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Microsaccades track shifting but not necessarily maintaining covert visual-spatial attention

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eLife Assessment

This **useful** study examines whether microsaccade direction primarily indexes shifts rather than the maintenance of covert spatial attention, offering a potentially informative account of inconsistencies in the prior literature. However, the evidence remains **incomplete** because the key effects are based on relatively sparse microsaccadic events, while concerns about event detection, fixation control, subject-level robustness, and the interpretation of gaze-density analyses remain unresolved. The correlational design and absence of a neutral condition or independent measure of attentional shifting further limit the central claim. The work will be of interest to researchers studying attention, eye movements, and visuomotor mechanisms.

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Abstract

Covert attention enables the brain to prioritise relevant visual information without directly looking at it. Ample studies have linked covert visual-spatial attention to the direction of small fixational eye movements called microsaccades, offering researchers and clinicians a potential non-invasive tool to track internal states of covert attention. However, other studies have reported only a weak link or no link at all. We now show that the link between covert visual-spatial attention and microsaccades critically depends on the stage of attentional deployment. Across two experiments—each employing a distinct but widely adopted approach to fixational control—we show that spatial microsaccade biases were more pronounced (Experiment 1) or even exclusively present (Experiment 2) during the initial stage of shifting covert spatial attention, even when covert attention was subsequently maintained as testified by enhanced visual discrimination. This shows that the involvement of the brain's oculomotor system in covert visual-spatial attention qualitatively changes over the course of attentional deployment, and how its peripheral fingerprint in the form of microsaccades reliably indexes shifting but not necessarily maintaining covert visual-spatial attention.

Significance statement

Covert attention is essential for efficient processing of information from our surroundings. Several studies have linked covert attention to the direction of miniscule eye-movements known as microsaccades. Other recent studies have questioned this link. We now explain this discrepancy by treating 'attention' not as a single static construct but as a dynamic process. We show that the link between microsaccades and covert attention critically depends on the stage of attention—with microsaccades robustly tracking shifting but not maintaining covert attention. This reveals how the contribution of the brain's oculomotor system to attention changes dynamically over time. These findings have implications for the use of microsaccades as an accessible marker of attentional (mal)function across psychological and neurological conditions.

Introduction

Visual-spatial attention refers to the dynamic process by which the human brain selects and prioritises the processing of currently relevant visual information (1–3). Visual-spatial attention can operate in two modes: overtly, by physically directing gaze towards relevant stimuli, and covertly, by attending to peripheral visual information outside of the current fixation. Extensive research has established that the neural circuitry that mediates shifts of overt visual-spatial attention, namely the oculomotor system, also contributes to covert visual-spatial attention (4–8). A canonical behavioural manifestation of this overlap in neural circuitry comes from the study of microsaccades—small eye movements that occur even during attempted fixation (9–13). In particular, many studies have reported an association between spatial biases in microsaccade direction and the allocation of covert visual-spatial attention.

This link between microsaccades and covert visual-spatial attention has been demonstrated repeatedly. Early studies linked the direction of microsaccades to the deployment of covert attention (14, 15) and these findings were later replicated and extended. For example, it has been demonstrated in both humans (14–25) and non-human primates (26, 27); during both externally directed perceptual attention (14, 15, 17, 20, 21, 24–27) and internally directed attention within visual working memory (16, 18, 19, 22, 23); and in both perception and action tasks following directional cues (28). Several studies have further linked the directional microsaccade bias to task performance (18, 21, 25, 28, 29). For example, following spontaneous microsaccades, perception of visual targets presented in the same direction is better (25), and visual discrimination benefits may start already prior to microsaccade execution (21). Recent evidence further suggests that microsaccades may even play a causal role in shaping the perception of peripheral stimuli (30).

At the same time, the link between covert visual-spatial attention and microsaccades is not always found (31–33), leading to mixed results in the literature. Notably, in a recent article, Willett & Mayo (33) reported no bias in microsaccade directions, despite clear behavioural and neural demonstrations that covert attention was allocated to the relevant visual stimulus.

One possible source underlying the mixed results that pervade the literature may be that 'attention' has been treated as a unitary and static construct. This is problematic under the hypothesis that the link between microsaccades and covert attention may only be reliable for specific stages associated with the deployment of spatial attention, such as when shifting between locations, but not when subsequently maintaining attention at the new location (34). Indeed, while most studies that reported a link used tasks that required *shifting* covert visual-spatial attention, the recent study by Willet & Mayo (33) that questioned this link used a task that required *maintaining* covert visual-spatial attention after it had already been shifted. Thus, whether—and how reliably—microsaccades track covert visual-spatial attention may fundamentally depend on whether one considers the period of shifting attention, or the ensuing period of maintaining attention after the shift (34).

This differentiation by stage of attention also naturally follows from the putative function of the brain's oculomotor system in attention. Consider the case of overt attention: when aiming to attend to an object by looking at it, the oculomotor system is initially engaged once to shift the eyes. Once the eyes have landed on the attended location, no further eye-movements are necessary, yet overt attention can remain at this location. We pose that the same may apply for covert attention: shifting covert attention may engage the oculomotor system particularly to relocate the focus of covert attention, leading to a bias in microsaccade direction particularly while the shift occurs. Once shifted, visual-spatial attention may effectively remain at the covertly attended location, but with a distinct contribution from the oculomotor system that does not necessarily result in a bias of microsaccade direction.

To address the hypothesis (34) that microsaccades may particularly track *shifting* covert visual-spatial attention—in comparison to *maintaining* attention at this location after the initial shift—we conducted two complementary experiments that each employed a distinct but widely adopted approach to gaze control during the task. Consistent with our hypothesis, microsaccades

predominantly (Experiment 1) or exclusively (Experiment 2) tracked shifting compared to maintaining covert visual-spatial attention, even though covert attention was maintained at the attended location after the initial shift, as evidenced by visual discrimination performance.

Results

Forty-eight healthy human volunteers performed a covert visual-spatial attention task in which a central colour cue indicated which of two laterally presented stimuli (positioned on the left and right below central fixation) would be most likely to show the target event: a slight orientation change that had to be judged as being clockwise or anticlockwise (Fig. 1A). To be able to study covert attention across both shifting and subsequent maintaining phases, we varied the interval between cue and target from 500 to 3200 ms. This necessitated shifting attention immediately in response to the cue (as the target event could already occur 500 ms after cue onset) as well as maintaining it on the cued stimulus (since the target event would often occur after longer cue-target intervals). We conducted two versions of this task, differing only in the approach to fixational control during the task. In Experiment 1, central fixation was instructed and monitored by the experimenter. Experiment 2 included gaze-contingent trial termination whenever gaze deviated more than 2 degrees visual angle from central fixation, as controlled by custom software. Adopting both approaches enabled us to relate our findings to a broader set of prior studies given that both are widely used in the vast literature on covert visual-spatial attention.

Attention was successfully shifted and maintained

We first verified that our task was successful in inducing early shifts of visual-spatial attention that were maintained even for target events occurring several seconds after the cue that prompted the initial attention shift. As shown in Figure 1B, responses were faster and more accurate for target events occurring at the cued (valid) versus the other (invalid) stimulus, with the cue-related attentional modulation extending across all cue-target intervals. Cue-validity effects are summarised in the bar graphs in Figure 1B, and were robust in both experiments, for both reaction times (E1: $t(23)=4.673$, $p=1.052e-04$, $d=0.540$; E2: $t(23)=4.098$, $p=4.407e-04$, $d=0.894$) and accuracy (E1: $t(23)= 3.281$, $p=0.003$, $d=0.383$; E2: $t(23)= 3.062$, $p=0.006$, $d=0.745$). (For cue-validity effects at each of the tested cue-target intervals, see Supplementary Table S1.)

Attention was accompanied by a bias in microsaccade direction

Building on prior demonstrations of a bias in microsaccade direction accompanying the spatial allocation of covert visual attention (see introduction), we assessed whether microsaccades are also biased towards the cued stimulus in our orientation change detection task. We were specifically interested in the *temporal dynamics* of such a microsaccade bias in our task, which invoked both shifting covert attention to the cued visual stimulus, as well as maintaining it there until the target event (the orientation change) would occur.

We exclusively analysed directional microsaccade biases prior to the orientation change, to ensure our results are uncontaminated from potential responses related to the target event itself. To ensure sufficient trials for analysing microsaccades, we included all trials where the orientation change occurred later than 1400 ms after cue onset. This provided a good trade-off between having sufficient trials, while also having sufficient time to capture microsaccade biases during the initial shifting phase and the ensuing phase in which attention was maintained on the cued stimulus after this shift. Importantly, our conclusions remain unaffected when investigating the entire time course through a complementary analysis (see Supplementary Figure S2).

Figure 2A shows the time-resolved rates of downwards saccades made either towards the cued stimulus or towards the other stimulus, for both experiments (for directional histograms and a visualisation of which saccades were included, see Supplementary Figure S3; a complementary analysis of upwards saccades can be found in Supplementary Figure S4). To zoom in on the 'attentional modulation', Figure 2B shows the spatial saccade bias, as the difference in saccade rates toward the cued versus the other stimulus. In both experiments, a significant saccade bias is present towards the cued item, starting around 200 ms after cue onset (E1: cluster $p < 0.001$; E2:

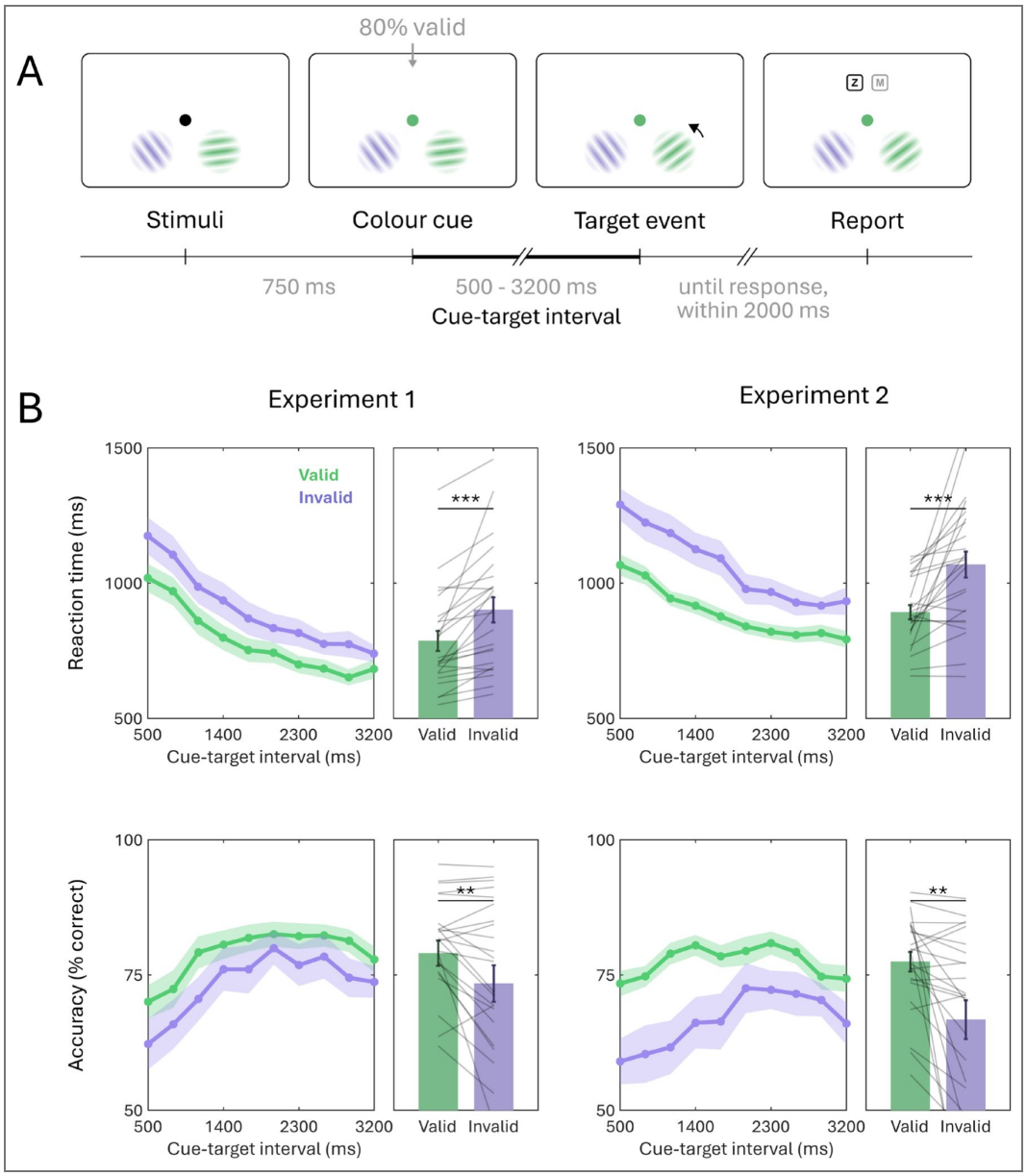

Figure 1. Attention was successfully shifted and maintained.

(A) Schematic showing the progression of a single example trial. A colour change of the central fixation dot cued which of two visual stimuli was most likely to change orientation (the visual target). After a variable delay, the target event (the orientation change) occurred in either the cued (80% of trials, valid) or other (20%, invalid) stimulus and participants reported whether it was clockwise or anticlockwise. Colours shown serve only as an example, for the experimental colours, see the methods section. Supplementary Figure S1 shows the stimulus screen and stimulus sizes and distances to scale. (B) Behavioural performance (reaction times above, accuracy scores below) as function of cue-target interval for validly vs. invalidly cued targets. Circular markers indicate mean values, with shaded areas indicating the standard error of the mean. Bars reflect the mean behavioural performance, with whiskers indicating the standard error of the mean. Grey lines indicate individual participants' data. Throughout the entire figure, the following significance levels were used: *: p< 0.05, **: p < 0.01, ***: p < 0.001, ****: p < 0.0001.

cluster p=0.036). While in Experiment 1 saccade directions remain significantly biased towards the cued stimulus throughout the entire investigated period, in Experiment 2—where fixation demands were more strict, as trials were terminated upon breaking fixation—this bias became short lived, occurring only within the typical window associated with attentional shifting (see 14, 16, 18, 20, 23, 35–37). When directly comparing this spatial saccade bias between experiments, we observed that the effect was significantly larger in Experiment 1 than in Experiment 2 from 535 to 745 ms (cluster p=0.013) and 1153 to 1309 ms after cue onset (cluster p=0.029). A complementary analysis further showed that the magnitude of the spatial saccade bias correlated with the magnitude of the cue-related attentional modulation in Experiment 1, but not in Experiment 2 (see Supplementary Figure S5).

Rather than classifying saccades as 'micro' or 'macro' based on an inherently arbitrary threshold, Figure 2C shows the attentional modulation (saccade bias) as a function of the size of the saccades that contributed to it. Consistent with our instructions to deploy attention covertly, the reported bias was not driven by participants looking directly at the cued stimulus at 5 degrees visual angle from central fixation, but rather by smaller saccades that were *biased* in the direction of the cued stimulus. Indeed, the saccade bias was driven predominantly (Experiment 1) or exclusively (Experiment 2) by saccades in the classic microsaccade range, i.e. below 1 degree. Note that Figure 2C does not show saccade landing positions. While it is theoretically possible for multiple small unidirectional saccades to lead to a larger change in gaze position, a complementary analysis shows that fixation was maintained during the period of peak microsaccade rate in both experiments (see Supplementary Figure S6).

Microsaccade directions are biased predominantly during attentional shifting

To summarise our findings, we finally quantified the observed spatial bias separately in two time windows that were chosen a-priori to capture either the period of 'shifting' or the ensuing period of 'maintaining' covert attention at the cued stimulus, after the initial shift. Specifically, we defined the shift period as the time period from 200 to 600 ms after cue onset, consistent with prior microsaccade studies (e.g., 14, 15, 18, 20, 23) as well as with complementary behavioural studies showing that voluntary attentional deployment unfolds between 200-600 ms after an endogenous attention cue (e.g., 35–37). Following the logic that participants were required to shift attention to the cued stimulus and retain it there, we defined the maintenance period simply as the interval immediately following the period of shifting: from 600 to 1400 ms after cue onset.

Figure 3 shows a quantification of the directional bias in both timeframes. This confirmed a significant directional bias during the 'shift' period in both experiments (E1: $t(23)=3.117$, $p=0.005$, $d=0.615$; E2: $t(23)= 2.543$, $p=0.018$, $d=0.502$), while the bias in the 'maintain' period was significantly only in Experiment 1 ($t(23)=2.838$, $p=0.009$, $d=0.560$) and no longer present in Experiment 2 ($t(23)=0.889$, $p=0.383$, $d=0.175$). To clarify this result, we found moderate Bayesian evidence for the absence of a bias during the maintenance period in Experiment 2 ($BF_{01}=3.26$). This corresponds with the absence of a significant cluster in Experiment 2 in Figure 2. Finally, complementing the findings for Experiment 1 shown in Figure 2—where we found a single cluster spanning both shift and maintenance periods—an additional targeted quantification revealed that in Experiment 1, the bias was still significantly larger during the shift than during the post-shift maintain period ($t(23)=2.300$, $p=0.031$, $d=0.329$).

Discussion

By investigating attention as a dynamic process, we reveal how the relationship between microsaccades and covert visual-spatial attention critically depends on the stage of attentional deployment. Specifically, our results show that microsaccade directions are predominantly (Experiment 1) or even exclusively (Experiment 2) biased during the initial process of shifting covert attention to a relevant visual stimulus, as opposed to the subsequent stage of maintaining attention on this stimulus after the initial shift. We observed this even though visual

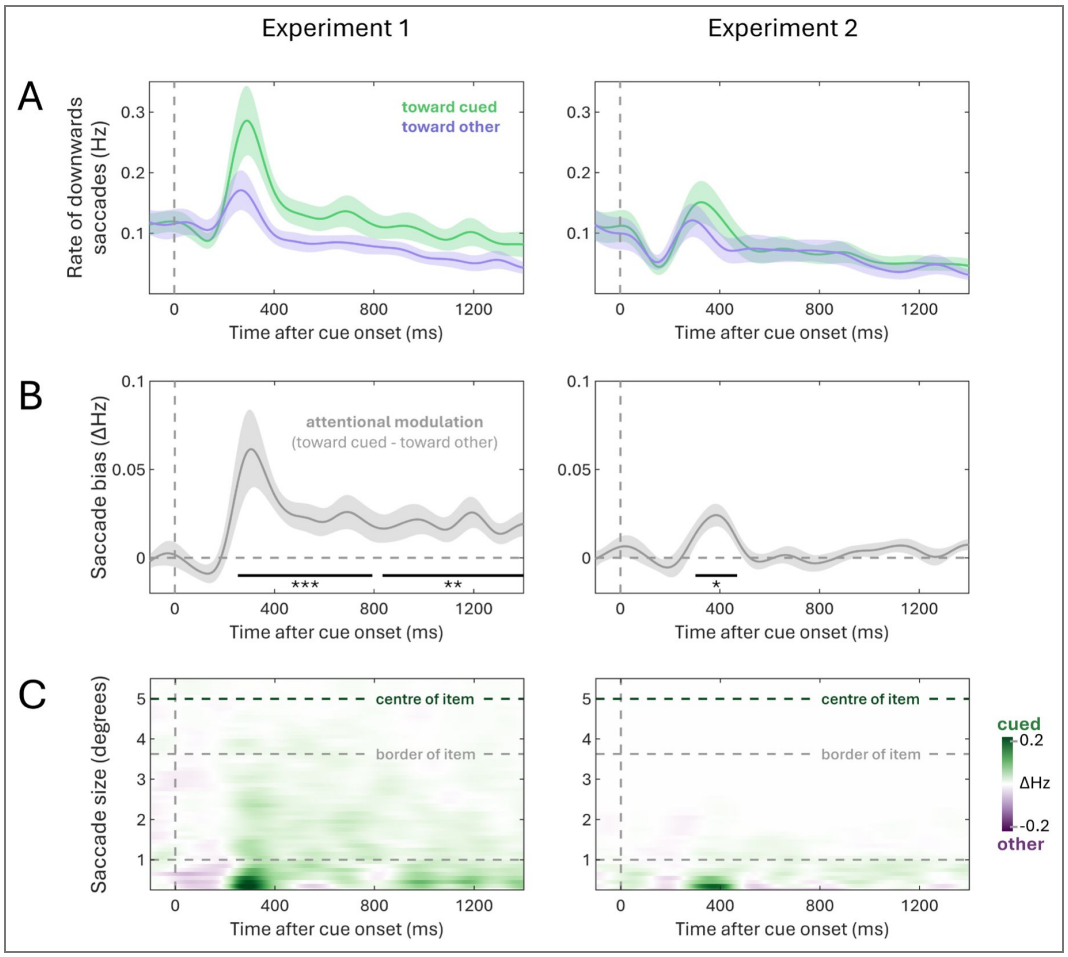

Figure 2. Attention was accompanied by a bias in microsaccade direction.

(A) Time courses of saccade rates for saccades towards the cued and the other item. This analysis only includes downward saccades, as stimuli were positioned at a 45° angle below fixation, allowing us to distinguish saccades towards the item from those returning to central fixation. The time courses indicate mean values, with shaded areas indicating the standard error of the mean. (B) Time courses of the attentional modulation of saccade direction (toward cued – toward other). The time courses indicate mean values, with shaded areas indicating the standard error of the mean. Black horizontal lines indicate significant temporal clusters (as determined by a cluster-based permutation test). (C) Time courses of attentional modulation (as shown in B) as a function of saccade size. For reference, dashed horizontal lines indicate 1 degree visual angle, as well as the locations of the centre and closest border of the visual stimulus. Throughout the entire figure, the following significance levels were used: *: p< 0.05, **: p < 0.01, ***: p < 0.001, ****: p < 0.0001.

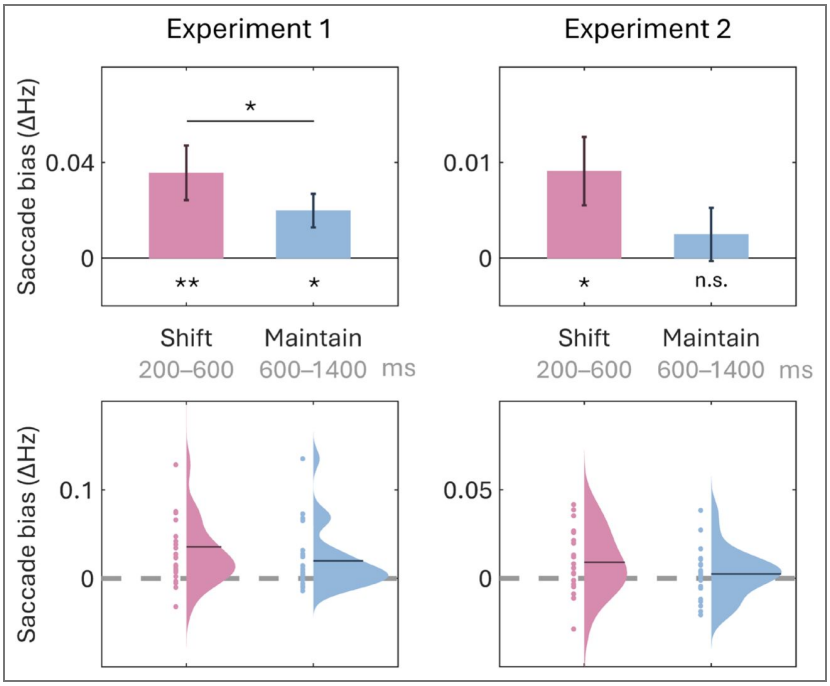

Figure 3. Microsaccade directions are biased predominantly during attentional shifting.

Bars represent the mean saccade towardness rate in the denoted timeframes, with whiskers indicating the standard error of the mean. Distribution and scatter plots show individual participants' data, with dark lines indicating the mean. Note that the y-axes have different scales between Experiment 1 and Experiment 2 and between bar graphs and distribution plots, for visualisation purposes. Throughout the entire figure, the following significance levels were used: *: p< 0.05, **: p < 0.01, ***: p < 0.001, ****: p < 0.0001.

discrimination performance confirmed the allocation of attention well beyond the initial shift window. The insight that the relation between microsaccade direction and covert visual-spatial attention depends on the stage of attention provides a critical nuance in our general understanding of the relationship between the oculomotor system and covert attention. This insight may not only clarify apparent inconsistencies in the literature (34), but also has important theoretical and practical implications, as we elaborate below.

When considering microsaccades as a peripheral marker of the oculomotor system (38, 39), our data suggest that role of the oculomotor system is likely distinct during different stages of covert attentional deployment. While the role of the wider oculomotor system in attention is well documented (4–8), the notion that this role may fundamentally differ across stages of attentional deployment (40) remains underappreciated. Yet, this dynamic stage-dependent participation is highly plausible when we consider that the same system is responsible for both covert and overt shifts of attention. In the case of overt attention, we make an eye movement to bring a new object into view once, and then simply keep this object foveated. By studying microsaccades, we provide evidence that a similar dynamic participation of the oculomotor system likely holds for covert visual-spatial attention. This does not necessarily imply that the oculomotor system contributes *more* during attentional shifts—it simply contributes differently. For example, shifting covert attention may principally recruit populations of motor and visual-motor neurons within the oculomotor system, while after attention has been shifted, covert attention may be held in place primarily by visually selective neurons in the same system that do not bias microsaccade directions (for the widely studied distinction between visual, motor, and visual-motor tuning, see e.g., 41, 42). Future work using direct neurophysiological recordings are required to asses this possibility.

Our findings also have practical implications. When using microsaccades as a marker to study covert attention, our data demonstrate that this marker is predominantly useful for tracking attentional shifts, but less so for tracking subsequent maintaining of visual-spatial attention (for at least one other recent study showing a transient effect likely confined to the attentional shift period, see 28). This does not necessarily negate its use to also track more sustained forms of attention (24, 43), but our data make clear how this is less reliable and may break down, especially when employing stricter fixational control (as was shown in Experiment 2). Furthermore, while our data confirm a robust link between microsaccade direction and shifts of covert visual-spatial attention on a trial average, it is important to note that microsaccades are not necessarily a reliable marker of attention shifts at the single-trial level. In fact, previous research has shown that microsaccades and attention shifts can decouple at the single-trial level (18, 31, 44, 45), and that attention shifts only influence the likelihood of microsaccades following the same direction as attention, without directly triggering any new microsaccades (19). Therefore, even if microsaccades may more reliably track shifting than maintaining attention, as our current findings show, our findings should not be taken as evidence that microsaccades reliably track attentional shifts at the single-trial level.

In this current work, we assessed the relationship between microsaccades and covert attention in two experimental settings with different approaches to fixation control: instructed fixation with monitoring by the experimenter (Experiment 1) or with gaze-contingent trial termination controlled by custom software (Experiment 2). Because both approaches are commonly employed in the field, this set-up allows our data to be comparable with a broader literature on covert attention. In both cases, microsaccades were more reliably associated with the initial shifting of attention. Moreover, during the stricter fixation demands of Experiment 2, the link between microsaccades and attention could exclusively be established during the initial shifting period. This was the case even though we observed clear modulations of performance at intervals far beyond the initial shift period. Together, these complementary experiments reinforce our central conclusion that the link between microsaccades and covert visual-spatial attention critically depends on the attention stage under investigation. In addition, they show how the exact mode of fixation control can shape the observed nature of this link.

The present study specifically examined microsaccade biases in relation to shifting and the ensuing period of maintaining covert visual-spatial attention in a task where the target event could occur anywhere between 500 and 3200 ms after cue onset. In future work, it will be valuable to extend our findings to situations where covert visual-spatial attention must be sustained over much longer timescales. Such situations may also naturally invoke lapses of attention that then require a re-establishing or re-shifting of covert visual-spatial attention. Whether such refocusing resembles the cue-induced shifts studied here—and is therefore similarly tracked by microsaccades—remains an interesting question for future research.

By considering attention not as a unitary and static construct but as a dynamic process, our data highlight how the involvement of the oculomotor system in covert visual-spatial attention fundamentally depends on the stage of attentional deployment, as reflected peripherally by microsaccades. This provides new insight into the intricate dynamics of covert visual-spatial attention and its oculomotor underpinnings and carries implications for the utility of microsaccades as an accessible peripheral marker of attention and its dysfunction in various neurological and psychiatric conditions.

Methods

All experimental procedures were reviewed and approved by the ethics committee of the Vrije Universiteit Amsterdam. Each participant provided written informed consent prior to participation and was reimbursed with 10 euros/hour or participation credits.

Participants

We performed two experiments that only differed in the approach to fixation control (detailed below under '*Experiment 1 vs. Experiment 2: Gaze-contingent trial termination*'). A sample size of twenty-four per experiment was set a-priori, as based on previous publications from our lab with similar experimental designs and outcome measures.

All participants were recruited from the Vrije Universiteit Amsterdam. Twenty-five healthy human volunteers participated in experiment one, one of which was excluded post-hoc due to the inability to keep stable fixation. This resulted in the a-priori determined sample size of twenty-four participants (age range: 18-37; 19 women, 4 men and 1 non-binary; 21 right-handed). Twenty-nine healthy human volunteers participated in experiment two, five of which were unable to finish the experiment due to the inability to keep stable fixation. This resulted in the a-priori determined sample size of twenty-four participants (age range: 18-28; 20 women, 4 men; 23 right-handed). Recruitment for both experiments was done independently, with the constraint that participants were not allowed to participate in both experiments.

Task and procedure

Experiment 1 and Experiment 2 used the same task that we depict in Figure 1A. This consisted of a covert visual-spatial attention task requiring participants to detect and discriminate a sudden orientation change in one of two presented Gabor patches. Prior to the orientation change, a colour cue was shown indicating with 80% reliability which Gabor patch would contain the target event (i.e., the orientation change).

Both experiments contained 20 blocks of 40 trials each. Before starting the experiment, participants practiced both the required response and entire trials until they felt acquainted with the task.

The stimuli would always be on the screen for 750 ms before the central fixation marker would change to match the colour of either stimulus serving as the cue. Left and right cues were equally likely and counterbalanced across trials. The interval between cue onset and the target event (the orientation change of either stimulus) varied from 500 to 3200 ms in discrete steps of 300 ms, resulting in ten possible cue-target intervals. Cue-target intervals were randomly drawn and

counterbalanced across trials. The inclusion of early target events ensured that participants had an incentive to shift attention upon cue onset, while the inclusion of later target events enabled us to also study the period of subsequently maintaining attention on the cued stimulus.

After the orientation change occurred, participants reported the direction of the orientation change (i.e. clockwise or anticlockwise) by pressing either the 'z' (anticlockwise) or 'm' key (clockwise). Clockwise and anticlockwise target events were equally likely and counterbalanced across trials.

The Gabor patches were each positioned at an angle of 45° below the central fixation dot (see Supplementary Figure S1 for a to-scale representation of the stimulus screen), one to the left and the other to the right. Critically, this positioning prevents conflating saccades associated with returning to fixation after moving in the direction of one stimulus with saccades directed towards the other stimulus.

Experimental set-up

Both tasks were programmed in Python 3.8.17 using the PsychoPy library (46) to generate the stimuli. Participants were seated 70 cm in front of a 24-inch LCD monitor, with a 1920 x 1080 pixel resolution and a 239 Hz refresh rate.

Gabor patches presented on a dark grey background (rgb: 64, 64, 64, luminance: 29.0 cd/m^2) could be one of four colours: blue (rgb: 19, 146, 206), magenta (rgb: 217, 103, 241), green (rgb: 101, 148, 14), orange (rgb: 238, 104, 60). Colours were randomly intermixed across trials with the constraint that the two Gabors in each trial had a different colour. All four colours were calibrated prior to the experiment to ensure equiluminance on the monitors used in the lab, with a luminance of 88.5 cd/m^2. All Gabor patches were 2.75 degrees visual angle in diameter, presented at a 5 degree distance to the fixation dot (centre to centre) (all distances are shown to scale in Supplementary Figure S1). The fixation dot was 0.2 degrees in diameter and near-white (rgb: 234, 234, 234). The orientation change that occurred as the target event was always 2° clockwise or anticlockwise. Orientations of Gabor patches were randomly generated from a 160°-space, where orientations from -85 to -5 and 5 to 85 degrees were allowed, to avoid cardinal orientations.

Experiment 1 vs. Experiment 2: Gaze-contingent trial termination

We conducted two complementary versions of the above described experiment that each employed a distinct but widely adopted approach to gaze control during the task. In Experiment 1, participants were instructed to fixate on the central fixation dot at all times and this was monitored by the experimenter who would repeat fixation instructions when necessary.

In contrast, during Experiment 2, trials were terminated when fixation strayed more than 2 degrees visual angle from the centre pixel in any direction. This set-up was attained by custom code that checked the current gaze position every 15 ms. Every gaze sample was checked in real-time and the trial was terminated as soon as any single sample was out of the allowed bounds. Upon trial termination, instructions to return to fixation were shown for 1 second. After this, the fixation dot was shown on its own for half a second, so participants could get their fixation back in order before the task continued with the next trial. Terminated trials were not replaced.

In Experiment 2, all trials where fixation was broken before the target event occurred were not included in both the behavioural and eye-tracking analyses. For the numbers of included trials and included saccades of both experiments, see Supplementary Table S2.

Eye-tracking acquisition and preprocessing

Horizontal and vertical gaze positions of the participant's right eye were continuously sampled using an EyeLink 1000 Plus at a rate of 1000 Hz throughout the experiment. The eye-tracker was positioned 10 cm in front of the monitor, and 60 cm away from the eyes. Participants used a chinrest to minimize head movements. Prior to recording, the eye tracker was calibrated and

validated using the built-in HV9 calibration module. The eye tracker was always re-calibrated exactly halfway through the experiment (after 10 blocks), and could additionally be re-calibrated in each break, if the signal was deemed of too poor quality.

Following acquisition, eye-tracker datafiles were converted from their original Eyelink Data Format (.edf) to an ASCII text file (.asc), and analysed in MATLAB R2024b using a combination of the Fieldtrip analysis toolbox (47) and custom code. Custom code was used to detect blinks and remove them from the signal for 100 ms before and after each blink (by replacing these data with NaNs). After blink removal, data were epoched relative to cue onset. Finally, all trials with a premature keyboard response were removed from the saccade data analyses. In Experiment 2, we additionally removed all trials that were terminated by a break in fixation before the target event had occurred. In total, this yielded an average of 752±45 (M±SD, out of 800) trials per participant for Experiment 1 and 633±74 for Experiment 2.

For our main analysis (the results of which are shown in Figure 2) we exclusively analysed directional microsaccade biases prior to the target event (i.e., the orientation change of either visual stimulus). This ensures our results are uncontaminated from potential responses related to the target event itself. To also ensure sufficient trials for analysing microsaccades, we included all trials where the orientation change occurred later than 1400 ms after cue onset. This provided a good trade-off between having sufficient trials, while also having sufficient time to capture microsaccade biases during the initial shifting phase and the ensuing phase in which attention was maintained on the cued stimulus after this shift.

Importantly, our conclusions remain unaffected when investigating the entire time course through a complementary analysis (see Supplementary Figure S2). In this analysis, all data occurring after the orientation change were removed on each trial (by replacing these data with NaNs), thereby also ensuring our results are uncontaminated from potential responses related to the target event itself. While this complementary analysis does allow for examination of the full time course, the disadvantage of this approach is that the number of available data points decreases progressively towards the end. Consequently, statistical inferences become less stable at later time points. Because of this limitation, we did not adopt this approach as our main analysis.

Saccade detection and visualisation

To detect saccades, a previously established and validated velocity-based method was employed (18) (building on (14)). In this approach, saccades are detected by finding those samples where the 2-dimensional velocity exceeds a certain trial-based threshold. Gaze velocity was obtained as the derivative of gaze position that was smoothed in the temporal dimension with a Gaussian-weighted moving mean filter with a 7-ms sliding window (using the built-in MATLAB function 'smoothdata'). The velocity threshold was set at 5 times the median velocity in any given trial, which is consistent with earlier work (16, 19, 23). A minimum delay of 100 ms between successive saccades was imposed to avoid counting the same saccade multiple times. No criteria were set for minimum microsaccade duration or amplitude, but a visualisation of the relationship between saccade amplitude and peak velocity shows that the detected saccades generally follow the expected main sequence (see Supplementary Figure S7). Crucially, we did not restrict our analyses to initial saccades; rather, all detected saccades were included. This allowed us to examine oculomotor behaviour during later trial phases, where initial saccades are unlikely to occur.

Saccade size (in degrees) and direction were calculated by estimating the difference between the pre-saccade gaze position (−50 to 0 ms before threshold crossing) and the post-saccade gaze position (50 to 100 ms after threshold crossing). Because stimuli were placed at a 45° angle *below* the central fixation dot we only included downwards saccades (saccades with an angle between 0° and 180°; for a visualisation of which saccades were included, see Supplementary Figure S3), following the same logic as in (33). This ensured that all saccades were either toward the cued stimulus or toward the other stimulus, while neglecting upward saccades back to the fixation marker. (Note how this deliberate analysis choice also renders saccade rates lower than in many other articles that include saccades in all directions).

Depending on the direction of the saccade and the side of the cued item, we thus classified saccades as 'towards the cued stimulus' or 'towards the other stimulus', where the 180°-space was divided in two, so for a rightwards cue, all saccades between 0° and 90° were classified as 'towards the cued stimulus'. Importantly, a complementary analysis shows our conclusions remain unaffected when the spatial saccade bias is investigated with narrower angular definitions of 'towards cued stimulus' and 'towards other stimulus' (see Supplementary Figure S8). After detecting and classifying the saccades, the participant's individual time courses of saccade rates (in Hz) were determined by using a sliding time window of 100 ms, advancing in steps of 1 ms. These participant-level average time courses were smoothed using a Gaussian sliding time window of 200 ms, before subjecting these time courses to second level statistics using cluster-based permutation analysis.

To also investigate the size of the saccades that contributed to our findings without imposing an arbitrary threshold, we additionally decomposed saccade rates into a time-size representation, showing the time courses of saccade rates as a function of the saccade size (as also done in 16, 18, 23). For this, we used successive saccade-size bins of 0.5 degrees visual angle in steps of 0.1 degree.

Statistical analysis

For the analysis of the behavioural data, we employed paired-sample Student's t-tests to compare average reaction times and accuracy scores between trials in which the target event occurred in the cued (valid cue) or other (invalid cue) stimulus, collapsed over all cue-target intervals. Effect sizes for each comparison were quantified using Cohen's d.

To statistically evaluate the time series gaze data, we employ a non-parametric cluster-based permutation approach (48). The permutation analysis was conducted using Fieldtrip with default clustering settings (grouping adjacent time points that were significant in a mass-univariate comparison). A permutation distribution of the largest cluster size was acquired by randomly permuting the condition labels of each participant's trial-averaged time course data (i.e. randomly flipping the sign of the difference in rate of toward vs. away saccades) 10,000 times and identifying the size of the largest clusters observed in these randomised data after each permutation. The p-values of the clusters observed in the original data were calculated as the proportion of random permutations where the largest cluster was equal to or larger than the cluster that we observed in the original data.

The cluster-based permutation approach only identifies temporal clusters of significance, but is not suited to directly compare modulations between the pre-defined time windows of shifting vs. maintaining attention. In order to compare the magnitude of the saccade bias between the two time windows of interest, we defined the shift window as the window between 200-600 ms after cue onset (determined a-priori, based on prior work, see 16, 18, 23 and consistent with ample related literature, see 35–37) and we defined the subsequent stage of attentional maintenance in the period following this (in the main analysis, this period lasts from 600 until 1400 ms after cue onset). This enabled us to average the saccade biases (defined as the difference in saccades rates toward the cued versus the other stimulus) in both a-priori defined time windows and to compare these biases using paired-sample Student's t-tests. Effect sizes for each comparison were again quantified using Cohen's d. As this analysis resulted in a non-significant p-value, we supplemented this analysis with a Bayesian analysis to also quantify evidence in favour of the absence of an effect. This was implemented in JASP using default settings (i.e., the prior distribution is a Cauchy distribution with location 0 and scale 0.707).

Supplementary information

Reaction time						
	Experiment 1			**Experiment 2**		
Cue-target interval (ms)	t-value	p-value (uncorrected)	p-value (Bonferroni)	t-value	p-value (uncorrected)	p-value (Bonferroni)
500	3.431	0.002**	0.02*	3.876	7.66e-04***	0.008**
800	3.482	0.002**	0.02*	3.211	0.004**	0.04*
1100	3.007	0.006**	0.06$^{n.s.}$	3.822	8.74e-04***	0.009**
1400	4.022	5.33e-04***	0.005**	3.864	7.88e-04***	0.008**
1700	3.299	0.003**	0.03*	3.384	0.003**	0.03*
2000	3.322	0.003**	0.03*	2.717	0.012*	0.12$^{n.s.}$
2300	3.278	0.003**	0.03*	3.347	0.003**	0.03*
2600	2.604	0.016*	0.16$^{n.s.}$	2.826	0.010*	0.1$^{n.s.}$
2900	3.629	0.001**	0.01*	3.422	0.002**	0.02*
3200	1.603	0.122$^{n.s.}$	1.00$^{n.s.}$	3.390	0.003**	0.03*
Accuracy						
	Experiment 1			**Experiment 2**		
Cue-target interval (ms)	t-value	p-value (uncorrected)	p-value (Bonferroni)	t-value	p-value (uncorrected)	p-value (Bonferroni)
500	2.389	0.025*	0.25$^{n.s.}$	3.505	0.002**	0.02*
800	2.322	0.029*	0.29$^{n.s.}$	2.713	0.012*	0.12$^{n.s.}$
1100	2.934	0.007*	0.07$^{n.s.}$	3.488	0.002**	0.02*
1400	2.310	0.030*	0.3$^{n.s.}$	3.190	0.004**	0.04*
1700	1.893	0.071$^{n.s.}$	0.71$^{n.s.}$	2.356	0.027*	0.27$^{n.s.}$
2000	1.363	0.186$^{n.s.}$	1.00$^{n.s.}$	1.728	0.097$^{n.s.}$	0.97$^{n.s.}$
2300	1.976	0.060$^{n.s.}$	0.6$^{n.s.}$	2.382	0.026*	0.26$^{n.s.}$
2600	1.399	0.175$^{n.s.}$	1.00$^{n.s.}$	1.804	0.084$^{n.s.}$	0.84$^{n.s.}$
2900	2.419	0.024*	0.24$^{n.s.}$	1.303	0.205$^{n.s.}$	1.00$^{n.s.}$
3200	1.783	0.088*	0.88$^{n.s.}$	2.039	0.053$^{n.s.}$	0.53$^{n.s.}$

Table S1. Cue-validity effects at each of the tested cue-target intervals. This table splits the cue-validity effects from Figure 1 into the ten possible cue-target intervals. The top half of the table shows the effects of cue-validity on reaction time; the bottom half of the table shows the effects of cue-validity on accuracy. Results from Experiment 1 and 2 are shown, respectively, on the left and right sides of the table. The table shows both uncorrected and Bonferroni corrected p-values, along with their significance levels: *: p< 0.05, **: p < 0.01, ***: p < 0.001.

	Trials included behaviourally per subject per condition (M±SD)		**Saccades per subject per condition** (M±SD)			
			Shift		Maintain	
	Valid	Invalid	Toward	Away	Toward	Away
Experiment 1	640±0	160±0	39±30	24±18	42±39	27±22
Experiment 2	529±54	133±14	18±18	16±16	19±20	18±21

Table S2. Average number of trials per participant per condition and average number of saccades per participant per condition. This table reflects the amount of data included in the main analyses shown in Figure 1, Figure 2 and Figure 3.

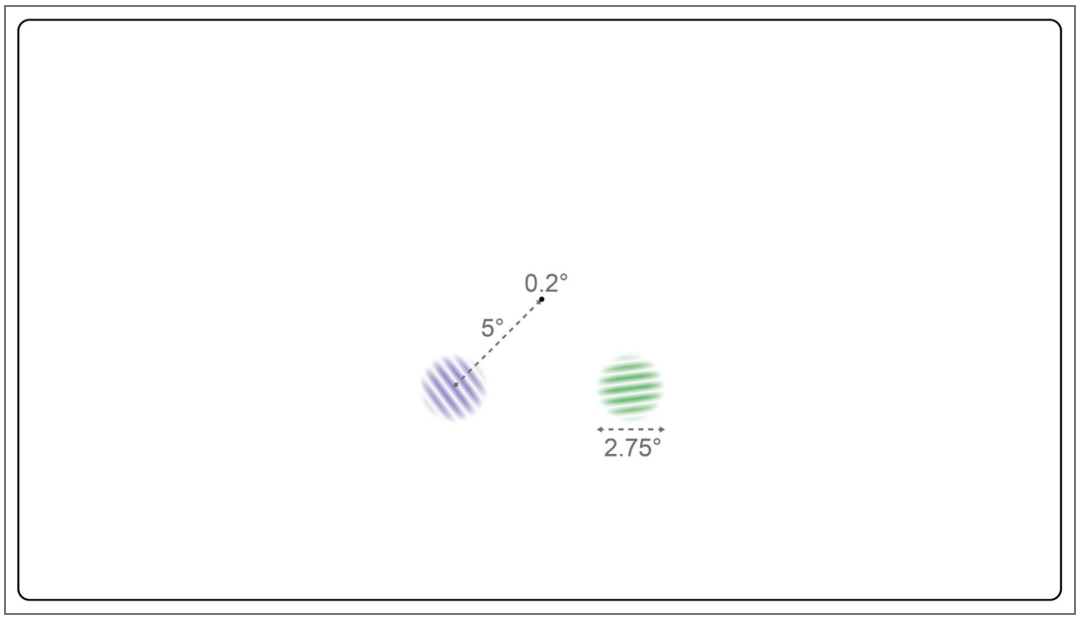

Figure S1. Size of the experimental screen, stimuli, and distances to scale.

Attention was successfully shifted and maintained throughout the entire trial period.

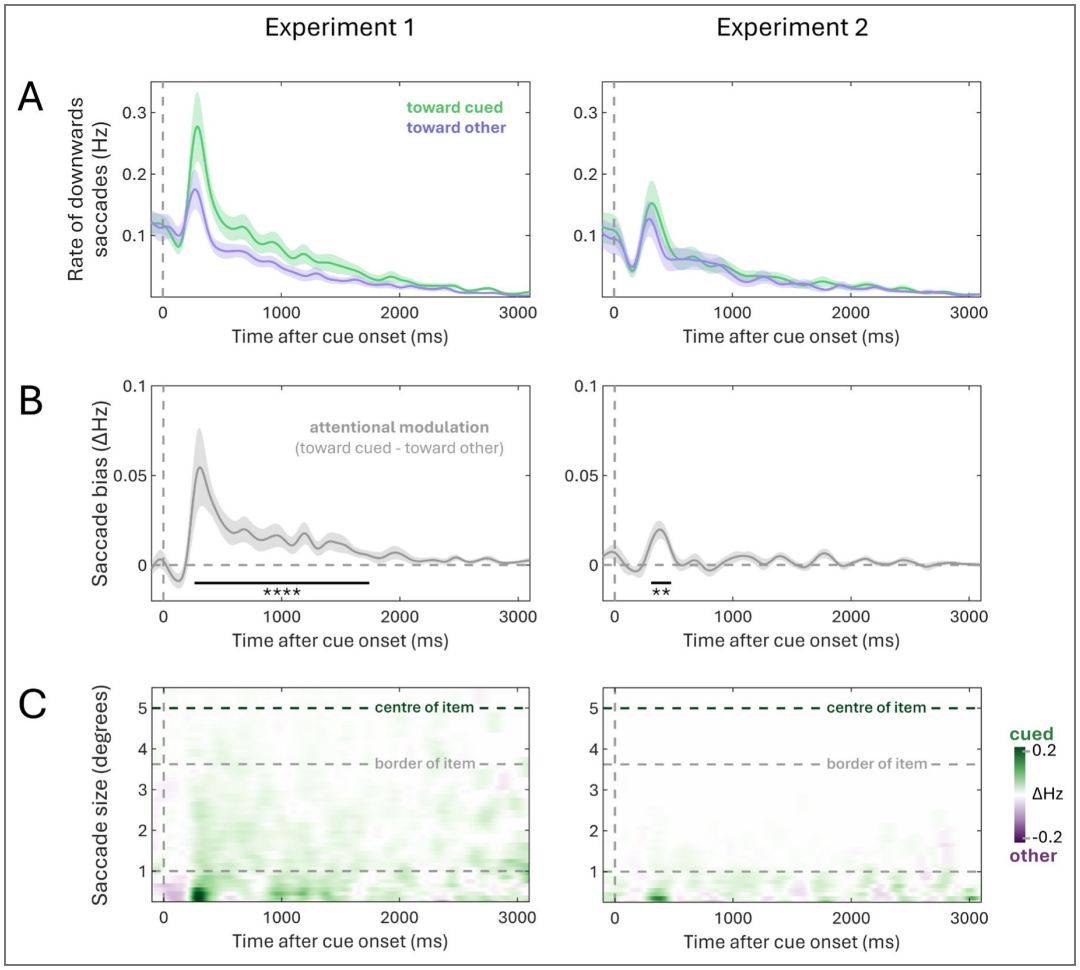

Figure S2. Full time courses of saccade data. This figure replicates the results shown in Figure 2 using the complementary analysis described in the Methods section. (A) Time courses of saccade rates for saccades towards the cued and the other item. This analysis only includes downward saccades, as stimuli were positioned at a 45°

angle below fixation, allowing us to distinguish saccades toward the item from those returning to central fixation. The time courses indicate mean values, with shaded areas indicating the standard error of the mean. (B) Time courses of the attentional modulation of saccade direction (toward cued – toward other). The time courses indicate mean values, with shaded areas indicating the standard error of the mean. Black horizontal lines indicate significant temporal clusters (as determined by a cluster-based permutation test). (C) Time courses of attentional modulation (as shown in B) as a function of saccade size. For reference, dashed horizontal lines indicate 1 degree visual angle, as well as the locations of the centre and closest border of the visual stimulus. Throughout the entire figure, the following significance levels were used: *: p< 0.05, **: p < 0.01, ***: p < 0.001, ****: p < 0.0001.

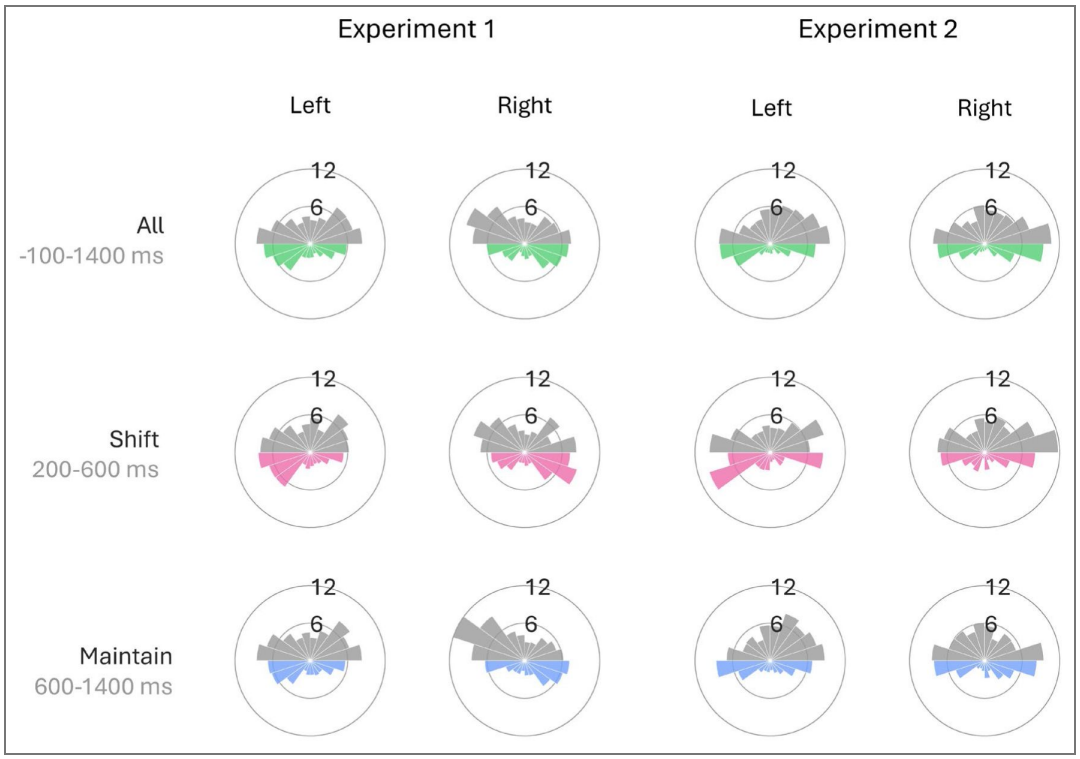

Figure S3. Polar histograms of all saccades included in the main analyses. This figure shows the percentage of detected saccades going in a specific direction for both experiments. This visualisation is repeated for the three main timeframes of interest: (1) the whole trial (from -100 to 1400 ms after cue onset), (2) the 'shift' period (from 200 to 600 ms after cue onset) and (3) the 'maintain' period (from 600 to 1400 ms after cue onset). The coloured saccades are those classified as 'downwards' saccades in the analyses of Figure 2. The radial axis shows the percentage of detected saccades going in one of twenty binned possible directions, which is calculated by weighting each participant equally (i.e., averaging the proportion of saccades detected for each participant per polar angle bin).

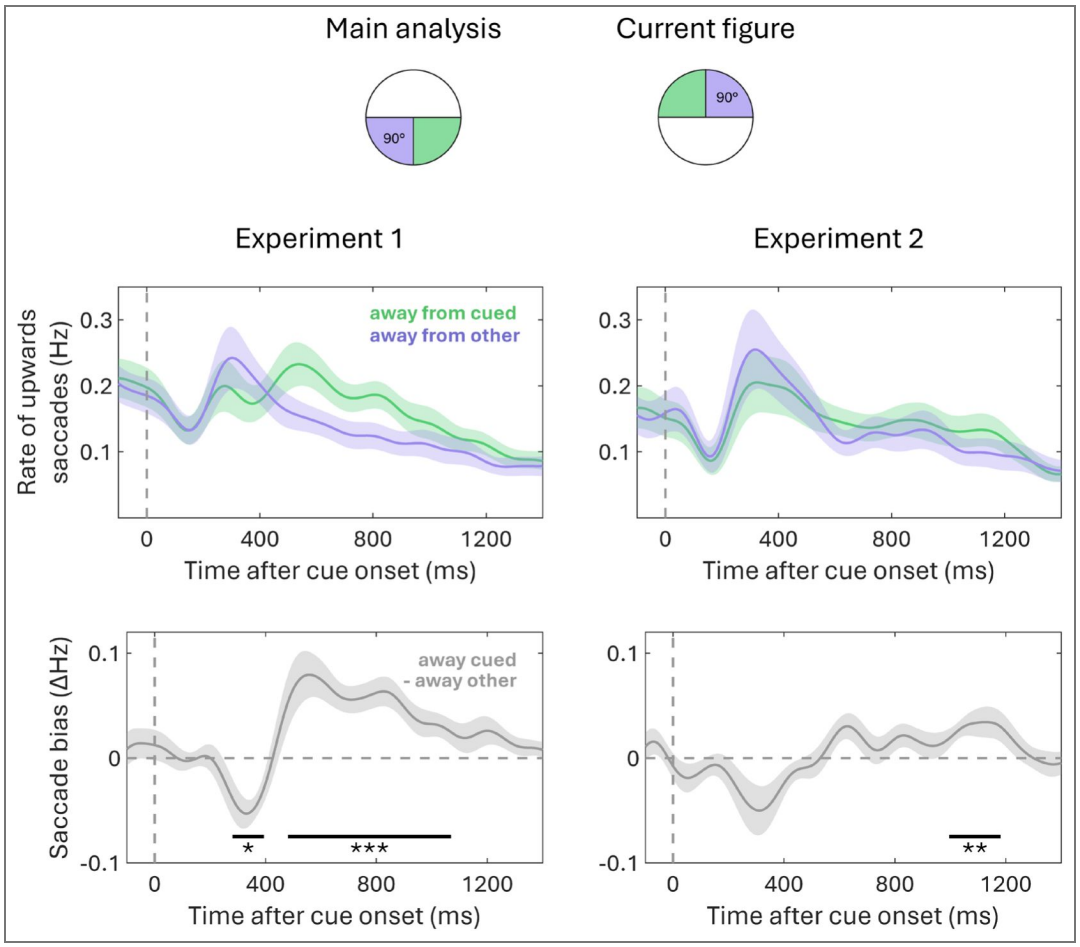

Figure S4. Attentional bias in upwards saccades. This figure repeats the analysis shown in Figure 2, but for upwards saccades instead of downwards saccades. Note that the visual items were always downward in our task. (A) Time courses of saccade rates for saccades away from the cued and the other item. This analysis only includes upwards saccades. As stimuli were positioned at a 45° angle below fixation, this mainly reflects a return to central fixation. The time courses indicate mean values, with shaded areas indicating the standard error of the mean. (B) Time courses of the attentional modulation of saccade direction (away cued – away other). The time courses indicate mean values, with shaded areas indicating the standard error of the mean. Black horizontal lines indicate significant temporal clusters (as determined by a cluster-based permutation test). Throughout the entire figure, the following significance levels were used: *: p< 0.05, **: p < 0.01, ***: p < 0.001, ****: p < 0.0001.

In Experiment 1 a positive correlation was found between the magnitude of the cue-related attentional modulation and the magnitude of the spatial saccade bias, but this did not replicate in Experiment 2.

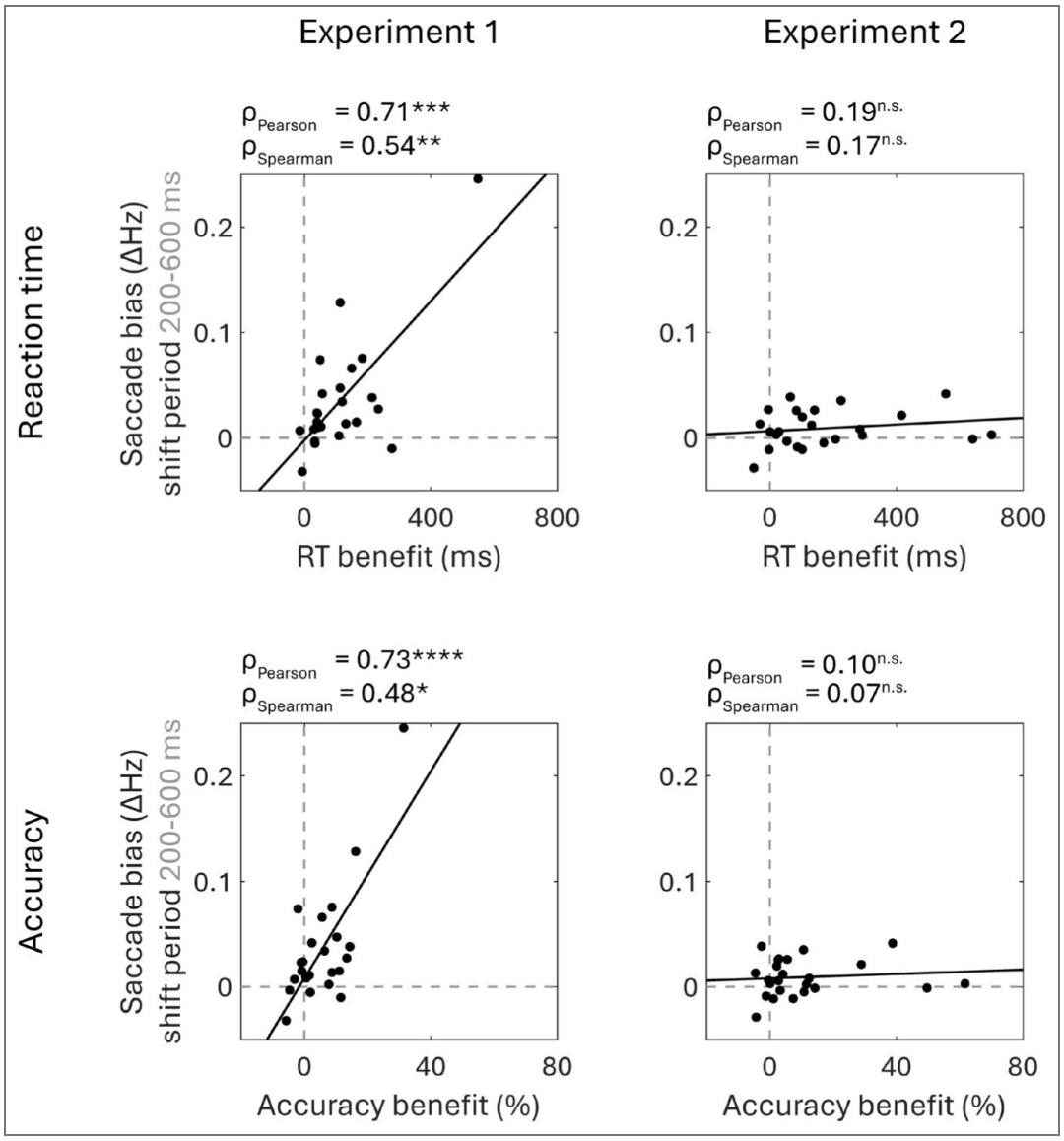

Figure S5. Relationship between the magnitude of the cue-related attentional modulation and the magnitude of the spatial saccade bias. This figure shows the relationship between the cue-related attentional modulation (referred to in the figure as a 'benefit' for simplicity) and the average saccade bias during the 'shift' period (from 200 to 600 ms after cue onset), for both reaction time and accuracy. Each dot represents one participant. Throughout the entire figure, the following significance levels were used: *: p< 0.05, **: p < 0.01, ***: p < 0.001, ****: p < 0.0001.

Fixational control was successful in both experiments.

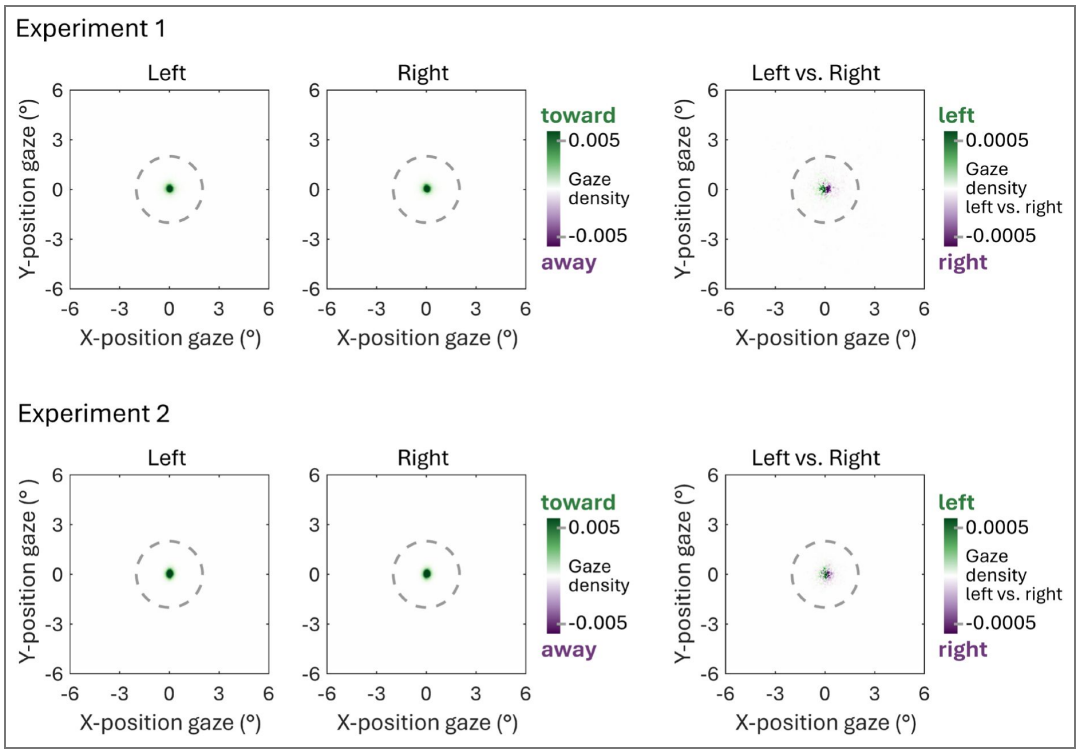

Figure S6. Gaze density during the 'shift' period. This figure shows the gaze density during the 'shift' period (from 200 to 600 ms after cue onset) for both experiments, separately for left cued trials and right cued trials. The very right column shows the difference in gaze density between left and right cued trials.

In both experiments, the saccade-detection algorithm worked well: detected saccades generally follow the main sequence.

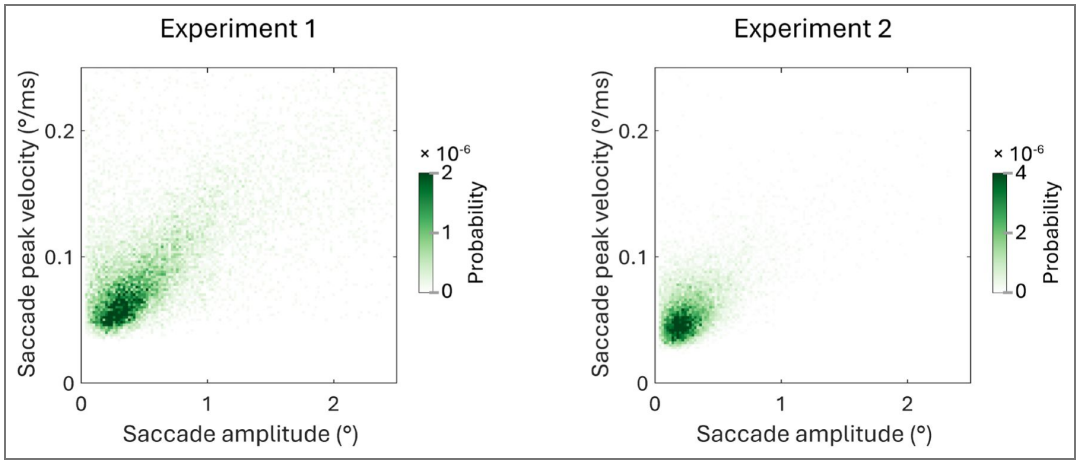

Figure S7. Relationship between amplitude and peak velocity of all detected saccades. This figure shows the relationship between the amplitude (in degrees visual angle) and peak velocity (in degrees visual angle / millisecond), for Experiment 1 and Experiment 2 separately. A visual inspection of both plots shows that the detected saccade generally follow the main sequence.

The main results of both experiments are replicated when using a narrower angular definition of 'toward cued' and 'toward other'.

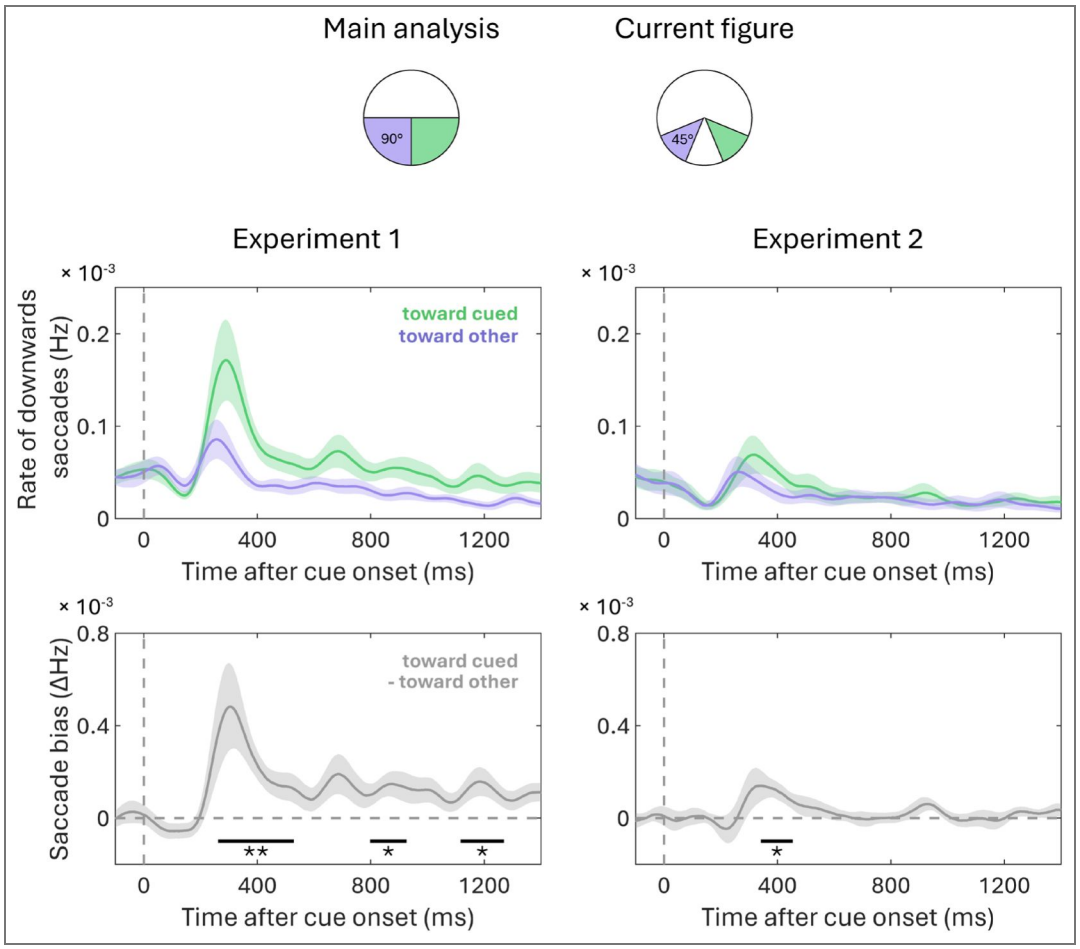

Figure S8. Re-analysis of saccade data with narrower angular definitions of 'toward cued' and 'toward other'. This figure repeats the analysis shown in Figure 2, but uses an angular definition of 45° around the direction of the visual stimulus to define the 'toward cued' and 'toward other' directions, instead of the original 90°. (A) Time courses of saccade rates for saccades towards the cued and the other item. This analysis only includes downward saccades, as stimuli were positioned at a 45° angle below fixation, allowing us to distinguish saccades towards the item from those returning to central fixation. The time courses indicate mean values, with shaded areas indicating the standard error of the mean. (B) Time courses of the attentional modulation of saccade direction (toward cued – toward other). The time courses indicate mean values, with shaded areas indicating the standard error of the mean. Black horizontal lines indicate significant temporal clusters (as determined by a cluster-based permutation test). Throughout the entire figure, the following significance levels were used: *: p< 0.05, **: p < 0.01, ***: p < 0.001, ****: p < 0.0001.

Data availability

All study data and analysis code will be made public prior to final publication. The code for the experimental task is already publicly available on GitHub (*https://github.com/annavanharmelen/Microsaccade-bias-experiment*).

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Peer reviews

Reviewer #1 (Public review):

Summary:

This manuscript describes a study examining the relationship between microsaccades and covert attention. This question has been widely investigated, with numerous studies showing that during sustained fixation, when subjects covertly attend to a peripheral stimulus, microsaccades tend to be biased toward the attended location. Here, the authors ask whether this microsaccade bias reflects a shift of covert attention or the maintenance of covert attention. They conclude that the bias is primarily driven by attention shifts, a finding that also helps reconcile the seemingly conflicting results of prior research, where the bias was questioned in paradigms that largely involved attention maintenance rather than shifting.

Strengths:

A large sample size was used.

Weaknesses:

The main weakness is that the authors' response does not adequately resolve concerns about the robustness of the microsaccade analyses. The newly reported event counts reveal that the number of microsaccades per participant is very low, especially in Experiment 2, and highly variable across subjects. Because the key analyses rely on proportions of microsaccades toward versus away from the attended location, estimates based on so few events are likely unstable and may not provide reliable subject-level measures.

A second major concern is that several additional analyses introduced in the revision appear to suffer from the same limitation. The permutation analyses and angle-partition analyses may give the impression of statistical rigor, but if the underlying averages are based on very few microsaccadic events, the resulting probabilities are difficult to interpret. Further subdividing already sparse data into narrower angular bins likely makes the estimates even less reliable.

A third concern is that the authors have not fully addressed issues related to microsaccade detection and fixation control. The presence of very small-amplitude events with relatively high velocities raises the possibility that some detected microsaccades may be artifacts. The authors also did not implement the requested exclusion of microsaccades smaller than 5 arcmin or the suggested reanalysis using stricter fixation criteria. These omissions leave open the possibility that the reported effects are influenced by detection errors.

A fourth weakness is that some of the requested analyses or clarifications were addressed only superficially. The comparison with Brandolani et al. remains minimal, despite being highly relevant to interpreting whether the observed microsaccade-direction effect is transient or sustained. Similarly, the gaze-density plots do not show the raw gaze-position distributions that were requested and may therefore be misleading, because difference maps cannot determine whether subjects were actually fixating centrally.

Overall, the revision raises additional concerns rather than resolving the original ones. The main conclusions remain insufficiently supported unless the authors can demonstrate that the effects are robust at the individual-subject level, based on adequate numbers of microsaccadic events, reliable detection criteria, and appropriate controls for fixation behavior.

https://doi.org/10.7554/eLife.108798.2.sa2

Reviewer #2 (Public review):

Summary:

This study aims to test the hypothesis that microsaccades are linked to the shifting of spatial attention, rather than the maintenance of attention at the cued location. In two experiments, participants were required to judge an orientation change at either a validly cued location (80% of the time) or an invalidly cued location (20% of the time). This change was presented at varying intervals (ranging from 500 to 3,200 ms) after cue onset. Accuracy and reaction times both showed attentional benefits at the valid versus invalid location across the different cue-target intervals. In contrast, microsaccade biases were time-dependent. The authors report a directional bias primarily observed around 400 ms after the cue, with later intervals (particularly in Experiment 2) exhibiting no biases in microsaccade direction towards the cued location. Noteworthy, it would have been interesting to observe whether

directional biases in microsaccades are also evident when compared to a neutral condition. The authors argue that this finding supports their initial hypothesis that microsaccade biases reflect shifts in attention, but that maintaining attention at the cued location after an attention shift is not correlated with microsaccade direction.

Strengths:

The results are straightforward given the chosen experimental design. The manuscript is clearly written, and the presentation of the study and its visualisations are of a high standard.

Weaknesses:

The link between attention and microsaccades has been the subject of extensive research over the past two decades. The authors present a potential solution to the conflicting past findings, arguing that attention should be considered a dynamic process that can be broken down into an attention shift and a sustained attention phase. To differentiate between the two components, the authors varied the interval between the onset of the attention cue and the test stimulus. It would have been nice to use a theory-driven criterion (or an independent measure), in addition to their data-driven approach, to distinguish between these components of a dynamic attention concept. Moreover, it is important to note that the current experiments take a purely correlational approach.

https://doi.org/10.7554/eLife.108798.2.sa1

Author response:

The following is the authors' response to the current reviews.

We thank the reviewers for their time and for their valuable inputs throughout the review process. We wish to clarify, one final time, the primary scope and empirical grounding of our work for prospective readers.

Our study was designed to evaluate whether microsaccades track (in a correlative manner) covert visual-spatial attentional shifting, maintenance, or both. We did so within a single dedicated paradigm, across a large sample (N = 48 human participants). Despite remaining criticisms concerning per-participant event counts and microsaccade classification criteria, the key observation remains a striking dissociation (of the link between microsaccades and covert attention) during the initial shifting and the subsequent maintenance of visual-spatial attention. Moreover, we note how the robust effect observed during shifting (but not maintaining) attention, mitigates residual concerns regarding microsaccade sparsity or signal-to-noise ratio.

We thus remain confident in the empirical foundation of our work and we invite readers to examine the full paper, supplementary materials, and open-access data to evaluate these findings independently.

The following is the authors' response to the original reviews.

We sincerely thank the reviewers and the editors for their careful evaluation of our article and for their valuable input. Building on these suggestions, we were able to further corroborate our main conclusions, make our article more comprehensive, and thereby substantially strengthen the manuscript.

We have one additional point of our own: we noticed that in our original submission, we had smoothed the data more than intended. Having caught this, we have now reduced the smoothing employed by 2.5 times compared to the original amount of smoothing (the exact

smoothing values have also been added to the methods section). Importantly, however, while this has affected how the results look, this has not affected any of our original conclusions.

General summary

We would like to first respond to the major points brought forward by both the editorial summary and the public reviews. As we understand, the two main points that were raised regard: (1) the novelty and theoretical importance of our work and (2) the (in)completeness of our results. We start by providing our response to both of these main points below.

Novelty and theoretical relevance of the work

Regarding the novelty of our work, we believe the reviews and, by extension, the editorial summary underappreciated the main theoretical value of the question we addressed. Our work set out to investigate whether microsaccades track covert attentional shifting, attentional maintenance, or both. We fully recognise that there are ample prior studies that investigated and reported a link between microsaccades and covert attention, but also underscore how other studies report seemingly contradicting evidence by reporting that there is no such link. One such example is a recent paper by Willett & Mayo in PNAS (2023). Prompted by the recent hypothesis that this seemingly conflicting evidence may be due to prior work investigating attention 'in different stages' (van Ede, PNAS, 2023), we set out to address precisely this using a dedicated task that we designed for this purpose. As acknowledged by the summary and public reviews, this helps to reconcile seemingly opposing views in the literature. In our view, such reconciliation has substantial theoretical value.

While we appreciate that our reported insights may resonate and appear plausible to those working on this topic, we are not aware of any prior studies that directly addressed whether the link between covert attention and microsaccades may fundamentally depend on the 'stage' of attentional deployment ('shift' vs. 'maintain'). To fill this key gap and address this timely issue, we developed a dedicated experiment designed to evaluate the relationship between microsaccades and the different stages of attention within a single paradigm. We did so by varying the cue-target intervals to uniquely incentivise early shifting (by having short intervals), while also being able to assess microsaccade biases during subsequent maintenance (in the longer trials). To our knowledge, no previous task has jointly examined these components in this manner.

Finally, our inclusion of two widely adopted approaches to fixational control provides yet another source of novelty. Together, we believe that these features position our work as a substantive advance that reconciles seemingly opposing theoretical views.

Completeness of results

Regarding the completeness of our results, the editorial summary points to "the absence of independent measures, single-trial analyses, and neutral-condition controls needed to substantiate the central claims". While the raised points are valuable, they pertain to issues that are tangential to our primary question and stem from unfortunate misunderstandings of key analytical choices, as we now better clarify. We consider our results complete and comprehensive with regards to the main question our studies set out to answer.

First, regarding the portrayed "need" for independent measures to define the 'shift window' of interest, we clarify how our main analysis is completely agnostic to predetermined time windows, as we employ a cluster-based permutation approach to assess our rich time-resolved data across the full time axis. For the complementary analyses that address the 'shift' and 'maintain' windows more directly, we use a priori defined windows that are based on ample prior literature (from prior literature studying microsaccade biases, as well as from prior literature on the time course of top-down attention as studied through SOA

manipulations). Accordingly, even these 'zoomed in' analyses rely on time windows that are empirically grounded in prior research.

Second, regarding the use of single-trial analyses, we want to emphasise that single-trial predictability is not where our theoretical question resides. We start from the perspective that the relationship between covert visual-spatial attention and microsaccades is inherently probabilistic. Our aim is not to address or question this. Rather, our aim is to determine whether this probabilistic relationship behaves similarly during attentional shifting and maintenance— an issue our analyses directly address. In addition, we also explicitly discuss how the link between microsaccades and attention is fundamentally probabilistic at the single-trial level in our discussion, and prompted by the valuable feedback, we have expanded on this important contextualisation as part of our revision.

Finally, regarding the portrayed "need" for a neural-attention control condition, we agree that inclusion of a neutral attention condition could be informative for disentangling the 'benefits' versus 'costs' of attentional cueing. However, such disambiguation is tangential to our central aim. Rather, our behavioural data primarily serve to verify attentional 'allocation' also at later cue-target intervals. Observing a difference between valid and invalid cues suffices for this central aim. We also note how inclusion of a neutral condition would have reduced trial numbers and statistical power for our critical conditions of interest. Accordingly, we do not see this as a limitation that challenges our main conclusions. Having clarified this, we embraced this valuable reflection and revised the article to ensure that we do not mention selective 'benefits' or 'costs' of our cueing manipulation, but refer to 'the presence of an attentional modulation' instead.

Taken together, the explicit design and analysis choices that we made align with the theoretical aims of our study, and the central question we set out to address. The raised points are valuable and we are grateful to have been able to leverage them to improve our article, but we hope to have also clarified how they do not render our findings "incomplete" (as currently portrayed) with regards to the key goal of our article.

> ***Public Reviews:***
>
> ***Reviewer #1 (Public review):***
>
> *Summary:*
>
> *This manuscript describes a study examining the relationship between microsaccades and covert attention. This question has been widely investigated, with numerous studies showing that during sustained fixation, when subjects covertly attend to a peripheral stimulus, microsaccades tend to be biased toward the attended location. Here, the authors ask whether this microsaccade bias reflects a shift of covert attention or the maintenance of covert attention. They conclude that the bias is primarily driven by attention shifts, a finding that also helps reconcile the seemingly conflicting results of prior research, where the bias was questioned in paradigms that largely involved attention maintenance rather than shifting.*
>
> *Strengths:*
>
> *The paradigm and conclusions appear sound and supported by the results. A large sample size was used.*

We thank the reviewer for this clear and supportive summary of our work.

> *Weaknesses:*
>
> *Weaknesses are mostly related to how the authors enforced fixation in the task, and clarifications are needed regarding some methodological details. A more direct*

> *comparison of the effect in the two experimental conditions is missing.*

We thank the reviewer for raising these valuable points. We have now added a direct statistical comparison of the main effect in the two experimental conditions (page 5: "When directly comparing this spatial saccade bias between experiments, we observed that the effect was significantly larger in Experiment 1 than in Experiment 2 from 535 to 745 ms (cluster p=0.013) and 1153 to 1309 ms after cue onset (cluster p=0.029)"). Regarding the fixation points, we will address them in our point-by-point replies below.

> ***Reviewer #2 (Public review):***
>
> *Summary:*
>
> *This study aims to test the hypothesis that microsaccades are linked to the shifting of spatial attention, rather than the maintenance of attention at the cued location. In two experiments, participants were required to judge an orientation change at either a validly cued location (80% of the time) or an invalidly cued location (20% of the time). This change was presented at varying intervals (ranging from 500 to 3,200 ms) after cue onset. Accuracy and reaction times both showed attentional benefits at the valid versus invalid location across the different cue-target intervals. In contrast, microsaccade biases were time-dependent. The authors report a directional bias primarily observed around 400 ms after the cue, with later intervals (particularly in Experiment 2) exhibiting no biases in microsaccade direction towards the cued location. The authors argue that this finding supports their initial hypothesis that microsaccade biases reflect shifts in attention, but that maintaining attention at the cued location after an attention shift is not correlated with microsaccade direction.*
>
> *Strengths:*
>
> *The results are straightforward given the chosen experimental design. The manuscript is clearly written, and the presentation of the study and its visualisations are both of a high standard.*

We thank the reviewer for this clear summary of our work.

> *Weaknesses:*
>
> *The major weakness of this paper is its incremental contribution to a widely studied phenomenon. The link between attention and microsaccades has been the subject of extensive research over the past two decades. This study merely provides a limited overview of the key insights gained from these papers and discussions. In fact, it attempts to summarise previous work by stating that many experiments found a link, while others did not, and provides only a relatively small number of references. To make a significant contribution, I believe the authors should evaluate the field more thoroughly, rather than merely scratching the surface.*

We thank the reviewer for this valuable reflection. For an elaborate response to the perceived novelty, please see our general summary reply above. In addition, we have added a more thorough evaluation of the field to the introduction (page 2, find relevant paragraph below). We hope that this will provide more context for the manuscript and strengthen its contribution to the field.

Revised paragraph from introduction:

"This link between microsaccades and covert visual-spatial attention has been demonstrated repeatedly. Early studies linked the direction of microsaccades to the deployment of covert attention [14, 15] and these findings were later replicated and extended. For example, it has been demonstrated in both humans [14–25] and non-human primates [26, 27]; during both

externally directed perceptual attention [14, 15, 17, 20, 21, 24–27] and internally directed attention within visual working memory [16, 18, 19, 22, 23]; and in both perception and action tasks following directional cues [28]. Several studies have further linked the directional microsaccade bias to task performance [18, 21, 25, 28, 29]. For example, following spontaneous microsaccades, perception of visual targets presented in the same direction is better [25], and visual discrimination benefits may start already prior to microsaccade execution [21]. Recent evidence further suggests that microsaccades may even play a causal role in shaping the perception of peripheral stimuli [30]."

> *The authors then present a potential solution to the conflicting past findings, arguing that attention should be considered a dynamic process that can be broken down into an attention shift and a sustained attention phase. Although the authors present this as a novel concept, I cannot think of anyone in the field who considers spatial attention to be a static entity. Nevertheless, I was curious to see how the authors would attempt to determine the precise timing of the attention shift and manipulate the different stages individually. However, the authors only varied the interval between the onset of the attention cue and the test stimulus, failing to further pinpoint their dynamic attention concept.*
>
> *The current version of the experiment, therefore, takes a correlational approach, similar to initial studies by Engbert and Kliegl (2003) and Hafed and Clark (2002). Meanwhile, we have learned a great deal about the link between microsaccades and attention. Below, I will list just a few of these findings to demonstrate how much we already know. It is important to note that, while the present study cites some of these papers, it does not provide a clear overview of how the current study goes beyond previous research.*
>
> *(1) Yuval-Greenberg and colleagues (2014) presented stimuli contingent on online-detected microsaccades. A postcue indicated the target for a visual task, and the target could be congruent or incongruent with the microsaccade direction. The authors showed higher visual accuracy in congruent trials. The authors cited that paper, but it is still important to emphasize how this study already tried to go beyond purely correlational links on a single trial level.*
>
> *(2) The Desimone lab (Lower et al., 2018) showed that firing rates in monkey V4 and IT were increased when a microsaccade was generated in the direction of the attended target.*
>
> *(3) However, attention can modulate responses in the superior colliculus even in the absence of microsaccades (Yu et al., 2022)*
>
> *(4) Similarly, Poletti, Rucci & Carrasco (2017) observed attentional modulations in the absence of microsaccades, or comparable attention effects irrespective of whether a microsaccade occurred or not (Roberts & Carrasco, 2019).*
>
> *Thus, in light of these insights, I believe the current study only adds incrementally to our understanding of the link between microsaccades and spatial attention.*

We thank the reviewer for this insightful comment, and for pointing out several important studies on this topic. While we appreciate that our reported insights may resonate and appear plausible to those working on this topic, we are not aware of any prior studies that directly addressed whether the link between covert attention and microsaccades may fundamentally depend on the 'stage' of attentional deployment ('shift' vs. 'maintain').

To fill this key gap and address this timely issue, we developed a dedicated experiment designed to evaluate the relationship between microsaccades and the different stages of attention within a single paradigm. We did so by varying the cue-target intervals to uniquely incentivise early shifting (by having short intervals), while also being able to assess

microsaccade biases during subsequent maintenance (in the longer trials). To our knowledge, no previous task has jointly examined these components in this manner. Moreover, our inclusion of two widely adopted approaches to fixational control provides yet another source of novelty. Together, we believe that these features position our work as a substantive advance that reconciles seemingly opposing theoretical views.

Regarding the use of single-trial analyses, we want to emphasise that single-trial predictability is not where our theoretical question resides. We start from the perspective that the relationship between covert visual-spatial attention and microsaccades is inherently probabilistic. Our aim is not to address or question this. Rather, our aim is to determine whether this probabilistic relationship behaves similarly during attentional shifting and maintenance— an issue our analyses directly and appropriately address. In addition, we also explicitly discuss how the link between microsaccades and attention is fundamentally probabilistic at the singletrial level in our discussion. Prompted by the reviewer's valuable feedback, we have expanded on this important contextualisation in our discussion section (page 8: "Therefore, even if microsaccades may more reliably track shifting than maintaining attention, as our current findings show, our findings should not be taken as evidence that microsaccades reliably track attentional shifts at the single-trial level."). We also incorporated the valuable reference suggestions in our revised manuscript, including in the revised paragraph in our introduction where we provide a more extensive overview of the prior literature, as shown in response to the preceding comment and in the discussion where we discuss the relationship between microsaccades and attention on a single-trial level.

> *In general, it is important to have an independent measure of the dynamics of an attention shift. I think a shift of 200-600 ms is quite long, and defining this interval is rather arbitrary. Why consider such a long delay as the shift? Rather than taking a data-driven approach to defining an interval for an attention shift, it would be more convincing to derive an interval of interest based on past research or an independent measure.*

We thank the reviewer for their question. We wish to clarify how our main analysis is completely agnostic to predetermined time windows, as we employ a cluster-based permutation approach to assess our rich time-resolved data across the full time axis. For the complementary analyses that address the 'shift' and 'maintain' windows more directly, we use a priori defined windows that are based on ample prior literature (from prior literature studying microsaccade biases, as well as from prior literature on the time course of top-down attention as studied through SOA manipulations). Accordingly, even these 'zoomed in' analyses rely on time windows that are empirically grounded in prior research.

> *The present analyses report microsaccade statistics across all trials, but do not directly link single-trial microsaccades to accuracy. Similarly, reaction times and accuracy were analyzed only with respect to valid vs. invalid trials. Here, it would be important to link the findings between microsaccades and performance on a single-trial level. For instance, can the authors report reaction times and accuracy also separately for trials with vs. without microsaccades, and for trials with congruent vs. incongruent microsaccades?*

We thank the reviewer for their sincere interest in our findings and for the great suggestion of an additional analysis. We have now investigated whether trials with a congruent, incongruent or no microsaccade in the shift window (where congruent or incongruent was determined as based on the first microsaccade within the shift window) have, on average, different reaction times. This analysis did not show significant differences between these three conditions (congruent microsaccade, incongruent microsaccade, no microsaccade).

In interpreting this observation, we would like to stress that our experiment is not particularly well-suited to this analysis, as the amount of time between cue onset and the

target events are highly variable across trials. Because of this clear drawback, we have decided not to include these analyses.

> *The study would benefit greatly from including a neutral condition to substantiate claims of attentional benefits and costs. It is highly probable that invalid trials would also demonstrate costs in terms of reaction times and accuracy. It would be interesting to observe whether directional biases in microsaccades are also evident when compared to a neutral condition.*

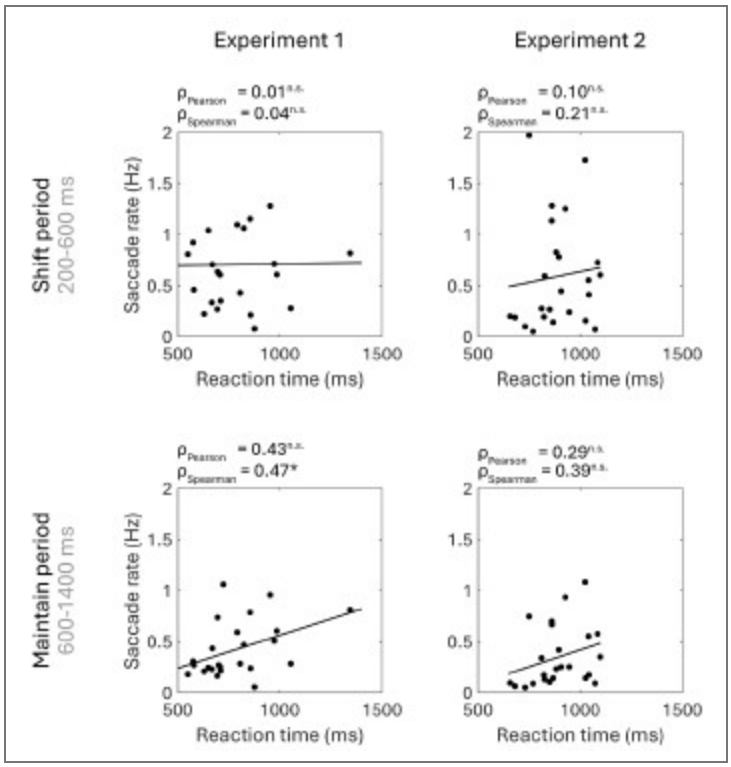
We thank the reviewer for this valuable reflection. We agree that the inclusion of a neutral attention condition could be informative for disentangling the 'benefits' versus 'costs' of attentional cueing. However, such disambiguation is tangential to our central aim. Rather, our behavioural data primarily serve to verify attentional 'allocation' at later cue-target intervals. Observing a difference between valid and invalid cues suffices for this central aim. We also note how inclusion of a neutral condition would have reduced trial-numbers and statistical power for our critical conditions of interest. Accordingly, we do not see this as a limitation that in any way challenges our main conclusions.

Prompted by this reflection, we have ensured to not mention selective 'benefits' or 'costs' of our cueing manipulation throughout the article, but refer to this only as 'the presence of an attentional modulation' instead (such changes were made on pages 3 and 9, and we kept this phrasing consistent in our additions on pages 5 and 22).

> ***Recommendations for the authors:***
>
> ***Reviewer #1 (Recommendations for the authors):***
>
> *(1) The results resemble recent findings by Brandolani et al. (2025), who also showed that the microsaccade bias-in a similar task and using a comparable analysis approach-was restricted to a narrow time window, primarily around the time of the attention shift. The authors should reference this work, discuss similarities and differences, and, given their larger sample size, consider whether they observe a similar correlation between response times and microsaccade rate.*

We thank the reviewer for pointing out this useful reference to us. We have included this article in our introduction, when sketching the current state of the field (page 2) and also mentioned this related article in the discussion (page 8).

In addition, prompted by this comment, we have investigated whether we observe a similar correlation between response times and microsaccade rate in valid trials. We have investigated this for both possible timeframes: (1) the 'shift' timeframe and (2) the 'maintain' timeframe. For both experiments, there was no consistent correlation between response times and overall microsaccade rate, as shown in Author response image 1.



Author response image 1. Relationship between the reaction time and the overall saccade rate. This figure shows the relationship between the average reaction time and the average overall saccade rate for both the 'shift' period (from 200 to 600 ms after cue onset) and the 'maintain' period (from 600 to 1400 ms after cue onset). Each dot represents one participant. Throughout the entire figure, the following significance levels were used: *: p< 0.05, **: p < 0.01, ***: p < 0.001, ****: p < 0.0001.

> *(2) I could not find information on the average number of trials per condition and the average number of microsaccades per subject per condition. Ideally, these numbers should be reported (e.g., in a supplementary table). Since the analysis is based on microsaccade direction, knowing how many microsaccades each subject contributed per condition is critical. Microsaccade rates vary substantially across individuals, and subjects with very few events may add noise to the analysis, as proportions of toward/away microsaccades become unreliable.*

We thank the reviewer for pointing out that this relevant information was missing. We have now included these numbers in a supplementary table as suggested (page 17).

> *(3) Relatedly, it was unclear whether the time-course analyses were based on collapsing all microsaccade events across subjects or on subject-level averages. In the Methods, the authors state that "the permutation distribution of the largest cluster size was acquired by randomly permuting the trial-average data at the group level 10,000 times," but this is ambiguous. Please clarify.*

We thank the reviewer for pointing out this ambiguity. We have changed the methods section to reflect more clearly that we first obtain time-courses of the microsaccade rate per participant, and subject these time courses to second level statistics using cluster-based permutation analysis (page 13). We have also reworded the sentence you quoted to remove any ambiguity (page 13): "A permutation distribution of the largest cluster size was acquired by randomly permuting the condition labels of each participant's trial-averaged time course data (i.e. randomly flipping the sign of the difference in rate of toward vs. away saccades) 10,000 times and identifying the size of the largest clusters observed in these randomised data after each permutation."

> *(4) The authors analyze only downward microsaccades, but the cutoff definition is not specified. Presumably, this includes all directions between 180{degree sign} and 360{degree sign}, which may also include nearly horizontal events. This should be clearly stated. In addition, the rate of upward microsaccades should still be shown, divided into up-left and up-right quadrants to parallel the toward/away analysis. This would provide informative context on whether upward microsaccade rates change systematically over time.*

We thank the reviewer for pointing out that this information was missing, and for suggesting this valuable additional analysis. In the methods section, we now explicitly state the angular cutoffs used for the main analysis (page 12). Additionally, we have added a supplementary figure that shows the time course of upwards microsaccades over time (page 21), please see Supplementary Figure S4.

> *(5) If the dataset contains enough microsaccadic events per subject, it would be useful to test more conservative angular cutoffs for defining "toward" versus "away."*

We thank the reviewer for this insightful suggestion. We have included an additional analysis, where only microsaccades were included with a direction within a 45° angle around the exacttoward and exact-away directions. This replicated our main finding. The results from this analysis are now included in the supplementary materials (page 24), please see Supplementary Figure S8.

> *(6) Figure 2C: It is unclear what the "Center" and "Border" lines represent. The figure is also potentially confusing because it shows microsaccade amplitudes rather than landing positions. Small amplitudes may still bring gaze close to the target; this distinction should be clarified.*

We thank the reviewer for pointing this out. We have changed the “centre” and “border” labels to include more information (pages 6 and 19). We have also added an in-text clarification of the distinction between saccade amplitude and landing position (page 5: “Note that Figure 2C does not show saccade landing positions. While it is theoretically possible for multiple small unidirectional saccades to lead to a larger change in gaze position, a complementary analysis shows that fixation was maintained during the period of peak microsaccade rate in both experiments (see Supplementary Figure S6).”). In addition, in response to the related comment below, we have now also added heatmaps of gaze showing that gaze overall remained close to fixation in our tasks.

> *(7) From the Methods, it appears that in Experiment 1, there was no automatic criterion for discarding trials in which gaze deviated from fixation. In Experiment 2, trials were terminated if gaze left a 2{degree sign} window, but given that the target was only 5{degree sign} from fixation, this seems a relatively loose criterion. It would be important to show the distribution of gaze positions during the task to assess whether fixation control was adequate.*

We thank the reviewer for this great suggestion. We have now added a figure to the supplementary materials (page 23) that shows the probability density of gaze position throughout the ‘shift’ period, for left cued trials and right cued trials separately, please see Supplementary Figure S6. We hope that this will further show that even in Experiment 1, fixational control was successful. We also show the difference between left cued and right cued trials, which again shows a gaze bias towards the cued item.

> *(8) Did the authors examine whether there was a response time benefit (e.g., RT in congruent microsaccade trials minus RT in incongruent microsaccade trials, as in Brandolani et al., 2025) or an accuracy benefit when microsaccades were directed toward the target?*

We thank the reviewer for their interest in our findings and for the great suggestion of an additional analysis. As we discussed also in response to the general summary from reviewer #2 above, we have now investigated whether trials with a congruent, incongruent or no saccade in the shift window (where congruent or incongruent was determined as based on the first saccade within the shift window) have, on average, different reaction times. This analysis did not show significant differences between these three conditions (congruent microsaccade, incongruent microsaccade, no microsaccade).

In interpreting this observation, we would like to again stress how our experiment is not particularly well-suited to this analysis, as the amount of time between cue onset and the target events are highly variable across trials. Because of this clear drawback, we decided not to include these analyses. However, please note that we did now include the outcomes of another analysis that more directly targeted the relation between the spatial modulations in microsaccades and task performance, as we turn to below.

> *(9) Was there a relationship between the size of the attentional effect and the magnitude of the microsaccade bias?*

We thank the reviewer also for this insightful question. We have investigated the relationship between the magnitude of the microsaccade bias during the 'shift' period and the behavioural benefit. We have done this separately for a response time benefit and an accuracy benefit. Experiment 1 shows a significant correlation for both reaction times and accuracy with the magnitude of the microsaccade bias, but for Experiment 2 both of these relationships did not survive. Because this relationship did not prove robust across both experiments, but is nonetheless a set of findings our readers will likely be interested in, we have included this figure in the supplementary materials (page 22). Please see Supplementary Figure S5.

> *(10) The criteria for minimum microsaccade amplitude and duration are not specified. This should be clarified. I recommend excluding events smaller than ~5 arcmin, as these are likely noise-especially since eye tracking was monocular. Monocular "microsaccades" can be spurious, but this can be determined only with binocular tracking. It is also unclear whether subjects used chin/head rests. A main-sequence plot in the supplementary material would be helpful.*

We thank the reviewer for pointing this out. We have included a main-sequence plot in the supplementary materials (page 23). The main-sequence plot can also be found in Supplementary Figure S7 and suggests that our saccade-detection algorithm worked well with detected saccades following the main sequence. We have also stated more clearly in the methods section that subjects used a chinrest (page 11).

> *(11) Please specify the asterisk convention in figure captions (i.e., what * vs. ** vs. *** correspond to in terms of p-values).*

We thank the reviewer for pointing out that these significance levels were not mentioned in every figure caption, so we have added this information to every figure caption where they were missing (page 4, 6, 7 and 19).

> *(12) The fact that stricter fixation criteria reduced the size of the effect suggests the possibility that gaze drift toward the target might have conferred an eccentricity advantage in this discrimination task. A direct comparison of the effect in the two*

> *experiments would be valuable. The authors should comment on this. It would be informative to plot the average gaze position around the time of peak microsaccade rate in both experiments. Reanalyzing the data post hoc with a stricter trial-selection criterion (e.g., excluding trials where gaze deviated more than 1{degree sign} from fixation) could also be very valuable, as it would systematically test how fixation control influences the observed microsaccade-attention relationship. This would be informative for the community studying this topic.*

We thank the reviewer for these valuable reflections. We have now added a direct statistical comparison of the main effect in the two experimental conditions (page 5: "When directly comparing this spatial saccade bias between experiments, we observed that the effect was significantly larger in Experiment 1 than in Experiment 2 from 535 to 745 ms (cluster p=0.013) and 1153 to 1309 ms after cue onset (cluster p=0.029)").

Regarding the average gaze position around the time of peak microsaccade rate: in response to reviewer #1, under point (7), we have included Supplementary Figure S6 that shows the probability distribution of gaze position throughout the 'shift' period (the same figure is found on page 23 in the article), which shows that fixational control was successful in both experiments. This period is also the period of peak microsaccade rate in both experiments.

We wholeheartedly agree that systematically investigating the effect of fixational control is important for the field as a whole, and this is also precisely why we set out to perform the same experiment in two different experimental settings with regards to fixational control, and why we decided to include the results from both experiment variants side-by-side in our article.

> ***Reviewer #2 (Recommendations for the authors):***
>
> *In addition to my general concerns in the public review, I have the following recommendations.*
>
> *(1) Did the authors distinguish between the initial and subsequent microsaccades during their analysis? Is it possible to produce multiple microsaccades when shifting attention, or do the authors only consider the first microsaccade to be linked to an attention shift?*

We thank the reviewer for pointing out this ambiguity. We have now stated more clearly in the methods section that we consider all microsaccades for our analyses (page 12: "Crucially, we did not restrict our analyses to initial saccades; rather, all detected saccades were included. This allowed us to examine oculomotor behaviour during later trial phases, where initial saccades are unlikely to occur."). We also believe this methodological choice is important, as otherwise it would be conceivable that no microsaccade bias can be found during the 'sustain' period, simply because no 'first' microsaccades occur anymore.

> *(2) Two microsaccades had to be separated by at least 100 ms. This is an unusually long delay.*
>
> *Could the authors please specify how many microsaccades were discarded using this criterion?*

This inter-saccade-interval is quite large on purpose, as we want to minimise the probability of counting the same microsaccade twice. We have re-analysed the data with a minimum ISI of 50 ms, and this led to an increase of found saccades of a, respectively, 5.1% and 1.9% increase for Experiments 1 and 2. However, two participants in Experiment 1 led to a much higher increase in saccades than all other participants (these participants had z-scores of 3.9 and 2.4 for the number of additionally found saccades with an ISI of 50 ms; all other z-scores for Experiment 1 were between -0.5 and 0.5). When those two participants were removed, in Experiment 1 only 1.6% more saccades were found.

> *(3) If I understand correctly, the authors did not use staircase procedures to eliminate differences in task difficulty between participants. Could the authors demonstrate how task difficulty relates to the link between microsaccades and performance? For example, is the time course of the microsaccade direction bias correlated with performance?*

We thank the reviewer for this suggestion (that overlaps with a comment of Reviewer 1). We have investigated the relationship between the magnitude of the microsaccade bias during the 'shift' period and the behavioural benefit. We have done this separately for a response time benefit and an accuracy benefit. Experiment 1 shows a significant correlation for both reaction times and accuracy with the magnitude of the microsaccade bias, but for Experiment 2 both of these relationships did not survive. Because this relationship did not prove robust across both experiments, but is nonetheless a set of findings our readers will likely be interested in, we have included this figure in the supplementary materials (page 22). Please see Supplementary Figure S5.

> *(4) The authors reported using equiluminant stimuli. Could the authors please specify the exact luminance?*

We thank the reviewer for pointing out this missing information. We have now included this information in the methods section (page 11: ", with a luminance of 88.5 cd/m^2."). We have also included the luminance of the background (page 11: "luminance: 29.0 cd/m^2").

> *(5) Could the authors please provide a full polar plot showing all microsaccade directions, and colour-code those included in the analysis?*

We thank the reviewer for this great suggestion on how to present our results even more clearly and comprehensively. We have included a supplementary figure showing the full polar histograms (with 20 radial bins), for all three timeframes of interest: the whole trial, the 'shift' period and the 'maintain' period (page 20). As requested, the saccades included in the main analyses are colour-coded. See Supplementary Figure S3

> *(6) Can the authors please directly compare the main effects between experiment 1 and experiment 2 (Figure 2B)?*

We thank the reviewer for this great suggestion (that was also made by reviewer 1). We have now added a direct statistical comparison of the main effect in the two experimental conditions (page 5: "When directly comparing this spatial saccade bias between experiments, we observed that the effect was significantly larger in Experiment 1 than in Experiment 2 from 535 to 745 ms (cluster p=0.013) and 1153 to 1309 ms after cue onset (cluster p=0.029)").

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