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Commercial Interest:

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Abstract

The interplay between attention, alertness and motor planning is crucial for our manual interactions. To investigate the neural bases of this interaction, and challenging the views that attention cannot be disentangled from motor planning, we instructed human volunteers of both sexes to plan and execute reaching movements while attending to the target, while attending elsewhere, or without constraining attention. We recorded reaction times to reach initiation and pupil diameter and interfered with the functions of the medial posterior parietal cortex (mPPC) with online repetitive transcranial magnetic stimulation to test the causal role of this cortical region in the interplay between spatial attention and reaching. We found that mPPC plays a key role in the spatial association of reach planning and covert attention. Moreover, we have found that alertness, measured by pupil size, is a good predictor of the promptness of reach initiation only if we plan a reach to attended targets, and mPPC is causally involved in this coupling. Different from previous understanding, we suggest that mPPC is neither involved in reach planning *per se*, nor in sustained covert attention in absence of a reach plan, but it is specifically involved in attention functional to reaching.

Significance Statement

Attention is required to perform dexterous arm movements. In this work we show the neural bases of the interplay between attention and reaching preparation, with the aim to provide information useful to address effective rehabilitation strategies to treat functional deficits observed in attention-related diseases. We discuss how brain areas are involved in orchestrating attention and reaching by signaling the alignment of their spatial coordinates. Moreover, we found that pupil size changes during reach preparation are related to reach initiation, suggesting a coordination between vigilance and reach promptness when preparing a reach to attended targets.

Introduction

Manual interactions with objects benefit from the concurrent involvement of attention. The posterior parietal cortex (PPC) integrates spatial attention-related information with movement-related one (Fattori et al., 2017; Sulpizio et al., 2023) to perform dexterous reaching movements. In the macaque, the medial portion of the PPC includes area V6A (Galletti et al., 1999) which contains cells modulated by covert shifts of attention (Galletti et al., 2010; Caspari et al., 2015) as well as by movement planning (Breveglieri et al., 2016; Fattori et al., 2017). A putative homolog of V6A has been found in the human brain (hV6A) (Pitzalis et al., 2013, 2015). Similarly to the monkey V6A, this cortical region is involved in the shifts of covert attention (Vandenberghe et al., 2001; Yantis et al., 2002; Molenberghs et al., 2007; de Haan et al., 2008; Kelley et al., 2008; Capotosto et al., 2013; Ciavarro et al., 2013; Tosoni et al., 2013; Caspari et al., 2018) and in motor planning (Cavina-Pratesi et al., 2010; Vesia et al., 2010; Breveglieri et al., 2021, 2024; Sulpizio et al., 2023).

Given that the hV6A is involved in both spatial attention and in movement planning (Galati et al., 2011), and since we commonly move spatial attention when planning a movement unless we are forced to act differently (Rizzolatti et al., 1987), the activation of hV6A during planning of a movement toward a spatial location could simply be the result of the attentional orienting towards that location. Is this true? Or does hV6A represent the motor plan and the direction of attention,

separately? To answer these questions we examined the role of hV6A in motor planning, either with or without a congruent spatial attention allocation.

It is known that PPC is also involved, besides orienting covert attention, in vigilance and arousal (Galletti et al., 1996; Greene et al., 2014; Lee et al., 2022). Pupil size is a reliable biomarker of the fluctuations of the alerting system, being it related to the activity of Locus Coeruleus (Rajkowski et al., 1994; Aston-Jones and Cohen, 2005; Laeng et al., 2012; Murphy et al., 2014; Reimer et al., 2016; Stitt et al., 2018; van der Wel and van Steenbergen, 2018; Keene et al., 2022; Strauch et al., 2022). Some studies showed that high vigilance states cause pupil dilation (Aston-Jones and Cohen, 2005; Reimer et al., 2016; Mathôt, 2018), and others that pupillary light response is enhanced when the bright stimulus is attended versus ignored (Binda et al., 2013; Naber et al., 2013; Binda and Gamlin, 2017; Koevoet et al., 2023a). Moreover, pupil size scales also with motor complexity (van der Wel and van Steenbergen, 2018; Koevoet et al., 2023b). Pupil size has been also used to study the link between arousal and motor actions. For instance, a presaccadic pupil dilation was observed in trials with faster saccadic reaction times (Jainta et al., 2011; Wang et al., 2015, 2016, 2017). It is unknown whether a similar correlation exists between the arousal level, measured by pupil size, and the reaction time of reaching. Neither is it known whether this association is attention-dependent, nor whether hV6A is involved in this process. If this correlation exists, and if it involves hV6A, then an impairment of hV6A should disrupt it.

To check the involvement of hV6A in attention-dependent modifications of pupil size, as well as in devising a motor plan with or without the congruent allocation of attention, we designed a task where motor planning and attention can be manipulated independently each other. We used transcranial magnetic stimulation (TMS) to establish a causal role for the hV6A. We found that hV6A is not involved in motor initiation *per se*, but specifically when attention is endogenously allocated on the reaching target during planning. Furthermore, we show here a correlation between the level of arousal and reaching initiation, and a causal role of hV6A in this coupling, only in case of reaching to attended locations.

Materials and Methods

Participants

Thirty-four healthy volunteers participated in this study. Seventeen of them took part in a transcranial magnetic stimulation (TMS) experiment (aged 23.12+/-3.37 years, age range 19-30 years, 5 males), whereas the remaining seventeen (aged 27.82+/-7.05, age range 23-48 years, 7 males) participated in a control experiment. The participants were classified as right-handed based on the Edinburgh Handedness Inventory (Oldfield, 1971), had normal or corrected-to-normal visual acuity in both eyes and were naïve as to the purposes of the experiment. None of the participants had neurological, psychiatric, or other medical problems, nor did the participants of the TMS experiment have any contraindications to TMS (Rossi et al., 2009). Participants provided written informed consent. The procedures were approved by the Bioethical Committee at the University of Bologna (Prot. 170133, Prot. 237243, Prot. 57635) and were in accordance with the ethical standards of the 2013 Declaration of Helsinki. No discomfort or adverse effects during TMS were reported or noticed.

TMS experiment: localization of brain sites

The coil position was identified on each participant's scalp using the Cortexplore Neuronavigator (Cortexplore, Linz, Austria)(Klink et al., 2021; Breveglieri et al., 2024).

We tested 2 active stimulation sites, the area of interest (left hV6A) and a control area (V1/V2), and one Sham condition. The Talairach coordinates for hV6A we used were x = −10, y = –78, z = 40 (Talairach and Tournoux, 1988; Ciavarro et al., 2013; Breveglieri et al., 2021, 2023a, 2023b, 2024), that were similar to those used for studying the anterior part of the superior parieto-occipital cortex (Vesia et al., 2010, 2017), a region that likely includes hV6A (Pitzalis et al., 2015) and that was

investigated in several imaging studies (Filimon et al., 2009; Cavina-Pratesi et al., 2010; Gallivan et al., 2011; Tosoni et al., 2015). To target V1/V2, the coil was centered 2 cm above the center of the inion, thus resulting in a bilateral stimulation (Romei et al., 2016; Chiappini et al., 2018). Sham stimulation was performed by placing the coil tilted at 90° over the vertex bilaterally, so that participants could feel coil–scalp contact and discharge noise as during active stimulation, but no current was induced in the brain (Lisanby et al., 2001; Sandrini et al., 2011). Bilateral control conditions are often performed (Vesia et al., 2010; Breveglieri et al., 2021, 2023b, 2024).

TMS protocol

Biphasic TMS pulses (10 Hz, 3 pulses, as performed in other studies on the medial PPC; (Vesia et al., 2010; Striemer et al., 2011; Breveglieri et al., 2024)) were delivered using a Deymed DuoMAG XT stimulator connected to a 70mm figure-of-eight coil. Stimulation of hV6A was carried out by placing the coil tangentially over the scalp site along a parasagittal line with the handle pointing downward (Vesia et al., 2010; Breveglieri et al., 2021, 2023a, 2023b, 2024). The active control area (V1/V2) was targeted by placing the coil tangentially over the scalp site along a parasagittal line with the handle pointing downward.

To set TMS intensity, the resting motor threshold (rMT) was estimated for all participants in a preliminary phase of the experiment using standard procedures (Sandrini et al., 2011). Motor evoked potentials (MEPs) induced by stimulation of the left motor cortex were recorded from the right first dorsal interosseous (FDI) by means of a 2-channel DuoMAG MEP amplifier. Electromyography (EMG) signals were FIR-filtered and digitized at a sampling rate of 5 kHz. Pairs of disposable pre-gelled Ag–AgCl surface electrodes were placed in a belly tendon montage with a ground electrode on the midpoint of the palmar surface of the wrist. The optimal scalp position for inducing MEPs from the right FDI was first localized, and the rMT was determined from that position. The rMT was defined as the minimal intensity of stimulator output that produced MEPs with an amplitude of at least 50 μV in the FDI with a probability of 50% (Rossini et al., 2015). For

149 both hV6A and V1/V2 stimulations, the intensity of magnetic stimulation was fixed at 120% of the
150 rMT, as in a previous study (Breveglieri et al., 2023b). The range of intensities was 50-71% of the
151 total stimulator output (mean value 61.53+/- 6.03). No phosphenes were perceived by the
152 participants.
153

154 *TMS experiment: apparatus and behavioral task*

155 We used a setup which consisted of a 19-inch touchscreen (37.5cm x 30cm, ELO IntelliTouch 1939
156 L, screen resolution 1280px x 1024px) set vertically at 43cm in front of the participants on a desk.
157 The screen displayed the targets of the reaching movements performed by the participants (grey
158 squares in Fig. 1A). For stimuli presentation, we used Matlab (Mathworks, USA,
159 RRID:SCR_001622) with the Psychophysics toolbox extension (Brainard, 1997). Participants were
160 seated on a comfortable chair in a darkened room, with their head stabilized by a head/chin rest to
161 minimize head movements. In all trials, the reaching movement started with the participant's right
162 hand on a button (home-button, HB, Fig. 1A) placed on the desk, centered to the touchscreen (Fig.
163 1A).
164 The task was designed to associate or separate the direction of spatial attention from the direction of
165 movement plan, and was adapted from a task firstly used in monkeys (Messinger et al., 2021). To
166 this aim, we used a cue whose color instructed participants about the direction of the movement to
167 plan and whose side about the deployment of attention. Each trial started, after an intertrial period
168 of 6s, with the onset of the fixation point (diameter 0.3cm, 0.4° of visual angle) in the center of the
169 screen between the two reaching targets. This indicated to the participants to press and hold down
170 the home-button. The two reaching targets (squares of 0.6cm side, 0.78°, located 10° lateral to the
171 fixation point) were displayed on the touchscreen during the entire duration of each trial. After a
172 fixation period (Fix, Fig. 1A) of 1.3-1.5 s (randomly chosen) a central, endogenous cue (0.6cm side,
173 0.76°) appeared around the fixation point, which informed the participant about the target to

174 covertly attend and the target to subsequently reach (motor-attention (MotorATN) trials, Fig. 1B,
175 where the direction of attention and of motor plan were constrained) *or* only about the target to
176 subsequently reach (motor (Motor) trials, Fig. 1B, where the direction of motor plan was instructed
177 whereas attention wasn't). After a randomly chosen period of 0.3-0.6-1s (Plan), a small vertical line
178 (Go) appeared for 0.08s in the center of one reaching target. Importantly, the 2 trials with duration
179 of the epoch Plan of 1s have been inserted (and then excluded from the analysis) in each
180 condition/stimulation area to guarantee the participants' attention to the cued side. In fact, the more
181 variable the duration of this epoch, the less the probability of time-locked behaviors of the
182 participants. TMS pulses were delivered during the Plan epoch, with the first pulse delivered after
183 50ms from the Cue onset. After a variable reaction time to the detection of the Go signal,
184 participants reached with their right hand the previously cued target (cued by the color, Reaching,
185 Fig. 1A). At the movement offset, the targets and the fixation point disappeared and another
186 intertrial period started.
187 In MotorATN trials (Fig. 1B, left), the color of the cue was informative about the location of the
188 target to subsequently reach, in order to make participants plan a reach towards the cued side
189 (red=reach planning to the right target, represented in orange in Fig. 1, green=reach planning to the
190 left target, represented in blue in Fig. 1), while the colored side of the cue was informative about the
191 location of the subsequent onset of the Go signal, in order to make participants covertly move the
192 spatial attention towards the cued side (right side colored= the Go signal will appear within the right
193 target; left side colored= the Go signal will appear within the left target). In MotorATN trials, the
194 Go signal appeared always within the attended target (instructed by the colored side of the cue, Fig.
195 1A), so all the trials were 'valid' as in the Posner paradigm (Posner, 1980). If the movement was
196 planned in the same location (as instructed by the cue color), the MotorATN trial was labeled
197 'congruent' (Fig. 1A, top and Fig. 1B, left); if movement was planned in the opposite, unattended
198 target, the MotorATN trial was labeled 'incongruent' (Fig. 1A, bottom and Fig. 1B, left). Thus, in
199 congruent trials, the participants had to plan a reach toward an attended location during the Plan

epoch, whereas in incongruent trials they planned a reach toward an unattended location. In MotorATN trials, the attention was constrained in one side of the screen.

In Motor trials (Fig. 1A, bottom and 1B, right), the central cue was a fully colored square informative only about the location of the movement plan (same color conventions as in MotorATN trials). In these trials, participants had to plan a reach without any constraints concerning the location where attention must be directed during the Plan epoch. Effectively, in these trials the cue was neutral regarding attention. To ensure that attention of the participants was not automatically directed to the location of the movement plan, we designed and inserted valid and invalid Motor trials in equal number. In valid trials, the Go signal appeared in the target of the planned movement (Fig. 1A, bottom). Conversely, in invalid trials the Go signal appeared in the opposite target (not shown in the figure). Overall, 8 conditions were tested (4 conditions with MotorATN trials and 4 for Motor trials, Fig. 1B). Importantly, attention was not constrained during the movement execution, either in MotorATN trials or in Motor ones.

The task was composed of 2 blocks of 48 trials each (6 trials per condition per block) per stimulation condition (Sham, V1/V2, hV6A, counterbalanced), for a total of 288 trials performed over the same experimental session. Each session lasted approximately 2h. The task was always performed with the right arm. We randomized the conditions of MotorATN trials and of Motor trials (they were interleaved in each block). A 48-trials training block was included at the beginning of the experimental session.

TMS experiment: data acquisition, analysis, and statistics

The kinematics of reaching movements was recorded using a motion tracking system (VICON motion capture system, 5 M cameras, 1024 × 1024 pixel resolution) by sampling the position of two markers at a frequency of 100 Hz; markers were attached to the right wrist (on the scaphoid bone) and to the nail of the right index finger (reaching finger). Reaching onset/offset was determined as

the time when the markers' velocities exceeded/fell and remained below 30 mm/s. The reaction time was defined as the interval between the "Go" signal offset and reaching onset. Participants were asked to move the hand without pauses or interruptions, at a fast but comfortable speed, and as accurately as possible.

Given the intrinsic difficulty of the task, a possibility existed that participants reached to the wrong target or started a wrong movement trajectory and amended it to get to the correct target. We excluded a trial from the subsequent analyses if the endpoints were in the opposite side of the cued target and if the first or the second half of the trajectories exceeded the 2 standard deviations calculated with all the trajectories of that participant. We also excluded trials with RTs shorter than 100ms (Ciavarro et al., 2013) or longer than 1000ms (Rizzolatti et al., 1987). We excluded around 6% of trials for at least one of these above-mentioned reasons. The 22% of excluded trials were congruent trials, the 28% incongruent trials, the 24% unconstrained trials, and the 32% invalid ones. We used an eye-tracker (EyeLink 1000, SR Research Ltd) to record real-time gaze position and pupil size at 1kHz. Before collecting data from each participant, the equipment was calibrated using a nine-point grid (horizontal distance= 8cm; vertical distance=5cm) that the participants were asked to fixate steadily (3 x 3° tolerance window) and to covertly attend the targets.

TMS experiment: analysis of behavioral variables

The influence of the stimulation on reaction times in the different trial types was evaluated separately in valid and invalid trials, because in valid trials no redirection of attention to different hemifields occurred, whereas it was the case in invalid trials at the appearance of the Go signal in the opposite hemifield than the one where participants were planning a movement.

In valid trials, we used a two-way repeated measures analysis of variance (ANOVA) with TMS (3 levels, Sham, V1/V2, hV6A) and Trial type (3 levels, MotorATN congruent, MotorATN incongruent and Motor valid trials) as factors. In invalid trials, we performed a two-way repeated

measures ANOVA with TMS (3 levels, Sham, V1/V2, hV6A) and Redirection side (2 levels, rightward and leftward) as factors.

In all the analyses, the threshold for significance was set at 0.05 and all post-hocs were carried out with the Duncan correction for multiple comparisons.

TMS experiment: analysis of pupil size

As the pupil size is considered an index of effort and attention (Morad et al., 2000; Paladini et al., 2016)·(Keene et al., 2022), we have tested the changes in pupil size during the Plan epoch. Following the procedures of baseline-correction used previously (Bala and Takahashi, 2000; Moresi et al., 2008; Cherng et al., 2020; Hsu et al., 2021) for each trial, a baseline value was determined by averaging pupil size from 100ms before the Cue onset. To rule out the influence of the color of the cue on pupil size, we averaged the pupil size of the two congruent conditions, of the two incongruent ones, and of the conditions where attention was not constrained. Data was not normally distributed (Shapiro-Wilk test, $p<0.05$), so we used a non-parametric analysis of variance (ANOVA) (SPM1d Matlab package, (Pataky, 2012), codes at www.spm1d.org) with factor TMS (3 levels, Sham, V1/V2, hV6A), to compare the pupil size during the Plan epoch of the different stimulation conditions in the different trial types.

To investigate whether the pupil response was predictive of the reaction time, we have used linear mixed-effects (LME) models as performed in Koevoet et al. (Koevoet et al., 2023a) in each trial type. To account for interindividual differences in pupil size and to isolate evoked pupil response from baseline pupil size, we robust zscored the pupil size (Rousseeuw and Hubert, 2011; Koevoet et al., 2023a) by subtracting the median baseline pupil size from the data of the last 100ms before the Go signal and subsequently dividing by the median absolute deviation per participant. We then included in the model the interaction between pupil response and TMS with a Matlab formula: reaction time ~ 1 + pupil response*TMS + (1 | Participant). Next, in case the interaction term was significant (see Results), we ran the model during Sham stimulation (formula: reaction time ~ 1 +

pupil response + (1 | Participant)). If this correlation was significant ($p<0.05$), we tested if it was still significant during V1/V2 or hV6A stimulation.

Control experiment.

In the control experiment, we collected the pupil size of participants using the same apparatus and visual stimuli used in the TMS experiment. The task sequence was the same as in the TMS experiment except for the timing of the Cue appearance and for the events after the Cue offset. In this experiment, the Cue appeared for 1s, and participants were instructed to detect its offset by releasing the home-button. Six conditions were tested (10 trials each), 4 of them with the same half-colored Cues of the MotorATN trials of the TMS experiment, and 2 with the full-colored Cue of the Motor trials of the TMS experiment. Nevertheless, in this experiment, the color and the shape of the Cue were neither informative about any attentional directing nor about any spatial motor plan, and the participants did not take part in the TMS experiment. This control experiment was conceived to see whether there are differences in pupil size dynamics for visual stimulation which instructed, or did not instruct, the direction of covert attention. Differences in pupil size dynamics between control experiment and the SHAM condition of TMS experiment were tested via non-parametric ANOVA (SPM1d Matlab package, (Pataky, 2012)) with factor experiment (2 levels, TMS or control) in each trial type.

Results

Effectiveness of the cue in directing attention

The double informative nature of the cue of our task had already been revealed effective in non-human primates (Messinger et al., 2021) in instructing attention and/or motor plans. However, as our task was a simplification of the task of Messinger (Messinger et al., 2021), we wanted to confirm whether the attention of participants was directed as instructed during the Plan epoch. To

do this, we used the reaction time as an indirect index of the direction of attention, as in the classic test of Posner (Posner, 1980).

Thus, we measured the reaction times of participants to the detection of Go signal (reach initiation) in the different trial types of TMS experiment during Sham stimulation (Fig. 2A). Reaction times turned out to be affected by trial type (1-way ANOVA, $F_{(3,48)}=12.30$, partial $\eta^2=0.43$, $p<0.001$) in that reaction times of congruent trials were significantly faster than the ones of all other trial types (all $p<0.02$). This confirmed the expectations that the common location of spatial attention and motor plan represents a gain that improves the detection of the Go signal. Participants were also slower in detection in invalid trials than in congruent and in incongruent trials (all $p<0.01$), again as expected. These results confirm the effectiveness of our attentional manipulation by demonstrating increased detection during congruent compared with incongruent, unconstrained and invalid trials. We thus confirm that the Cue features (side and color) were effective in directing attention of participants as instructed in our task design.

Valid trials

hV6A stimulation affected reach initiation in congruent motor-attention trials.

As showed in Fig. 2B, the stimulation of hV6A and V1/V2 produced significant effects on reaction times in valid trials (interaction TMS by Type of trial, $F_{(4,64)}=2.98$, partial $\eta^2=0.16$, $p=0.03$, individual participants' data in Fig. 6). The gain in reaction time brought by the co-localization of the motor plan and of attention seen during Sham stimulation (see black columns in Fig. 2B, all $p<0.01$) was cancelled by the stimulation of either V1/V2 or of hV6A (see white and grey columns, Fig. 2B). After V1/V2 or hV6A stimulations, all the reaction times were similar and did not depend on the trial type (all $p>0.20$). Moreover, in congruent trials, the reaction times during Sham stimulation were different from those after hV6A ($p=0.04$) or V1/V2 stimulation ($p=0.04$), whereas reaction times after hV6A or after V1/V2 stimulation were not different ($p=0.85$). In all the other trial types (incongruent attention-reach plan and reaching with unconstrained attention), the

stimulation did not affect reaction times (all $p>0.05$), suggesting that neither hV6A nor V1/V2 were causally involved in reach initiation when the movement was planned in an unattended location or when attention was not constrained to the target.

In summary, either stimulation of hV6A or of V1/V2 led to an increase in reaction times to the detection of a visual peripheral target (Go signal), specifically when attention and motor plan were on the same side. This suggests that these areas are causally involved in sending information to the motor cortex about the alignment of the spatial coordinates of attention and motor plans.

hV6A stimulation did not affect pupil size.

We wanted to test whether the stimulation of hV6A affected the arousal level, measured through pupil size. Pupil size was not affected by the stimulation (non-parametric repeated measures ANOVA with factor TMS, $p>0.05$) and this was true in all the trial types (Fig. 3). This suggests that neither hV6A nor V1/V2 are causally involved in modulating pupil size *per se*.

Pupil size changed during the Plan epoch (Fig. 3) with a pupil constriction due to the pupillary light response, with a well-known time course (see for example (Wang et al., 2015)). The comparison of the pupil size during the Sham stimulation with the pupil size of a group of participants of a control experiment (where participants looked at the cues which conveyed the same illumination as in the TMS experiment, but neither being informative about the attentional orienting nor instructing a reach planning) revealed that the pupil size of participants looking at the uninformative cue (control experiment, yellow traces of Fig. 3, left) was significantly lower than during the observation of the informative cue during Sham stimulation (TMS experiment, black traces in Fig. 3, left; the differential values are plotted in Fig. 3, right). Moreover, in the TMS experiment, before pupil contraction (which occurred after 300ms from the Cue onset) a slight pupil enlargement was observed, that was significant from around 220ms after the cue onset in Congruent and Incongruent trials, and even before in unconstrained trials ($p<0.05$)(Fig. 3, left). As larger pupil size have been

associated with orienting responses (Wang et al., 2015; Hsu et al., 2021), this suggests that the task used in the TMS experiment was very effective in orienting the attention of the participants.

hV6A stimulation affected coordination between arousal and reaching in congruent motor-attention trials.

To test whether a correlation arousal-reach initiation does exist and, in this case, the role of hV6A in this coupling, we performed a trial-by-trial correlational analysis using linear mixed-effects models (LME) between pupil response right before the Go signal and reaction time. In congruent MotorATN trials, the LME model showed a significant pupil response main effect (β=12.87+/- 5.73, t=2.24, p=0.03) and a significant interaction pupil response by TMS (β=-6.65+/- 2.65, t=-2.51, p=0.01). Thus, we ran the model for each TMS condition to evaluate whether pupil size was a good predictor of reaction time. During Sham stimulation, pupil response significantly predicted the reaction time (β=10.41+/- 3.36, t=3.10, p=0.002, Fig. 4A), in that larger constrictions (lower z-scored values) led to faster reaction times. This was expected, because larger constrictions signal stronger attentional orienting (Naber et al., 2013; Binda and Gamlin, 2017; Koevoet et al., 2023a), and stronger attentional orienting causes faster reaction times. The stimulation affected this correlation, but with different effects depending on the stimulated area. After V1/V2 stimulation, the significant correlation remained, but with an opposite trend (β=-12.64+/- 5.01, t=-2.52, p=0.01, Fig. 4B) in that larger dilation led to faster reaction times. Instead, after hV6A stimulation, the correlation between pupil size and performance was no more significant (β=-3.26+/- 4.06, t=-0.80, p=0.42, Fig. 4C). In incongruent MotorATN and unconstrained (Motor) trials, pupil size did not significantly predict the reaction time in any stimulation condition, because neither the main effect of pupil response nor the interaction pupil size by TMS was significant (incongruent trials: main effect of pupil response: β=4.12+/- 5.00, t=0.84, p=0.40; interaction pupil response by TMS: β=-

2.09+/- 2.27, t=-0.92, p=0.36, unconstrained trials: main effect of pupil response: β=0.50+/- 4.91, t=0.10, p=0.92; interaction pupil response by TMS: β=-0.71+/- 2.22, t=-0.32, p=0.74).

To summarize, there is a coordination mechanism between arousal level (indicated by the pupil size) and reaction time of reaching toward attended locations, where greater pupil constrictions predict faster reaching onsets. Both V1/V2 and hV6A seem to be causally involved, even if with different roles, in instructing this coordination.

Invalid motor trials

hV6A stimulation impaired the redirection of covert attention.

Invalid Motor trials forced participants to automatically disentangle attention after the Go onset to bring it to the opposite hemifield. Reaction times of invalid trials were significantly affected by the interaction TMS by Redirection side ($F(2, 32)=5.55$, $p=0.008$, partial $\eta^2=0.26$, Fig. 5, individual participants' data in Fig. 7), an effect driven by the slower reaction time when attention was redirected leftward specifically after hV6A stimulation compared to Sham ($p<0.01$) and V1/V2 stimulation ($p<0.01$), that in turn were not different one another ($p=0.32$). During rightward redirection of attention, reaction times were not affected by TMS (all $p>0.52$). As shown in Fig.5, rightward redirections of covert attention during Sham stimulation caused slower reaction times than leftward redirections ($p=0.01$), an effect evident also during V1/V2 stimulation ($p=0.002$), but impaired specifically during hV6A stimulation ($p=0.43$). Overall, the analysis of reaction times of invalid trials revealed a specific involvement of hV6A in disentangling attention from the contralateral hemifield (right in our case). In invalid trials, no significant correlations between pupil responses and performance were observed (non-significant main effect of pupil response: β=0.74+/- 5.75, t=0.13, p=0.89; non-significant interaction pupil response-TMS, β=0.28+/- 2.56, t=0.11, p=0.91).

Discussion

Medial PPC is causally involved in attentional orienting and in disentangling attention before reaching.

We here find that hV6A is causally involved in reach initiation only if covert attention is allocated on the reaching target. To our knowledge, this is the first study on the PPC where participants were instructed to reach for unattended targets. The independent control of attention and reach plan was possible because, in our task, the cue was informative about the location to orient covert spatial attention and the location of motor plan, that could be the same or different. This design instructed participants to allocate their resources in multiple target locations simultaneously, an ability that was repeatedly demonstrated in humans (Baldauf et al., 2006; Hanning et al., 2018; Schonard et al., 2022). It required additional resources than the control task, as suggested by the increase of pupil size when looking at the informative cue (black line, Fig. 3) compared to the uninformative one (yellow line, Fig. 3), in all the trial types.

Because the spatial congruence of the attention-reach plan was essential for hV6A, one could argue that the activations previously seen in monkey and human V6A during reach planning (Cavina-Pratesi et al., 2010; Breveglieri et al., 2014; Hadjidimitrakis et al., 2014) are only the result of allocation of attention, given that primates naturally allocate attention on reaching targets unless they are forced to behave differently. If this were the case, hV6A should be involved in the process of maintaining attention on a target in absence of a reach plan. Other studies demonstrated that this is not the case (Capotosto et al., 2013; Ciavarro et al., 2013). Thus, we suggest that the activations during reach planning may not be attributed solely to the allocation of sustained attention. Rather, we suggest that the activations seen in hV6A during reach planning are the result of an allocation of attention that is functional to reach that location. The same phenomenon was observed before saccades ('presaccadic attention' (Li et al., 2021)~~(Carrasco et al 2011).~~ We thus propose that hV6A has a causal role in 'pre-reaching attention". In a single-cell monkey study (Breveglieri et al., 2014) the animals were trained to overtly attend a target and, in different trials, to overtly attend and plan a reach to the same targets. The most common modulations of V6A cells in this study were related to

both overt attention and reach planning, and this in keeping with the current results. Therefore, we suggest that hV6A might send information to frontal areas (Gamberini et al., 2009; Tosoni et al., 2015) about the alignment of spatial coordinates of attention and reach planning, and the frontal cortex may use this information to initiate a reach more promptly, resulting in a gain in the system (Fig. 2A-B, black columns). Accordingly, after hV6A stimulation this gain is lost (Fig. 2B, grey columns). Present findings are also in agreement with studies (Rolfs and Carrasco, 2012; Li et al., 2016, 2019, 2021; Messinger et al., 2021; Schonard et al., 2022), that go against obligatory coupling of attention and motor plans postulated by the Premotor Theory of Attention (Rizzolatti et al., 1987). As the effects of TMS were observed on reaction times, one could argue that the role of hV6A is solely related to the perceptual detection of the Go signal. We think we can discard this interpretation, because we have observed a TMS effect only in congruent trials, whereas the Go signal detection was required in all the trial types. We also found that hV6A has a causal involvement in redirecting attention in invalid trials (Fig. 5), in agreement with other results where the medial PPC was involved in shifts of attention in *exogenous* Posner paradigms (Vandenberghe et al., 2001; Molenberghs et al., 2007; Ciavarro et al., 2013), and we extend this concept to the *endogenous* attentional orienting mode used here. Differently from other TMS studies (Capotosto et al., 2013; Ciavarro et al., 2013), we show here that the effect of hV6A stimulation is direction-sensitive. Specifically, during Sham stimulation participants are slower in reach initiation after a rightward redirection of attention than after a leftward redirection (Fig. 5). This 'leftward gain' is in line with the right hemisphere's dominance in directing spatial attention (Heilman and Van Den Abell, 1980; Reuter-Lorenz et al., 1990; Benwell et al., 2014) and with lesser efforts required when attending to the left visual field compared to the right (Meyyappan et al., 2023). It is also in line with the concept of 'pseudoneglect', a phenomenon where healthy participants tend to place a bisection marker to the left of the real midpoint on a horizontal line, reflecting a natural trend to attend leftward (Bowers and Heilman, 1980). By stimulating the left hV6A, we found that this 'leftward gain' is lost, as suggested by the increase in the reaction time after hV6A stimulation than

Sham and V1/V2 stimulation. We thus suggest that hV6A has also a causal role in the disentangling the focus of attention from contralateral targets and in the pseudoneglect.

Occipital cortex is causally involved in attention orienting but not in its reorienting.

We here wanted to investigate the role of hV6A, and the stimulation of bilateral V1/V2 was an active control condition, so the investigation of occipital areas was out of the scope of this research. Nevertheless, our data suggest that V1/V2 are involved, together with hV6A, in reach initiation specifically when movements are directed towards attended location (Fig. 2B). Like for hV6A, we can rule out that the effects we found are merely perceptual (detection of the Go signal), so due to visual masking, a typical effect of V1/V2 stimulation (Amassian et al., 1989). In fact, the effect was restricted to congruent trials. Actually, the concept of V1/V2 as a mere perceptual region has been repeatedly dropped out, because early visual cortices are more active when a stimulus is attended (Brefczynski and DeYoe, 1999; Gandhi et al., 1999; Martínez et al., 1999; Somers et al., 1999), and directing attention even in absence of a visual stimulation activates these regions (Kastner et al., 1999; Ress et al., 2000; Silver et al., 2007; Murray, 2008). It has also been demonstrated that during attentional allocation before saccades, feedback signals are sent from Frontal Eye Fields (FEF) and Superior Colliculus to early visual cortices to enhance visual processing (Ekstrom et al., 2008; Bisley and Mirpour, 2019; Li et al., 2021; Hanning et al., 2023). Interestingly, early visual cortex is also involved in reaching, in that reaching directions can be decoded from occipital fMRI activity in sighted (Monaco et al., 2017, 2020) and even in blind (Bola et al., 2023) humans, suggesting that action representation in the occipital lobe is independent of vision and is reach-related. Thus, the role of early visual cortices in orienting attention before reaching shown here is in keeping with these studies. Our data show that, differently from hV6A, V1/V2 is not involved in shifts of attention. Although fMRI studies show that early visual areas are involved in shifts of attention (Dugué et al., 2020; Parisi et al., 2020), it has been reported that they are not causally involved in endogenous attention (Fernández et al., 2023). Thus, our results suggest a strict collaboration

between occipital and posterior parietal regions and hV6A seems to deal with higher computational loads. Further studies are required to clarify this interpretation.

hV6A has a specific role in coordinating arousal and reaching initiation.

Here we found that hV6A is not involved in modulating pupil size *per se* (Fig. 3). This is not surprising, given the absence of direct connections between the medial PPC and the subcortical structures (Locus Coeruleus included) that control the Edinger-Westphal nucleus (Gamberini et al., 2016, 2020, 2021a, 2021b; van der Wel and van Steenbergen, 2018).

We here demonstrate the existence of a coordinative machinery between arousal and reach initiation, in that larger pupil constrictions predict faster reach initiation (Fig. 4A). This correlation was significant only in trials where spatial attention was allocated on the reaching target, and was impaired after hV6A stimulation. The role of hV6A in the coordination arousal-reaching parallels the same role in pre-reaching attention shown here for congruent trials. Stimulation of the active control site (occipital cortex) led to an opposite coordination, instead of an absence of a coordination, and this demonstrates a functional specialization of hV6A and V1/V2.

A similar orchestration was also found for saccades (Jainta et al., 2011; Wang et al., 2015, 2016, 2017). According to a recent study (Hsu et al., 2021), TMS over FEF impairs the orchestration pupil size-saccade initiation, revealing the causal role of FEF in this coordination. We here found the functional counterpart of this process in the reaching domain. Again, and in line with the reaction time modulations, a specific role of hV6A was seen only when covert attention was directed on the reaching target.

After V1/V2 stimulation, an opposite correlation pupil constriction/reaction time compared to Sham stimulation was observed (Fig. 4B). A possible explanation for this effect may be found considering that a negative correlation between pupil size and activation of occipital areas was demonstrated (Bombeke et al., 2016; Lubinus et al., 2022). So, the larger is the pupil size, the lower is the

activation of the visual cortex. We thus can suggest that the interference given by the stimulation to the occipital lobe performed here could lead to a reduction in visual cortex activity and this may have increased the pupil size, as seen in figure 4B, where pupil constriction to light has been reduced after V1/V2 stimulation.

Future applications

The neural bases of the interplay between attention and reach planning shown here can inform the development of rehabilitation strategies to address deficits like attention-deficit hyperactivity disorder, that often involve impairments in both attention and motor control, ultimately improving functional outcomes.

References

Amassian VE, Cracco RQ, Maccabee PJ, Cracco JB, Rudell A, Eberle L (1989) Suppression of visual perception by magnetic coil stimulation of human occipital cortex. Electroencephalogr Clin Neurophysiol Evoked Potentials 74:458–462.

Aston-Jones G, Cohen JD (2005) An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. Annu Rev Neurosci 28:403–450.

Bala AD, Takahashi TT (2000) Pupillary dilation response as an indicator of auditory discrimination in the barn owl. J Comp Physiol A 186:425–434.

Baldauf D, Wolf M, Deubel H (2006) Deployment of visual attention before sequences of goal-directed hand movements. Vision Res 46:4355–4374.

Benwell CSY, Harvey M, Thut G (2014) On the neural origin of pseudoneglect: EEG-correlates of shifts in line bisection perfor- mance with manipulation of line length. Neuroimage 86:370–380.

Binda P, Gamlin PD (2017) Renewed Attention on the Pupil Light Reflex. Trends Neurosci 40:455–457.

Binda P, Pereverzeva M, Murray SO (2013) Attention to bright surfaces enhances the pupillary light reflex. J Neurosci 33:2199–2204.

Bisley JW, Mirpour K (2019) The neural instantiation of a priority map. Curr Opin Psychol 29:108–112.

Bola Ł, Vetter P, Wenger M, Amedi A (2023) Decoding Reach Direction in Early "Visual" Cortex of Congenitally Blind Individuals. J Neurosci 43:7868–7878.

Bombeke K, Duthoo W, Mueller SC, Hopf J-M, Boehler CN (2016) Pupil size directly modulates the feedforward response in human primary visual cortex independently of attention. Neuroimage 127:67–73.

Bowers D, Heilman KM (1980) Effects of hemispace on a tactile line bisection task. Neuropsychologia 18:491–498.

Brainard DH (1997) The Psychophysics Toolbox. Spat Vis 10:433–436 Available at: http://www.ncbi.nlm.nih.gov/pubmed/9176952 [Accessed September 12, 2019].

Brefczynski JA, DeYoe EA (1999) A physiological correlate of the "spotlight" of visual attention. Nat Neurosci 2:370–374.

Breveglieri R, Borgomaneri S, Bosco A, Filippini M, De Vitis M, Tessari A, Avenanti A, Galletti C, Fattori P (2024) rTMS over the human medial parietal cortex impairs online reaching corrections. Brain Struct Funct 229:297–310.

Breveglieri R, Borgomaneri S, Diomedi S, Tessari A, Galletti C, Fattori P (2023a) A Short Route for Reach Planning between Human V6A and the Motor Cortex. J Neurosci 43:2116–2125.

Breveglieri R, Borgomaneri S, Filippini M, Tessari A, Galletti C, Davare M, Fattori P (2023b) Complementary contribution of the medial and lateral human parietal cortex to grasping: a repetitive TMS study. Cereb Cortex 33:5122–5134.

Breveglieri R, Bosco A, Borgomaneri S, Tessari A, Galletti C, Avenanti A, Fattori P (2021)

Transcranial Magnetic Stimulation Over the Human Medial Posterior Parietal Cortex Disrupts Depth Encoding During Reach Planning. Cereb Cortex 31:267–280.

Breveglieri R, Bosco A, Galletti C, Passarelli L, Fattori P (2016) Neural activity in the medial parietal area V6A while grasping with or without visual feedback. Sci Rep 6:28893.

Breveglieri R, Galletti C, Dal Bò G, Hadjidimitrakis K, Fattori P (2014) Multiple aspects of neural activity during reaching preparation in the medial posterior parietal area V6A. J Cogn Neurosci 26.

Capotosto P, Tosoni A, Spadone S, Sestieri C, Perrucci MG, Romani GL, Della Penna S, Corbetta M (2013) Anatomical segregation of visual selection mechanisms in human parietal cortex. J Neurosci 33:6225–6229.

Caspari N, Arsenault JT, Vandenberghe R, Vanduffel W (2018) Functional Similarity of Medial Superior Parietal Areas for Shift-Selective Attention Signals in Humans and Monkeys. Cereb Cortex 28:2085–2099.

Caspari N, Janssens T, Mantini D, Vandenberghe R, Vanduffel W, Caspari XN, Janssens T, Mantini XD, Vandenberghe XR, Vanduffel XW (2015) Covert Shifts of Spatial Attention in the Macaque Monkey. J Neurosci 35:7695–7714.

Cavina-Pratesi C, Monaco S, Fattori P, Galletti C, McAdam TD, Quinlan DJ, Goodale MA, Culham JC (2010) Functional magnetic resonance imaging reveals the neural substrates of arm transport and grip formation in reach-to-grasp actions in humans. J Neurosci 30:10306–10323.

Cherng Y-G, Baird T, Chen J-T, Wang C-A (2020) Background luminance effects on pupil size associated with emotion and saccade preparation. Sci Rep 10:15718.

Chiappini E, Silvanto J, Hibbard PB, Avenanti A, Romei V (2018) Strengthening functionally specific neural pathways with transcranial brain stimulation. Curr Biol 28:R735–R736.

Ciavarro M, Ambrosini E, Tosoni A, Committeri G, Fattori P, Galletti C (2013) rTMS of Medial Parieto-occipital Cortex Interferes with Attentional Reorienting during Attention and Reaching Tasks. J Cogn Neurosci:1–10.

584 de Haan B, Morgan PS, Rorden C (2008) Covert orienting of attention and overt eye movements
585 activate identical brain regions. Brain Res 1204:102–111.
586 Dugué L, Merriam EP, Heeger DJ, Carrasco M (2020) Differential impact of endogenous and
587 exogenous attention on activity in human visual cortex. Sci Rep 10:21274.
588 Ekstrom LB, Roelfsema PR, Arsenault JT, Bonmassar G, Vanduffel W (2008) Bottom-up
589 dependent gating of frontal signals in early visual cortex. Science 321:414–417.
590 Fattori P, Breveglieri R, Bosco A, Gamberini M, Galletti C (2017) Vision for Prehension in the
591 Medial Parietal Cortex. Cereb Cortex 27:1149–1163.
592 Fernández A, Hanning NM, Carrasco M (2023) Transcranial magnetic stimulation to frontal but not
593 occipital cortex disrupts endogenous attention. Proc Natl Acad Sci U S A 120:e2219635120.
594 Filimon F, Nelson JD, Huang RS, Sereno MI (2009) Multiple parietal reach regions in humans:
595 Cortical representations for visual and proprioceptive feedback during on-line reaching. J
596 Neurosci 29:2961–2971.
597 Galati G, Committeri G, Pitzalis S, Pelle G, Patria F, Fattori P, Galletti C (2011) Intentional signals
598 during saccadic and reaching delays in the human posterior parietal cortex. Eur J Neurosci
599 34:1871–1885.
600 Galletti C, Breveglieri R, Lappe M, Bosco A, Ciavarro M, Fattori P (2010) Covert shift of attention
601 modulates the ongoing neural activity in a reaching area of the macaque dorsomedial visual
602 stream. PLoS One 5.
603 Galletti C, Fattori P, Battaglini PP, Shipp S, Zeki S (1996) Functional demarcation of a border
604 between areas V6 and V6A in the superior parietal gyrus of the macaque monkey. Eur J
605 Neurosci 8:30–52.
606 Galletti C, Fattori P, Kutz DF, Gamberini M (1999) Brain location and visual topography of cortical
607 area V6A in the macaque monkey. Eur J Neurosci 11:575–582.
608 Gallivan JP, McLean A, Culham JC (2011) Neuroimaging reveals enhanced activation in a reach-
609 selective brain area for objects located within participants' typical hand workspaces.

610 Neuropsychologia 49:3710–3721.
611 Gamberini M, Bakola S, Passarelli L, Burman KJ, Rosa MGP, Fattori P, Galletti C (2016) Thalamic
612 projections to visual and visuomotor areas (V6 and V6A) in the Rostral Bank of the parieto-
613 occipital sulcus of the Macaque. Brain Struct Funct 221:1573–1589.
614 Gamberini M, Passarelli L, Fattori P, Galletti C (2020) Structural connectivity and functional
615 properties of the macaque superior parietal lobule. Brain Struct Funct.
616 Gamberini M, Passarelli L, Fattori P, Zucchelli M, Bakola S, Luppino G, Galletti C (2009) Cortical
617 connections of the visuomotor parietooccipital area V6Ad of the macaque monkey. J Comp
618 Neurol 513:622–642.
619 Gamberini M, Passarelli L, Filippini M, Fattori P, Galletti C (2021a) Vision for action: thalamic and
620 cortical inputs to the macaque superior parietal lobule. Brain Struct Funct 226:2951–2966.
621 Gamberini M, Passarelli L, Impieri D, Montanari G, Diomedi S, Worthy KH, Burman KJ, Reser
622 DH, Fattori P, Galletti C, Bakola S, Rosa MGP (2021b) Claustral Input to the Macaque Medial
623 Posterior Parietal Cortex (Superior Parietal Lobule and Adjacent Areas). Cereb Cortex
624 31:4595–4611.
625 Gandhi SP, Heeger DJ, Boynton GM (1999) Spatial attention affects brain activity in human
626 primary visual cortex. Proc Natl Acad Sci U S A 96:3314–3319.
627 Greene CM, Flannery O, Soto D (2014) Distinct parietal sites mediate the influences of mood,
628 arousal, and their interaction on human recognition memory. Cogn Affect Behav Neurosci
629 14:1327–1339.
630 Hadjidimitrakis K, Bertozzi F, Breveglieri R, Bosco A, Galletti C, Fattori P (2014) Common neural
631 substrate for processing depth and direction signals for reaching in the monkey medial
632 posterior parietal cortex. Cereb Cortex 24:1645–1657.
633 Hanning NM, Aagten-Murphy D, Deubel H (2018) Independent selection of eye and hand targets
634 suggests effector-specific attentional mechanisms. Sci Rep 8:9434.
635 Hanning NM, Fernández A, Carrasco M (2023) Dissociable roles of human frontal eye fields and

636 early visual cortex in presaccadic attention. Nat Commun 14:5381.
637 Heilman KM, Van Den Abell T (1980) Right hemisphere dominance for attention: the mechanism
638 underlying hemispheric asymmetries of inattention (neglect). Neurology 30:327–330.
639 Hsu T-Y, Hsu Y-F, Wang H-Y, Wang C-A (2021) Role of the frontal eye field in human pupil and
640 saccade orienting responses. Eur J Neurosci.
641 Jainta S, Vernet M, Yang Q, Kapoula Z (2011) The Pupil Reflects Motor Preparation for Saccades -
642 Even before the Eye Starts to Move. Front Hum Neurosci 5:97.
643 Kastner S, Pinsk MA, De Weerd P, Desimone R, Ungerleider LG (1999) Increased activity in
644 human visual cortex during directed attention in the absence of visual stimulation. Neuron
645 22:751–761.
646 Keene PA, deBettencourt MT, Awh E, Vogel EK (2022) Pupillometry signatures of sustained
647 attention and working memory. Atten Percept Psychophys 84:2472–2482.
648 Kelley TA, Serences JT, Giesbrecht B, Yantis S (2008) Cortical mechanisms for shifting and
649 holding visuospatial attention. Cereb Cortex 18:114–125.
650 Klink PC et al. (2021) Combining brain perturbation and neuroimaging in non-human primates.
651 Neuroimage 235:118017 Available at:
652 https://www.sciencedirect.com/science/article/pii/S1053811921002949.
653 Koevoet D, Naber M, Strauch C, Somai RS, Van der Stigchel S (2023a) Differential aspects of
654 attention predict the depth of visual working memory encoding: Evidence from pupillometry. J
655 Vis 23:9.
656 Koevoet D, Strauch C, Naber M, der Stigchel S Van (2023b) The Costs of Paying Overt and Covert
657 Attention Assessed With Pupillometry. Psychol Sci 34:887–898.
658 Laeng B, Sirois S, Gredebäck G (2012) Pupillometry: A Window to the Preconscious? Perspect
659 Psychol Sci a J Assoc Psychol Sci 7:18–27.
660 Lee M, Sanz LRD, Barra A, Wolff A, Nieminen JO, Boly M, Rosanova M, Casarotto S, Bodart O,
661 Annen J, Thibaut A, Panda R, Bonhomme V, Massimini M, Tononi G, Laureys S, Gosseries

662 O, Lee S-W (2022) Quantifying arousal and awareness in altered states of consciousness using
663 interpretable deep learning. Nat Commun 13:1064.
664 Li H-H, Barbot A, Carrasco M (2016) Saccade Preparation Reshapes Sensory Tuning. Curr Biol
665 26:1564–1570.
666 Li H-H, Hanning NM, Carrasco M (2021) To look or not to look: dissociating presaccadic and
667 covert spatial attention. Trends Neurosci 44:669–686 Available at:
668 https://www.sciencedirect.com/science/article/pii/S0166223621000965.
669 Li H-H, Pan J, Carrasco M (2019) Presaccadic attention improves or impairs performance by
670 enhancing sensitivity to higher spatial frequencies. Sci Rep 9:2659.
671 Lisanby SH, Gutman D, Luber B, Schroeder C, Sackeim HA (2001) Sham TMS: Intracerebral
672 measurement of the induced electrical field and the induction of motor-evoked potentials. Biol
673 Psychiatry 49:460–463.
674 Lubinus C, Einhäuser W, Schiller F, Kircher T, Straube B, van Kemenade BM (2022) Action-based
675 predictions affect visual perception, neural processing, and pupil size, regardless of temporal
676 predictability. Neuroimage 263:119601.
677 Martínez A, Anllo-Vento L, Sereno MI, Frank LR, Buxton RB, Dubowitz DJ, Wong EC, Hinrichs
678 H, Heinze HJ, Hillyard SA (1999) Involvement of striate and extrastriate visual cortical areas
679 in spatial attention. Nat Neurosci 2:364–369.
680 Mathôt S (2018) Pupillometry: Psychology, Physiology, and Function. J Cogn 1:16.
681 Messinger A, Cirillo R, Wise SP, Genovesio A (2021) Separable neuronal contributions to covertly
682 attended locations and movement goals in macaque frontal cortex. Sci Adv 7.
683 Meyyappan S, Rajan A, Mangun GR, Ding M (2023) Top-down control of the left visual field bias
684 in cued visual spatial attention. Cereb Cortex 33:5097–5107.
685 Molenberghs P, Mesulam MM, Peeters R, Vandenberghe RRC (2007) Remapping attentional
686 priorities: differential contribution of superior parietal lobule and intraparietal sulcus. Cereb
687 Cortex 17:2703–2712.

688 Monaco S, Gallivan JP, Figley TD, Singhal A, Culham JC (2017) Recruitment of Foveal
689 Retinotopic Cortex During Haptic Exploration of Shapes and Actions in the Dark. J Neurosci
690 37:11572–11591.
691 Monaco S, Malfatti G, Culham JC, Cattaneo L, Turella L (2020) Decoding motor imagery and
692 action planning in the early visual cortex: Overlapping but distinct neural mechanisms.
693 Neuroimage 218:116981.
694 Morad Y, Lemberg H, Yofe N, Dagan Y (2000) Pupillography as an objective indicator of fatigue.
695 Curr Eye Res 21:535–542.
696 Moresi S, Adam JJ, Rijcken J, Van Gerven PWM, Kuipers H, Jolles J (2008) Pupil dilation in
697 response preparation. Int J Psychophysiol Off J Int Organ Psychophysiol 67:124–130.
698 Murphy PR, O'Connell RG, O'Sullivan M, Robertson IH, Balsters JH (2014) Pupil diameter
699 covaries with BOLD activity in human locus coeruleus. Hum Brain Mapp 35:4140–4154.
700 Murray SO (2008) The effects of spatial attention in early human visual cortex are stimulus
701 independent. J Vis 8:2.1-11.
702 Naber M, Alvarez GA, Nakayama K (2013) Tracking the allocation of attention using human
703 pupillary oscillations. Front Psychol 4:919.
704 Oldfield RC (1971) The assessment and analysis of handedness: The Edinburgh inventory.
705 Neuropsychologia.
706 Paladini RE, Müri RM, Meichtry J, Nef T, Mast FW, Mosimann UP, Nyffeler T, Cazzoli D (2016)
707 The Influence of Alertness on the Spatial Deployment of Visual Attention is Mediated by the
708 Excitability of the Posterior Parietal Cortices. Cereb Cortex 27:233–243 Available at:
709 https://doi.org/10.1093/cercor/bhw390.
710 Parisi G, Mazzi C, Colombari E, Chiarelli AM, Metzger BA, Marzi CA, Savazzi S (2020)
711 Spatiotemporal dynamics of attentional orienting and reorienting revealed by fast optical
712 imaging in occipital and parietal cortices. Neuroimage 222:117244.
713 Pataky TC (2012) One-dimensional statistical parametric mapping in Python. Comput Methods

714 Biomech Biomed Engin 15:295–301.
715 Pitzalis S, Fattori P, Galletti C (2015) The human cortical areas V6 and V6A. Vis Neurosci 32,
716 e00X,:E007.
717 Pitzalis S, Sereno MI, Committeri G, Fattori P, Galati G, Tosoni A, Galletti C (2013) The human
718 homologue of macaque area V6A. Neuroimage 82:517–530.
719 Posner MI (1980) Orienting of attention. Q J Exp Psychol 32(1), 3–25 32:3–25 Available at:
720 https://doi.org/10.1080/00335558008248231.
721 Rajkowski J, Kubiak P, Aston-Jones G (1994) Locus coeruleus activity in monkey: phasic and tonic
722 changes are associated with altered vigilance. Brain Res Bull 35:607–616.
723 Reimer J, McGinley MJ, Liu Y, Rodenkirch C, Wang Q, McCormick DA, Tolias AS (2016) Pupil
724 fluctuations track rapid changes in adrenergic and cholinergic activity in cortex. Nat Commun
725 7:13289.
726 Ress D, Backus BT, Heeger DJ (2000) Activity in primary visual cortex predicts performance in a
727 visual detection task. Nat Neurosci 3:940–945.
728 Reuter-Lorenz PA, Kinsbourne M, Moscovitch M (1990) Hemispheric control of spatial attention.
729 Brain Cogn 12:240–266.
730 Rizzolatti G, Riggio L, Dascola I, Umiltá C (1987) Reorienting attention across the horizontal and
731 vertical meridians: Evidence in favor of a premotor theory of attention. Neuropsychologia
732 25:31–40.
733 Rolfs M, Carrasco M (2012) Rapid simultaneous enhancement of visual sensitivity and perceived
734 contrast during saccade preparation. J Neurosci Off J Soc Neurosci 32:13744-52a.
735 Romei V, Chiappini E, Hibbard PB, Avenanti A (2016) Empowering Reentrant Projections from
736 V5 to V1 Boosts Sensitivity to Motion. Curr Biol 26:2155–2160.
737 Rossi S, Hallett M, Rossini PM, Pascual-Leone A, Safety of TMS Consensus Group (2009) Safety,
738 ethical considerations, and application guidelines for the use of transcranial magnetic
739 stimulation in clinical practice and research. Clin Neurophysiol 120:2008–2039.

Rossini PM et al. (2015) Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. Clin Neurophysiol 126:1071–1107.

Rousseeuw PJ, Hubert M (2011) Robust statistics for outlier detection. WIREs Data Min Knowl Discov 1:73–79.

Sandrini M, Umiltà C, Rusconi E (2011) The use of transcranial magnetic stimulation in cognitive neuroscience: A new synthesis of methodological issues. Neurosci Biobehav Rev 35:516–536.

Schonard C, Heed T, Seegelke C (2022) Allocation of Visuospatial Attention Indexes Evidence Accumulation for Reach Decisions. eNeuro 9:ENEURO.0313-22.2022.

Silver MA, Ress D, Heeger DJ (2007) Neural correlates of sustained spatial attention in human early visual cortex. J Neurophysiol 97:229–237.

Somers DC, Dale AM, Seiffert AE, Tootell RB (1999) Functional MRI reveals spatially specific attentional modulation in human primary visual cortex. Proc Natl Acad Sci U S A 96:1663–1668.

Stitt I, Zhou ZC, Radtke-Schuller S, Fröhlich F (2018) Arousal dependent modulation of thalamo-cortical functional interaction. Nat Commun 9:2455.

Strauch C, Wang C-A, Einhäuser W, Van der Stigchel S, Naber M (2022) Pupillometry as an integrated readout of distinct attentional networks. Trends Neurosci 45:635–647.

Striemer CL, Chouinard PA, Goodale MA (2011) Programs for action in superior parietal cortex: A triple-pulse TMS investigation. Neuropsychologia 49:2391–2399.

Sulpizio V, Fattori P, Pitzalis S, Galletti C (2023) Functional organization of the caudal part of the human superior parietal lobule. Neurosci Biobehav Rev 153:105357.

Talairach J, Tournoux P (1988) Co-planar stereotaxic atlas of the human brain: 3-dimensional proportional system: an approach to cerebral imaging (Thieme Medical, ed). Stuttgart, NY.

Tosoni A, Pitzalis S, Committeri G, Fattori P, Galletti C, Galati G (2015) Resting-state connectivity

and functional specialization in human medial parieto-occipital cortex. Brain Struct Funct 220:3307–3321.

Tosoni A, Shulman GL, Pope ALW, McAvoy MP, Corbetta M (2013) Distinct representations for shifts of spatial attention and changes of reward contingencies in the human brain. Cortex 49:1733–1749.

van der Wel P, van Steenbergen H (2018) Pupil dilation as an index of effort in cognitive control tasks: A review. Psychon Bull Rev 25:2005–2015.

Vandenberghe R, Gitelman DR, Parrish TB, Mesulam MM (2001) Location- or feature-based targeting of peripheral attention. Neuroimage 14:37–47.

Vesia M, Barnett-Cowan M, Elahi B, Jegatheeswaran G, Isayama R, Neva JL, Davare M, Staines WR, Culham JC, Chen R (2017) Human dorsomedial parieto-motor circuit specifies grasp during the planning of goal-directed hand actions. Cortex 92:175–186.

Vesia M, Prime SL, Yan X, Sergio LE, Crawford JD (2010) Specificity of human parietal saccade and reach regions during transcranial magnetic stimulation. J Neurosci 30:13053–13065.

Wang C-A, Blohm G, Huang J, Boehnke SE, Munoz DP (2017) Multisensory integration in orienting behavior: Pupil size, microsaccades, and saccades. Biol Psychol 129:36–44.

Wang C-A, Brien DC, Munoz DP (2015) Pupil size reveals preparatory processes in the generation of pro-saccades and anti-saccades. Eur J Neurosci 41:1102–1110.

Wang C-A, McInnis H, Brien DC, Pari G, Munoz DP (2016) Disruption of pupil size modulation correlates with voluntary motor preparation deficits in Parkinson's disease. Neuropsychologia 80:176–184.

Yantis S, Schwarzbach J, Serences JT, Carlson RL, Steinmetz MA, Pekar JJ, Courtney SM (2002) Transient neural activity in human parietal cortex during spatial attention shifts. Nat Neurosci 5:995–1002.

792 **Figure legends**

793

794

795 Figure 1

796 A) Timeline of Attention/reaching task. Fix=fixation time, Cue=cue onset, Plan= delay between cue
797 on and go signal, Go=go signal (a small vertical line), Reaction time and Reaching. The Cue is
798 depicted larger than the targets for the reader's convenience (real dimensions are stated in the
799 Methods section) and colored in orange and blue (color-blinded people's convenience, real colors
800 are stated in the Methods). The timeline is shown for MotorATN Congruent trials (top), in which
801 attention and motor plan were directed toward the same hemifield, for MotorATN Incongruent trials
802 (middle), in which attention and motor plan were directed toward opposite hemifields, and for
803 Motor valid trials (bottom), in which attention was not constrained, the direction of the motor plan
804 was instructed, and the Go signal appeared in the same hemifield as the motor plan. The same
805 timeline also applied to Motor invalid trials (not shown for conciseness). B) Types of trials,
806 according to the information received by the central cue: MotorATN trials and Motor trials. The
807 MotorATN trials were only valid (Go signal in the target where attention was directed by the
808 colored side of the cue) and could be congruent (attention and movement plan directed toward the
809 same location) or incongruent (attention and movement plan aimed in opposite directions. The
810 Motor trials could be valid (Go signal in the target where movement was planned) or invalid (Go
811 signal in the opposite target).

812

813

814 Figure 2

815 A) Reaction times during Sham stimulation in the different types of trials of the TMS experiment.
816 Bars represents standard error, asterisk represents significant ($p<0.05$) posthoc comparisons. Grey
817 lines connect points that represent data of individual participants. These data show that the task

elicited attention in the expected way. B) Reaction times of different types of valid trials in the different stimulation conditions (Sham=black, V1/V2=white, hV6A=grey). It is evident the effect of the stimulation in slowing down the detection of the Go signal for reaching. This figure contains only valid trials (MotorATN congruent, MotorATN incongruent and Motor unconstrained trials). Individual participants' data are in Figure 6. Data regarding Motor invalid trials are shown in Figure 5.

Figure 3

Pupil size dynamics during the Plan epoch of the different trial types of valid trials in the TMS experiment and in the control experiment. Left) Pupil size is represented as baseline corrected values (see Methods). Different colors represent different stimulation conditions (as in Fig. 2B) and yellow trace represents pupil size dynamics during the conditions of the control experiment with the Cue of the same features of the corresponding TMS trial. Black thick lines represent the time when pupil size of control trials was significantly different from the one during Sham stimulation. Right) Differential values between pupil size during each stimulation condition and pupil size of the control experiment are plotted over time. The pupil constriction is evident in all the trials, but it is more intense during the control trials, when participant payed attention to the Cue. No effect of stimulation was found.

Figure 4

Pupil responses predict reaching reaction times in congruent trials. Significant prediction after Sham stimulation (A), after V1/V2 stimulation (B) and non-significant prediction after hV6A stimulation (C). Light grey lines are linear regression fits to data per participant. Thick lines show the correlations pooled over all trials. *$p \leq 0.01$; n.s., $p > 0.05$.

Figure 5

Distribution of the reaction times of invalid trials. Leftward redirection of cover attention is impaired after hV6A stimulation. Same conventions as in Fig. 2. Individual participants' data are in Figure 7.

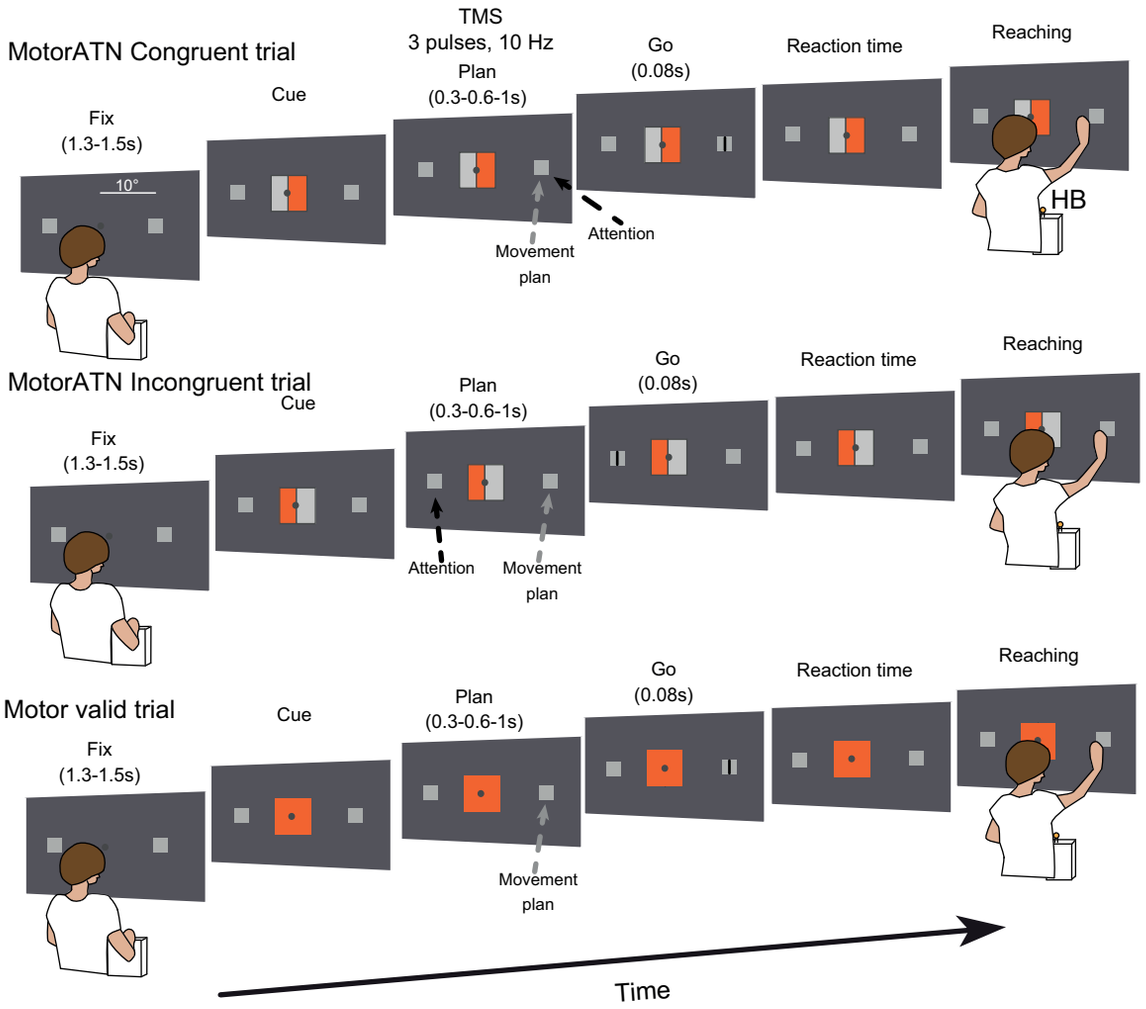
Figure 6

Mean population reaction times with data of individual participants in valid trials. Same conventions as in Fig. 2.

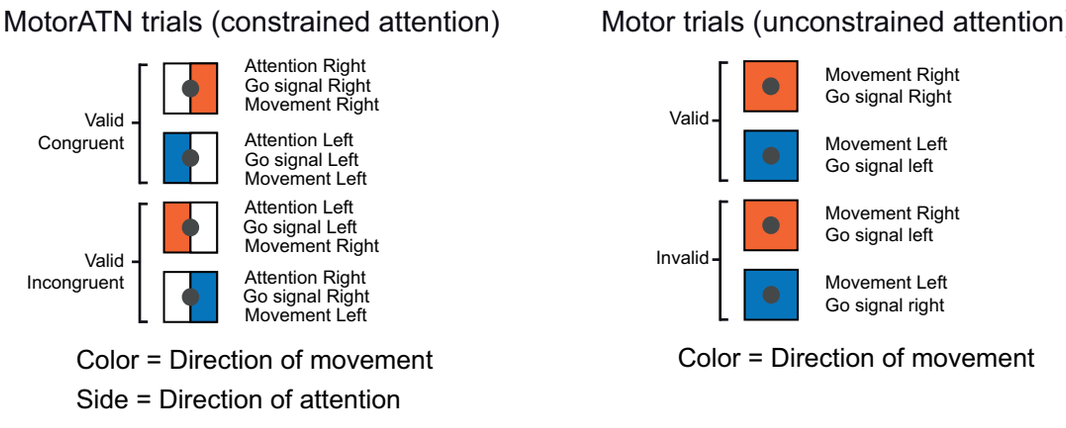
Figure 7

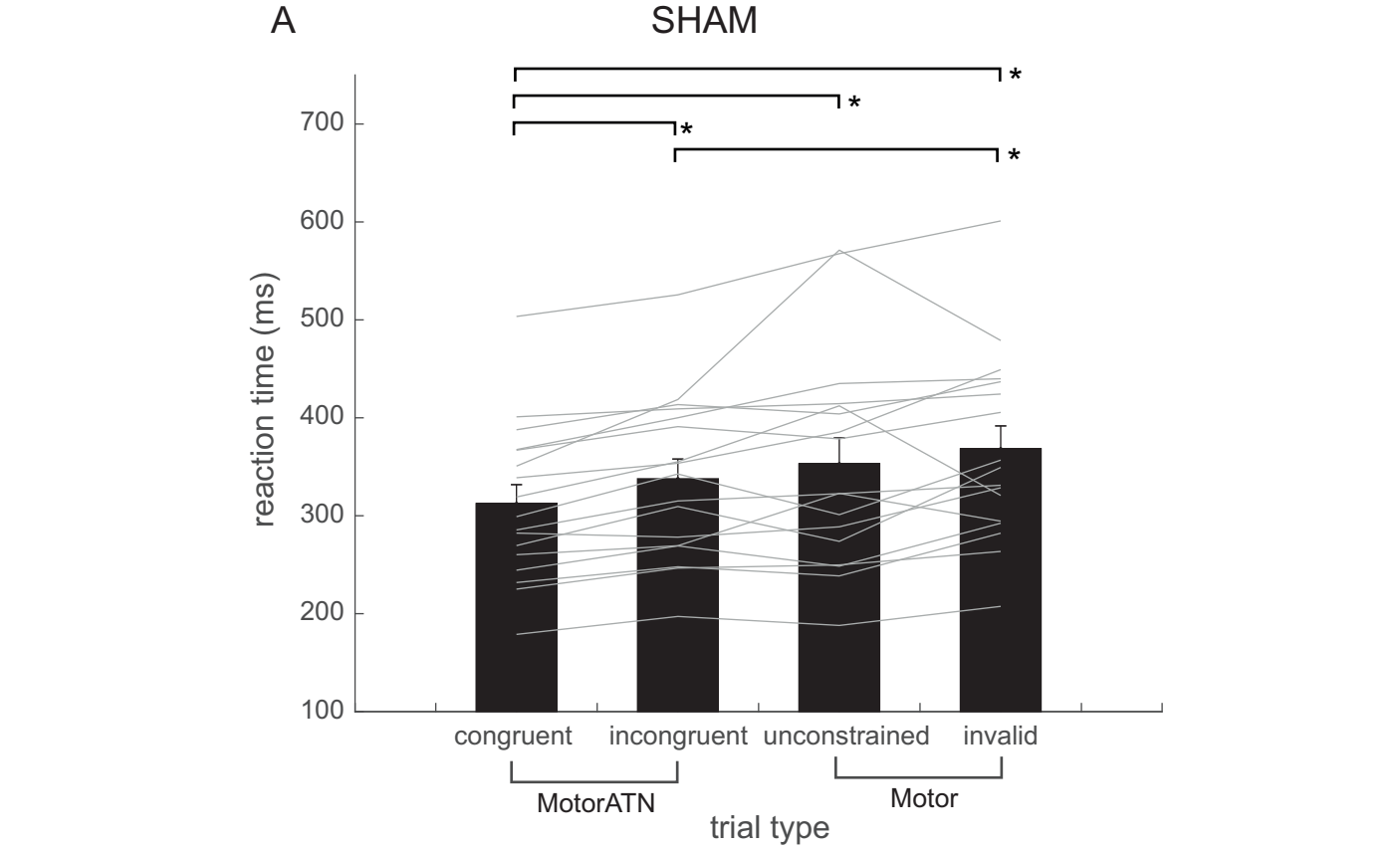
Mean population reaction times with data of individual participants in invalid trials. Same conventions as in Figs. 2 and 5.

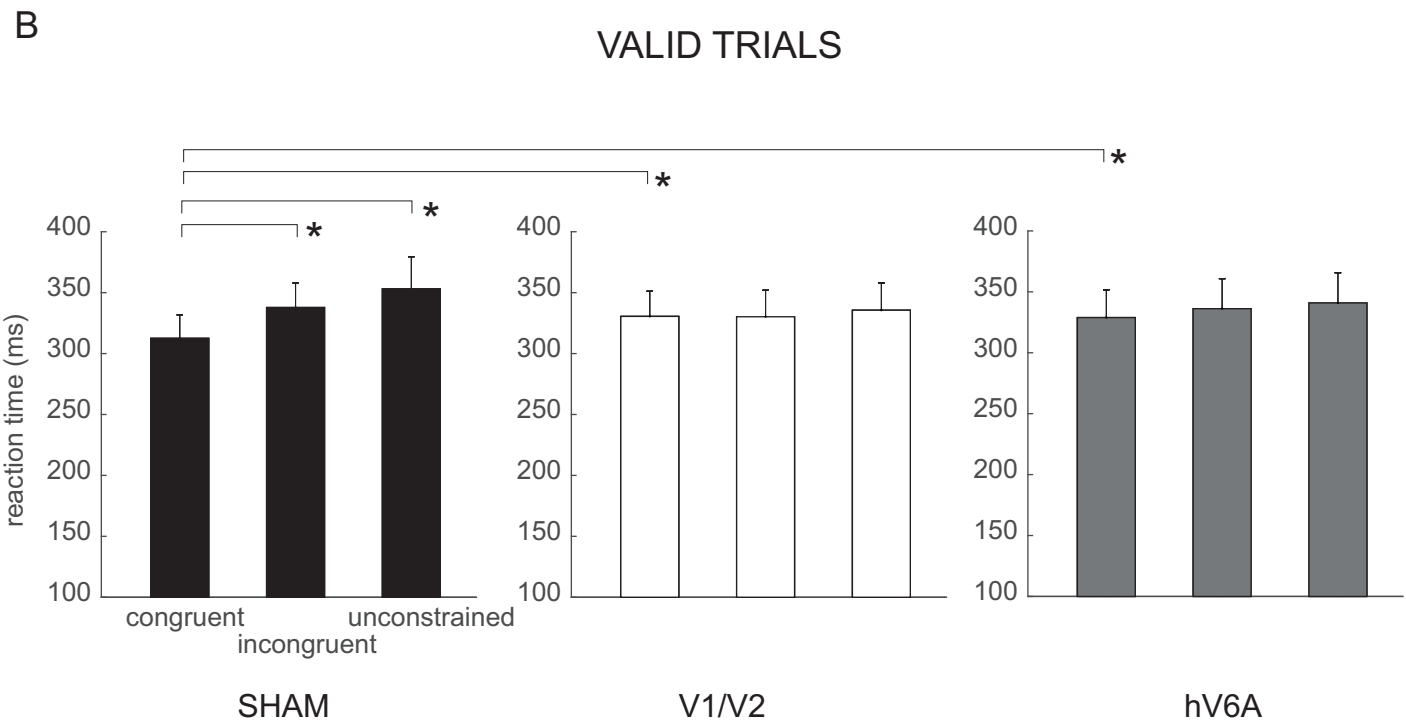
A

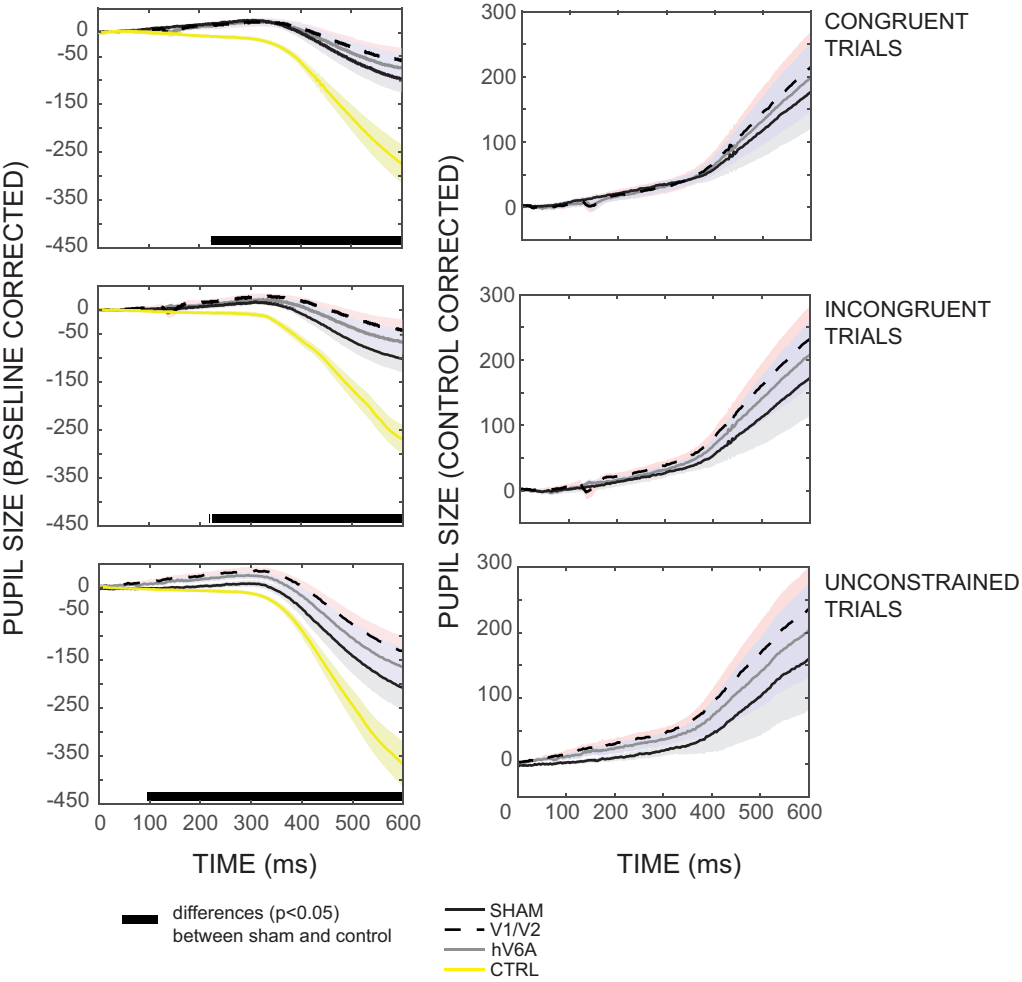

MotorATN Congruent trial
TMS
3 pulses, 10 Hz
Fix
(1.3-1.5s)
Cue
Plan
(0.3-0.6-1s)
Go
(0.08s)
Reaction time
Reaching
10°
Movement
plan
Attention
HB
MotorATN Incongruent trial
Fix
(1.3-1.5s)
Cue
Plan
(0.3-0.6-1s)
Go
(0.08s)
Reaction time
Reaching
Attention
Movement
plan
Motor valid trial
Fix
(1.3-1.5s)
Cue
Plan
(0.3-0.6-1s)
Go
(0.08s)
Reaction time
Reaching
Movement
plan
Time

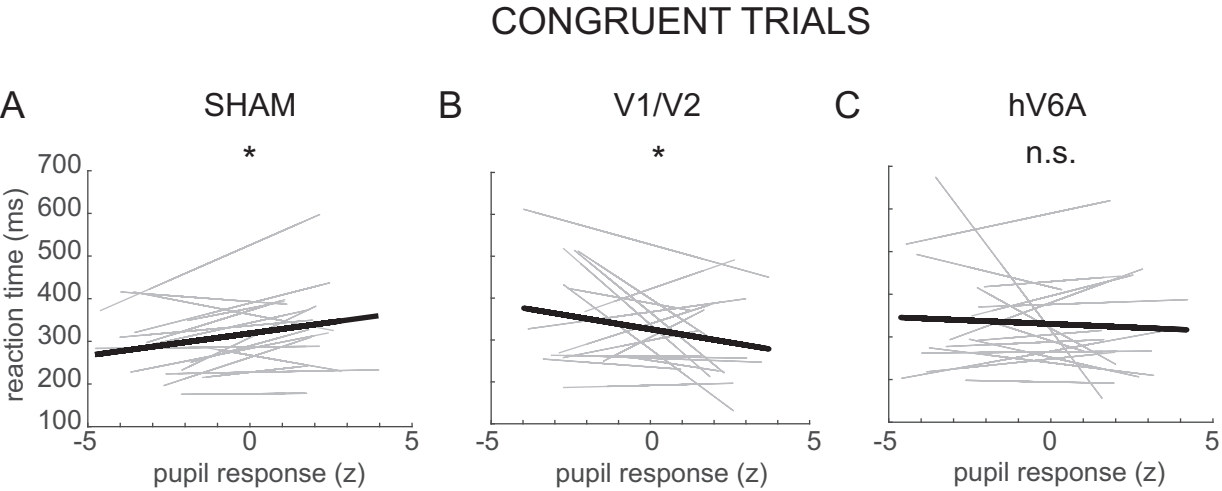
B

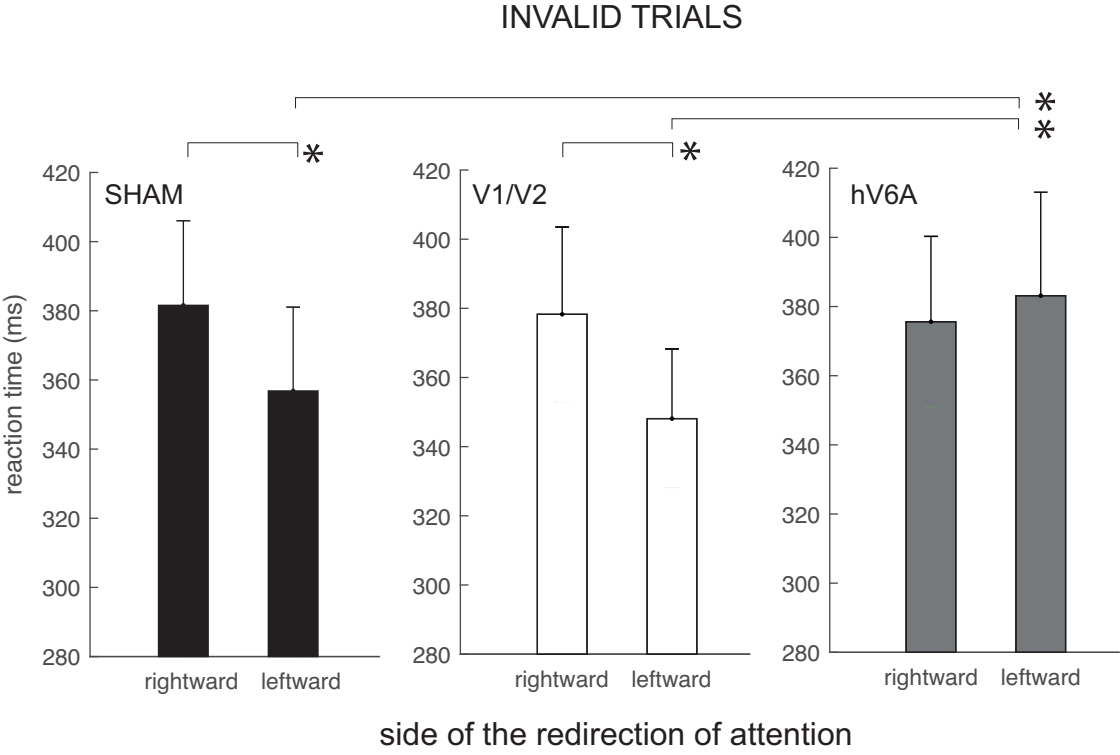

MotorATN trials (constrained attention)
Valid
Congruent
Attention Right
Go signal Right
Movement Right
Attention Left
Go signal Left
Movement Left
Valid
Incongruent
Attention Left
Go signal Left
Movement Right
Attention Right
Go signal Right
Movement Left
Color = Direction of movement
Side = Direction of attention
Motor trials (unconstrained attention)
Valid
Movement Right
Go signal Right
Movement Left
Go signal left
Invalid
Movement Right
Go signal left
Movement Left
Go signal right
Color = Direction of movement

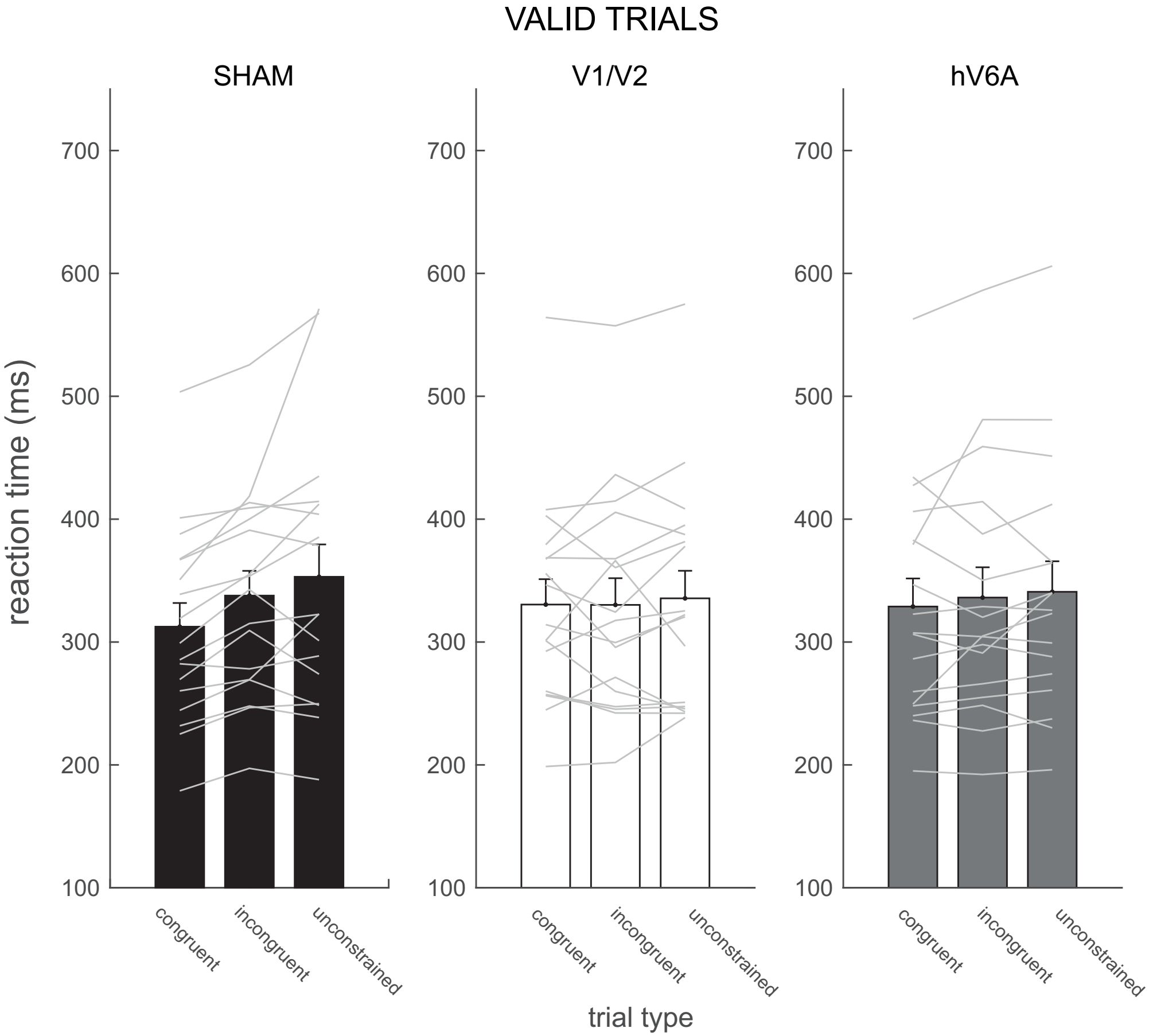

A
SHAM
reaction time (ms)
700
600
500
400
300
200
100
*
*
*
*
congruent
incongruent
unconstrained
invalid
MotorATN
Motor
trial type

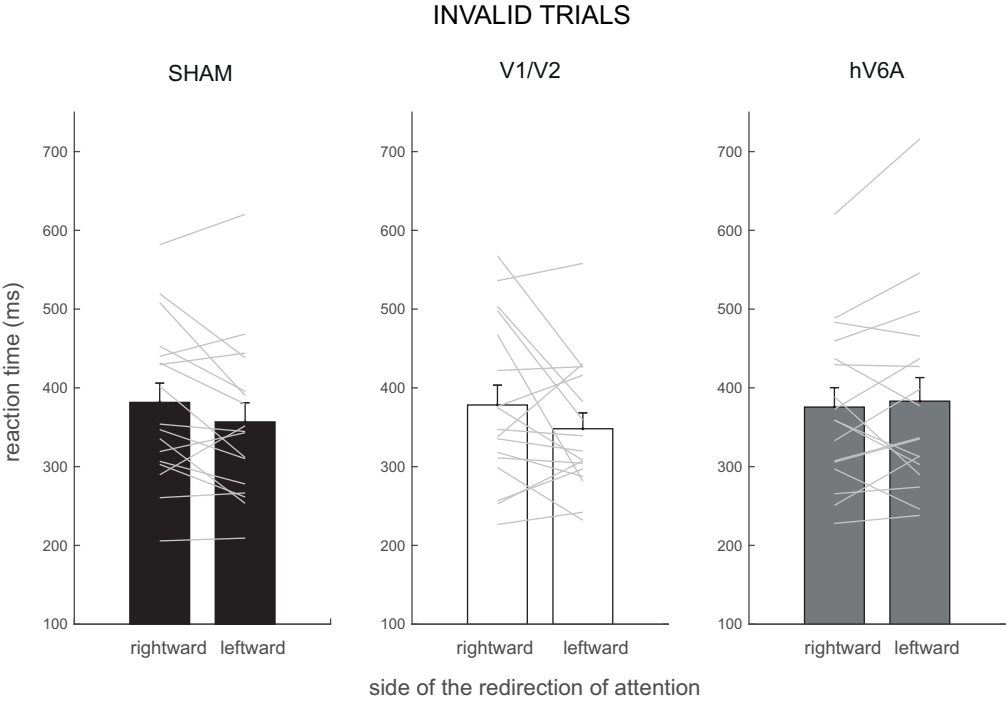

B
VALID TRIALS
reaction time (ms)
400
350
300
250
200
150
100
*
*
*
*
congruent
incongruent
unconstrained
SHAM
V1/V2
hV6A


PUPIL SIZE (BASELINE CORRECTED)
PUPIL SIZE (CONTROL CORRECTED)
CONGRUENT TRIALS
INCONGRUENT TRIALS
UNCONSTRAINED TRIALS
TIME (ms)
TIME (ms)
differences (p<0.05) between sham and control
SHAM
V1/V2
hV6A
CTRL


CONGRUENT TRIALS
A
SHAM
*
B
V1/V2
*
C
hV6A
n.s.
reaction time (ms)
700
600
500
400
300
200
100
-5
0
5
pupil response (z)
-5
0
5
pupil response (z)
-5
0
5
pupil response (z)


INVALID TRIALS
SHAM
V1/V2
hV6A
reaction time (ms)
420
400
380
360
340
320
300
280
rightward
leftward
*
*
*
side of the redirection of attention


VALID TRIALS
SHAM
V1/V2
hV6A
reaction time (ms)
700
600
500
400
300
200
100
congruent
incongruent
unconstrained
trial type


INVALID TRIALS
SHAM
V1/V2
hV6A
reaction time (ms)
700
600
500
400
300
200
100
rightward
leftward
side of the redirection of attention