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Disentangling gaze shifts from preparatory ERP effects during spatial attention

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Abstract

After a cue directing attention to one side, anterior event-related potentials (ERPs) show contralateral negativity (anterior directing attention negativity, ADAN). It is unclear whether ADAN effects are contaminated by contralateral negativity arising from residual gaze shifts. Conversely, it is possible that ADAN-related potentials contaminate the horizontal electrooculogram (HEOG), via volume conduction. To evaluate these possibilities, we used high-resolution infrared eye tracking while recording EEG and HEOG in a cued spatial-attention task. We found that, after conventional ERP and HEOG preprocessing exclusions, small but systematic residual gaze shifts in the cued direction can remain, as revealed by the infrared measure. Nevertheless, by using this measure for more stringent exclusion of small gaze shifts, we confirmed that reliable ADAN components remain for preparatory spatial attention in the absence of any systematic gaze shifts toward the cued side.

Descriptors: ADAN, ERP, Spatial attention, HEOG, Eye movements

Many event-related potential (ERP) studies of spatial attention use cuing paradigms, in which a cue event on each trial instructs the participant about where to attend for a subsequent imperative peripheral stimulus on that trial (e.g., Eimer, van Velzen, & Driver, 2002; Harter, Miller, Price, LaLonde, & Keyes, 1989; Mangun & Hillyard, 1991; Nobre, Sebestyen, & Miniussi, 2000; Talsma, Slagter, Nieuwenhuis, Hage, & Kok, 2005). Numerous studies, across several paradigms, have shown that sensory ERPs triggered by a peripheral stimulus can be modulated in amplitude, as a function of whether spatial attention was directed toward that stimulus or elsewhere (for reviews, see Eimer & Driver, 2001; Hillyard & Anllo-Vento, 1998; Hopfinger, Woldorff, Fletcher, & Mangun, 2001). It is well established that visual P1 and N1 components can be larger for the same visual stimulus when spatially attended than when ignored (e.g., Di Russo, Martínez, Sereno, Pitzalis, & Hillyard, 2002; Eason, Harter, & White, 1969; Gunter, Wijers, Jackson, & Mulder, 1994). Analogous attentional modulations have also been found for the auditory N1 component (e.g., Alho et al., 1987; Schröger & Eimer, 1993; Talsma & Woldorff, 2005) and for the somatosensory N140 component (e.g., Forster & Eimer, 2005; García-Larrea, Bastuji, & Mauguiere, 1991; Kida, Nishihira, Wasaka, Nakata, & Sakamoto, 2004).

In addition, ERP research has also considered the possible *preparatory* attentional control processes that might lead to such modulations of sensory ERP components. One approach investigates ERP components time-locked to presentation of an instructional cue event (e.g., an arrow pointing to the left or right), indicating to which side to attend for an upcoming peripheral stimulus and presented well ahead of that stimulus. ERPs time-locked to the instructional cue can then be compared as a function of the side toward which the cue directs attention (e.g., Eimer et al., 2002; Eimer, van Velzen, Forster, & Driver, 2003; Harter et al., 1989; Mangun & Hillyard, 1991; Nobre et al., 2000; van der Lubbe, Neggers, Verleger, & Kenemans, 2006; Yamaguchi, Tsuchiya, & Kobayashi, 1995b). To date, ERP studies of this type have revealed three characteristic cue-locked preparatory effects. An early negativity at posterior sites contralateral to the cued side (early directing attention negativity, EDAN) has been observed in studies using an arrow cue to direct attention (Harter & Anllo-Vento, 1991; Harter et al., 1989; Hopf & Mangun, 2000; Talsma et al., 2005; van der Lubbe et al., 2006; Yamaguchi, Tsuchiya, & Kobayashi, 1994). But this particular effect might reflect processing of the asymmetrical arrow-cue stimulus, rather than preparatory spatial attention per se (van Velzen & Eimer, 2003). Enhanced negativity has also been observed at anterior electrodes contralateral to the cued side, typically in the period 300–600 ms after cue onset (anterior directing attentional negativity, ADAN; see Eimer et al., 2002; Hopf & Mangun, 2000; Nobre et al., 2000; van Velzen, Forster, & Eimer, 2002; Yamaguchi et al., 1995b). Later enhanced

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This experiment was realized using Cogent (www.vislab.ucl.ac.uk/Cogent) and analyzed using SPM (www.fil.ion.ucl.ac.uk/spm). Stefan Kiebel, James Kilner, and Rik Henson gave advice on using SPM functions for EEG analysis. This research was supported by Wellcome Trust grants to J.D. and M.E. J.D. and M.E. also hold Royal Society-Wolfson Research Merit Awards.

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contralateral positivity has also been found more posteriorly in some studies (late directing attentional positivity, see Eimer et al., 2002; Hopf & Mangun, 2000; Nobre et al., 2000; van Velzen et al., 2002). It has been proposed that the ADAN and late-directing attentional positivity preparatory effects might reflect different aspects of attentional control. The more frontal ADAN might reflect underlying attentional control of spatial selection (e.g., Eimer et al., 2002; Nobre et al., 2000), whereas the more posterior late-directing attentional positivity might reflect changes in excitability for some sensory areas anticipating upcoming target events (e.g., Harter et al., 1989; Hopf & Mangun, 2000) or allocation of attention within visual space (Green, Teder-Sälejärvi, & McDonald, 2005; van Velzen, Eardley, Forster, & Eimer, 2006).

ADAN (and late-directing attentional positivity) preparatory components have now been observed in cue–target intervals following a variety of symbolic cues instructing where to attend, including abstract sounds (Eimer & van Velzen, 2002) as well as more conventional visual cues, such as arrowheads (Harter & Anllo-Vento, 1991; Harter et al., 1989; Hopf & Mangun, 2000; Talsma et al., 2005; Yamaguchi et al., 1994) or more symmetrical triangles (e.g., Eimer et al., 2002; Nobre et al., 2000; van Velzen et al., 2002). Moreover, these preparatory components can be found in experiments where the subsequent peripheral stimulus may be either visual, auditory, or tactile (Eimer & van Velzen, 2002; Eimer et al., 2002). Indeed, the ADAN preparatory component can appear very similar regardless of the sensory modality of the anticipated peripheral target, apparently generalizing across visual, auditory, or tactile experiments. This has been taken to indicate that the ADAN might reflect "supramodal" mechanisms of attentional control (Eimer & van Velzen, 2002; Eimer et al., 2002; but see also van Velzen et al., 2006).

Most experiments to date on such preparatory differential components used HEOG criteria for assessing whether participants shifted their gaze toward the expected target location on some trials, rather than directing purely *covert* spatial attention as instructed (though see Griffin, Miniussi, & Nobre, 2002). Individual trials are typically excluded from further analysis if they show evidence of horizontal eye movements, as revealed by large HEOG deviation from a precue baseline (ranging from $\pm 75\ \mu$V down to ± 30 V, e.g., Eimer & van Velzen, 2002; Eimer et al., 2002; Nobre et al., 2000; van Velzen et al., 2002; Yamaguchi, Tsuchiya, & Kobayashi, 1995a) and/or via trial-by-trial human inspection (Hopf & Mangun, 2000; Nobre et al., 2000). Although a systematic deviation of 30 μV is thought to equate approximately to $\pm 2.5^\circ$ (Mangun & Hillyard, 1991), it might be difficult in practice to apply a more stringent HEOG exclusion criterion than this, given typical noise levels in HEOG. A further check may then be made (e.g., Eimer & van Velzen, 2002; Eimer et al., 2002; Nobre et al., 2000; van Velzen et al., 2002) on "residual" HEOG waveforms in individual participants, constructed by averaging trials that survive the initial exclusions, as a function of cued side. Individuals might then be excluded from the study if their residual HEOG signal deviates from baseline above a certain criterion, typically $\pm 2\ \mu$V on each side, or, alternatively, a difference of 4 μV between cue-left and cue-right trials (e.g., Eimer & van Velzen, 2002; Eimer et al., 2002; Nobre et al., 2000; van Velzen et al., 2002).

It is often assumed, either explicitly or implicitly, that after such "conventional" HEOG-based exclusions, the remaining data should then be free from possible contamination by gaze shifts. However, some small residual HEOG signal, below the exclusion criterion, could, in principle, still be present in several or most participants, and might even be systematic as a function of cued side. Other approaches apply an off-line correction of EEG data based on concurrent HEOG signal (e.g., Talsma et al., 2005;for a review, see Croft, Chandler, Barry, Cooper, & Clarke, 2005) or analysis of independent components across the whole EEG/HEOG data set (Jung et al., 2000).

However, it might be worthwhile to be even more cautious when excluding participants or trials contaminated by gaze shifts when studying a *contralateral negativity* (or, equivalently, an ipsilateral positivity) such as the preparatory ADAN component. HEOG gives an indication of eye position because the eyeballs are dipoles, causing relative negativity at the electrode contralateral to gaze direction. Thus, if participants systematically moved their eyes toward the cued side, even slightly, an ADAN-like effect (relative negativity contralateral to that cued side, but caused by a systematic gaze shift) might arise at HEOG electrodes. Moreover, via volume conduction, any such signal originating at the eyes could, in principle, be picked up also at anterior scalp electrodes and thereby leak into the ADAN measure. Conversely, if the ADAN effect is indeed a true EEG effect reflecting covert attentional preparation, rather than contamination from HEOG, as is widely held (e.g., by Eimer et al., 2002; Hopf & Mangun, 2000; Nobre et al., 2000; Praamstra, Boutsen, & Humphreys, 2005; van Velzen et al., 2002; Yamaguchi et al., 1995b), the HEOG signal itself might, in principle, become cross-contaminated to some extent by signals from the brain (Croft et al., 2005; Jung et al., 2000). Indeed, given the spatial proximity of the electrode sites often used to measure HEOG or ADAN, it would be rather surprising if absolutely no relationship were observed between them, regardless of the direction(s) of any cross-contamination (i.e., from EOG to EEG or vice versa or both), even if they ultimately have different sources, as is widely thought (e.g., Praamstra et al., 2005; van der Lubbe et al., 2006). Moreover, in addition to potential electrical leakage of one signal (ADAN or HEOG) into the other, one must also consider the important effects on cognitive and sensory processing that small but systematic nonexcluded eye movements might have in studies purporting to index purely covert attentional processes.

To address these issues, we used high-resolution *infrared* eye movement tracking, thus sidestepping volume conduction. We combined infrared eye tracking with EEG and HEOG recording in a standard spatial cueing paradigm. The current experiment employed a central symbolic cue to indicate which side participants should attend to for a visual judgment on a trial-by-trial basis (see below for further details). This should generate the typical ADAN preparatory component, in the cue–target interval (as closely similar cues did in Eimer et al., 2002; Eimer, Forster, & van Velzen, 2003; van Velzen et al., 2002). We concurrently measured any such ADAN effect, plus HEOG, together with the infrared signal. By comparing ADAN with HEOG, we could observe any relationship between these two signals. By comparing HEOG with infrared eye tracking, we could determine whether any HEOG signal was due to genuine eye position deviation (as revealed by the independent infrared eye tracking) or might instead represent leakage of lateralized EEG brain signals into HEOG. We could also examine whether any small but systematic gaze shifts (as assessed by infrared tracking) remain after "conventional" trial exclusions based on HEOG. Finally, we could use the better signal-to-noise ratio of the infrared eye tracking to eliminate any trials with small gaze shifts more stringently, rather than using just HEOG for this as conventionally done, to assess whether ADAN effects remained present when any systematic residual gaze shifts were eliminated.

We implemented successively more stringent criteria for eliminating trials based on the infrared measure, to examine how these successive criteria might affect the observed relationship between ADAN and residual eye position (as assessed both with infrared tracking and via HEOG). It seemed important to resolve this issue definitively, given the increasing number of studies investigating the ADAN in order to assess mechanisms of putatively *covert* spatial attention. Moreover, the dependence or independence of ADAN effects from eye position has theoretical as well as empirical implications. For example, the apparently "supramodal" nature of the ADAN in some reports (Eimer & van Velzen, 2002; Eimer et al., 2002) might become less surprising if, in fact, this component simply reflected small but systematic residual gaze shifts in some circumstances.

Unlike the HEOG signal, the infrared tracking obviously *cannot* be contaminated by potential electrical interaction with EEG. Nevertheless, the infrared signal from the ASL504 eye tracker (details below) will still contain some fluctuating noise. Gaze devaitions of well below 1° of visual angle can be detected (as we confirm below), but noise levels will still fluctuate somewhat across and within participants. A balance therefore needs to be struck between retaining enough trials within the ERP and HEOG analyses for statistical power, yet eliminating trials where infrared tracking indicates some gaze deviations away from central fixation. We adopted a titration approach to the single-trial rejection procedure here. We set increasingly stringent central-fixation thresholds for the infrared eye position signal, which first started at ± 1° and were then reduced in steps of 0.1°. Four such iterations were performed. Importantly, at each iteration of this process, analyses were performed to assess the remaining ADAN effect, any *residual* HEOG effect, and the *residual* infrared eye tracking signal for those trials that still passed the increasingly stringent infrared tracking exclusion criteria.

Methods

Participants

Twenty-two healthy volunteers, naïve as to the purpose of the experiment, were paid £15 each for their time. One was excluded because of exceptionally poor eye fixation. A second was excluded due to eye tracker malfunction. A third was excluded due to excessive alpha-wave activity. Thus 19 participants (7 women, 12 men, one left-handed), aged 20–39 years (mean age: 26 years) remained in the sample. All had normal or corrected vision by self-report.

Stimuli and Apparatus

Stimuli and procedures were very similar to those employed in several previous ADAN studies (Eimer et al., 2002; Eimer, Forster & van Velzen, 2003; van Velzen et al., 2002). Participants sat in a dimly lit experimental chamber, with head movement precluded by an adjustable chin rest. A computer monitor was placed 58 cm in front. A centrally located gray fixation cross, ~ 0.3° in size, was continuously present on the screen, 10 cm below horizontal straight ahead. The cue stimulus took the form of two arrowheads formed by open triangles, one red, one blue, pointing in opposite directions (i.e., either "<+>" or ">+<"; see Figure 1 for details). Equiprobably, either arrowhead could be red, with the other blue. Thus there were four equally likely cue stimuli, all subtending 1.9° × 0.75° (see Figure 1). Of the 19 participants included in the following analysis, 11 used the red arrowhead and the remainder used the blue arrowhead as the instruction for which side to attend for the upcoming peripheral stimuli (see also Nobre et al., 2000).

Figure 1. Types of cue stimuli used in the current study. Each cue had one blue and one red arrowhead. Half of the participants attended to the side indicated by the blue arrowhead, half followed the red arrowhead.

Peripheral visual stimuli were presented at the end of the cue–target interval, by illuminating an ensemble of green light-emitting diodes (LEDs; consisting of six 5-mm LEDs arranged in a circle plus one central LED) on the left or right. One LED ensemble was placed on each side at the same height and distance as the fixation cross, at a visual angle of 23° to the left or right of central fixation. Standard ("nontarget") stimuli consisted of the continuous illumination of one LED ensemble for 200 ms. For the rare "deviant" events, which had to be reported for the cued side only, one LED ensemble was illuminated for 75 ms, turned off for a 50-ms gap, and illuminated again for 75 ms (total duration: 200 ms). Participants rested one arm on their lap and the other arm on the table top with that hand at the midline, resting on a response key. The active hand alternated throughout the experiment (see Procedure). The task was to respond by pressing the response key with that hand only for a target (i.e., deviant) visual event and only if such an event appeared on the side that had been cued for that trial. Standard events on either side required no response and likewise for any deviant event on the uncued side.

Procedure

The experiment consisted of eight experimental blocks. Participants were instructed to respond to target events (deviant) on the cued side only, as indicated by the arrowhead in their nominated color at the start of each trial while keeping their gaze at central fixation throughout. Although this fixation instruction was emphasized, gaze did nevertheless tend to shift slightly but systematically on some trials, as will be shown below.

The cued side was indicated on a trial-by-trial basis by the appearance of one of the four cue stimuli (see above and Figure 1), which were equally likely and presented in pseudorandom order. Trials began with a cue stimulus for 100 ms, followed at a stimulus onset asynchrony (SOA) of 885 ms by a peripheral visual stimulus for 200 ms. After a further intertrial interval of 1000 ms, the next trial started. Each block consisted of 68 trials. For 56 trials, a standard peripheral visual nontarget (continuous LED illumination for 200 ms) was presented on either the left or right side with equal likelihood. In the remaining, randomly intermingled 12 trials, a single visual "deviant" target (LED illumination for 200 ms, with gap) was presented. Of these, eight were on the cued side (four on the left, four on the right) and four on the uncued side (two on each side). The instructed task was to attend covertly to the cued side without shifting gaze and to respond to deviant events on the cued side only by pressing the response key as quickly and accurately as possible. The active hand was changed after each block. Of the 19 participants analyzed below, 10 began with their left hand and 9 with their right hand. To familiarize them with the specific task requirements, participants performed two training blocks prior to the first experimental block.

Recording and Data Analysis

EEG was recorded with Ag-AgCl electrodes and linked-earlobe reference from Fpz, Fz, Cz, Pz, Oz, F7, F8, F3, F4, FC5, FC6, T7, T8, C3, C4, CP5, CP6, P3, P4, P7, P8, PO7, and PO8 (according to the extended 10–20 system). HEOG was recorded bipolarly from the outer canthus of each eye. The impedance for all electrodes was kept below 5 kΩ. The amplifier bandpass was 0.1 to 40 Hz and signals were sampled with a digitization rate of 200 Hz and stored on disk. Eye position was further monitored using an infrared ASL504 tracker (Applied Science Laboratories, Bedford, MA), which uses relative positions of the pupil and the reflection from the corneal surface to infer gaze direction. The eye tracker was placed centrally on the desk below the computer monitor. Horizontal eye position and pupil diameter were sampled at 60 Hz. Prior to each block of trials, participants were instructed to follow a cross with their eyes as it jumped to five known horizontal locations at (± 15°, ± 7.2°, 0°). This calibration procedure allowed the infrared eye tracker setup to be verified or recalibrated, and allowed the infrared eye tracking signal to be converted into degrees of visual angle.

EEG and HEOG were epoched off-line into 985-ms periods starting 100 ms prior to the cue onset and ending at the onset of the peripheral visual stimulus that participants had to judge (on the cued side) or ignore (on the uncued side). The infrared eye tracking signal was converted into degrees of visual angle using the calibration data obtained before each experimental block. It was then converted to 200 Hz by linear interpolation to allow for its analysis alongside the EEG data, using the same software. Finally, it was epoched just as for EEG and HEOG.[1]

Trials with blinks (VEOG exceeding ± 60 μV in the interval from cue onset to peripheral stimulus onset, relative to 100 ms precue baseline), with frank horizontal eye movements (HEOG exceeding ± 30 μV relative to baseline, approximately equal to ± 2.5°; see Mangun & Hillyard, 1991), or other artifacts (a voltage exceeding ± 60 μV at any electrode location in the same interval relative to baseline) were excluded from further analysis. In addition, all trials following those with a response or an error were removed (11.9% of all trials) to avoid any overlap of ERP waveforms with response-related or error-related processes. Taking all of the above typical preprocessing exclusion criteria together resulted in removal of 26.4% of all trials. Additionally, any trials in which the infrared eye position signal was lost were also omitted (a further 2.9% of all trials).

Four parallel analyses proceeded from this point, using increasingly stringent infrared eye position criteria to exclude trials based on eye position, as planned a priori. These analyses were identical except for the infrared eye position threshold used to reject individual trials. Trials were rejected if the signal exceeded the particular threshold relative to baseline for a continuous 50-ms period in the interval from the baseline start to peripheral stimulus onset 885 ms later, compared to a 100 ms precue baseline. A 50-ms period of continuous infrared eye tracker signal deviation was chosen to avoid excluding trials with brief noise fluctuations. The four successive eye position thresholds were 1.0°, 0.9°, 0.8°, and 0.7° from central fixation.

Table 1. *Percentage Additional Excluded Trials due to Excessive Infrared Eye Deviation ($>0.7°$) after Conventional Artifact/Error Rejection Procedures*[a]

Cued side	Left		Right	
Arrowhead side	Left	Right	Right	Left
Cue stimulus	**<**>	>**<**	<**>**	**>**<
Infrared deviation				
Left	4.9	2.7	2.1	3.2
Right	2.5	3.6	5.0	3.2

[a]Data are broken down by saccade/infrared signal direction, cued side, and cue stimulus design. Bold arrowhead denotes imperative cue.

Following these increasingly stringent steps of eye position trial exclusion, there remained respectively 91.8%, 87.5%, 81.5%, and 72.9% of those trials that had survived the preceding conventional preprocessing rejections. In each case, surviving trials were then collapsed across cue shape ("<+>" vs. ">+<"; see van Velzen & Eimer, 2003) to leave just two conditions (left- vs. right-side cued) for further analysis. Table 1 shows the percentage of trials rejected due to eye deviations as detected by infrared eye tracking, displayed according to which direction the infrared eye position signal deviated and which cue–stimulus was displayed, with the rejection threshold set at 0.7°. Note that, overall, more gaze deviations were detected with infrared eye position toward the cued side (despite the instructions to maintain central fixation and despite the fact that trials with substantial frank saccades had already been excluded via the conventional HEOG criteria, *prior* to the further infrared eye tracking rejections listed in Table 1). Additionally, more gaze deviations were detected overall toward the side of the imperative arrowhead (i.e., the one in the relevant color for the participant). Hence, gaze deviations (despite prior HEOG exclusions) were most common toward the cued side and especially when the imperative arrowhead also fell toward that side. Note that, even with more conventional arrow cues presented centrally (e.g., Praamstra et al., 2005; Talsma et al., 2005), the arrowhead itself is usually placed slightly toward the cued side.

Results

All ERPs and infrared eye position signals were measured relative to the mean signal of the 100-ms precue baseline interval; all numerical latencies are defined relative to onset of the cue stimulus. Figure 2 plots the grand averaged waveforms for electrode pair FC5 and FC6 in the cue-left and cue-right conditions, after the most stringent eye position trial-exclusion criterion. This illustrates the evoked responses to the cue at the electrode sites with the maximal ADAN effect. To visualize the ADAN effect more clearly, a double-subtraction procedure was undertaken (as in Eimer et al., 2002; Harter et al., 1989; van Velzen et al., 2002), though all statistical analyses were conducted on the averaged waveforms prior to any subtractions. For all six pairs of lateral anterior and lateral central electrodes (F7-F8, F3-F4, FC5-FC6, T7-T8, C3-C4, and CP5-CP6), the waveforms for the difference between cue-left and cue-right trials was calculated (cue left minus cue right). Then, using these resulting difference

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[1] At the request of a referee, we also reran all of our analyses after the raw infrared eye-tracking data had been initially highpass filtered at 0.1 Hz (in the same way as the EEG data had been). All results after adding this filtering step agreed with those presented below.

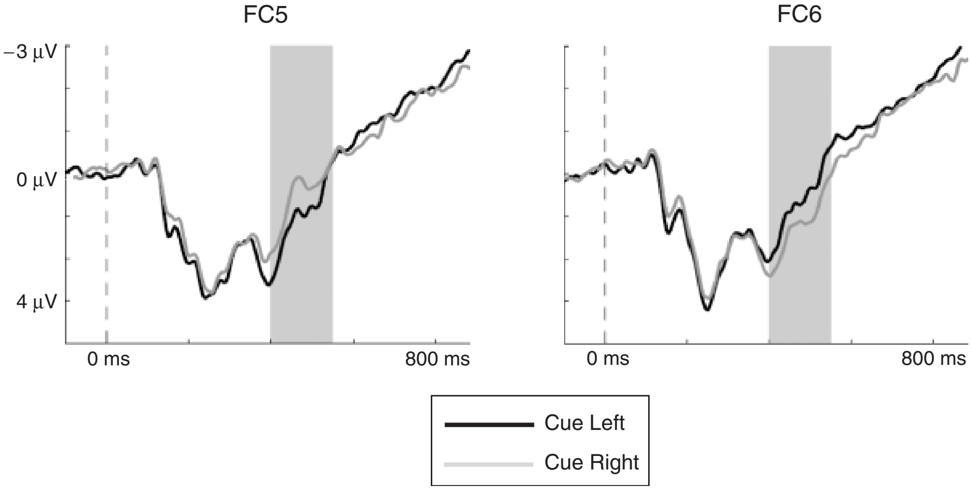

Figure 2. Grand averaged ERPs from the most stringent eye movement trial-rejection criterion (i.e., 0.7°) elicited at electrodes FC5 and FC6 in the interval between cue onset (dashed line) and onset of the peripheral stimulus, after a cue to attend to the left or right side. In the interval 400–550 ms after cue onset (shaded area), anterior directing attentional negativity (ADAN) can be observed, as expected; the trace is more negative following a cue to the right for FC5, but after a cue to the left for FC6.

waveforms, the further difference between opposing scalp sites was calculated (left electrode minus right electrode). The effect of this double subtraction is to add together the amplitudes of the relative contralateral negativity for cue-right and cue-left trials. The resultant waveforms plot the ADAN effect positive going, with an amplitude double that measured at any single electrode site, though note that this does not change the statistical analyses below in any way (Eimer et al., 2002; Harter et al., 1989; van Velzen et al., 2002).

Figure 3 plots this grand-mean ADAN effect for all six anterior and central electrode pairs after the most stringent eye position exclusion criterion. Note that the ADAN amplitude was maximal at FC5-FC6, consistent with several previous observations (Eimer et al., 2002; Eimer, Forster, et al., 2003; Eimer, Forster, van Velzen, & Prabhu, 2005; Nobre et al., 2000; van Velzen et al., 2002), slightly more lateral and posterior than the maximal location found in a recent higher-density investigation of ADAN scalp topography (Praamstra et al., 2005). Figure 4 (black lines) plots this grand-mean ADAN effect obtained at FC5-FC6 across four separate graphs for the successively more stringent infrared eye position exclusion thresholds.

HEOG and infrared eye position difference waveforms for cue-left minus cue-right trials were calculated to allow direct comparison with the ADAN for the same trials. Figure 4 shows the grand mean HEOG effects (dark gray lines) on the same axes as the ADAN effects (black lines), and the infrared eye position signals (pale gray; see right y-axis in each graph) are also shown for each of the four successive infrared eye position trial-rejection thresholds. Note that the infrared eye position signals shown here do not reveal abrupt phasic changes because they represent trials remaining *after* trials with larger eye movements were removed via conventional HEOG-based exclusion. Moreover, these infrared eye position signals average over many trials and participants, with any small gaze deviations typically being jittered in time.

The ADAN effect can clearly be seen 400–550 ms after cue onset in Figure 4. Interestingly, for liberal infrared eye position rejection thresholds and also for more stringent thresholds, the HEOG signal closely follows the ADAN recorded from FC5-FC6 (presumably because, as noted in the Introduction, each of these signals can leak into the other to some extent via volume conduction). The infrared eye position signal showed a residual, small, *but systematic* gaze-direction shift toward the cued side, after conventional trial exclusion by HEOG alone had been applied. This observation was confirmed by a one-way ANOVA conducted on the mean residual infrared eye position signal within the time window 400–550 ms after cue onset (the ADAN time window as used in the further analyses below). The factor of Cued Direction (left vs. right) was significant, $F(1,18) = 20.1$, $p < .001$. This residual gaze deviation persists even when the infrared eye tracking signal was used to reject additional trials with saccades larger than 1.0°, 0.9°, and 0.8°. Residual infrared eye position deviation diminishes and is eventually eliminated as stricter trial-exclusion thresholds are applied. Importantly, the ADAN effect remains reliable even when the residual gaze deviation as measured by infrared eye tracking is no longer systematic; see below for statistical confirmation.

To evaluate the reliability of these observations, within-participant ANOVAs were performed on each of the four data sets (i.e., those with successively more stringent infrared eye position exclusion thresholds). ADAN analyses were conducted on the mean value of the averaged waveforms (now without application of the double-subtraction procedure used for visualization), in the time window 400–550 ms after cue onset. Each three-way ANOVA had factors of Cued Direction (left vs. right), Hemisphere (left vs. right), and Electrode Site (F7/8 vs. F3/4 vs. FC5/6 vs. C3/4). A reliable ADAN is revealed by a significant interaction of Cued Direction with Hemisphere (see Eimer et al., 2002; van Velzen et al., 2002). This term was significant across all four analyses, all $Fs(1,18) > 13.3$, all $ps < .002$. Although the ADAN was numerically larger at FC5-FC6 and C3-C4 than at F7-F8 and T7-T8 (see Figure 3), three-way interactions (Electrode Site × Cued Direction × Hemisphere) failed to reach statistical significance in any of the four analyses here, all $Fs(3,54) < 2.9$, all $ps > .07$. Higher-density electrode arrays are needed for detailed topography of the ADAN and other ERP components (e.g., Praamstra et al., 2005). Further ANOVAs, which were performed separately for each electrode pair (F7-F8, F3-F4, FC5-FC6, C3-C4) and each infrared eye position

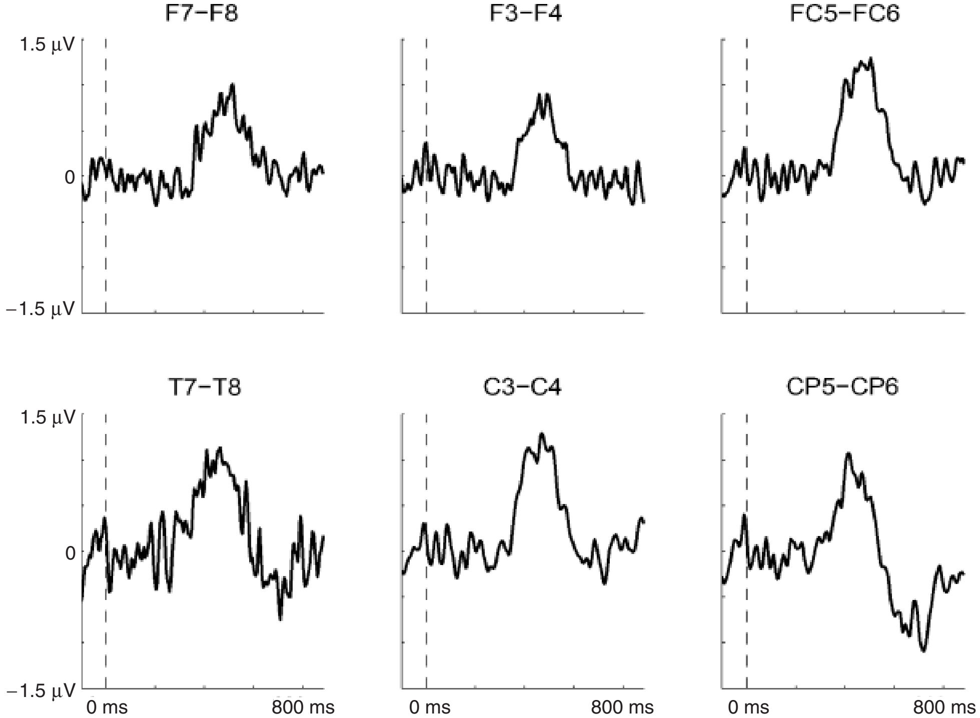

Figure 3. Difference waveforms, showing ADAN, obtained at lateral anterior (F3/4, F7/8, FC5/6; top) and lateral central (T7/8, C3/4, CP5/6) electrode pairs in the 885-ms interval following cue onset, after exclusion of eye movement trials using the most stringent infrared eye position threshold (0.7°). These difference waves were generated by subtracting ERPs in response to right cues from ERPs in response to left cues and then subtracting the resultant difference waveforms at right electrodes from those at corresponding left electrodes. Enhanced contralateral negativity is revealed as positive values in the resulting waveform.

rejection criterion, confirmed the presence of a significant ADAN throughout, all *F*s(1,18) > 9.0, all *p*s < .008. Thus, the ADAN remained reliable even with our most stringent infrared eye position deviation exclusions. Figure 5A (black line) plots *F* values for the maximal ADAN effect (i.e., at FC5-FC6) under the successive trial-exclusion thresholds. Significant *F* values fall above the horizontal dotted line that represents a significance level of $p = .05$, confirming the robustness of the ADAN effect despite increasingly stringent infrared eye position rejection.

Average HEOG and infrared eye tracking signals were analyzed for the same time window with ANOVAs analogous to those used for the EEG data, except that there was no Hemisphere factor, thus leaving Cued Direction as the relevant factor. These analyses yielded contrasting results for HEOG and infrared eye tracking. The residual HEOG effect (see Figure 5A, dark gray line, for *F* values) remained significant for all four trial-rejection thresholds, *F*s(1,18) > 8.8, *p*s < .009. The residual infrared eye position effect (see Figure 5A, pale gray line, for *F* values) was significant for the three most liberal trial-selection regimes, *F*s(1,18) > 4.7, *p*s < .043, but not for the 0.7° eye-movement rejection criterion, $F(1,18) = 2.7$, $p = .12$. Figure 5B shows normalized effect sizes for all three signals and for all four analyses, corresponding to the statistical reliability shown in Figure 5A. These effect sizes have been normalized to allow visual comparison. This normalization started with each effect size, as calculated by taking the mean size of the ADAN, residual HEOG, and residual infrared eye position waveforms plotted in Figure 4, within the time window used in the above statistical analyses (i.e., 400–550 ms after cue onset). Each value was then divided by its respective value from the first analysis (i.e., 1.0° infrared eye position trial-exclusion criterion). Hence, all effects have a value of exactly 1 for the most liberal analysis. It is clear from Figure 5B that HEOG and ADAN effect sizes tended to remain similar (consistent with the anticipated leakage between these; see Introduction), whereas the infrared eye position effect size decreases steadily with more stringent exclusion criteria, until no systematic shift of eye position toward the cued side remains (as confirmed by the *F* values in Figure 5A).

To further confirm these observations statistically, additional ANOVAs were conducted on the ADAN obtained at FC5-FC6, the residual HEOG, and residual infrared eye position. The ADAN analysis had factors of Infrared Eye Position Criterion (1.0° vs. 0.9° vs. 0.8° vs. 0.7°), Cued Direction, and Hemisphere. The analogous analyses for the residual HEOG and infrared eye position effects had factors of Infrared Eye Position Criterion and Cued Direction. These analyses confirmed the patterns shown in Figure 5. ADAN size did not significantly vary with Infrared Eye Position Criterion, $F(3,54) = 0.4$, $p = .6$. Similarly, the residual HEOG effect was also invariant across Infrared Eye Position Criterion, $F(3,54) = 0.9$, $p = .4$, most likely because an ADAN effect was picked up at HEOG electrodes. In contrast, the residual infrared eye position effect did significantly change with Infrared Eye Position Criterion, $F(3,54) = 13.2$, $p < .001$.

Finally, given the utmost importance of even minor gaze deviations for our present concerns, we returned to consider the two different cue designs (i.e., <+> vs. >+<), which we had pooled over thus far, as in all previous studies using such counterbalanced symmetrical cues (Eimer et al., 2002;Eimer, Forster, et al., 2003; van Velzen et al., 2002). With outward-pointing cue stimuli (<+>), whichever of the two arrowheads was imperative was inevitably placed slightly toward the cued side (see Figure 1), whereas with inward-pointing cue stimuli (>+<),

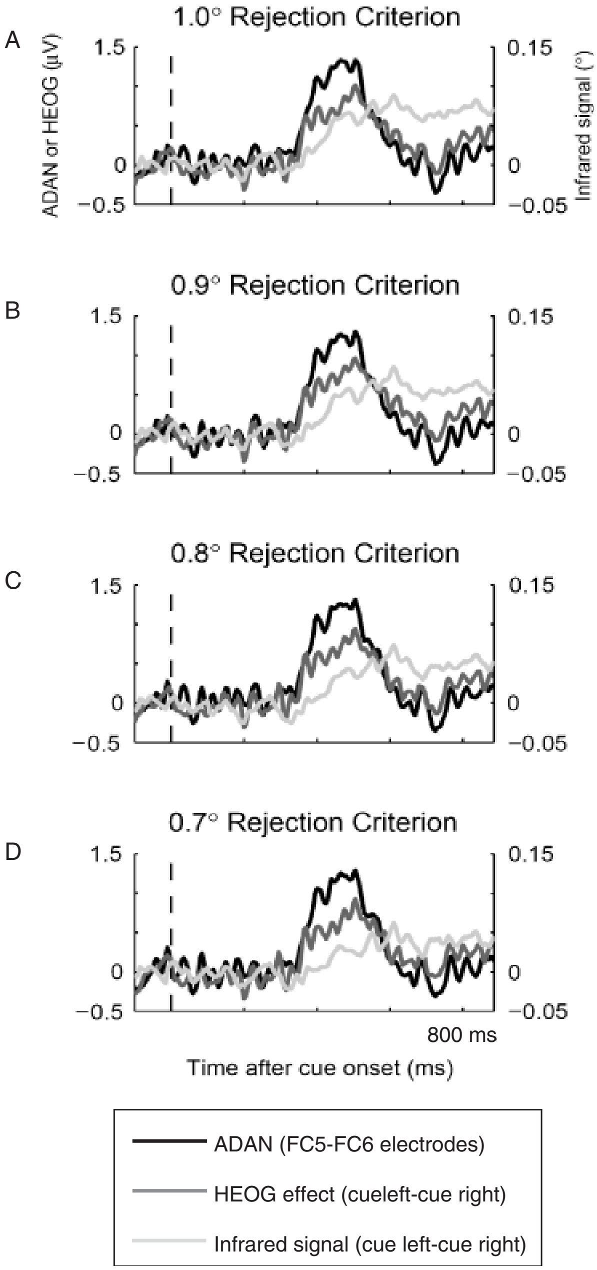

Figure 4. Difference waveforms showing the ADAN at FC5/6 electrodes, residual HEOG, and residual infrared eye position effects for the four increasingly stringent infrared eye position trial-rejection thresholds (1.0°, 0.9°, 0.8°, 0.7°). The ADAN difference waves were calculated as in Figure 3. The residual HEOG and infrared eye position effects were calculated by subtracting the waveforms from cue-right trials from the waveform obtained in cue-left trials. Thus the three waveforms can be compared directly. ADAN and HEOG are plotted on the same (left) *y*-axis; see right *y*-axis for infrared eye position scale.

the currently imperative arrowhead inevitably fell slightly toward the *un*cued side. Even with our most stringent infrared eye position exclusions, the mean *residual* eye position effect still differed very slightly (by a mean of 0.13° within the ADAN time window) between outward- and inward-pointing cue types, $t(18) = 4.7$, $p < .001$, falling slightly but systematically toward the *un*cued side for the inward-pointing cues. Our ability to pick up this tiny infrared eye position effect again confirms the sensitivity of the infrared eye tracking measure. Moreover, this allowed a further and final test of whether the ADAN remains when gaze certainly does *not* deviate toward the cued side. When considering only those trials with inward-pointing cues, on which any residual small but systematic gaze shifts are toward the *un*cued side (see above), a significant ADAN still remained over three electrode pairs (FC5/6, $F[1,18] = 7.7$, $p = .01$; T7/8, $F[1,18] = 5.8$, $p = .03$; C3/4, $F[1,18] = 5.7$, $p = .03$), even though the average eye position for these trials was now slightly *away* from the cued side.

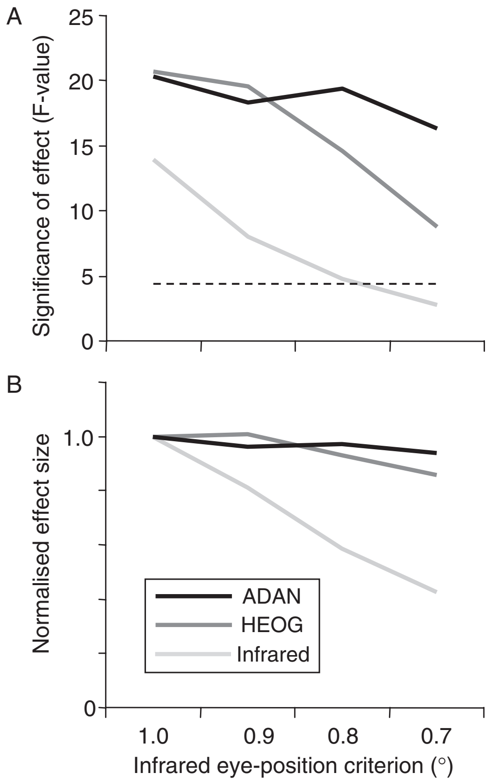

Figure 5. A: *F* values (all with $df = 1,18$) for analyses of the ADAN effect, of any residual HEOG effect, and of the residual infrared eye position, all in the time window 400–550 ms after cue onset, for four successive levels of increasingly stringent infrared eye position trial-rejection criterion (marked along the *x*-axis). Higher *F* values correspond to more significant effects; horizontal dotted line denotes significance level of $p = .05$. B: Normalized effect amplitudes for the ADAN, residual HEOG, and residual infrared eye position, corresponding to the statistical reliability data shown in A. Normalized effect amplitudes were calculated from the mean ADAN, residual HEOG, and residual infrared eye position, as averaged across the time window 400–550ms after cue onset. Normalization arbitrarily sets each signal's magnitude equal to one for the most liberal analysis (i.e., 1.0° infrared eye position trial-exclusion criterion). This allows fair comparison of effect magnitude (*y*-axis) of the three signals across the four trial-exclusion thresholds (see *x*-axis). Note how ADAN and residual HEOG effect sizes vary together, whereas the infrared eye position, unsurprisingly, decreases monotonically as the infrared eye position trial-exclusion criterion becomes more stringent.

Discussion

We investigated the potentially problematic relationship between any residual eye movements and the ADAN. The latter arises contralateral to the side cued for attention, and putatively reflects covert attention rather than gaze deviation. In principle, any residual overt eye movements toward the cued side could result in ADAN-like ERP lateralizations being detected at anterior scalp electrode sites, due to cross-contamination from HEOG signals. Conversely, any true ADAN effect might, in principle, also contaminate HEOG measures to some extent (as our data indicate). Here we employed infrared eye tracking as an additional independent measure. This allowed us to completely sidestep the cross-contamination issue when considering eye position. Although other more sophisticated approaches to separating HEOG from other ERP components are possible (e.g., as in some biophysical-model or independent-component approaches to EEG analysis; Croft et al., 2005; Jung et al., 2000; Talsma et al., 2005), here our aim was to separate the ADAN from eye position itself, for which a nonelectrical eye tracking measure provides the most direct approach.

Our first main finding was that even after "conventional" HEOG trial-exclusion procedures, which are usually thought to eliminate any residual gaze deviation, infrared eye tracking signals still showed a significant *residual* eye position effect, with gaze tending to shift slightly but reliably toward the cued side on average. This remained the case even after additional trial exclusions, based on all but the most strict infrared eye-tracking-based eye position thresholds applied here (see Figures 4 and 5). Most conventional ERP preprocessing in cued attention studies will typically overlook such small but potentially confounding gaze deviations. Any residual HEOG deviations after initial HEOG-based exclusions often go unreported (e.g., Eimer, Forster, Fieger, & Harbich, 2004; Eimer, Forster, et al., 2003; Eimer et al., 2005; Green et al., 2005; Praamstra et al., 2005; van der Lubbe et al., 2000; van Velzen & Eimer, 2003; van Velzen et al., 2002) or are shown graphically to be only a fraction of the visual angle toward any peripheral stimuli but not tested further (e.g., van der Lubbe et al., 2006; Verleger, Vollmer, Wauschkuhn, van der Lubbe, & Wascher, 2000). Nevertheless, small gaze deviations toward the cued side might have cognitive or sensory consequences, as well as electrical effects. It might be important to exclude these consequences when seeking to study covert attention. Assessing this could, in principle, be readily done by performing statistical tests on the *residual* HEOG signal itself (or arguably better still, on an infrared eye tracking signal) *after* exclusions, as here. As we found here, even very small residual HEOG effects (e.g., $< 1\ \mu V$) or tiny residual infrared eye position effects (e.g., $< 0.04°$ degrees) can prove statistically reliable and might be important when seeking to distinguish covert attention from any small but nevertheless systematic residual gaze deviations.

Given the likely presence of such residual eye movements toward cued locations in previous ADAN studies, the question arises of whether those previous effects might predominantly or occasionally have been caused by systematic, time-locked overt gaze shifts in response to the cue. Here we took the direct approach of using nonelectrical, independent, high-resolution infrared tracking. This allowed us to show unequivocally that the ADAN cannot solely be attributed to gaze deviations. Treating the infrared infrared eye tracking signal to an "ERP-style" analysis here (i.e., epoching and averaging signals over many trials) proved to be very sensitive. Using the 1.0°, 0.9°, and 0.8° infrared eye position rejection thresholds still left a significant detectable difference in eye position between cue-left versus cue-right trials, even though the grand-mean *residual* infrared eye position effect was very small in absolute terms (see Figure 4A–C, right *y*-axis). Moreover, making the infrared eye position rejection threshold increasingly stringent, in successive 0.1° steps, produced a revealing pattern (see different graphs in Figure 4 and functions in Figure 5A,B). Using increasingly stringent infrared eye position thresholds did not eliminate either the ADAN or the residual HEOG effect (see Figures 4 and 5). At the most stringent threshold, there was no longer any systematic gaze deviation toward the cued side in infrared eye position, yet a comfortably significant ADAN still remained (see Figure 5A), thereby demonstrating that the ADAN effect is not exclusively caused by small but systematic eye movements toward the cued location. As a further confirmation of this, we were able to show not only that a reliable ADAN remains even after our most stringent gaze-related exclusions, but also that the ADAN effect remains even on those trials with "inward-pointing" symmetrical cues, when any small deviation in gaze was *away* from the cued side, toward the imperative arrowhead.

This result corroborates previous indirect arguments that ADAN is not solely due to confounding gaze deviations. ADAN effects are often more pronounced at central sites, not just at more anterior electrodes (Eimer et al., 2002), as we also found here, thus suggesting that electrically confounding influences from HEOG are unlikely to account for the complete ADAN pattern (see also van der Lubbe et al., 2006,for an account of the ADAN in terms of saccade suppression mechanisms). Moreover, the best evidence on topography and source localization (e.g., Praamstra et al., 2005; van der Lubbe et al., 2006) indicates that true ADAN effects and true HEOG effects do have different sources. Nevertheless, ADAN amplitudes might, in principle, still be artifactually enlarged and/or made significant in some studies due to HEOG leakage, which could also somewhat distort scalp topographies.

The fact that ADAN-like effects have now been found not only during putatively "covert" attentional orienting but also during the covert preparation of *manual* responses in the absence of any explicit attentional instruction (Eimer, 1995; Eimer et al., 2005; Praamstra et al., 2005; van der Lubbe et al., 2000; Verleger et al., 2000) might be interpreted as further indirect evidence that such effects cannot reflect undetected eye movements. However, small, undetected but systematic gaze deviations toward the responding hand could potentially arise in those manual tasks also.

Our data also show clearly that ADAN and residual HEOG effects show some relation (see Figure 5). Although applying the strictest infrared eye-tracking-based exclusion criterion eliminated any significant residual infrared eye position effect, the residual HEOG effect remained reliable (see Figures 4 and 5). Taking these two findings together suggests that the residual HEOG that was still found presumably reflects the ADAN signal as measured at the HEOG electrodes (rather than vice versa), but this might have been difficult to establish unequivocally without the independent infrared eye tracking measure used here. ADAN and HEOG might be hard to disentangle completely by data-driven or model-based analytical approaches (although see Croft et al., 2005; Jung et al., 2000; Talsma et al., 2005), because both have the same polarity, and both might in principle be time-locked to the cue if gaze shifts are cue triggered. Any such problem was avoided here by using the independent, nonelectrical eye tracker.

The possibility that lateralized EEG activity might contaminate the HEOG (as apparently is the case for ADAN leaking into HEOG here) may have important implications for some HEOG-correction procedures used to model and remove contaminating effects of eye movements from EEG signals (e.g., Croft et al., 2005; Talsma et al., 2005). Applying such procedures may be relatively unproblematic when studying effects that do not concern cue-locked (or potentially gaze-locked) lateralized ERP effects. However, when studying the ADAN effect, which takes exactly this form, HEOG-correction procedures might tend to *over*estimate the contribution of eye movements and thereby remove some genuine EEG activity (a possibility pointed out previously by Croft et al., 2005; Jung et al., 2000). This can be avoided by using a nonelectrical measure of eye position, as here.

In summary, we showed that "conventional" EEG and HEOG preprocessing on its own might not always be sufficient to eliminate confounding residual gaze deviations in spatial cuing paradigms. Lateralized EEG activity and HEOG signals may cross-contaminate each other, but an independent nonelectrical measure of eye deviation can disentangle the direction of causality for any such contamination. By using high-resolution infrared eye tracking, and then analyzing the resulting infrared eye tracking signals in an ERP-like manner, we were able to show that the preparatory ADAN effect can indeed reflect the direction of strictly *covert* spatial attention, surviving in the absence of systematic gaze deviations toward the cued side, and even (for the inward-pointing cues) in the presence of small gaze deviations away from that side.

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