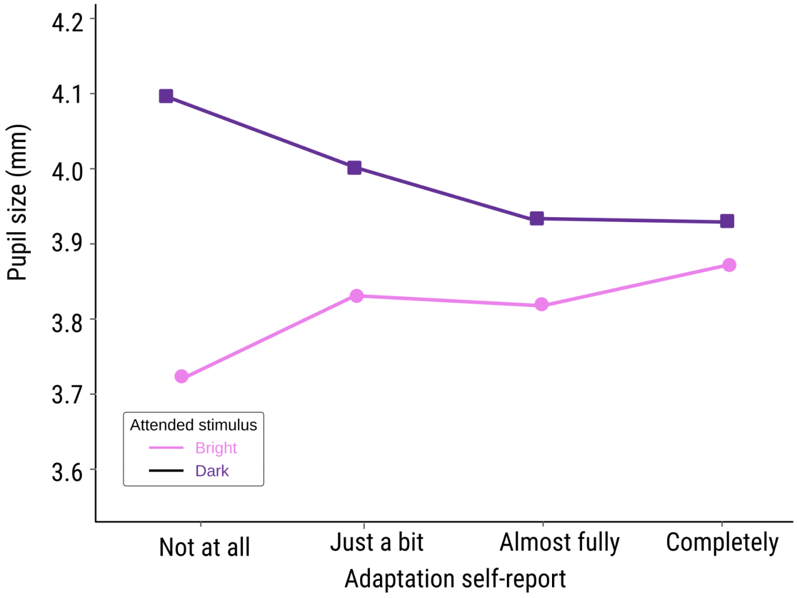

Figure 4. Predicted results. The interaction between Adaptation self-report measure and Covertly attended brightness (bright/ dark) on Pupil size in the Fading block. This pattern confirms hypothesis 5. *Note*: The plot is generated based on hypothetical data.

Hypothesis 6: The accuracy of responses is higher in validly cued trials compared to invalidly cued trials. We predict a main effect of Cue Validity on Accuracy in both Fading and Non-Fading blocks. The cueing effect is well-established and would serve as a confirmation of Posner's theory of spatial attention (Posner, 1980) and attentional deployment in our study.

Data analysis and preprocessing

Data exclusion

Participants will be excluded (with replacement) from the dataset if the experiment is not fully completed. Participants for whom the staircase procedure did not converge will be excluded and replaced. Since it is very difficult to predict staircase behavior, convergence will be determined post-hoc and transparently reported.

Experiment: data preprocessing

As regards the analysis of the experimental data, we will first preprocess pupillary data as follows (Mathôt & Vilotijević, 2022): we will epoch the pupil data, that is, segment it into the time window of interest. The epoch of interest will start at the fixation display onset and end just before the target's offset; this will result in epochs of maximum 7s or minimum of 5s. Next, segments of data that contain eye blinks will be reconstructed using the `blinkreconstruct()` function from Python `DataMatrix.` (Even though blinks may counteract perceptual fading to some extent, we will not exclude those trials as the window of interest is too long, and blinks will inevitably occur. Therefore, we will rely on subjective reports as mentioned above to ensure that this effect is minimal). All pupil data will be down-sampled from 1000 to 100 Hz. Trials on which baseline pupil size deviates ±2 SD from the participant's mean baseline pupil size (calculated during the first 50ms after the fixation onset for each block separately) will be excluded from the analysis. Finally, we will exclude trials in which the horizontal deviation from the center of the screen is larger than 7.42° (distance to the inner edge of the patches) for more than 10 ms consecutively.

Experiment: analyses

General statistical approach. For all analyses below, we will run a cross-validation in combination with linear mixed effects (LME) to localize and test effects using the Python library `time_series_test`. This is a preferred approach in analyzing pupillary data that we explain in detail in Mathôt & Vilotijević (2022). We will use four-fold cross-validation, such that 75% of the data will be used as a training set and the remaining 25% as a test set. As the dependent measure we will use pupil size during the covertly attended period, that is, from cue onset until 4 s later. We will use a maximal random-effects structure, which means that we include by-participant random intercepts and slopes for all fixed effects and interactions. We will use an alpha level of .02.

For hypotheses that predict a null result (3.1 and 4), we will also perform a Bayesian linear mixed effects model; this model will have the same fixed- and random-effects structure as the regular model, and use the same dependent variable (i.e. as determined by cross-validation); we will use the default parameters for Bayesian linear mixed models as implemented in JASP. These hypotheses will be confirmed if the BF in favor of the null hypothesis for the predicted effect exceeds 3.

Testing hypothesis 1. For this analysis, we will include only the trials from the Non-Fading block. The model will include Pupil size as dependent variable, Covertly attended brightness as fixed effect, and Participant as random effect. Hypothesis 1 will be confirmed by an effect of Covertly attended brightness on Pupil size on the Non-Fading block trials in the predicted direction.

Testing hypothesis 2. For this analysis, we will include all trials from both blocks. The model will include Pupil size as the dependent variable, Block type as fixed effect, and Participant as random effect. Hypothesis 2 will be confirmed by an effect of Block Type on Pupil size in the predicted direction.

Testing hypothesis 3. For this analysis, we will use all trials from the Non-Fading block and trials from the Fading block where participants reported high levels of adaptation (ratings of 2 and 3), to ensure full perceptual fading. The model will include Pupil size as the dependent variable, Covertly attended brightness, Block type (with Fading block as reference), and their interaction as fixed effects, and Participant as random effect. Hypothesis 3.1 and 3.2 will both be confirmed by an interaction between Block Type and Covertly attended brightness in the predicted direction (Figure 2). Hypothesis 3.1 will be confirmed by the absence of a main effect of Covertly attended brightness (which will relate to the Fading block because it is the reference) in the predicted direction, and evidence for the null hypothesis for this effect as indicated by the corresponding Bayesian linear mixed model (both are required). Hypothesis 3.2 will be confirmed by the presence of a main effect of Covertly attended brightness.

Testing hypothesis 4. For this analysis, we will include all trials. The model will include Pupil size as the dependent variable, Block type (with Fading as reference), Covertly attended brightness, Trial number, and their interactions as fixed effects, and Participant as random effect. Hypothesis 4 will be confirmed by the presence of a three-way interaction between Covertly attended brightness, Trial number, and Block type on Pupil size in the predicted direction (Figure

3). Hypothesis 4 will be confirmed by the presence of a two-way interaction between Covertly attended brightness and Trial number (which will relate to the Fading block because it is the reference). Finally, Hypothesis 4 will be confirmed by the absence of a two-way interaction between Covertly attended brightness and Trial number for Non-Fading blocks (which we will test using a post-hoc contrast, because Non-Fading is not the reference), and evidence for the null hypothesis for this effect as indicated by the corresponding Bayesian linear mixed model (both are required).

Testing hypothesis 5. For this analysis, we will include the trials from the Fading block. The model will include Pupil size as the dependent variable, Covertly attended brightness, Fading self-report, and their interaction as fixed effects, and Participant as random effect. Hypothesis 5 will be confirmed by the presence of an interaction between Covertly attended brightness and Fading self-report in the predicted direction (Figure 4).

Testing hypothesis 6. For this analysis, we will include all trials. To test the behavioral cueing effect, we will run Generalized Linear Mixed Models (GLMM) to test a model that includes Accuracy as dependent variable, Cue validity and Block type as the fixed effects, and and Participant as random effect. Hypothesis 6 will be confirmed by the presence of a main effect of Cue validity on Accuracy in the predicted direction. To ensure that this effect exists in both blocks, we will run this analysis twice: once with each Block Type as reference value.

Table 1. Study design template

Research question	
Do cognitive modulations of the PLR disappear along with the subjective experience of brightness or darkness?	
The design	
Experimental design	Within-subjects design
Dependent variables	Pupil size
Independent variables (with respective levels)	Covertly attended brightness (bright vs. dark); Block type (Fading vs. Non-Fading); Trial number
Non-independent predicting variables (with respective levels)	Fading Self-Report (0,1,2,3)
Counterbalancing	- Block order will be counterbalanced across participants. - The side of bright/ dark patches in the Fading block will be counterbalanced across participants.
Hypotheses, analyses plan and prospective interpretation	

Research question	**Hypothesis**	**Sampling plan**	**Analysis plan**	**Rationale for deciding the sensitivity of the test for confirming or disconfirming the hypothesis**	**Interpretation given different outcomes**	**Theory that could be shown wrong by the outcomes**
	Quality check hypotheses					
	Neutral hypotheses					
	Of interest hypotheses					

Does covert visual attention to bright/ dark affect pupil size?	**Hypothesis 1:** Covert visual attention to bright/ dark modulates the PLR in the Non-Fading block.	We estimated sample size using the `simR` package (Green & MacLeod, 2016). Specifically, we used pilot data (*N* = 2) and selected all the trials from the Non-fading block and trials from the Fading block where participants reported high levels of adaptation (ratings of 2 and 3) to test the interaction effect between Covertly attended brightness and Block type on Pupil size (Hypothesis 3). The model included Mean pupil size as a dependent variable, Covertly attended brightness, Block type, and their interaction as fixed	We will include only the trials from the Non-Fading block. The model will include Pupil size as dependent variable, Covertly attended brightness as fixed effect, and Participant as random effect.	For all analyses below, we will run a cross-validation in combination with linear mixed effects (LME) to localize and test effects using the Python library time_series_test. This is a preferred approach in analyzing pupillary data that we explain in detail in Mathôt & Vilotijević (2022). We will use four-fold cross-validation, such that 75% of the data will be used as a training set and the remaining 25% as a test set. As the dependent measure we will use pupil size during the covertly attended	Hypothesis 1 will be confirmed by an effect of Covertly attended brightness on Pupil size on the Non-Fading block trials in the predicted direction. This finding serves as a sanity check in our study and would replicate previous studies on the effect of covert attention on pupil size.	
Does covert visual attention to bright/ dark affect pupil size differently when the subjective experience of brightness or darkness is eliminated (during perceptual fading)	**Hypothesis 2:** Pupil size is smaller in the Non-Fading as compared to the Fading block.		We will include all trials from both blocks. The model will include Pupil size as the dependent variable, Block type as fixed effect, and Participant as random effect.		Hypothesis 2 will be confirmed by an effect of Block Type on Pupil size in the predicted direction. This finding serves also as a sanity check because it is	

and when it is maintained?		effects, and random effects for participants. The power analysis revealed that a sample of 30 participants would be required in order for the interaction effect to be detected with a power of 100% and an alpha level of .02. Therefore, our target sample size will be 30 participants (psychology students at the University of Groningen) for the experiment.		period, that is, from cue onset until 4 s later. We will use a maximal random-effects structure, which means that we include by-participant random intercepts and slopes for all fixed effects and interactions. We will use an alpha level of .02. For hypotheses that predict a null result (3.1 and 4), we will also perform a Bayesian linear mixed effects model; this model will have the same fixed- and random-effects structure as the regular model, and use the same dependent variable (i.e. as determined by cross-validation);	a strong prediction based on the general properties of the pupil response to visual change.	
Does covert visual attention to bright/ dark affect pupil size during perceptual fading?	**Hypothesis 3.1.:** Covert visual attention to bright/ dark does not modulate pupil size in the Fading block during full perceptual fading.		We will use all trials from the Non-Fading block and trials from the Fading block where participants reported high levels of adaptation (ratings of 2 and 3), to ensure full perceptual fading. The model will include Pupil size as the dependent variable, Covertly attended brightness, Block type (with Fading block as reference), and their interaction as fixed effects, and Participant as random effect.		Hypothesis 3.1 and 3.2 will both be confirmed by an interaction between Block Type and Covertly attended brightness in the predicted direction. Hypothesis 3.1 will be confirmed by the absence of a main effect of Covertly attended brightness (which will relate to the Fading block because it is the reference) in the predicted direction, and evidence for the null hypothesis for this effect as indicated by the corresponding Bayesian linear mixed model (both	Theoretically, the notion that modulations of pupil size by covert visual attention reflect a high level of visual processing could be (un)supported by our proposed analysis. If neither of 3.1. Or 3.2. Hypothesis comes out, the idea that modulations of pupil size by covert visual attention reflect high levels of visual processing will be refuted.
	Hypothesis 3.2.: Covert visual attention to bright/ dark does modulate pupil size in the Fading block during full perceptual fading, but the effect is smaller than in the Non-Fading block.					

				we will use the default parameters for Bayesian linear mixed models as implemented in JASP. These hypotheses will be confirmed if the BF in favor of the null hypothesis for the predicted effect exceeds 3.	are required). Hypothesis 3.2 will be confirmed by the presence of a main effect of Covertly attended brightness. Confirming Hypothesis 3.1. would imply that PLR is not modulated by covert attention once the subjective experience of brightness or darkness is lacking. In broader terms, this would suggest that cognitive modulations of the PLR are dependent on subjective perception and rely solely on a high level of visual processing. However, confirming Hypothesis 3.2 would imply that PLR is modulated	

					by covert attention, but the effect decreases as the subjective experience of brightness or darkness decreases. In broader terms, this would suggest that cognitive modulations of the PLR reflect a mixture of high-level and lower-level visual processing.	
Does the effect of covert visual attention to bright/dark on pupil size differ over time during perceptual fading?	**Hypothesis 4:** The effect of covert visual attention on the pupil size decreases over time in the Fading block but not in the Non-Fading block.		We will include all trials. The model will include Pupil size as the dependent variable, Block type (with Fading as reference), Covertly attended brightness, Trial number, and their interactions as fixed effects, and Participant as random effect.		Hypothesis 4 will be confirmed by the absence of a two-way interaction between Covertly attended brightness and Trial number for Non-Fading blocks (which we will test using a post-hoc contrast, because Non-Fading is not the reference), and	

			Hypothesis 4 will be confirmed by the presence of a three-way interaction between Covertly attended brightness, Trial number, and Block type on Pupil size in the predicted direction.		evidence for the null hypothesis for this effect as indicated by the corresponding Bayesian linear mixed model (both are required). This finding would imply that the effect of perceptual fading strengthened over the Fading block course and that covert attention effect decreases as participants’ vision gets more adapted.	
Does the effect of covert visual attention to bright/dark on pupil size during perceptual fading depend on a self-reported level of perceptual fading?	**Hypothesis 5:** The effect of covert visual attention on pupil size decreases with increasing self-reported perceptual fading in the Fading block.		We will include the trials from the Fading block. The model will include Pupil size as the dependent variable, Covertly attended brightness, Fading self-report, and their interaction as fixed effects, and		Hypothesis 5 will be confirmed by the presence of an interaction between Covertly attended brightness and Fading self-report in the predicted direction. This finding would imply that the effect	

			Participant as random effect.		of covert attention decreases as participants' vision gets more adapted. In broader terms, this would suggest that cognitive modulations of the PLR are dependent on subjective perception and rely on a high level of visual processing.	
Does the cue validity have an effect on accuracy?	**Hypothesis 6:** The accuracy of responses is higher in validly cued trials compared to invalidly cued trials.		We will include all trials. To test the behavioral cueing effect, we will run Generalized Linear Mixed Models (GLMM) to test a model that includes Accuracy as dependent variable, Cue validity and Block type as the fixed effects, and and Participant as random effect.		Hypothesis 6 will be confirmed by the presence of a main effect of Cue validity on Accuracy in the predicted direction.	Posner's theory of spatial attention would be confirmed.

Quality-check measures
Participants for whom the staircase procedure did not converge will be excluded and replaced. Since it is very difficult to predict staircase behavior, convergence will be determined post-hoc and transparently reported.

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