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# MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

„The Pupillary Response to Multiple Target Color  
Templates“

verfasst von / submitted by

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angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of  
Master of Science (MSc)

Wien, 2020 / Vienna 2020

Studienkennzahl lt. Studienblatt /  
degree programme code as it appears on  
the student record sheet:

UA 066 840

Studienrichtung lt. Studienblatt /  
degree programme as it appears on  
the student record sheet:

Masterstudium Psychologie UG2002

Betreut von / Supervisor:

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## **Abstract**

In multiple item visual search, it is controversial whether one or more attentional templates can be maintained simultaneously. Recent evidence suggests that we switch between multiple single-item templates. The present study addressed this question on the basis of the pupillary light reflex (PLR), as previous studies have shown that pupil size reflects the luminance of an object in early visual working memory (VWM). In this experiment ( $N = 30$ ), we compared performance in single-color and dual-color conditions of a visual search task.

Same sized cuing effects in all three conditions suggest that two target colors can guide search simultaneously. We found a significant effect for stimulus brightness during encoding and early retention in single-color trials, but only during encoding in dual-color trials. Surprisingly, we did not find a switching mechanism in the PLR. It therefore seems that the PLR reproduces maintained VWM content in single-color search, but not in dual-color search. We suggest that multiple target color retention in VWM uses a different mechanism, that does not rely on early sensory channels.

*Keywords:* visual attention, pupillometry, visual working memory

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Attention and perception are an absolute necessity in mastering many tasks in everyday life. The visual modality plays a central role in our society, because it is a key component in processing information – whether in road traffic, in social interactions or when we are searching for something. Because visual attention has such a high impact on our mind, and thus how we experience the world and interact with it, it has been researched in various fields of cognition – from language processing, to decision making, sexual attraction and memory. But not only does what we see shape our thoughts and behavior; our eyes can also mirror the processes that are taking place in our minds. The eyes are often called windows to the soul. Speaking less poetic, they might actually be considered windows to the brain: The retina and optic nerve develop from embryonic forebrain tissue, and are thus considered part of the central nervous system.

This also shows in the fact that there are many reflexes of the pupil that biologists and cognition researchers have observed. The best known one is called the Pupillary Light Reflex (PLR) which serves in adjusting light influx by contraction and dilation of the pupil relative to a specific stimulus brightness or overall environment lighting. It is one of the most fundamental necessities for accurate human vision. It enables us to adapt to lighting changes and moreover, object brightness and brightness differences, ultimately giving us the ability to perceive contrast. But the pupil is involved in more processes than just reaction to different light conditions: many cognitive factors modulate pupil size as well, such as attention (e.g., Kahneman, 1973), emotions (e.g., Bradley et al., 2008; Nagai et al., 2002) or decisions (Simpson & Hale, 1969). Pupil size also increases with cognitive load (Hess & Polt, 1964) and moreover, increased working memory (WM) and task demands (Granholm et al., 1996; Kahneman & Beatty, 1966). In a recent review, Mathôt (2020) describes three broad classes of pupil responses: aforementioned PLR, the pupil near response as another biological adaptation mechanism that accommodates the pupil size to focal distance, and finally, the psychosensory pupil response. The latter comprises pupil responses that are not mainly involved in adjustment of object recognition, but rather driven by additional cognitive processes and demands as mentioned above. We will refer to the psychosensory pupil response as PLR as it is

the more common term in literature. It stands to reason that most research on the relationship between cognition and the PLR comes from visual attention, though even within this field, many roads lead to the connection of pupillometry and attention. I will focus on the interplay of covert attention, visual working memory (VWM) and PLR here.

### **The pupil response in attention**

Early important studies on the relationship between attention, pupil response and luminance (= perceived brightness) stem from the field of binocular rivalry, where different content is presented to each eye. As the attention switches from one visual field to the other, for example from a dark stimulus in the left eye to a bright stimulus in the right eye, pupillary constriction takes place (Lowe & Ogle, 1966). Work in this field raised the idea that the pupil does not only react to what is presented to the eyes, but rather to what we attend to. This act of having an attentional focus other than what we look at with the fovea, the center of the pupil, is called covert attention. The concept of covert attention itself, however, is older: it was Helmholtz (1894) who found evidence that attention could be oriented without eye movements. Later, Posner (1980) extended this finding in his famous studies on attention and signal detection where he showed that attention shifts do not necessarily need to involve saccadic eye movements. Interestingly, he also found that the retinal areas – whether foveal or peripheral – are equipotential in luminance detection, in other words, equally sensitive to luminance changes. For experiments on the PLR this is especially useful since attention can thus be guided despite fixated gaze. Naturally, if participants are to move their eyes relative to a stimulus with changing distance, they will optimize light influx with the pupil near reflex. Even if the stimuli are presented within a constant distance, pupil dilation reflecting preparation of eye movements occur if attentional shifts are accompanied by saccadic eye movements (Jainta, 2011; Mathôt et al., 2014b).

Recent research has successfully proven that the pupillary response is closely linked to the locus of attention. Binda et al. (2013) showed that the pupillary dilation and constriction reflects the luminance of a single object, even in covert attention.

Their participants attended to one of two large disks while maintaining a fixated gaze on a central point and never looking directly at the disks. In line with their expectations, Binda et al. (2013) found that pupil constriction occurred when participants switched their attention from the dark disk to the bright one. Later studies replicated and extended this finding (see also Mathôt et al., 2013; Naber et al., 2013).

These studies all focused on attentional guidance and objects' influence of the PLR in present stimuli – when they were actually displayed. But there is evidence that even absent stimuli influence the pupil size. Picture the following scenario. It is a warm, bright summer day, but you are sitting in the University's basement in a dark room, having participants do color search tasks. While you sit there, you think of the sun and how good it would feel on your skin. Not only do you now want to be outside, but something else happens in this moment: your pupil constricts, just as if you were outside, even though you are still in that dark room. Laeng and Sulutvedt (2014) conducted an experiment with exactly this task, instructing their participants to think of either a dark room or the sunlight. They found that pupil dilation or constriction, respectively, occurred relative to the stimulus' luminance. What makes this finding so surprising is that Laeng and Sulutvedt (2014) did not show any bright or dark pictures, the participants' imagination was sufficient to evoke a pupil response. These results are consistent with the finding that similar visual sensory areas are active during perception and mental imagery of visual objects (Ganis et al., 2004).

Current research supports the hypothesis that the pupil reflects working memory items and their luminance. Blom et al. (2016) showed that the pupil adjusts to the relevant target's luminance during encoding of content to the VWM, that is, where visual stimuli are stored for a short time. Lately, Hustá et al. (2019) provided evidence that the PLR also reflects maintenance of a stimulus in the VWM. They created a similar study design as Blom et al. (2016), but with retro cues, and found a brightness-related PLR in encoding and in the maintenance phase as well (which Blom and colleagues did not find). Zokaei et al. (2019) argue this to be true even when stimulus brightness is not required for the working memory task. Strikingly, in Hustá et al. (2019), pupil size reflected VWM content only briefly. This suggests that a

shift of attention within VWM momentarily activates an active memory representation, but that this representation quickly transforms into a hidden state that does not rely on sensory areas. In conclusion, there has been sturdy proof that the pupil changes relative to WM items' luminance; even if only for a short time and this pupil response is usually smaller than the common PLR (Mathôt, 2020). Recent studies (Heitz et al., 2008; Tsukahara et al., 2016) have furthermore found a positive correlation between average resting-state pupil sizes and individuals' working memory capacity. In a follow-up study, Aminihaibashi et al. (2019) found that it is in fact a higher variability in resting-state pupil size that correlates with higher WM capacity. Could different pupil sizes be related to multiple objects in VWM? Answering this question is not trivial and requires more elaboration on what we know so far about VWM in general and especially in visual search.

### **Attentional templates and (visual) working memory**

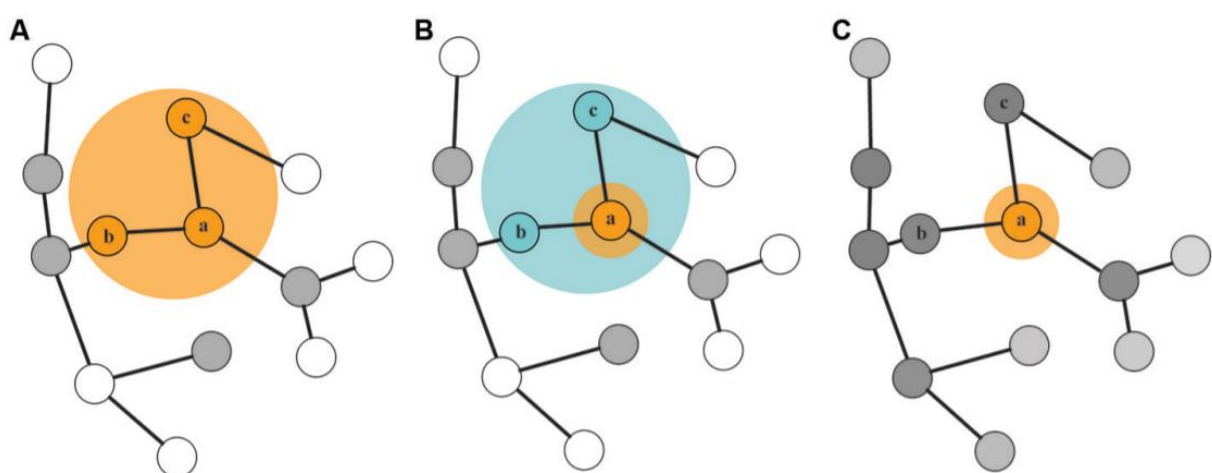
What allows us to find specific target items within milliseconds of search? Folk et al. (1992) carried out major investigations on attentional capture as a mechanism where stimuli that match VWM content draw attention. They used visual cues that appeared before searching for a target color item. These can match the target color (matching) or not (non-matching) and be in the same place as the target (valid) or elsewhere (invalid) (e.g., Büsel et al., 2019). Shorter response times (RTs) on valid than invalid trials are referred to as cueing effect and prove that attention was captured by the cue. Folk et al. (1992) showed that cueing effects are only observed when cue and target properties match. This is usually interpreted as proof that an attentional template in VWM has been established, wherein we save target items and their features (e.g., Desimone & Duncan, 1995). These attentional templates or search templates facilitate the attentional shift toward the memorized color. Thus, only stimuli whose features match the attentional template capture attention. However, theories disagree on how many VWM items can bias attention simultaneously. In other words, there is an ongoing debate whether we can uphold multiple items in attentional templates. How are multiple items stored in WM in general?



Modern WM models more or less agree on a distinction between active and passive states of items, but differ in how many items can be activated at once (see Figure 1). Cowan (1995) coined the term Focus of Attention (FoA), describing a prioritized (activated) state in short term memory that up to four items can concurrently enter. Items in the FoA receive resource prioritization until they are no longer immediately relevant and thus enter the state of activated long term memory (aLTM). McElree (1998) proposes a more smoothly transcending continuum of memory strength stages and a single-item FoA as the strongest state of activation. Finally, Oberauer (2002) identifies a narrow FoA which can select information from a state of direct access – that is a state between FoA and aLTM (for a review, see LaRocque et al., 2014). While all these approaches share the idea of attention as main actor in item activation in WM, none of them can answer the most intriguing questions alone: How big exactly is the item range of the FoA? And consequently, how many items can we use in attentional templates at a time when only items in a more or less active state can bias attention?

**Figure 1**

*State-based working memory models*



*Note.* The state-based models by (A) Cowan (1995), (B) Oberauer (2002) and (C) McElree (1998). Adapted from "Multiple neural states of representation in short-term

memory? It's a matter of attention " by J. J. LaRocque, J. A. Lewis-Peacock and B. R. Postle, 2014, *Frontiers in Human Neuroscience*, 8, 5.

An even more recent view on WM addresses these questions by saying that instead of a fixed number of (active) items, our WM capacity is defined by a limited resource pool that can be distributed flexibly according to task demands (Ma et al., 2014). This could explain why the results in research on attentional templates differ from each other, depending on which study design is used: There is an ongoing debate concerning the number of items that can be implemented in templates to guide visual search.

Supporters of the single-item-template (SIT) hypothesis hold that only one item can be in an active state at once and bias attention (Olivers et al., 2011). As such, although it is possible to store multiple items in VWM, only one item would affect visual search. Later, Van Moorselaar et al. (2014) added another assumption to the SIT hypothesis: When multiple items are held in VWM, competition between the items would arise and none could reach an active state. In contrast, the multiple-item-template (MIT) hypothesis states that multiple VWM items can be activated simultaneously (Beck et al., 2012). Beck et al. (2012) instructed participants to search for two target colors simultaneously or sequentially (i.e., one color at a time). They found that participants frequently alternated between differently colored targets. Also, fixating the same target color twice in a row was just as efficient as shifting between two differing target colors. Based on the lack of switch costs (worse performance when target properties changed from trial to trial), the authors concluded that maintaining two active templates in VWM is possible.

On the contrary, Luck and Hillyard (1994) conducted an EEG study where participants had to memorize one or two colors in a search task. An N2pc component was triggered when the target singleton was presented within an array of distractors, but not if the array could be rejected as nontarget. Thus, the N2pc is concluded to represent orienting of attention. They also found the N2pc component when participants had to hold two colors in VWM. However, in that case, the N2pc was smaller in amplitude and emerged a bit later in time (about 30 ms). This suggests

that guidance by multiple VWM items is possible, but less effective as the number of VWM items increases (Grubert et al., 2016). Other authors have started to give more credit to the idea that allocation of VWM resources is task dependent. Kristjánsson and Kristjánsson (2018) found a linear increase of switch costs with increased target number. They concluded that instead of use of one versus two attentional templates, the load amount determines VWM performance. Chetverikov et al. (2020) propose that attentional templates represent spatial probabilities in VWM.

Kerzel and Witzel (2019) conclude on these contradictory results by differentiating two major lines of research, that both claim to investigate maintenance of two stimuli in VWM. In dual task studies, a second task is embedded within the main task. That way, the initial target stimulus is maintained in the VWM while another task has to be completed. On the other hand, there are dual target search studies, where two stimuli are to be maintained and only one of them will appear as target. While dual task studies mainly support the idea of SIT, dual target search studies find more evidence in favor of MIT (see Kerzel et al., 2019). Therefore, it is unclear if keeping in mind multiple objects and their features at once (in one attentional template) is possible. Even though there is mixed evidence on multiple templates, many studies (e.g., Ansorge & Horstmann, 2007; Ansorge et al., 2005; Irons et al., 2012; Worschech & Ansorge, 2012) find contingent capture for two memorized colors. Thus, it is safe to say that at least under certain conditions in dual-color search, two items can simultaneously guide visual attention.

However, the questions remain if we store multiple items in multiple attentional templates or in a single one, and whether attentional guidance by multiple items is less effective than guidance by a single item. Lately, the idea that we very quickly switch between single items held in different attentional templates is gaining more attention (e.g., Ort et al., 2017). This view can also integrate the finding of alternation between differently colored targets by Beck and colleagues (2012). Büsel et al. (2019) wanted to investigate whether in dual-color search the two colors are held in one shared attentional template or in two separate templates. They found switch costs and mixing costs (Monsell, 2003), meaning that participants performed worse when target properties changed from trial to trial than when they stayed the

same. This attentional switch therefore demanded activation of multiple VWM items. Büsel et al. (2019) concluded that these costs are caused by a switch between two attentional templates.

### **Fluctuation in attention**

Selective attention prioritizing one item out of many at a time and switching between them if required is not an entirely new idea. Landau and Fries (2012) showed that the covert sampling of multiple targets occurs rhythmically, fluctuating between two targets with a frequency of 8 Hz and thus attending one object at approximately 4 Hz. They used a salient flash event to mark onset of attentional capture and then asked participants to react to visual events that occurred rhythmically in the right and left visual field. Performance was best if the events matched the 8 Hz frequency.

Re et al. (2019) recently added that attentional sampling operates independent of spatial position, indicating that encoding of objects is feature-based: they found oscillation at 8 Hz for overlapping, moving cloud patterns. Re et al. (2019) suggest that because of overlapping, their stimuli had no spatial separation. Therefore, attentional oscillation seems to reflect switching between two mental objects rather than two locations. According to Büsel et al. (2019) and Ort et al. (2017), multiple color items are stored in separate attentional templates VWM and we switch between them. Since sensory processing, mental imagery and the mechanisms of the VWM share many properties (e.g., Ganis et al., 2004), it is very likely, that we switch between attentional templates in a comparable manner as in spatial attention itself. If this happens at the same frequency of 4 Hz per item has still to be investigated. Since the PLR is able to reproduce the VWM item that is currently in an active state, it is suited to address this question.

In a second experiment, Hustá et al. (2019) tested for a PLR in the maintenance of multiple color targets, but did not find a significant effect. However, the results were in the same direction as in their set-size-one condition, where they found a tendency towards smaller pupils when maintaining bright circles as compared to dark. In their set-size-two condition, they used multiple targets of the

same brightness levels, meaning that the two targets would for example be two dark circles. While they probably intended to ensure a large PLR, it is not possible to differentiate between two distinct PLRs that could have developed for the single items. Naber and Alvarez (2013) studied how fast PLRs can alternate and found that pupil size adapted to a flickering screen with a frequency up to 3 Hz. Since the classical fluctuation rate per object is 4 Hz, it should just be enough to leave a visible PLR.

We constructed a task similar to Kerzel and Witzel (2019), in which the participants were asked to memorize two colors of highly different brightness levels, and where colors are chosen randomly with equidistance to each other. The attentional capture paradigm was used because it serves well in showing the active attentional template. Cuing effects were measured to make sure that both target colors have been successfully translated into templates. In accordance with Hustá (2019), the PLR should reflect encoding and maintaining of the active attentional template by pupil size relative to the stimulus brightness, at least in single-color search. In dual-color search, the PLR should show oscillations, reflecting a rhythmical switch between attentional templates during encoding and maintaining of the two colors with different brightness in VWM.

## **Method**

### **Participants**

The PLR during VWM maintenance of multiple colors has not been reported in the literature so far other than by Hustá et al. (2019), therefore we aimed for the same sample size of 30 subjects. Participants were undergraduate psychology students at the University of Vienna that participated voluntarily (in exchange for partial course credit). All participants had normal or corrected-to-normal visual acuity and intact color vision. The experiment was conducted in accordance with the Declaration of Helsinki and APA ethical standards in the conduct of research. Written informed consent was obtained from all participants. The study was approved by the

Ethics Committee of the University of Vienna. Participants received a short introduction to the study goals online beforehand and extended verbal debriefing after their testing.

In total, 30 students participated in the experiment. Data from two participants were excluded from further analysis because no reliable pupil recordings could be obtained and the task was not completed. The 28 participants (22 women, 6 men) used for data analysis were aged 19 to 46 years ( $M=22.5$ ,  $SD=5$ ,  $Mdn=20$ ).

### **Stimuli and Apparatus**

All stimuli were chosen from CIE Lab (CIELAB) space because it describes all the colors visible to the human eye and serves as a device-independent reference model. In CIELAB, color values are defined on three axes: one achromatic axis, perceived lightness  $L$ , and two chromatic axes, green-red dimension  $a$  and blue-yellow dimension  $b$ . The polar coordinates of the chromatic axes ( $a$  and  $b$ ) correspond to hue (color intensity) and chroma (difference from grey). In particular, distances in CIELAB approximate perceived color differences (Fairchild, 2005). Therefore, distances on the  $L^*$  axis mirror luminance distances and allow exact manipulations of perceived brightness. For white point reference, we used the D65 illuminant.

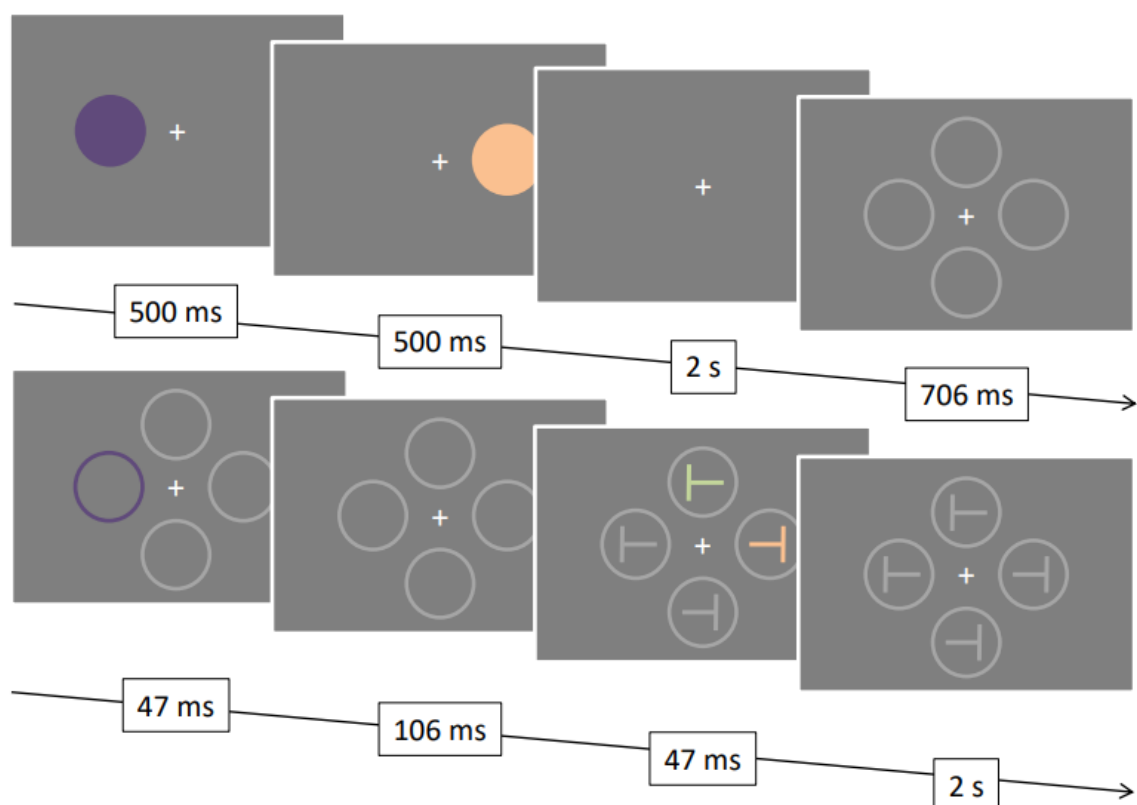
The bright and dark target colors were selected from a specified brightness range each. The bright range extended from  $L=74$  to  $L=78$ , and the dark range extended from  $L=32$  to  $L=34$ . To determine the two target colors each trial, two different color triplets were chosen from these ranges, always one bright and one dark. Coordinates on the  $a$ - and  $b$ -axis were chosen randomly to match an overall predetermined hue level. The background was dark grey ( $L = 66$ ,  $a = 19$   $b = -44$ ), placeholders light grey ( $L = 74$ ), the fixation cross and instructions white ( $L = 109$ ,  $a = 16$   $b = -55$ ). All stimuli except for the memory color disks were  $0.19^\circ$  thick. The participants had a fixated distance of 59 cm to the RCT monitor (1280 x 1024, 85 Hz). Their dominant eye was tracked with an SR Eyelink 1000 plus eye tracker which sampled the data at 1000 Hz. The experiment was programmed in MATLAB with Psychtoolbox-3 and Eyelink toolbox.

## Procedure

For the task procedure (see Figure 2 for time stamps), we adapted the task from Kerzel et al. (2019), but with spatially and temporally separated color displays and a longer retention interval to allow for the relatively slow pupil response. In three blocks, the participants were asked to memorize either the first or the second color (single-color blocks) or both colors (dual-color block). Subsequently, participants were asked to report orientation of the target color stimulus.

**Figure 2**

*Task procedure*



*Note.* Task procedure from top left to bottom right. Numbers indicate the stimulus timings. This figure represents one arbitrary trial sequence in the dual-color block with an irrelevant, invalid cue.

First, one disk (f.e. bright, radius of  $3.2^\circ$ ) appeared on the left side of the fixation cross, followed by a disk (f.e. dark, respectively; radius of  $3.2^\circ$ ; both placed with a distance of  $0.78^\circ$  to the screen center) to the right of the fixation cross ( $0.39^\circ \times 0.39^\circ$ , placed in the screen center). After that, there was a retention period where only the fixation cross was shown. The retention period was followed by onset of placeholder rings (all  $3.2^\circ$  big with a distance of  $0.78^\circ$  to the screen center) for the cue, which was realized as coloring of one placeholder. The cue could be of the same color as the target (matching) or the third, unrelated color (nonmatching), but never the non-target memory color. The cue's position could be identical to the target (valid) or not (invalid). The target window consisted of the light grey placeholders (same position and size) plus four "T"s, tilted  $90^\circ$  to the left or to the right, one of them in the target color, one in a distractor color and two in light grey ( $2.24^\circ \times 2.24^\circ$ , placed centrally within placeholder rings). The distractor could have the irrelevant second color or an unrelated random color in single-color blocks and was always a random unrelated color in the dual-color block. In the subsequent answer window, participants were asked to report the orientation of the target by using the keys Y (left) or M (right) on the computer keyboard. After participants' report, a feedback was given if the answer was correct or not. In case of response times over two seconds or an invalid key press (a key other than Y or M), participants were asked to answer faster or were reminded of the valid keys, respectively. Finally, there was an inter trial interval to allow for a pupillary refractory period. Blocks consisted of 768 trials each, so in total, each participant completed 2304 trials. Breaks of self-chosen length were possible between blocks and within blocks at 25%, 50% and 75% of trials completed. All trials within blocks were randomized, as was order of blocks with equal weight.

## Results

### Response times

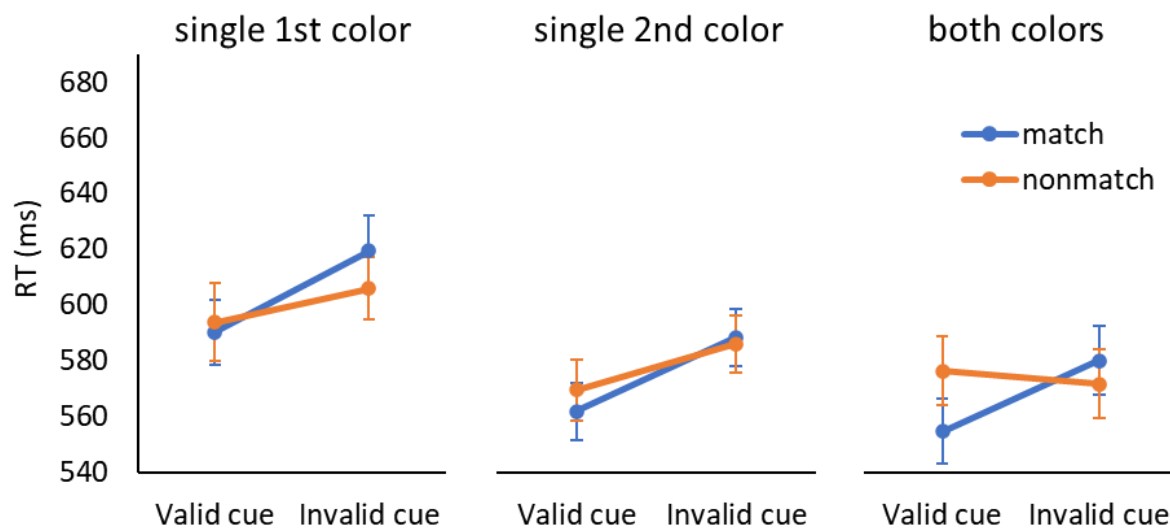
As stated above, data of two participants was excluded from analysis due to a technical issue with the eye tracking. Reaction times greater than 1 s and shorter than 200 ms were excluded from further analysis (3.06% of trials).



We first carried out a 3 (memorized target color: first, second, both)  $\times$  2 (cue color: matching, non-matching)  $\times$  2 (cue validity: valid, invalid) repeated-measures ANOVA on the individual mean trimmed RTs. Figure 3 shows the mean RTs for every task condition. The main effect of memorized target color was  $F(2) = 3.987, p < .05^*$ ,  $\eta_p^2 = .129$ , indicating differences between the blocks. In general, participants responded slower when they had to remember the first color ( $M = 602$  ms,  $SD = 65.66$ ) than the second ( $M = 577$  ms,  $SD = 55.55$ ) or both colors ( $M = 571$  ms,  $SD = 63.89$ ). We found a significant validity effect with  $F(1) = 58.892 (p < .001^{***}, \eta_p^2 = .686)$  over all conditions. There was also a 2-way interaction between cue color and validity, which modulated the main effects:  $F(1) = 22.784 (p < .001^{***}, \eta_p^2 = .458)$ , while cue color alone had no significant effect on RTs [ $F(1) = 0.680, p > .05$ ].

**Figure 3**

*Mean RTs as a function of task conditions*



*Note.* Mean RTs as a function of task (memorize the 1<sup>st</sup>, the 2<sup>nd</sup>, or both target colors), match between memorized target color and cue color, and cue validity. Error bars represent  $\pm 1$  SEM.

The 3-way-interaction between memorized target color  $\times$  validity  $\times$  cue color was not significant [ $F(2) = 1.947, p = .153$ ], showing that the cue color  $\times$  validity

interaction occurred independently in single-color and two-color trials. This was also reflected in post-hoc comparisons which showed that the cue color dependent validity effect size was similar in all three memory color conditions, as reported in Table 1 below.

**Table 1**

*The cue color dependent validity effect*

matching cue color	valid	invalid	<i>M</i> difference	<i>t</i>	<i>p</i>
first	590.25 (62.04)	619.53 (66.18)	-29.287	-5.698	<.001***
second	561.95 (54.80)	588.59 (54.24)	-26.636	-5.183	<.001***
both	554.85 (61.11)	580.27 (64.32)	-25.414	-4.945	<.001***
nonmatching cue color					
first	593.92 (73.03)	606.05 (60.12)	-12.130	-2.360	.740
second	569.66 (57.86)	586.10 (53.63)	-16.444	-3.200	.273
both	576.47 (64.54)	571.86 (65.99)	4.611	2.677	.415

*Note.* Mean RTs (SD) in ms for valid and invalid cues for each memorized target color condition (first, second, both) by matching and nonmatching cue color trials. Mean differences were tested in post hoc t-tests.

This interaction of validity and cue color shows that valid cues led to shorter RTs than invalid cues when the cue matched the memorized target color, but not when the cue did not match the memorized target color. This commonly referred to as contingent capture effect. Summarized over all conditions, participants responded faster in trials with valid, matching cues than in trials with invalid, matching cues (27 ms slower,  $t = -8.918$ ,  $p < .001^{***}$ ), followed by invalid, nonmatching cues (19 ms slower,  $t = -6.603$ ,  $p < .001^{***}$ ) and valid, nonmatching cues (11 ms slower,  $t = -4.142$ ,  $p < .001^{***}$ ). Furthermore, RTs in trials with invalid, matching cues were 16 ms slower ( $t = 5.603$ ,  $p < .001^{***}$ ) than in trials with valid, nonmatching cues and 8 ms slower than in trials with invalid, nonmatching cues ( $t = -3.060$ ,  $p < .01^{**}$ ). Finally, for nonmatching cues, valid trials were 8 ms faster than invalid trials ( $t = -2.627$ ,  $p < .05^{*}$ ).

### Inverse efficiency scores

Additionally, to have a look at possible speed-accuracy tradeoffs, inverse efficiency scores (IES) were calculated as  $IES = RT / \text{accuracy}$ , with accuracy = 1 - error rate (Townsend & Ashby, 1978). The same 3-way ANOVA was carried out for IES as for RTs. In summary, the results were in the same direction as the RTs. The validity effect was significant with  $F(1) = 18.660$ ,  $p < .001^{***}$ ,  $\eta_p^2 = .409$ . We also found the 2-way interaction of validity and cue color match with  $F(1) = 12.959$ ,  $p < .01^{**}$ ,  $\eta_p^2 = .324$ . The main effect for memorized target color, however, disappeared [ $F(1.65) = 2.344$ ,  $p > .05$ ]. For this comparison, the assumption of sphericity was violated (Mauchly's test,  $p > .05$ ) and thus, Greenhouse-Geisser correction was applied. Still, the means were biggest for first color ( $M = 680$  ms,  $SD = 131.89$ ), followed by the second color ( $M = 629$  ms,  $SD = 103.61$ ) and both colors ( $M = 627$  ms,  $SD = 104.47$ ). The 3-way interaction of memorized target color x validity x cue color was not significant [ $F(1.823) = 1.191$ ,  $p = .309$ ]. Participants were more efficient in valid, matching trials than in invalid matching trials (30 ms slower,  $t = -5.613$ ,  $p < .001^{***}$ ), invalid, nonmatching trials (26 ms slower,  $t = -3.466$ ,  $p < .01^{**}$ ) and valid, nonmatching trials (22 ms slower,  $t = -3.025$ ,  $p < .05^*$ ).

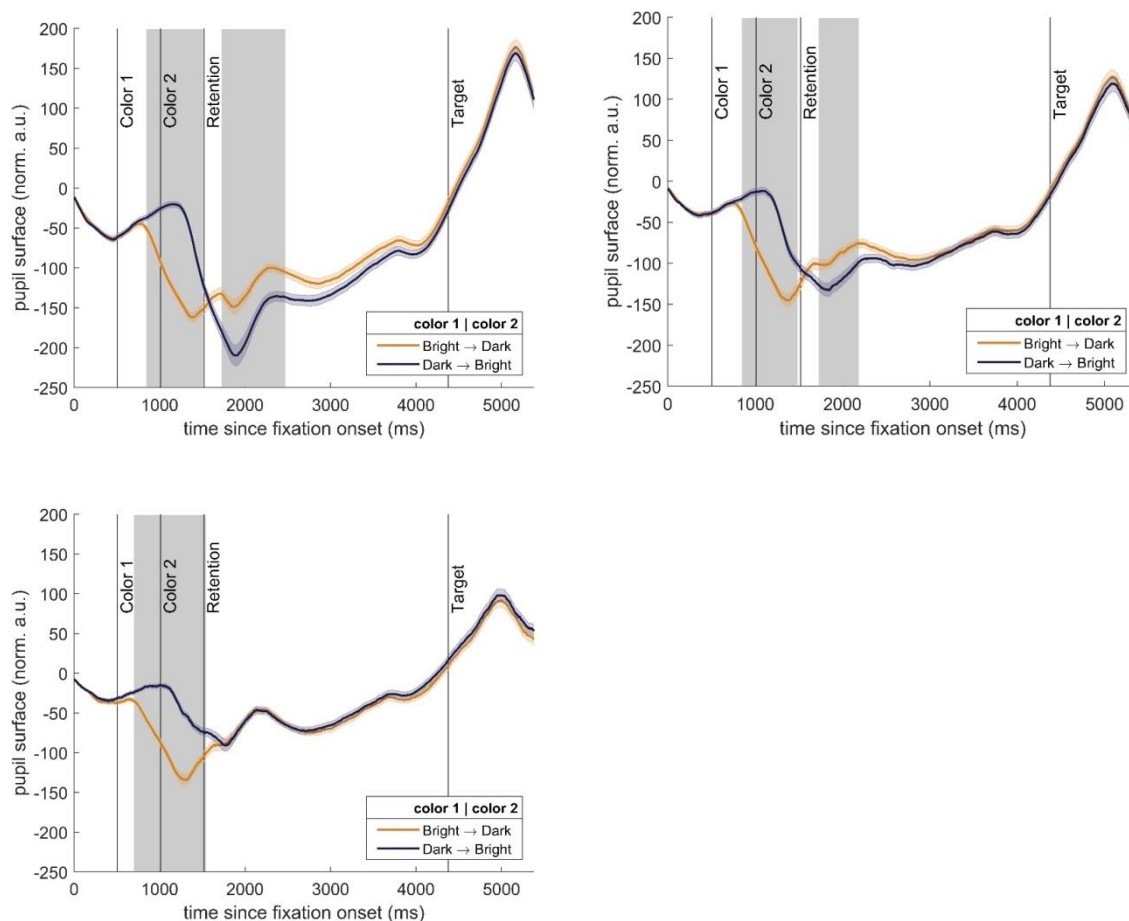
### Pupil Traces

Pupil traces from each participant were smoothed using a 50-point Gaussian window. Next, an algorithm to detect blinks was constructed (similar to Mathôt, 2013): using the smoothed signal a velocity profile was generated where velocity in each sample is the difference in pupil surface from the current to the previous sample. Blink onsets were defined as the moment when velocity dropped below a threshold of -10 (a.u.) while blink offsets were defined as the moment when velocity returned to 0 after a reversal period where velocity reached at least 6. As this algorithm tends to underestimate blink duration, a 10 ms margin was added to the beginning and end of each blink period. After detection, blinks were interpolated using cubic spline interpolation (Mathôt, 2013) and the signal was detrended.

Pupil surface was analyzed for epochs spanning the entire duration of each trial (only trials with correct responses were included in the analysis) relative to a

baseline period of 100 ms prior to onset of the fixation cross. The mean baseline pupil surface was subtracted from each sample in the epoch (Mathôt et al., 2018). To examine differences in pupil traces, linear mixed-effects (LME) analyses were used, with a criterion of  $t > 2$  for significant effects, which is comparable to  $p < .05$  (Baayen et al., 2008). LME models were constructed for each 1-ms pupil trace sample and only sequences of at least 200 ms (i.e., 200 consecutive samples) for which  $t > 2$  were considered significant (Mathôt et al., 2013). The LME models were of the form *pupil size*  $\sim$  *order* (*order* | *participant*), where the fixed effect *order* refers to the order of presentation for the memory colors (bright  $\rightarrow$  dark vs. dark  $\rightarrow$  bright). Participant was a random effect on the intercept and pupil size the dependent measure. The analysis was performed for each of the three memory conditions separately: first color, second color and both colors (see Figure 4).

Overall, all conditions shared a preparatory pupil constriction before onset of the first memory color as well as rapid pupil dilation at around 4000 ms (after fixation onset), which is typically related to the higher cognitive demand of the search task. Also, the quality of the PLR was determined by the order of brightness related stimuli rather than by the target color. There was, however, a big difference in the quantitative course of the PLR between conditions. Typically, in trials with the order bright  $\rightarrow$  dark, we found constriction until 1300 ms (shortly before retention onset), dilation until 1700 ms, short constriction of about 100 ms and finally dilation until 2500 ms. In trials with the order dark  $\rightarrow$  bright, PLR course was dilation until 1200 ms, constriction until 1900 ms, dilation until 2500 ms. In single-color conditions, PLR towards target brightness differed significantly in the late memory color display and in the early retention period. Dual-color trials differed only in the late memory color display.

**Figure 4***Pupil traces by memorized target color condition*

*Note.* Pupil traces from each of the three target color conditions by brightness order.

**Top left:** single-color condition, first color. **Top right:** single-color condition, second color. **Bottom:** dual-color condition, both colors. Each panel shows pupil size as a function of the order of stimulus presentation (first bright, then dark: orange line; first dark, then bright: blue line). Data from the entire trial from fixation onset until 1 s after target display onset. Pupil surface size is shown relative to baseline size during the 100 ms prior to fixation cross onset (0 on the x-axis). Gray shading indicates periods of significant differences between the conditions ( $t > 2$  for at least

200 consecutive samples). Error shading indicates  $\pm 1$  *SEM* pooled across all participants and trials within a condition. Data reflects the grand mean signal.

## Discussion

In this study, eye tracking was combined with behavioral data to investigate the nature of multiple items in VWM. Special focus was on activation states of color items in attentional templates. Particularly, we asked if it was possible to establish two attentional templates that hold a single target color in an active state in the early sensory VWM. Thus, the first question addressed the ongoing debate whether one single or multiple attentional templates can simultaneously control attention. This was investigated via PLR throughout encoding, retention and reproduction of brightness-controlled color stimuli in a search task.

### Pupil sizes

Consistent with previous studies (Binda et al., 2013; Hustá et al., 2019; Laeng & Sulutvedt, 2014; Mathôt et al., 2013), we showed that VWM content is reflected in the PLR during encoding of target colors. The three different target color conditions (first, second, both) clearly differed from each other. There was a significant difference between luminance orders bright  $\rightarrow$  dark and dark  $\rightarrow$  bright. During memory color display, PLR reflected stimuli brightness of both memory colors in the presented order, indicating that the irrelevant target also elicited a PLR.

We also found a significant difference in the PLR between dark and bright target color stimuli in the beginning of the retention interval in single-color trials. However, there were major differences between conditions in this phase. In the first condition, initial pupil constriction was followed by dilation for the bright  $\rightarrow$  dark luminance order; but there was no constriction in the second condition, only dilation in both luminance levels with bigger pupil sizes for dark targets. Our conclusion is that PLR in the beginning of the retention period has to be interpreted in respect to brightness order. The effects were in the expected direction; in that internally shifting

attention toward bright stimuli elicits smaller pupils than internally shifting attention toward dark stimuli, but this effect was influenced by the last presented color. After pupil dilation, there was always relative pupil constriction and vice versa, no matter the memory color (we will refer to this as reverse effect). Still, we present profound evidence that the PLR shows the focus of attention in stimuli encoding in general as well as in an early retention period in single-color search.

Surprisingly, against the hypothesis, we did not find an oscillating PLR in the dual-color condition. One could argue that the higher cognitive task demands caused a bias in the PLR. Even though average pupil size was bigger in the retention period in dual-color trials than in single-color trials, it was still below the baseline. Pupil dilation associated with task demands is usually seen shortly before and after target onset, indicating the (expected) search effort. This rapid increase in pupil size appears also in the dual-color condition. In fact, the PLR during retention in the dual-color condition is more similar to that in the single-color conditions than to the later search performance. Therefore cognitive demands do not seem to have had an impact before target onset. Hustá et al. (2019), who also found an effect in the single-color condition but not in the dual-color condition, interpreted their similar results as a lack of compelling dissociation between maintenance of one or two brightness-related color stimuli. However, the fact that single item conditions led to a significant PLR during stimulus maintenance and the dual-color condition did not, is a notable difference. Interestingly, the active attentional template in VWM affected pupil size only briefly, rather than throughout the entire retention interval. It is likely that this may represent the item's active state as VWM content, reflected in pupil size, which quickly transitioned to a hidden state not reflected in pupil size (Hustá et al., 2019). It seems that maintaining multiple items works in a different way than maintaining single items.

However, PLR during retention might not simply represent the VWM items, since pupil size during that interval did not depend on target brightness alone, but instead on the order in which the memory colors were presented. Mathôt et al. (2014a) found a similar reverse effect in the PLR when participants had to react to exogenous allocation of attention. In their study, this reflected an inhibition of

detection and discrimination in the brightness related area. Mathôt et al. (2014a) conclude that this brightness dependent reverse effect in the PLR signifies inhibition of return (IOR), where information processing in a certain visual area is impaired shortly after it was attended to (Posner & Cohen, 1984). Since we presented 2 colors only which also just differed in the color dimension, it is possible that in the single-color conditions, active suppression of the presented but not searched for color was necessary. This could have become apparent in pupil size, as an IOR to the PLR of the irrelevant target color. What makes this explanation likely is that our search task was relatively easy, with presentation of two items differing in one feature dimension. Under these conditions, the classic theory of four item slots in WM of Cowan (1995) would predict that we necessarily encode both items (and probably even two additional ones). In terms of resource models (Ma et al., 2014), the VWM resource pool might just be bigger than what was needed by the task. Average accuracies of 80-90% underline the simplicity of our task. Finally, even the encoding interval in single-color trials shows a PLR for both presented colors. A follow-up study should address this by using more than four colors.

Taken altogether, it seems that single-color search makes use of sensory VWM representations, while dual-color search does not or it does not become apparent in the same way as it does in single-color search. However, since single colors stay in an active state only for a short amount of time and then enter a hidden state, there seems to be at least two distinct mechanisms for retention in VWM: one that relies on early sensory channels and one that does not. Multiple relevant color targets might not enter an active state in sensory parts of the VWM during retention because they do not make use of this sensory mechanism. Consistent with Kristjánsson and Kristjánsson (2018), there could be task-dependent or load-dependent VWM mechanisms. Thus, even though in single-color search keeping up a visual image of the target might be a parsimonious retention mechanism in VWM, that does not seem to be the case with multiple objects.

### **Attentional templates**

We found classical contingent capture effects across single and dual target color conditions, which were in their size unaffected by the number of memorized



target colors. Only colors that matched the memory resulted in cuing effects, whereas the irrelevant shown color or unrelated colors did not. The size of the cuing effects was similar for one and two memorized target colors. In total, our findings are along the lines of those by Kerzel et al. (2019) and further support simultaneous control of attention by multiple attentional templates. Like them, we used randomly drawn target colors for cues and targets as described in the Methods section. This procedure made sure that participants could not load stimuli on aLTM but instead had to load them on VWM anew every trial. In the vast majority of previous dual target search studies, it was possible that participants simply offloaded target representations into aLTM because the memorized target colors were chosen from a very small range of colors.

We found a difference in RTs for the three memorized target color conditions that evened out when we calculated inverse efficiency scores. That indicates there was a small speed accuracy tradeoff in the third condition where two target colors had to be memorized. Interestingly, there were no overall costs for multiple colors, and if, rather towards lower RTs in the dual-color condition. In accordance with our inhibition hypothesis described in the discussion of pupil traces, two colors could actually facilitate search relative to search for one out of two colors: Because we worked with very low VWM load (maximum two items that were only defined on the color dimension), it is likely that we are, by nature, drawn to encode both presented stimuli. Lower RTs in the dual-color condition might thus reflect absence of active suppression for one color which in turn led to slightly higher error rates through interference by that color.

## **General discussion**

Traditional SIT theories predicted that the PLR would reflect VWM content only when one item had to be maintained, leading it to be in a high-priority state. This would not work when participants maintained two items, since these would then compete with each other and both take on a low-priority state (Olivers et al., 2011; van Moorselaar et al., 2014). RTs in this study, however, showed no disadvantage for the third condition where both colors had to be maintained. So, it seems unlikely that the absence of a PLR during retention was only due to competition of two color items

within one template, but rather due to competition between two templates. Cuing effects were present in dual-color search, showing that both colors were established as attentional templates. That was true even though colors changed from trial to trial and therefore, it was not possible to load on aLTM. As mentioned before, two items competing within one template would fail to reach a high-priority state and thus not lead to cuing effects.

Büsel et al. (2019) reported first evidence for multiple templates with single slots, but finding a clear answer to this question has proven tricky. It seems that at least a strong feature-based differentiation between the stimuli is necessary to promote establishment of multiple templates (see Hustá et al., 2019). Here, we present more support towards different VWM mechanisms for single-color search and dual-color search. Our results provide sturdy evidence supported by RTs and pupil data for attentional guidance by multiple single-item-templates. While recently evidence has been growing that at least under specific conditions, we are able to use multiple templates in search for targets differing in a certain feature, it is not clear how many items these templates may hold. With respect to state-based WM models (for a review, see LaRocque et al., 2014) multiple items within VWM enter different activation states. Future research should hence investigate how the VWM items transition from one state to another.

The possibility of color items being retained equally strong in a hidden state in VWM that is not reflected by the pupil should be highlighted. Against our expectation, we did not find an oscillating PLR for maintaining multiple target colors in the VWM. In fact, while we found a strong PLR during retention of single-color search items that could be related to active item suppression, there was no evidence for a VWM mechanism measurable with PLR in dual-color search. The old question remains: what makes single-item search and multiple-item search different? It also puts up some new questions for future research. What other mechanism is used for multiple-item search that is not reflected in the pupil? And finally, how can we learn more about this mechanism behind hidden states?

Despite the limits we have encountered in dual-color search, we believe that measuring the PLR is a great way to study visual attention because it offers an

alternative to classic approaches such as RTs alone. When studying covert attention, it is possible to predict which stimulus is currently in the focus of attention from pupil size, as Zokaei et al. (2019) showed. More advantages of using the PLR include its easy analysis, that it is suited for any participant and most importantly, reports reaction times with less interference from other mental tasks – because no oral or manual response must be given. We believe that establishing the PLR as an instrument in visual search can open up new possibilities for investigating VWM mechanisms.

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## Abbreviations

aLTM.....	activated long term memory
CIELAB.....	CIE Lab
FoA.....	Focus of Attention
IES.....	inverse efficiency scores
IOR.....	inhibition of return
LME.....	linear mixed-effects
MIT.....	multiple-item-template
PLR.....	Pupillary Light Reflex
RT.....	response time
SIT.....	single-item-template
VWM.....	visual working memory
WM.....	working memory

## Appendix

### Abstract (English)

In multiple item visual search, it is controversial whether one or more attentional templates can be maintained simultaneously. Recent evidence suggests that we switch between multiple single-item templates. The present study addressed this question on the basis of the pupillary light reflex (PLR), as previous studies have shown that pupil size reflects the luminance of an object in early visual working memory (VWM). In this experiment ( $N = 30$ ), we compared performance in single-color and dual-color conditions of a visual search task.

Same sized cuing effects in all three conditions suggest that two target colors can guide search simultaneously. We found a significant effect for stimulus brightness during encoding and early retention in single-color trials, but only during encoding in dual-color trials. Surprisingly, we did not find a switching mechanism in the PLR. It therefore seems that the PLR reproduces maintained VWM content in single-color search, but not in dual-color search. We suggest that multiple target color retention in VWM uses a different mechanism, that does not rely on early sensory channels.

*Keywords:* visual attention, pupillometry, visual working memory

**Abstract (German)**

Bei der visuellen Suche nach mehreren Objekten ist es umstritten, ob eine oder mehrere Suchschablonen gleichzeitig aufrechterhalten werden können. Neuere Erkenntnisse deuten darauf hin, dass wir zwischen mehreren Suchschablonen wechseln. Die vorliegende Studie befasste sich mit dieser Fragestellung auf der Grundlage des Pupillenlichtreflexes (PLR), da frühere Studien gezeigt haben, dass die Pupillengröße die wahrgenommene Helligkeit eines Objektes im frühen visuellen Arbeitsgedächtnis (VWM) widerspiegelt. In diesem Experiment (N = 30) haben wir die Leistung unter Einfarben- und Zweifarbenbedingungen einer visuellen Suchaufgabe verglichen.

Gleich große Cueing-Effekte in allen drei Bedingungen legen nahe, dass zwei Zielfarben die Suche gleichzeitig leiten können. Wir fanden einen signifikanten Effekt für die Stimulushelligkeit während der Kodierung und der frühen Aufrechterhaltung in einfarbigen Durchgängen, in zweifarbigen Durchgängen aber nur während der Kodierung. Entgegen unserer Hypothese fanden wir keinen Wechselmechanismus im PLR. Es scheint daher, dass der PLR den aufrechterhaltenen VWM-Inhalt zwar bei der Einfarbensuche wiedergibt, nicht aber bei der Zweifarbensuche. Wir vermuten, dass die Beibehaltung von mehreren Zielfarben im VWM einen anderen Mechanismus verwendet, der sich nicht auf frühe sensorische Verarbeitungswege stützt.

*Schlüsselwörter:* visuelle Aufmerksamkeit, Pupillometrie, visuelles Arbeitsgedächtnis





