Microsaccade-related brain potentials signal

the focus of visuospatial attention

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ABSTRACT

Covert shifts of visuospatial attention are traditionally assumed to occur in the absence of oculomotor behavior. In contrast, recent behavioral studies have linked attentional cueing effects to the occurrence of microsaccades, small eye movements executed involuntarily during attempted fixation. Here we used a new type of electrophysiological marker to explore the attention-microsaccade relationship, the visual brain activity evoked by the microsaccade itself. By shifting the retinal image, microsaccades frequently elicit neural responses throughout the visual pathway, scalp-recordable in the human EEG as a microsaccade-related potential (mSRP). Although mSRPs contain similar signal components (P1/N1) as traditional visually-evoked potentials (VEPs), it is unknown whether they are also influenced by cognition. Based on established findings that VEPs are amplified for visual inputs at currently attended locations, we expected a selective gain-modulation also for mSRPs. Eye movements and EEG were coregistered in a classic spatial cueing task with an endogenous cue. Replicating behavioral findings, the direction of early microsaccades 200–400 ms after cue onset was biased towards the cued side. However, for microsaccades throughout the cue-target interval, mSRPs were systematically enhanced at occipital scalp sites contralateral to the cued hemifield. This attention effect resembled that in a control condition with VEPs and did not interact with the direction of the underlying microsaccade, suggesting that mSRPs reflect the focus of sustained visuospatial attention, which remains fixed at the cued location, despite microsaccades. Microsaccades are not merely an artifact source in the EEG; instead, they are followed by cognitively modulated brain potentials that can serve as non-intrusive electrophysiological probes of attention.

Keywords: visual attention, fixational eye movements, Posner cueing, EEG, P1, saccade-related potential

1   INTRODUCTION

Studies on visuospatial attention traditionally assume that covert orienting occurs in the absence of oculomotor behavior. For example, in the classical Posner task, participants are instructed to maintain fixation, while they move their attention to a location indicated by a cue (Posner, 1980). Recent studies, however, suggest that covert attention shifts often correlate with the occurrence of microsaccades, small (typically less than 1°) fixational eye movements that occur on average once or twice per second during attempted fixation (Martinez-Conde, Macknik, & Hubel, 2004; Rolfs, 2009). Following the presentation of an endogenous cue (e.g., a central arrow), microsaccades are temporarily inhibited, but then tend to move into the cued direction 200–400 ms after cue onset (Engbert & Kliegl, 2003; Horowitz, Fine, Fencsik, Yurgenson, & Wolfe, 2007). After exogenous cues (e.g., peripheral flashes) this cue-congruency effect starts even earlier (Hafed & Clark, 2002) and is often followed by cue-opposing microsaccades (e.g., Rolfs, Engbert, & Kliegl, 2005).

Despite considerable debate (e.g., Hafed, 2013; Horowitz et al., 2007; Laubrock, Kliegl, Rolfs, & Engbert, 2010; Pastukhov & Braun, 2010; Pastukhov, Vonau, Stonkute, & Braun, 2013), the strength and nature of the link between attention and microsaccades have not been fully resolved. Do microsaccades provide a real-time index of an observer's changing attentional focus? While some authors have questioned this notion (Horowitz et al., 2007), others have suggested that behavioral cueing benefits may even be fully explained as a corollary of the execution of cue-congruent microsaccades (Hafed, 2013). An intermediate position (e.g., Laubrock et al., 2010) holds that the direction of microsaccades shortly after cue onset indexes attention, whereas that of later microsaccades is dissociated from attention and instead determined by oculomotor needs (e.g., compensation of drift).

In the current study, we introduce a new electrophysiological marker to track the focus of visuospatial attention and to investigate its relationship to microsaccades: the visually-evoked brain response generated by the microsaccadic gaze shift itself.

As microsaccades rapidly shift the gaze during fixation, the resulting changes in the retinal input evoke a volley of neural feed-forward activity throughout the visual pathway (Martinez-Conde et al., 2004; Martinez-Conde, Otero-Millan, & Macknik, 2013; Rolfs, 2009). Accordingly, single-cell recordings in monkeys show modulated firing rates in striate and extrastriate areas after microsaccades (Bair & O'Keefe, 1998; Kagan, Gur, & Snodderly, 2008; Leopold & Logothetis, 1998; Martinez-Conde, Macknik, & Hubel, 2000, 2002; Snodderly, Kagan, & Gur, 2001).

With regard to human electrophysiological research, microsaccades have been mainly discussed as a source of measurement artifacts (Carl, Acik, Konig, Engel, & Hipp, 2012; Hassler et al., 2013; Jerbi et al., 2009; Yuval-Greenberg, Tomer, Keren, Nelken, & Deouell, 2008), because eye muscle spikes at movement onset can distort the gamma band. In addition, however, each microsaccade evokes a genuine brain response that can be scalp-recorded in the EEG over visual cortex (Gaarder, Krauskopf, Graf, Kropfl, & Armington, 1964; Dimigen, Valsecchi, Sommer, & Kliegl, 2009). In previous studies, this

microsaccade-related potential (mSRP) has been related to a bottom-up processing of the physical properties of the fixated pattern (Armington & Bloom, 1974; Armington, Gaarder, & Schick, 1967; Gaarder et al., 1964).

While there is evidence that brain signal contributions from microsaccades are omnipresent in at least some experimental paradigms commonly used in cognitive neuroscience (Dimigen et al., 2009), a possible link between microsaccade-related brain activity and cognitive processes has not yet been investigated. Nevertheless, behavioral studies suggest that microsaccadic behavior is affected by task demands and contributes to perceptual processing during fixation (for reviews see Martinez-Conde et al., 2004; 2013; Rolfs, 2009). For example, microsaccades have been related to the scanning of small regions (Ko, Poletti, & Rucci, 2010; Otero-Millan, Macknik, Langston, & Martinez-Conde, 2013), the resolving of visual ambiguities (Laubrock, Engbert, & Kliegl, 2005, 2008; van Dam & van Ee, 2006), the informativeness of natural scences (McCamy, Otero-Millan, Di Stasi, Macknik, & Martinez-Conde, 2014), processes of stimulus categorization (Valsecchi, Betta, & Turatto, 2007; Valsecchi, Dimigen, Kliegl, Sommer, & Turatto, 2009), and task difficulty (Siegenthaler et al., 2014). Understanding whether and how the mSRP is modulated by cognitive task demands should not only provide new insights into the functionality of microsaccades during fixation, but may allow researchers to treat these potentials as signal rather than noise.

Thus, the primary goal of the present study was to investigate whether brain potentials co-occurring with microsaccades merely reflect the bottom-up processing of low-level stimulus attributes or whether they are also sensitive to aspects of higher-level cognition, specifically top-down attention. In such a case, our secondary goal was to use these mSRPs to assess the spatiotemporal profile of covert attention in comparison to biases in microsaccade direction.

To this end, eye movements and EEG were simultaneously recorded, while participants covertly attended to one side of a bilateral stimulus indicated by a central cue. In our analysis, we made use of the fact that mSRPs contain similar signal components (P1 and N1) as traditional visually-evoked potentials (VEPs) resulting from passive retinal stimulation. For VEPs, it is well-established that they are amplified for stimuli presented at cued locations (e.g., Eimer, 1993; Hillyard, Vogel, & Luck, 1998; Van Voorhis & Hillyard, 1977); for example, a concurrent stimulus presentation to the left and right hemifield results in a larger occipital P1 and/or N1 component over the hemisphere contralateral to the covertly attended hemifield (Drysdale, Fulham, & Finlay, 1998; Heinze, Luck, Mangun, & Hillyard, 1990). Since each microsaccade refreshes the retinal image, we hypothesized that mSRPs should be similarly enhanced for stimuli in the currently attended hemifield and thereby provide an objective marker of a person's momentary attentional focus.

2   MATERIALS & METHODS

2.1 Participants

Sixteen right-handed students (mean age: 28.5 years, range: 23–44 years, seven females) with uncorrected normal acuity (tested with Bach, 1996) volunteered after providing written informed consent. They received course credit or 8 € per hour for taking part in the ~2.5 hour experiment. No participants were excluded.

2.2 Stimuli and Procedure

Participants were seated in an electrically and acoustically shielded cabin, at a viewing distance of 60 cm from a 22 inch monitor (Iiyama Vision Master Pro 510, vertical refresh: 60 Hz, resolution: 1024×760 pixel). Eye movements and EEG were recorded while participants performed a spatial cueing task requiring a speeded manual choice reaction.

Stimuli and trial sequence are illustrated in Figure 1. A *regular* trial began with the presentation of an empty black screen for 500 ms. Afterwards, a fixation display was shown, consisting of a small red fixation point (diameter: 0.19°) in the screen center and two empty white boxes centered at an eccentricity of ±7° to its left and right. Boxes were quadratic with 2° side length and 0.35° line thickness.

After a random fixation interval between 1500–2000 ms, an additional cue stimulus appeared around the fixation point, signaling the likely position of the upcoming target stimulus with a validity of 80%. In line with previous cueing studies (Engbert & Kliegl, 2003; Horowitz et al., 2007), the cue consisted of a left- or right-pointing white arrow head (height: 0.97°, width: 0.60°, line thickness: 0.11°). Location of the arrow head was adjusted so that the fixation point remained visible in its center and an equal number of cue pixels were presented to the left and right hemifield. The cue-target interval (CTI) varied randomly between 2000–2500 ms (drawn from a uniform distribution) and was terminated by the presentation of the target in one of the boxes for 150 ms. The target was, equiprobably, a filled white circle (diameter: 0.75°) or diamond (side length: 0.63°).

Participants were instructed to maintain central fixation throughout the trial and to shift their attention covertly towards the cued box. Their task was to report the shape of the target as quickly and accurately as possible by pressing one of two response buttons operated with the left or right index finger. Assignment of target symbol (circle vs. diamond) to response hand (left vs. right) was counterbalanced across participants.
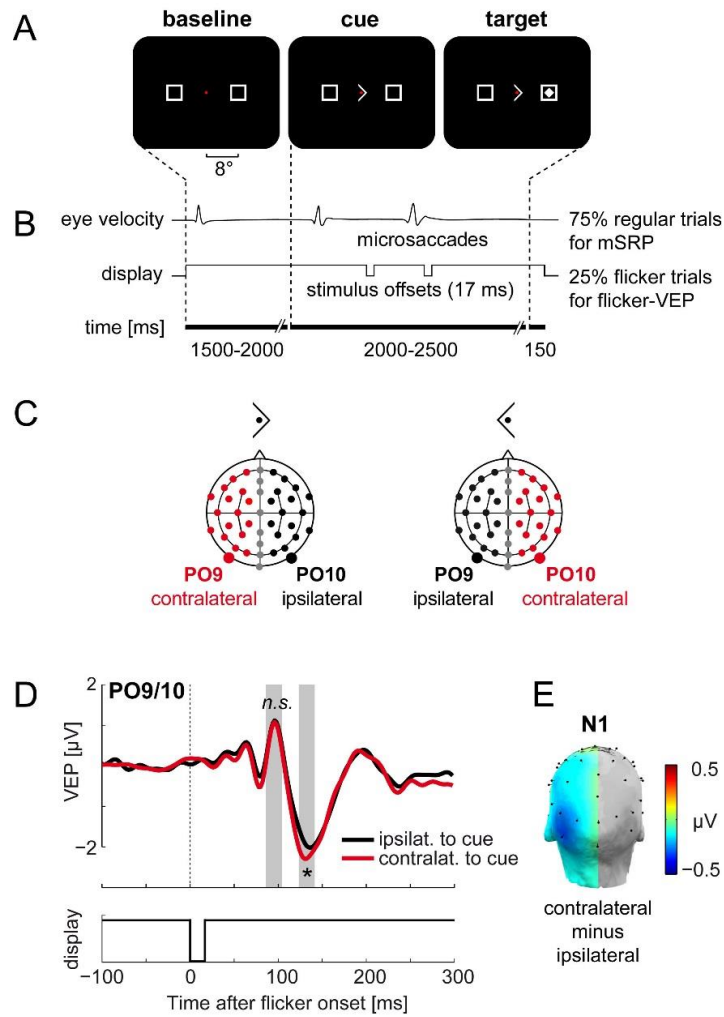
Figure 1. Stimuli, paradigm, and flicker-VEP results. (A) Participants performed a classic spatial cueing task. After a baseline interval, a central arrow cue signaled the likely location of a target stimulus, which appeared in one of the peripheral boxes 2–2.5 s later. The task was to maintain fixation and to classify the target shape (circle or diamond) with a button press. Display items are not shown true to scale, but have been enlarged for clarity. (B) In regular trials, the EEG was time-locked to the occurrence of involuntary microsaccades during the cue-target interval (CTI). The randomly intermixed flicker trials served as a control condition with passive stimulation. In these trials, the display flickered occasionally during the CTI. Each flicker consisted of 17 ms display offset (empty black screen), which served as time-locking point for VEP analysis. (C) To study attention effects on VEPs and microsaccade-related potentials, occipital electrodes located ipsilateral to the cued visual field were compared to contralateral electrodes. (D) Effect of attentional cueing on VEPs in flicker trials. The N1 component was significantly larger over the hemisphere located contralateral to the cued visual field. (E) Scalp topography of the N1 attention effect (at 134 ms). Note, that the topography depicts only the difference between lateral EEG channels (contralateral minus ipsilateral to cued direction). The difference at midline channels is zero by necessity.

To compare attention effects on mSRPs to those on traditional VEPs, we introduced a slight variation of the trial sequence in 25% of the trials: In these so-called *flicker trials*, the entire display flickered 1–3 times (on average twice) during the CTI to produce a passive visual stimulation. Each flicker consisted of a single offset of the entire stimulus (blank black screen) lasting one display cycle

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(16.7 ms) after which the previous stimulus display continued. The brief blank interval was intended to roughly approximate the visual changes generated by an eye movement, with a peri-saccadic "offset" due to retinal blurring followed by a renewed stimulation. Flickers occurred at pseudo-random latencies between 300 ms after cue onset and 300 ms before target onset and with a minimum inter-flicker interval of 300 ms. Participants were informed about the occasional flickering of the display and told to ignore it. Eye movements during flicker trials were not analyzed.

Following 20 practice trials, participants completed 360 regular trials (75%) and 120 flicker trials (25%, containing 240 flickers), presented randomly intermixed. To test for effects of attentional cueing on manual behavior, reaction times (RTs) and response accuracies were subjected to two-way repeated measures ANOVAs on the factors *cueing* (target at cued vs. uncued location) and *trial type* (regular vs. flicker).

2.3 Eye movement recording

Binocular gaze position was recorded at a sampling rate of 500 Hz and an instrument spatial resolution of up to 0.01° using an infrared video-based eye tracker (iView X Hi-Speed 1250, Sensomotoric Instruments GmbH, Germany). Head movements were counteracted by the forehead and chin rest of the eye tracker. At least every 40 trials, the system was recalibrated with a standard 9-point grid. To ensure compliance with the fixation instruction, binocular gaze position was assessed at the start of every trial, 300–400 ms after the onset of the fixation display. If gaze was not detected within a $2° \times 2°$ region around the fixation point, the trial was automatically restarted.

2.4 EEG recording

EEG and electrooculogram were recorded from 45 Ag/AgCl electrodes placed at standard positions of the International 10-10 System. Electrodes of the EEG were mounted in an elastic electrode cap (Easycap, Herrsching, Germany) and electrooculogram (EOG) electrodes were affixed below and at the outer canthus of each eye. Electrode impedances were kept below 10 kΩ. Data was sampled at 500 Hz with a time constant of 10 s using BrainAmp amplifiers (BrainProducts GmbH, Gilching, Germany). Electrodes were referenced against the left mastoid (M1) and an electrode at FCz served as ground. Offline, the EEG was re-referenced against the mean of all electrodes (average reference) and filtered with a bandpass from $0.1 - 40$ Hz using a zero-phase finite response filter.

To synchronize recordings, trigger pulses were sent from the presentation PC (running *Presentation* software, Neurobehavioral Systems Inc, Albany, CA) to the eye tracking and EEG hardware on every trial. Data records were aligned offline using the EYE-EEG extension (http://www2.hu-berlin.de/eyetracking-eeg; Dimigen, Sommer, Hohlfeld, Jacobs, & Kliegl, 2011) for EEGLAB (Delorme & Makeig, 2004). Subsequent analyses were performed with custom MATLAB scripts (The Mathworks Inc., Natick, MA).

2.5 Trial selection

For data analysis, we excluded trials with incorrect manual responses or RTs exceeding the lower or upper 2.5% of the individual RT distribution. Furthermore, the data from the eye tracker was used to remove trials in which the participant blinked or executed a saccade larger than 2° (at any time between 1000 ms before cue onset until 300 ms after target presentation). These criteria not only eliminated trials with ocular artifacts due to blinks and large saccades, but also ensured that only trials entered the analysis in which the participant complied with the fixation instruction.

For the remaining trials, eye tracking and EEG data was segmented into cue-locked epochs ranging from -1000 ms before cue onset until target presentation, yielding segments with a variable length between 3000–3500 ms. To exclude data with voltage drifts, segments were rejected during which voltages at any EEG or EOG channel varied by > 150 µV. After all exclusions (due to incorrect manual responses, blinks, large saccades, and EEG voltage drifts), 74% of regular trials (186–326 trials per participant) and 73% of flicker trials (58–110 trials per participant containing 116–225 flicker events) entered analysis. All analyses (RTs, microsaccades, EEG) were based on this selection of trials.

2.6 Flicker-evoked potentials (flicker-VEP)

In order to analyze flicker-VEPs, short EEG segments (-200 to 300 ms) were cut around the onset of each flicker event (i.e., the display offset), baseline-corrected with a 100 ms pre-flicker baseline, and averaged. If not otherwise stated, EEG averages (VEPs and mSRPs) were first calculated within participant and then averaged across participants.

By the brief dis- and reappearance of the two peripheral boxes, the flicker event provides a bilateral stimulation at both the cued and uncued location. Effects of attending to one side of such a bilateral probe stimulus are typically studied by comparing voltages at electrodes located contralateral to the attended visual field to those at ipsilateral electrodes (cf., Heinze et al., 1990). Since such attention effects are strongest at lateral occipital scalp sites (e.g., Eimer, 1993; Hillyard & Anllo-Vento, 1998), the occipito-parietal electrodes PO9 (left hemisphere) and PO10 (right hemisphere) were used for all analyses. For this electrode pair, P1 and N1 amplitude of the VEP were determined in single-subject averages. Amplitudes of the P1 and N1 were defined as the mean voltage within two 20 ms time windows (P1: 86-106 ms; N1: 124-144 ms) centered on the observed peak latency in the grand-average VEP (P1: 96 ms; N1: 134 ms). Single-subject P1/N1 amplitudes were determined for the electrode contra- and ipsilateral to the cued hemifield and subjected to one-tailed paired *t*-tests, testing the hypothesis that peaks are enhanced over the hemisphere contralateral to the cued visual field.

2.7 Microsaccade detection

Involuntary microsaccades were detected in the cue-locked epochs of regular trials as binocular outliers in two-dimensional (horizontal and vertical) eye velocity space using the algorithm of Engbert

and Kliegl (2003). The adaptive velocity threshold was set at 4 SDs (median-based) of all eye velocity samples recorded within a given epoch. Furthermore, eye movements were considered as microsaccades only if they occurred with temporal overlap in both eyes and lasted for 3 samples or more. If multiple microsaccade events were detected less than 20 ms apart, only the largest saccade of each temporal cluster was kept to avoid detecting saccadic overshoots as new microsaccades. For plotting, microsaccade rates were smoothed with a symmetrical boxcar-shaped moving average with a half-width of 50 ms.

2.8 Analysis of cueing effects on microsaccade direction

To investigate cueing effects on microsaccade direction, we compared the relative frequencies of cue-congruent and cue-incongruent microsaccades during the CTI (cf., Laubrock et al., 2010; Pastukhov & Braun, 2010). A cue-congruent microsaccade is one that moves towards the direction indicated by the cue, whereas a cue-incongruent microsaccade moves in the opposite direction. As in previous studies (Hafed, 2013), this analysis considered only a subset of microsaccades with a predominantly horizontal orientation (max. deviation of ±45° from the horizontal, 88% of all microsaccades).

Previous studies using arrow cues have reported cue-congruency effects on microsaccade direction 200–400 ms after cue onset, during the rebound in microsaccade probability (Engbert & Kliegl, 2003; Horowitz et al., 2007; Laubrock et al., 2010). Consequently, the directed hypothesis of an orientation bias towards the cued side was tested by comparing the rate of cue-congruent and cue-incongruent microsaccades within this interval with a one-tailed *t*-test.

2.9 Microsaccade-related potentials

Processing steps for mSRPs were analogous to those described above for the flicker-VEP. Around each microsaccade detected during regular trials (from 800 ms before cue onset until 300 ms before target onset), a 500 ms data segment (-200 to 300 ms relative to microsaccade onset) was cut from the simultaneously recorded EEG and baseline-corrected by subtracting the mean voltages in the 100 ms interval before movement onset.

In addition to a small corneoretinal artifact at frontolateral electrodes (Dimigen et al., 2009; Keren, Yuval-Greenberg, & Deouell, 2010), initial analyses revealed a main effect of microsaccade direction on the scalp distribution of the mSRP (see *3.6 Attentional enhancement of mSRPs*). Importantly, both effects could be controlled by a two-step averaging procedure: mSRPs were separately averaged as a function of cue direction (for electrodes located ipsilateral vs. contralateral to the cued visual field) and microsaccade direction (for electrodes located ipsilateral vs. contralateral to the movement direction). For these latter averages, an ipsilateral electrode is located on the hemisphere towards which the gaze is moving (e.g., left-hemisphere electrode in case of a leftward microsaccade), while a contralateral electrode is located over the opposite hemisphere (e.g., right-hemisphere electrode

in case of a leftward microsaccade). To compute attention effects, the sub-averages for electrodes ipsilateral versus contralateral to the cue were first calculated separately for each microsaccade direction and then collapsed across the effect of microsaccade direction. Thus, contrary to previous behavioral studies in which it was possible to directly compare attention effects between cue-congruent and cue-incongruent microsaccades (Engbert and Kliegl, 2003; Hafed, 2013), both types of microsaccades entered the average with an equal weight in the present study, irrespective of their relative frequency.

For our main analysis of attention effects on mSRPs, we analyzed microsaccades that occurred between 300 ms after cue onset and 300 ms before target onset, that is, in the same interval during which flicker events happened in flicker trials. Analogous to the flicker-VEP, single-subject P1 and N1 amplitudes were determined at PO9/PO10 within 20 ms time windows centered on the P1 (108 ms) and N1 peak (162 ms) in the grand average. Two-factor repeated-measures ANOVAs were run to test for effects of cue direction and microsaccade direction on the P1 and N1 of the mSRP.

2.10 Sliding window analysis

In a final analysis, we tested the possibility to derive a continuous measure of visuospatial attention from the brain activity co-occurring with microsaccades. For this purpose, a symmetrical sliding window with a half-width of 250 ms was moved sample-by-sample (in 2 ms steps) across the entire trial duration, from -800 ms before until 2200 ms after cue onset. At each of the resulting 1500 window positions, we selected all microsaccades (from all participants) that fell into the current time window and averaged their neural responses to obtain a grand-average mSRP waveform, using analogous processing steps as described above (e.g., cue-congruent and -incongruent saccades were given an equal weight in each average). Scalp lateralization of the mSRP was then quantified as the difference between peak amplitudes at scalp sites located contralateral minus those located ipsilateral to the cued direction in the trial. At each window location, this *lateralization index* was computed for the P1, the N1, and additionally for the peak-to-peak difference (P1 minus N1 amplitude).

Two procedural adjustments were necessary: First, to account for the fact that some participants made only a few microsaccades in certain time windows, participants entered the grand-average with an unequal weight depending on their number of microsaccades in a given window. Second, instead of aggregating over fixed intervals to quantify P1 and N1 amplitude, component peaks were detected in the grand-average mSRP curves by searching for the maximum (P1) and minimum (N1) voltage within broad, pre-defined time windows (80–130 ms for the P1 and 120–180 ms for the N1). This procedure accounted for the fact that peak latencies differed slightly for mSRPs in the baseline interval and in the CTI.

Statistical reliability was assessed with a resampling procedure (e.g., Maris & Oostenveld, 2007; Welch, 1990). At each window position and for each laterality index (P1, N1, and P1–N1) separately, an empirical distribution expected under the null-hypothesis (no difference between hemispheres) was

estimated by randomly permuting the labels indicating the cue direction in the respective experimental trial 1000 times. For each permutation sample, a grand-average mSRP was again computed, peaks were detected, and the laterality index for P1, N1, and the P1–N1 difference was determined. Attention effects were considered reliable if the measured lateralization index for a given window position exceeded the 95% confidence interval of the permutation distribution in the expected direction (contralateral > ipsilateral to cue).

3   RESULTS

3.1 Cueing benefit in manual responses

The analysis of manual response behavior confirmed robust attentional cueing benefits. While response accuracy was high (96%) and did not differ between conditions, average RT was 44 ms shorter when targets appeared at the cued rather than the uncued location as reflected in a main effect of cueing, $F[1,15] = 18.37$, $p < .001$. This benefit was significant both for regular trials (cued: $M = 430$ ms with SD of ± 82 ms, uncued: $M = 477$ ms ± 107 ms; $t[15] = 4.94$, $p < .001$, Cohen's $d = 0.50$) and flicker trials (cued: $M = 443$ ms ± 91 ms, uncued: $M = 484$ ms ± 113 ms; $t[15] = 3.05$, $p < .01$, $d = 0.40$). Although participants tended to respond slower in flicker trials ($M = 463$ ms ± 102 ms) than regular trials ($M = 453$ ms ± 96 ms, $F[1,15] = 4.53$ $p = .05$), the size of the cueing benefit (41 ms and 47 ms, respectively) was not different (non-significant interaction cueing $\times$ trial type, $p = .57$).

3.2 Attentional modulation of flicker-VEP

In a control condition with flicker trials, brief display offsets were inserted into the CTI to imitate the stimulation introduced by microsaccades. Figure 1D shows the grand-average VEP time-locked to the onset of display flickers for electrodes located ipsi- and contralateral to the cued side. While the P1 to the brief display offset was generally small and unaffected by the cueing condition, $t[15] = 0.63$, $p = .73$ (one-tailed test), the N1 peak showed an attentional enhancement with a significantly larger amplitude at electrodes contralateral to the cued hemifield (ipsilateral to cue: $M = -1.89$ µV ± 2.05; contralateral to cue: $M = -2.18$ µV ± 2.06; $t[15] = 2.0$, $p < .05$, one-tailed test, $d = 0.15$). Voltage maps for the attention effect in the N1 time window (see Figure 1E) confirmed its occipito-parietal scalp distribution. These results show that a brief stimulus offset is sufficient to produce a lateralized attention effect in the EEG, suggesting that brain potentials from microsaccades could be amplified in a similar manner.

3.3 Microsaccade characteristics

Despite the fixation instruction in the cueing task, participants exhibited significant oculomotor activity at a small spatial scale. Over all participants, a total of 9,147 microsaccades was detected during regular trials (range across participants: 197–1109), with 6,057 of these movements occurring during

the CTI. Two-thirds of the trials (65%) contained at least one miniature saccade while attention was cued to the peripheral target location.

Kinematic properties of the detected microsaccades fell into the ranges previously reported (Martinez-Conde et al., 2009) with a median movement amplitude of $0.39° \pm 0.11$, a median peak velocity of $52.7°/s \pm 11.5$, and a median duration of 10 ms ± 0.9. Furthermore, saccade amplitude and saccadic peak velocity were highly correlated ($r = .81$, $p < 0.0001$, see Figure 2B), suggesting that the events marked by the detection algorithm were in fact microsaccades rather than measurement noise (Zuber & Stark, 1965).
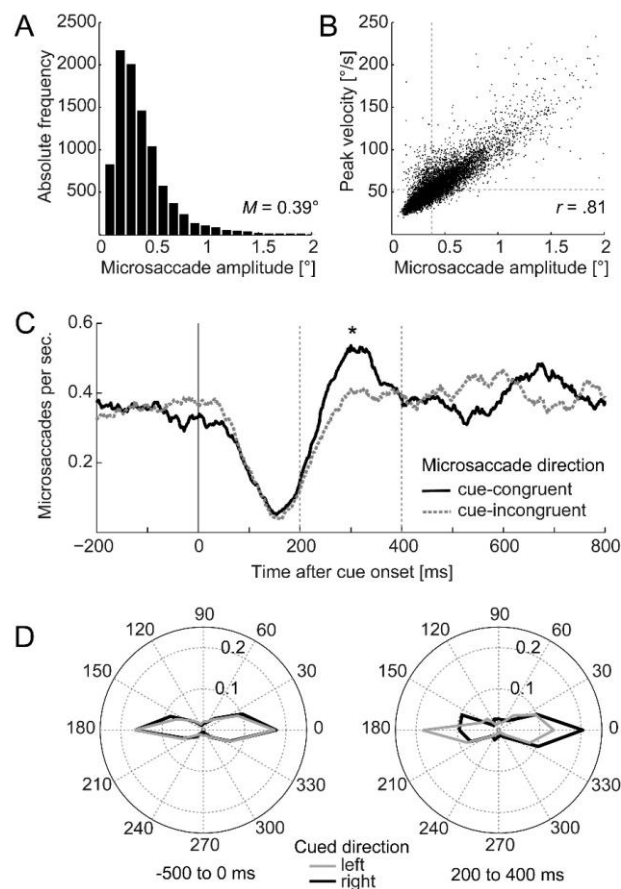

Figure 2. Properties of microsaccades detected during regular trials. (A) Amplitude distribution of microsaccades. (B) Amplitude-velocity relationship. As expected, both properties were highly correlated. (C) Grand-average rate of microsaccades after cue onset, plotted separately for eye movements going into the cued direction (cue-congruent microsaccades) and those going into the opposing direction (cue-incongruent microsaccades). After cue onset, microsaccades showed the characteristic stimulus-driven inhibition followed by a rebound around 200–400 ms. During the rebound, there was a significant bias towards cue-congruent microsaccades, followed by an oscillatory pattern in the average rate. (D) Polar histograms show the directional distribution of microsaccades (but not microsaccade amplitude) as a function of cue direction for a baseline interval before cue onset (left plot) and for the rebound (right plot). Results replicate the previously reported microsaccade-attention link in behavior (e.g. Engbert & Kliegl, 2003). Note, plot shows probability of microsaccade direction but not microsaccade amplitude.

3.4 Cueing effects on microsaccade orientation

Figure 2C depicts the rate of microsaccades over the course of the trial. After cue onset, microsaccade rate showed the previously reported biphasic modulation (Engbert & Kliegl, 2003; Galfano, Betta, & Turatto, 2004) with an early stimulus-driven inhibition followed by a rebound in microsaccade probability around 300 ms. Afterwards, the rate returned slowly to baseline level. As in previous studies, the vast majority of microsaccades was horizontally oriented (see Figure 2D; Rolfs, 2009).

Importantly, the direction of microsaccades initiated during the rebound interval (200–400 ms) was systematically biased towards the cued visual field, with a higher rate of cue-congruent ($M = 0.43$ microsaccades/s $\pm$ 0.26) than cue-incongruent microsaccades ($M = 0.34$ microsaccades/s $\pm$ 0.24). In contrast, the rebound was smaller for microsaccades moving against the cued direction. This pattern, also shown in Figure 2D, was confirmed by a significant effect of cue-congruency on microsaccade rate between 200–400 ms, $t[15] = 2.21$, $p < .05$, one-tailed test, $d = 0.34$. Thus, in line with previous studies (Engbert & Kliegl, 2003; Horowitz et al., 2007; Laubrock et al., 2010), early microsaccades tended to follow the direction signaled by the cue. Cue-congruent and cue-incongruent microsaccades did not differ in terms of their amplitude (median: 0.42° and 0.43°, respectively, $t[15]= 1.52$, $p = 0.15$).

3.5 Microsaccade-related potentials

Figure 3 shows the average microsaccade-related potentials. Aligning the EEG to microsaccade onset during the CTI revealed distinct muscle and brain potentials in each case (cf. Dimigen et al., 2009). Movement onset was accompanied by the biphasic spike potential strongest at facial electrodes (Keren et al., 2010). This potential is most likely generated by the extraocular muscles and responsible for saccade-related gamma-band artifacts in the EEG (Yuval-Greenberg et al., 2008). Importantly, the spike potential was followed by a genuine P1-N1 complex over occipital cortex: A positive deflection of around 3 µV peaking 108 ms after microsaccade onset reflected the P1 or *lambda response* (Armington & Bloom, 1974; Armington et al., 1967; Dimigen et al., 2009; Gaarder et al., 1964) also seen after larger saccades. The following negative peak after 162 ms resembled the N1 component known from conventional VEPs.

As Figure 3A shows, a P1/N1 complex was not only observed for microsaccades during the CTI, but smaller and delayed peaks were also seen for microsaccades during the preceding fixation interval, with only the fixation point and peripheral boxes visible. This pattern suggests that the post-saccadic response is jointly determined by stimulation of foveal (fixation point, arrow cue) and peripheral regions (white boxes) of the visual field (Rémond, Lesevre, & Torres, 1965).

3.6 Attentional enhancement of mSRPs

The key finding of the present study is that brain potentials from involuntary microsaccades are

amplitude-modulated by attention in a similar manner as VEPs. Figure 3C depicts the grand-average mSRP for microsaccades during the CTI, separately for electrodes located ipsilateral and contralateral to the cued hemifield. Importantly, both P1 and N1 of the mSRP were enhanced by visuospatial attention. The P1 was significantly more positive over the contralateral ($M = 2.60$ µV ± 1.24) rather than the ipsilateral hemisphere ($M = 2.27$ µV ± 1.11) relative to the visual field indicated by the arrow cue, $F[1,15] = 11.99$, $p < .01$, $\eta_p^2 = .44$. Similarly, the following N1 was more negative at contralateral as compared to ipsilateral electrodes (ipsilateral: $M = -0.99$ µV ± 0.90, contralateral: $M = -0.63$ µV ± 0.94; $F[1,15] = 12.10$, $p < .01$, $\eta_p^2 = .45$). In case of the N1, the attentional enhancement resembled that seen for the N1 of the flicker-VEP (compare Figures 1E and 3D, see also Supplementary Figure S1). Generally, scalp voltage distributions of the attention effect on the P1/N1 complex confirmed that it was restricted to occipital and occipito-parietal electrodes (Figure 3D).
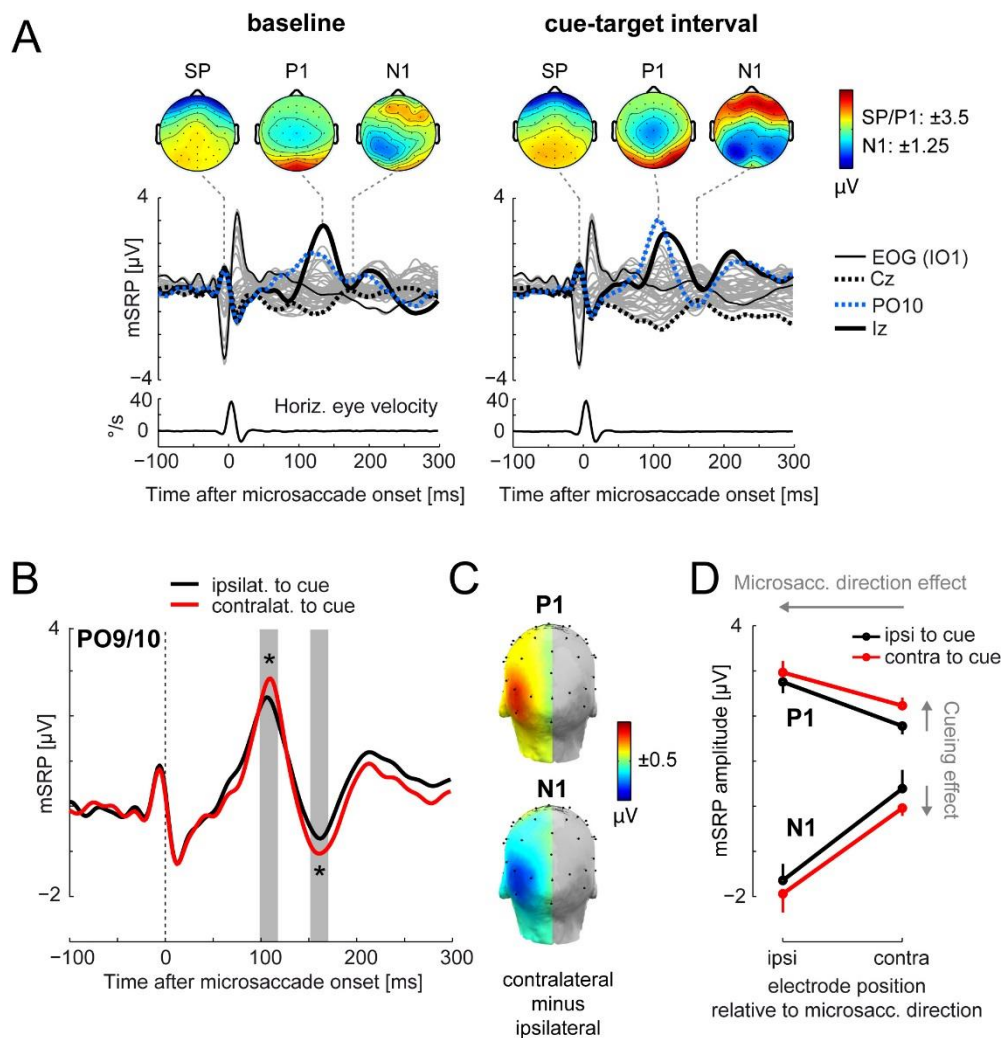

Figure 3. Microsaccade-related potentials (mSRPs) and their modulation by visuospatial attention. (A) Grand average mSRPs for microsaccades detected during the baseline (left) and the cue-target interval (right). Time zero indicates movement onset. All recording channels are plotted superimposed. Microsaccade onset was accompanied by the spike potential (SP). This muscle artifact was followed by genuine visually-driven brain potentials at occipital

electrodes. Topographies show scalp distributions at the SP, P1, and N1 peaks. (B) Effect of attention on mSRPs following microsaccades in the CTI. Both the positive P1 and the negative N1 component were amplified at occipital electrodes located contralateral to the cued visual field, suggesting that information in this hemifield was preferentially processed. (C) Scalp distribution of attention effects on P1 (at 108 ms) and N1 (at 162 ms), see Fig. 1E for details. (D) Joint influences of cue direction and microsaccade direction on the mSRP. In addition to the main effect of cue direction, the direction of the movement had a main effect on the lateralization of the mSRP. Peaks were larger at electrodes located ipsilateral to the direction of the microsaccade, likely due to saccade-related changes in low-level stimulation (see *4.3 Other influences on microsaccadic brain potentials*). Importantly, both effects were strictly additive.

In addition, the direction of the microsaccade had a main effect on the scalp lateralization of the mSRP. Note that in this analysis the factor microsaccade direction indicates the position of an electrode relative to the direction of the saccade (ipsilateral vs. contralateral), but not whether the microsaccade itself pointed left- or rightwards. As a result, visual components were enhanced over the hemisphere towards which the microsaccade was aiming. Both P1 (ipsilateral: $M = 2.86$ µV ± 1.26; contralateral: $M = 2.00$ µV ± 1.19; $F[1,15] = 20.34$, $p < .001$, $\eta_p^2 = .58$) and N1 (ipsilateral: $M = -1.80$ µV ± 1.16, contralateral: $M = 0.18$ µV ± 1.12; $F[1,15] = 30.54$, $p < .001$, $\eta_p^2 = .67$) were larger at occipital electrodes located ipsilateral rather than contralateral to the movement direction. Crucially, as shown in Figure 3D, the main effects of microsaccade direction and cueing did not interact with each other, but were additive. An interaction was found neither for the P1, $F[1,15] = 0.22$, $p = .65$, nor for the N1 component, $F[1,15] = 0.06$, $p = .81$.

3.7 Control analyses

Two control analysis were conducted. First, we repeated all analysis steps reported above for microsaccades occurring in the baseline interval before cue onset. As expected, P1 and N1 for these microsaccades were influenced by microsaccade direction, but not by the cue direction in the respective trial (see also Figure 4D). There was also no interaction.

In a second control analysis, we tested whether attention effects on mSRPs might be simply explained by the presence of a long-lasting and lateralized event-related potential (ERP) elicited by the initial presentation of the cue stimulus (Harter, Miller, Price, Lalonde, & Keyes, 1989; Hopf & Mangun, 2000). For this purpose, mSRP analyses were repeated after subtracting the average cue-locked ERP for each participant (separately for cue-left vs. cue-right trials) from each single-trial cue-locked EEG segment before the extraction of microsaccade-locked epochs. This subtraction had no effect on the pattern of results: Stable effects of attentional cueing remained for the P1 (cueing effect: $F[1,15] = 15.14$, $p < .01$, $\eta_p^2 = .50$; microsaccade direction effect: $F[1,15] = 19.93$, $p < .001$, $\eta_p^2 = .57$) and the N1 (cueing effect: $F[1,15] = 6.94$, $p < .05$, $\eta_p^2 = .32$; direction effect: $F[1,15] = 31.62$, $p < .0001$, $\eta_p^2 = .68$) without an interaction between both effects ($p = 0.55$ and $p = .90$ for P1 and N1, respectively).

In summary, eye movement direction had a global effect on the brain responses following microsaccades, both during fixation periods and during the CTI. Beyond that, we observed an

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independent cueing-related enhancement of the P1 and N1 peak over lateral-occipital recording sites that was specific for periods when covert shifts of attention were demanded but not present during mere central fixation. This attentional lateralization closely resembled that classically reported for VEPs and was also similar to the N1 enhancement seen for the flicker-VEP in the present study. Hence, visuospatial attention modulates microsaccade-related activity in visual cortex.

3.8 Temporal evolution of attention effects on mSRPs

Under conditions of prolonged fixation, the oculomotor system typically generates 1–2 microsaccades per second. Given this ubiquity of microsaccades, their brain potentials might provide an electrophysiological marker of attention with a relatively fine temporal resolution. This hypothesis was tested by calculating the cue-dependent lateralization of the P1, N1, and P1-N1 difference within a sliding time window. Again, all EEG effects reported in the following are independent of microsaccade direction due to the stepwise averaging procedure described above.

Results are depicted in Figure 4D. Systematic scalp lateralization was absent in the baseline interval, but built up 150–300 ms after cue presentation in all three measures. Consistent with an attentional enhancement at electrodes contralateral to the cued hemifield, the P1 and P1-N1 difference were more positive at contralateral sites, while the N1 was more negative. As expected, lateralization was strongest in the peak-to-peak difference, which combines the enhancement of the P1 and N1 in one measure. Importantly, while the behavioral bias in microsaccade direction was most pronounced in a short interval after cue onset (Figure 4B), a reliable lateralization of the mSRP (with only a few non-significant time points) was observed in at least one of the EEG measures across the entire duration of the CTI.
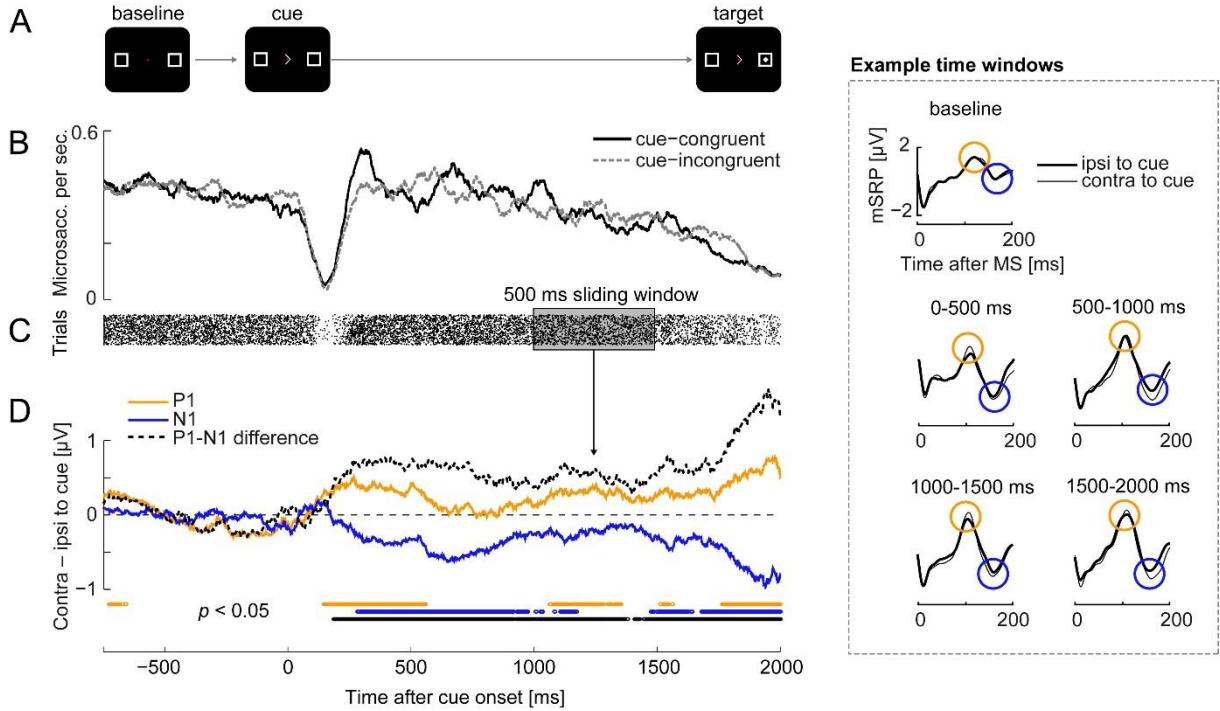
Figure 4. Temporal evolution of attention effects on microsaccade-related brain potentials in regular trials. (A) Simplified trial scheme. (B) Rate of cue-congruent and cue-incongruent microsaccades across the full trial, same data as in Figure 2C. (C) The scatter plot depicts all 9,147 detected microsaccades. To obtain a continuous electrophysiological measure of attention, a sliding window was moved sample-by-sample across the trial. Microsaccades falling into each window position were averaged to obtain a mSRP waveform and the lateralization of the P1, N1, and P1-N1 difference was computed. Mean mSRP waveforms for five exemplary time windows are shown on the right. (D) mSRP lateralization (peak amplitude contra- minus ipsilateral to cue) as a function of time. Orange, blue, and black lines depict the lateralization index for the continuously computed P1, N1, and P1-N1 difference, respectively. Colored bars indicate time points at which lateralization deviated reliably from zero (non-parametric permutation tests, $p < 0.05$). Cue-related enhancement of mSRP peaks was absent during the baseline, built up about around 150-300 ms after cue onset, and then persisted throughout much of the CTI until target presentation.

4   DISCUSSION

Signals from microsaccades have been primarily viewed as measurement artifacts in human cognitive neuroscience (e.g. Yuval-Greenberg et al., 2008). However, by shifting the gaze, microsaccades also refresh visual representations about once or twice per second. In the present study, we investigated whether the resulting brain potentials reflect aspects of higher-level cognition, focusing on influences of visuospatial attention. Fixational eye movements and EEG were co-registered in a classic spatial cueing task. We expected that the execution of a microsaccade over a bilateral stimulus would evoke lateralized occipital potentials when attention is covertly deployed to one display side. This was in fact the case: The visual transients produced by microsaccadic gaze shifts are not a pure bottom-up phenomenon, but amplitude-modulated by top-down attention in similar ways as VEPs to passive

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stimulation.

4.1 Microsaccade-related potentials

Contrary to the assumption that covert orienting occurs in the absence of eye movements (Carrasco, 2011), small-amplitude saccades (median: 0.39°) were detected in a majority of trials confirming previous behavioral studies (e.g., Engbert & Kliegl, 2003; Hafed & Clark, 2002). These abrupt gaze shifts are known to evoke transient visual signals and, thereby, to contribute to perceptual processing during fixations (Martinez-Conde et al., 2009; 2013; Rolfs, 2009). In the present study, each microsaccade was accompanied by sizeable scalp-recordable activity, characterized by a muscle spike at movement onset (Armington, 1978; Keren et al., 2010) and a subsequent P1-N1 complex of cortical origin (Dimigen et al., 2009). Previous results strongly suggest that the P1 – and likely also the N1 – reflect a sweep of activation through early visual areas following small displacements of the retinal image. Specifically, the visual nature of the microsaccadic P1 is implied by its sensitivity to the luminance-contrast of the fixated pattern (Armington & Bloom, 1974; Armington et al., 1967; Gaarder et al., 1964), strong correlation with the electric response at the retina (Armington & Bloom, 1974), and dipole sources in striate or early extrastriate cortex (Dimigen et al., 2009).

4.2 Effects of top-down attention on VEPs and mSRPs

Paying attention to a stimulus is known to enhance neural responses at the single-cell level (Luck, Chelazzi, Hillyard, & Desimone, 1997; Moran & Desimone, 1985; Recanzone, Wurtz, & Schwarz, 1997), in hemodynamic recordings (Gandhi, Heeger, & Boynton, 1999; Heinze et al., 1994; Somers, Dale, Seiffert, & Tootell, 1999), and also in the EEG, where the effect manifests as a larger VEP to attended stimuli (Eimer, 1993; Hillyard & Anllo-Vento, 1998; Van Voorhis & Hillyard, 1977). In a control condition, we replicated this attentional enhancement with brief offsets of the display, mimicking the stimulation generated by a microsaccade. Although the short offsets evoked only a small P1 in the flicker-VEP, the subsequent N1 component showed the expected attention-dependent lateralization: A significantly larger N1 arose over the contralateral hemisphere which processed the attended visual field, suggesting a facilitated processing of the box-shaped stimulus presented at this location.

A key finding of the present study is that microsaccade-related brain potentials are enhanced in a similar manner: In mSRPs, cueing attention to a peripheral location was associated with both larger P1 and N1 amplitudes at occipital scalp sites located contralateral to the cued visual field. This pattern of results closely resembles established attention effects on the P1 (Heinze et al., 1990; Luck, Heinze, Mangun, & Hillyard, 1990) and N1 component (Drysdale et al., 1998; Griffin, Miniussi, & Nobre, 2002; Kasai, Moriya, & Hirano, 2011) of VEPs elicited by bilateral stimulation. In the case of VEPs, increased peak amplitudes are assumed to reflect a sensory gain-control mechanism by which attention increases the sensitivity of neurons whose receptive fields cover the attended location (e.g., Hillyard et al., 1998).

To our knowledge, these results provide the first demonstration that a similar process amplifies the visual transients measurable after saccadic eye movements. During periods of fixation, the amplification of microsaccadic responses may serve as a mechanism to maintain a stable representation of privileged locations or objects within the visual field.

4.3 Other influences on microsaccade-related brain potentials

In addition to the attentional enhancement, the mSRP was influenced by the direction of the movement itself: P1 and N1 were larger at occipital scalp sites located ipsilateral to microsaccade direction. While the origin of this effect remains to be determined, a likely explanation is that the predominantly horizontal microsaccades tend to move the foveal parts of the stimulus further into the hemifield opposite to the movement direction. This displacement results in a stronger post-saccadic activation of the newly stimulated hemisphere; i.e., the hemisphere ipsilateral to microsaccade direction. Importantly, this main effect of microsaccade direction did not interact with the effect of covert attention (Figure 3D) and could be controlled by means of a step-wise averaging procedure.

We can also rule out two alternative explanations for the attention effect. Firstly, kinetic properties of the saccade itself – in particular its size – are known to affect overall mSRP amplitude (Armington & Bloom, 1974; Dimigen et al., 2009). In the present study, this influence was controlled by comparing the activity between hemispheres for the same microsaccades. Secondly, the presentation of a directional cue is known to elicit lateralized ERP components, in particular a sustained *late directing attention positivity* at electrodes contralateral to the cue (Harter et al., 1989; Hopf & Mangun, 2000). Importantly, the lateralization effect reported here is not the result of such a global voltage shift, because it (1) consisted of a more positive P1 peak and a more negative N1 peak within less than 100 ms, (2) was found for microsaccades throughout the CTI, even in late time intervals after cue onset, and (3) persisted even when the cue-locked ERP was subtracted from the data epochs before extracting mSRPs.

4.4 The microsaccade-attention link

While under normal circumstances saccades and attention are tightly linked (e.g., Deubel, 2014; Deubel & Schneider, 1996; Kowler, Anderson, Dosher, & Blaser, 1995; Rolfs & Carrasco, 2012), the strength of this relationship is more controversial for the case of involuntary microsaccades (Hafed, 2013; Horowitz et al., 2007; Laubrock et al., 2010). In the present study, we replicated a previously reported tendency for microsaccades to point towards the cued direction shortly after cue onset (Engbert & Kliegl, 2003).

As many trials contained both cue-congruent and cue-incongruent microsaccades, a perfect coupling between microsaccades and attention would imply an unstable attentional focus, moving back and forth with each microsaccade. If we assume such an extreme scenario, where every microsaccade goes along with a corresponding attention shift, we would not only expect cue-congruent microsaccades

to be associated with enhanced neural processing of the cued location, but also that cue-incongruent microsaccades (caused, for example, by "faulty cue processing or imprecise control of attention", Horowitz et al., 2007, p. 357) would lead to a similar benefit for the uncued location. In other words, the mSRP evoked by cue-incongruent microsaccades should show the same effect of cueing, but with an opposite polarity (larger peaks over the hemisphere ipsilateral to cue direction). Averaging over cue-congruent and cue-incongruent microsaccades should therefore cancel out any cueing effect in the EEG. However, this was clearly not the case. Instead, a "net effect" of cueing persisted, with enhanced responses contralateral to the cue (Figure 3D).

Furthermore, by aggregating mSRPs within a sliding time window, we were able to derive a continuous measure of the hemifield that was preferentially processed at different times after cue onset. Consistent with the notion of a relatively stable attentional focus, we found systematic lateralization of the mSRP, which started around 150–300 ms after cue onset, that is, during the bias in microsaccade direction, but then persisted until the presentation of the target (Figure 4D).

Based on these observations, we propose that the oculomotor bias and the amplification of mSRPs reflect different aspects of attention (Kinchla, 1992): The direction of microsaccades early in the trial appears to be mainly sensitive to the initial shifting of attention after cue presentation (Laubrock et al., 2010; Pastukhov & Braun, 2010). At the neural level, this temporary coupling might be mediated by a short-lived, cue-related imbalance (Engbert, 2012) within the activation map of the superior colliculus (Hafed, Lovejoy, & Krauzlis, 2013), a key structure in (micro-)saccade generation. Microsaccades late during the CTI would be largely independent of this shifting process, and tend to restore central fixation (Engbert & Kliegl, 2004). In contrast, the sustained lateralization of microsaccade-related potentials appears to reflect the focus of sustained visuospatial attention, which builds up after 150–300 ms and then remains fixed at the attended location, despite occasional cue-incongruent microsaccades (see also Pastukhov et al., 2013 for behavioral evidence in support of this conclusion).

4.5 Tracking attention with (micro-)saccadic brain potentials

Due to their non-intrusive nature, microsaccades have potential advantages over established electrophysiological methods of studying attention. A common way of tracking visuospatial attention is to measure the amplitude of the VEP to "probe" stimuli flashed or flickered during the CTI (Hillyard et al., 1998; Lalor, Kelly, Pearlmutter, Reilly, & Foxe, 2007; Vialatte, Maurice, Dauwels, & Cichocki, 2010). However, such external stimulation is potentially attention-grabbing by itself and may interfere with cognitive processes of interest (Müller & Rabbitt, 1989) – as also indicated by a trend towards longer RTs for flicker trials in the present experiment. The present findings indicate that an alternative may be to exam the visual transients produced by involuntary gaze shifts over stationary stimuli.

In conclusion, the current study shows that microsaccades are not just a source of eye muscle artifacts in human electrophysiological recordings (Carl et al., 2012; Jerbi et al., 2009; Yuval-Greenberg

et al., 2008), but carry information about a subject's cognitive state. As microsaccades occur frequently, unconsciously, and at varying latencies during any fixation task, the associated brain responses may be useful as self-generated neural probes of attention, allowing a non-intrusive tracking of an observer's attentional focus over time.

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Supplementary information
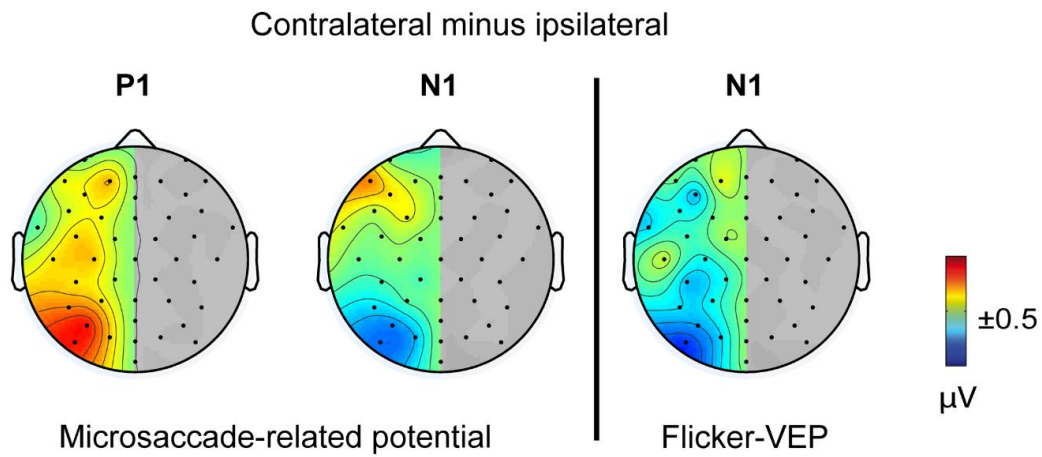
Supplementary Figure 1: Scalp topography for the effect of spatial attention on the mSRP and the flicker-VEP. Each topographical plot shows the difference between EEG channels located contralateral to the cued direction minus the ipsilateral channel, plotted onto the left side of the head. The difference at midline channels is zero by necessity. The attention effect on P1 (at 108 ms) and N1 (at 162 ms) of the mSRP are depicted in the left and middle plot, respectively. The N1 attention effect (at 134 ms) of the flicker-VEP is shown in the plot on the right.