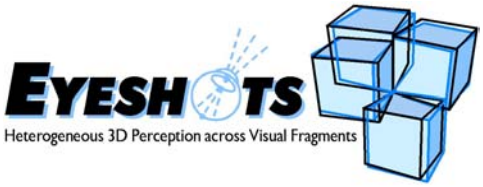

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Abstract:

Link across single visual fragments can be obtained in many physiological situations. Commonly, in natural conditions, when we catch with vision a target of a potential reaching action, we move the eyes toward it and then the hand, or we can "capture" our target with the focus of attention. Attention is used to enhance neural processing of selected parts of a visual scene. It increases neural responses to stimuli near target locations and is usually coupled to eye movements. Covert attention shifts, however, decouple the attentional focus from gaze, allowing to direct the attention to a peripheral location without moving the eyes. We found that covert attention shifts modulate ongoing neuronal activity in the medial parieto-occipital area V6A, an area that provides a bridge between visual signals and arm-motor control. This modulation in an area primarily involved in visuo-motor transformation for reaching may form a neural basis for linking eye movements and arm movements across fragments.

Contents

1- Executive summary

This deliverable describes the research work performed by the Neurophysiology Lab of UNIBO in collaboration with WWU, with regard to Work Package 5 of the EYESHOTS project.

We performed single cell recordings from area V6A in monkeys trained to fixate straight-head, while shifting attention outward to a peripheral cue and inward again to the fixation point.
We found that neurons in V6A are influenced by spatial attention. The attentional modulation occurs without gaze shifts and cannot be explained by visual stimulations. Visual, motor, and attentional responses can occur in combination in single neurons. This neural behaviour can form a neural basis for coupling attention to the preparation of reaching movements.
Our results show that cortical processes of the generation of attention are related not only to eye-movement control, as many studies have shown, but also to the control of arm movements, a finding that has been suggested by some previous behavioral findings of our lab. Therefore, the widely-held view that spatial attention is tightly intertwined with - and perhaps directly derived from - motor preparatory processes should be extended to a broader spectrum of motor processes than just eye movements.

1.1 Relation to previous work in EYESHOTS

Link across single visual fragments can be obtained in many physiological situations. Commonly, in natural conditions, when we catch with vision a target of a potential reaching action, we move the eyes toward it and then the hand. Due to less inertia of the eyes, the eyes land on the target well before the hand starts to move. In area V6A of the medial parieto-occipital cortex, we have found neurons discharging in this interval, that is in the first 500 ms of fixation of a target in the dark. Interestingly, this kind of cells in V6A strongly prefer targets to be fixated in the peripersonal space, that is in the reachable space (Hadjidimitrakis et al., 2010). A neuron like the one reported in Fig.1 is an example of such early-fixation signal, with a strong preference for near space.

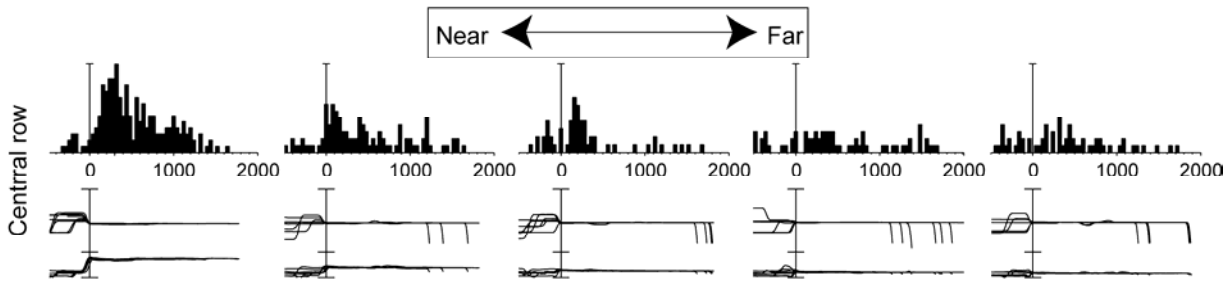

Fig. 1. Example of a neuron modulated in depth in the early fixation epoch. From top to bottom: neural responses and eye traces (version, top trace; vergence, bottom trace) to the five LEDs located on a midsagittal row, arranged from near (left) to far (right). The eye movement traces are aligned at the saccade onset. Scale bars for spike histograms and version and vergence traces were 80sp/s, 100 deg, and 20 deg, respectively.

We interpreted this neural behaviour as the neuronal correlate of a calibration between the eye and the arm systems and we proposed in the second year of the EYESHOTS project that the strong preference for reachable targets in early fixation period could reflect the shift of the attentional spotlight for the purpose of highlighting the location of the target of eye and hand movements in reaching an object (see Hadjidimitrakis et al., 2010).

Attention is important for providing the link across single visual fragments, attention is used to select targets in a visual scene for prioritized processing and for preparing appropriately directed actions. Our study intended to measure the influence of covert attention toward different parts of the visual world in neurons of area V6A. We induced in the monkey covert shifts of attention in absence of any effector movement, neither of the eyes nor of the arm. We performed single cell recording in V6A, while controlling the monkey focus of attention addressing it toward several positions in the workspace. In this way, we could study the influence of spatially directed attention on neurons in area V6A.

2- Introduction

When we want to recognize an object in the field of view, or want to reach and grasp it, we typically direct our gaze towards the object. The shift of gaze is the consequence, and the overt evidence as well, of the shift of our attention towards the object of interest. Although under normal circumstances the direction of attention and the direction of gaze are aligned, we are able to disengage attention from the point of fixation. This ability, known as covert spatial attention, allows us to select and acquire peripheral visual information without shifting the gaze (von Helmholtz, 1867; Posner, 1980).

Attention enhances both behavioral and neuronal performances (Spitzer et al., 1988). Reaction to attended targets is faster than to unattended targets (Posner, 1980), and responses of neurons to covertly attended stimuli enhance above those of unattended ones (Fischer and Boch, 1985; see Desimone and Duncan, 1995 for a review; Colby et al., 1996; Connor et al., 1997; Kodaka et al., 1997). Thus, attention modulates the processing of information in visual cortical maps, and selects parts of the scene to locally dedicate/recruit more processing resources.

The selection of the part of the scene to receive attention, i.e. the control of the focus of attention, is driven by the saliency of the stimuli and by the requirements of the task that is currently performed. If motor actions are to be performed on the selected targets, the focus of attention is closely related to these actions. The initiation of a saccade, for instance, is preceded by a mandatory shift of attention towards the saccade goal (Hoffman and Subramaniam, 1995; Kowler et al., 1995; Deubel

and Schneider, 1996; Awh et al., 2006). The deployment of attention is linked to the mechanisms of selecting a saccade target and preparing the saccade even for covert attention shifts (Moore et al., 2003; Cavanaugh and Wurtz, 2004; Ignashchenkova et al., 2004; Hamker, 2005; Thompson et al., 2005; but see also Lui et al., 2010).
The link between attention and goal-directed motor action is not confined to eye movements. Also the preparation of reaching movements is paralleled by a shift of attention to the goal of the reach (Castiello, 1996; Deubel et al., 1998). Therefore, one might expect that, similar to oculomotor areas that provide signals for overt and covert shifts of attention, also cortical areas that are involved in arm movements may contribute to shifts of attention, or may use spatial attentional signals to prepare arm movement or direct the hand towards the object to be grasped.

2.1 Aim of the present study

The medial posterior-parietal area V6A acts as a bridge between visual processing and arm motor coding (Galletti et al., 2003). Our aim in this study was to find out whether the activity of single cells in V6A is influenced by shifts of covert attention. Since, usually, the direction of gaze and the direction of attention are aligned, and since area V6A contains a high percentage of gaze-dependent neurons (Galletti et al., 1995), we had to disengage attention from the point of fixation (covert attention) in order to demonstrate that the direction of attention, and not the direction of gaze, modulates V6A neurons. In a task specifically designed for this, we found that the neural modulation was still present when the covert attention was shifted without any concurrent shift of the direction of gaze. We suggest that this attentional modulation is helpful in guiding the hand during reach-to-grasp movements, particularly when the movements are directed towards non-foveated objects.

3- Materials and Methods

3.1 Experimental procedures

Experiments were carried out in accordance with National laws on care and use of laboratory animals and with the European Communities Council Directive of 24th November 1986 (86/609/EEC), and were approved by the Bioethical Committee of the University of Bologna.
Three trained male Macaca fascicularis (4- 6, 5 kg) sat in a primate chair and performed an attentional task with their head restrained. We performed single microelectrode penetrations using home-made glass-coated metal microelectrodes with a tip impedance of 0.8-2 MOhms at 1 KHz, and multiple electrode penetrations using a 5 channel multielectrode recording minimatrix (Thomas Recording, GMbH, Giessen, Germany). The electrode signals were amplified (at a gain of 10,000) and filtered (bandpass between 0.5 and 5 kHz). Action potentials in each channel were isolated with a dual time-amplitude window discriminator (DDIS-1, Bak electronics, Mount Airy, MD, USA) or with a waveform discriminator (Multi Spike Detector, Alpha Omega Engineering, Nazareth, Israel). Spikes were sampled at 100 KHz and eye position was simultaneously recorded at 500 Hz. Eye position was recorded using an infrared oculometer (ISCAN, Inc) at a sampling rate of 100 Hz and was controlled by an electronic window (5 x 5 degrees) centered on the fixation target. Behavioral events were recorded with a resolution of 1 ms. We performed extracellular recordings on all the 3 animals; on two of them we also performed behavioral recordings.
Surgery to implant the recording apparatus was performed in asepsis and under general anesthesia (sodium thiopenthal, 8 mg/kg/h, i.v.). Adequate measures were taken to minimize the animal's pain or discomfort. Specifically, analgesics were used postoperatively (ketorolac trometazyn, 1mg/kg i.m. immediately after surgery, and 1.6 mg/kg i.m. on the following days). Extracellular recording techniques and procedures to reconstruct microelectrode penetrations were similar to those described in other reports (Galletti et al., 1995).

3.2 The attentional task

Data were collected while monkeys were performing a task specifically designed to study the effect of covert spatial displacements of the spotlight of attention on neural responses. The monkeys sat in front of a fronto-parallel panel which was located 14 cm from the animal's eyes. The panel contained 3 green/red light emitting diode (LED; 4 mm in diameter; 1.6° of visual angle) that served as fixation point and target to be detected. The fixation point was the green/red LED located in the straight-ahead position. Two circular rings (12 mm in diameter; 4.8° of visual angle), illuminated by a yellow LED, served as a cue that indicated the spatial position of the subsequent target to be detected. The cue and target LEDs were located 15° peripherally on opposite sides from the fixation point.

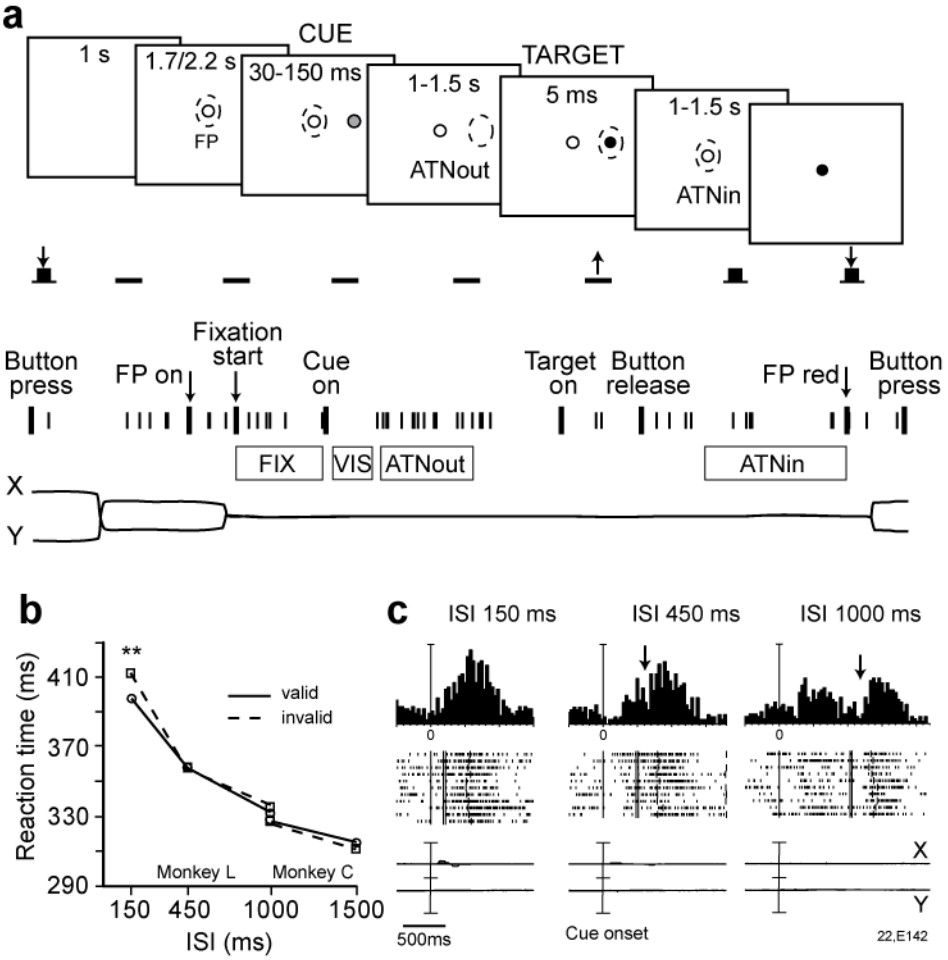

Fig 2. **a**) Schematic representation of the task. Top: Sequence of events in a single trial. After button pressing, the monkey maintained fixation on the central fixation point (white dot, FP) all throughout the trial while covertly shifting attention (dashed circle) towards the cued location (grey dot). After target (black dot) detection, the animal released the button, continuing to gaze the fixation point until it changed in color (from green to red). Color-change detection was reported by the animal by button pressing. Bottom: typical example of neural activity and eye traces during a single trial. Short vertical ticks are spikes. Long vertical ticks among spikes indicate the occurrence of behavioral events (markers). Below the neural trace, time epochs during a typical trial are indicated. ATNout: outward attention epoch, ATNin: inward attention epoch. **b**) Performance of 2 monkeys expressed as reaction time to detect the target at different inter-stimulus-intervals (ISIs). Results from valid (continuous) and invalid (dashed) trials are shown. Significant difference in reaction times between valid and invalid trials at ISI 150 shows that attention is directed towards the peripheral cue location at this time.
c) Peri-stimulus time histograms of an example neuron recorded with different ISIs. Trials are aligned to cue onset. The neuron shows two discharges (after cue onset and button release, respectively) that separate (arrow) clearly at longer ISIs.

The time sequence of the task is shown in Figure 2**a**. A trial began when the monkey decided to press the home-button near its chest. After pressing the button, the animal waited for instructions in complete darkness. It was free to look around and was not required to perform any action. After 1000 ms, the fixation LED lit up green. The monkey was required to look at the fixation target and to maintain the button press while waiting for an instructional cue.
After 1700-2200 ms, another LED (the CUE) lit up for 30-150 ms in one out of the two peripheral positions located 15° apart from the fixation point. After 1000-1500 ms a red flash (TARGET) of 5 ms occurred in the cued position. The monkey had to release the home-button as soon as it detected the target. The maximum time allowed to release the button was 1000 ms. If the monkey did not release the button during this period the trial was marked as error trial. After 1000-1500 ms, the fixation point changed in color from green to red. The monkey had to press the home-button again (maximum time to press was 1000 ms) to drink the reward. Home-button pressing ended the trial, issued monkey reward, and started the next trial.
The correctness of the animal's performance was evaluated by a software supervisor system (Kutz et al., 2005) which checked the status of microswitch (monopolar microswitches, RS components, UK) mounted under the home-button. Button presses/releases were checked with 1 ms resolution.
Displacements of the spotlight of attention towards the two peripheral positions were typically tested as a randomized sequence in order to collect trials in one position intermingled with the other. Up to ten trials for each position were collected (20 trials in total). The panel could be rotated in 4 different positions (horizontal, vertical, and 2 oblique positions in between the two), allowing us to test up to 8 spatial displacements of the spotlight of attention.
The task was performed in darkness. Eye fixation was always maintained in the straight ahead position within an electronic window of 5° amplitude. Fixation had to remain within this window throughout each trial until the fixation point switched off, otherwise the trial was aborted and a new one began without any reward. Off line inspection of eye records allowed to check for actual performance of fixation.

3.3 Neuronal data analysis

We divided the trial into functional epochs, defined as follows (see bottom part of Figure 2a):
• FIX: steady fixation of the LED from its appearance to the cue onset; it contains the baseline activity of the neuron, used to compare the cell activity during the other epochs.
• VIS: from 40 to 150 ms after the cue onset; it could contain the passive visual response evoked by the cue appearance.
• outward attention epoch (ATNout): from 200 to 500 ms after the cue onset; it could contain the response due to the covert, peripheral displacement of the spotlight of attention.
• inward attention epoch (ATNin): from 400 ms after button release to the change in color of the fixation point; during this epoch the animal concentrates attention on the fixation point, as it has to detect the fixation point's change in color.
For behavioral analysis, the reaction time between target onset and button release was determined.
Only units which were tested in at least 7 trials for at least two target positions were included in the analysis. This is a conservative choice connected to the implicit high variability of biological responses (see Kutz et al., 2003 for detailed explanation).
For each neuron, the mean firing rate was calculated for each trial in outward attention epoch and inward attention epoch, and statistically compared with the mean firing rate in epoch FIX (two-tailed Student's t-test; significance level, $p < 0.02$ with Bonferroni correction for multiple comparisons). This comparison was performed for each spatial location. Units with a significant discharge during at least one of the two attentional epochs were considered task-related and were further analyzed.

The spatial tuning of activity in the task-related cells was analyzed statistically by comparing the mean firing rate in each target position (one-way ANOVA, F-test; significance level, $p < 0.05$) for each of the functional epochs described above. A neuron was defined as 'spatially tuned' when it showed a statistically significant difference in mean firing rate in the same epoch in different spatial locations. Direction selectivity of neurons modulated during outward attention epoch and/or during inward attention epoch was quantified by a preference index (PI) for each functional epoch as follows:

$$PI = abs(D - OD)/(D + OD)$$

where D is the maximal discharge for cells excited with respect to FIX or minimal discharge for cells inhibited with respect to FIX, and OD is the discharge for the opposite position. PI ranged from 0 to 1.

Population activity of tested neurons was calculated as averaged spike density functions (SDFs). A SDF with a Gaussian kernel of half-width 40 ms was calculated for each neuron included in the analysis, averaged across all the trials for each tested condition, and normalized to the peak discharge of the neuron in the behavioral epochs of interest. The normalized SDFs were then averaged to derive population responses. We statistically compared the population SDFs with a permutation test with 10,000 iterations comparing the sum of squared errors of the actual and randomly permuted data.

3.4 Behavioral data

We performed psychophysical measurements in separate sessions on 2 animals. In these sessions we collected reaction times of the monkeys in valid trials, in which the target appeared in the cued position, and in invalid trials, in which the target appeared in the uncued position. These reaction times were recorded separately from the physiological data because the physiological recordings contained only valid trials. We recorded behavior during batteries of trials containing 10% (for Monkey C) or 20% (for Monkey L) of invalid trials randomly interleaved with valid trials. We tested two opposite target positions, to the right and to the left of the fixation point.

Various inter-stimulus-intervals (ISIs) were tested: for monkey L, we used ISIs = 150 ms, 450 ms, 1000 ms (similar to the ISIs tested in Bowman et al., (1993)); for monkey C, we tested ISIs = 1000 ms, 1500 ms. A repeated measures ANOVA ($p<0.05$) with factors: validity (2 levels) and ISI (3 levels) was used to assess the effect of validity, of ISI, and of the interaction between the two, on reaction time to target detection. To assess the validity effect for each ISI, post hoc comparisons using the Duncan correction were used.

4- Results

We performed extracellular recordings on 182 single cells of area V6A in 3 *Macaca fascicularis*. Cells were ascribed to V6A following the functional criteria described in Galletti et al. (1999), and on cytoarchitectonic criteria according to Luppino et al. (2005).

Animals were trained to fixate a light-emitting diode (LED) in the straight-ahead position in darkness while pressing a button located outside their field of view. While fixating, the monkeys had to detect a target (5 ms red flash) in one out of several peripheral positions and respond to it by releasing the button without moving the eyes (Fig. 2a). The target position was cued by a yellow flash (30-150 ms) preceding the target onset by 1-1.5 s. The cue signal prompted the monkeys to covertly displace attention towards the periphery. After target detection, the monkeys shifted attention back towards the straight-ahead position to detect the change in color of the fixation LED. This change in color had to be reported by pressing the button again. The monkeys were trained to maintain gaze in the straight-ahead position all throughout the trial. Their fixation was checked using an electronic window and off line inspection of recorded eye traces.

We quantified each cell's discharge during three time epochs (see Fig. 1a): the starting fixation epoch before cue onset (baseline activity, FIX), the epoch from 200 to 500 ms after cue onset (covert attention shifted towards the cue location, 'outward attention'), and the epoch from 400 ms after button release to the change in color of the fixation LED, when attention is again directed towards the central fixation point ('inward attention'). We also analyzed passive visual response to the cue appearance in an epoch from 40 to 150 ms after the cue onset (VIS).

4.1 Behavioral bases of covert attention shift

To check whether our experimental conditions induced covert attention shifts, we measured reaction times (RTs) between target onset and button release in two monkeys. These measurements were collected in separate behavioral testing sessions before the onset of single unit recording. These sessions contained valid trials as described above, and invalid trials in which the cue was misleading because the target appeared on the opposite side. It is well known that effects of covert attention shifts are reflected in differences in the reaction times between valid and invalid trials both in human (Posner, 1980) and monkey (Bowman et al., 1993). In valid trials, especially with brief inter-stimulus-interval (ISI), the reaction time are expected to be shorter than during invalid trials because the location where the target appears benefits from attentional enhancement evoked by cue appearance.
As reported in Figure 2**b**, reaction times for target detection in valid and invalid trials were recorded at ISIs of 150, 450 and 1000 ms (for Monkey L) and 1000 and 1500 ms (for Monkey C). Mean reaction times were 400.01 ms (ISI 150), 360.01 ms (ISI 450) and 335.90 ms (ISI 1000) for valid trials, and 412.89 ms (ISI 150), 357.35 ms (ISI 450) and 336.16 ms (ISI 1000) for invalid trials for monkey L; for Monkey C reaction times were 326.85 ms (ISI 1000), 314.86 ms (ISI 1500) for valid trials and 324.95 ms (ISI 1000), 309.09 ms (ISI 1500) for invalid trials. Data were entered into a 2 repeated measures ANOVA with ISI and validity (Valid vs invalid trials) as within factors. The ANOVA has revealed a significant interaction ISI × validity ($F(2,36)=5.47$, $p=0.008$) for monkey L with a difference in reaction time between valid and invalid trials occurred for the ISI of 150 ms ($p=0.0009$, Newman-Keuls *post hoc* test). The shorter RT for valid trials is an index of attention allocated to the cue, and confirms that the experimental paradigm we used elicited covert attention shifts in our monkey subjects. For longer ISIs, the validity effect was no longer significant, although reaction time for both trial types decreased with increasing ISI (repeated measures ANOVA, main effect of factor ISI, $F(2,36)=72.87$, $p=0.000001$) suggesting an increase of alertness when the ISI is longer.

4.2 Single-unit recordings

Since significant RT difference between valid and invalid trials was observed for ISI of 150 ms but not for ISIs of 450 ms and higher, and because we wanted to exclude from the analysis the effect of putative visual responses to cue onset, we restricted the analysis of the effect of outward attention shifts to a time epoch from 200 and 500 ms after cue appearance. However, we performed also the analysis with a time window from 150 ms to 450 ms and the results were the same. Below, we report the results of the former analysis as a more conservative approach.
Since key-press and key-release actions elicited neural responses in V6A (Galletti et al., 1997; Marzocchi et al., 2008), we wanted to separate in time the responses related to inward shifts of attention from the responses related to the button press. To this aim, in preliminary experiments we varied ISI during cell recordings. Figure 2c shows an example of a cell recorded with different ISIs (150, 450 and 1000 ms, tested in randomly interleaved trials) and a cue duration of 30 ms. When the ISI was 150 ms (Fig. 2c left), the cell had a strong and long discharge starting immediately after the cue onset. An increase of the ISI to 450 ms (Fig. 2c, center) caused the tendency of the discharge to separate in two components (see arrow in Fig. 2c, center). These two components became further

separated and distinguishable at an ISI of 1000 ms (see arrow in Fig. 2c, right), the first component related to the cue, the second to the button release. Since these components were clearly separable only at an ISI of 1000 ms, when recording from neurons we used ISIs of 1000 and 1500 ms, to be able to correlate each discharge component with the different events occurring during the trial.
Of 182 recorded cells, 83 (46%) showed neural discharges during the outward and/or inward attention epochs that were significantly different from the baseline (epoch FIX) as assessed by Student's t-test (with Bonferroni correction, $p<0.02$). From now on, we will refer to these cells as 'task-related cells'.

4.3 Neural responses during outward attention

Fifty-one task-related cells were modulated during outward attention epoch (Student' t-test, $p<0.05$). In particular, 24 cells (47%) were inhibited (i. e. the discharge during outward attention epoch was weaker than during FIX), and 27 cells (53%) were excited (i. e. the discharge during outward attention epoch was stronger than during FIX).
Figure 3 shows a cell with a typical outward attention response for cues presented in the lower space. The spatially-tuned outward attention activity had a very long latency (on average 283 ms). The cell discharged strongly after cue onset and continued to discharge well after cue offset. In some trials, the response lasted until target onset, that is 1 s or more later than the cue onset. Although we cannot rule out completely that what we call outward attention response was a visual response to the cue enhanced by attention, the observed discharge was very different from a typical V6A visual response. First, the duration of the outward attention response was much longer than the visual stimulus, contrary to what happens in typical visual responses where stimulus and response durations are nearly the same. Second, the latency of outward attention response was much longer and less strictly time locked than the latency of a typical visual response.

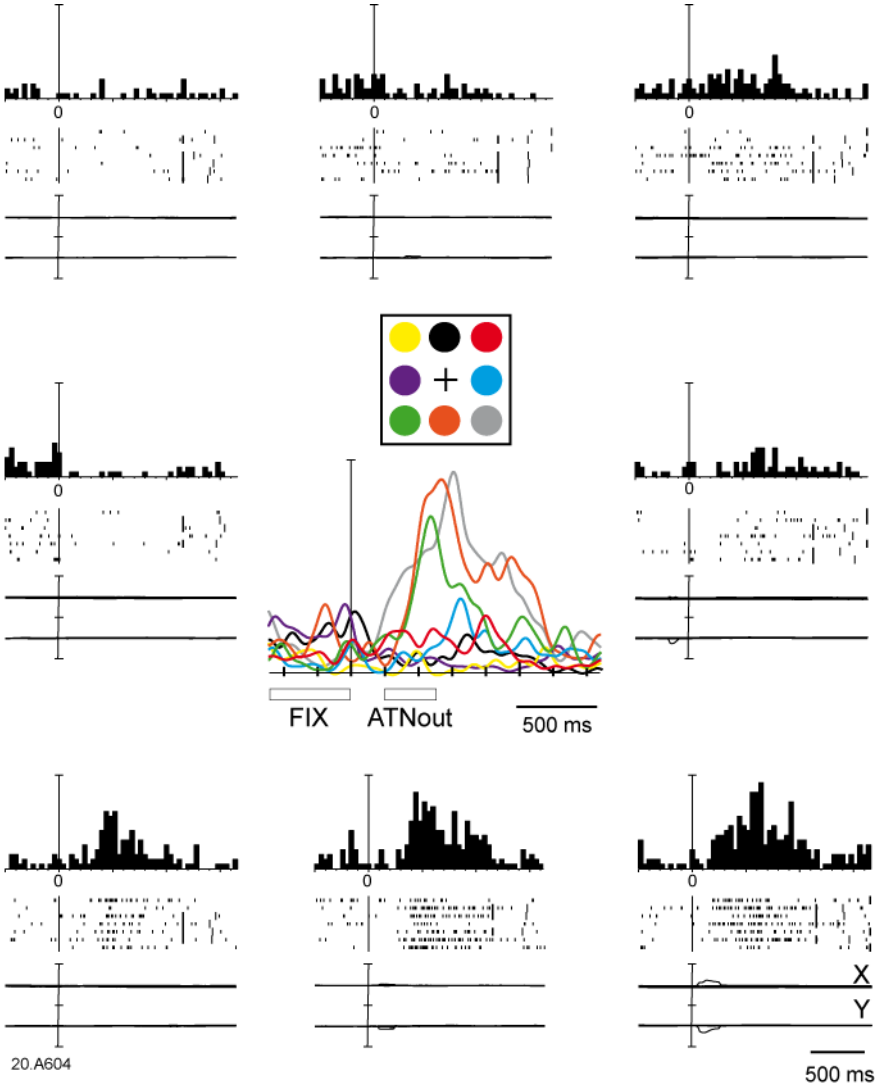

Fig. 3. Example of spatially-tuned modulations of neural activity during outward attention epoch.
The neuron shows a strong discharge during outward attention epoch preferring covert shifts of attention towards the bottom part of the space. Each inset (positioned in the same relative position as the cue on the panel) contains the peri-event time histogram, raster plots and eye position signals. In the central part of the figure, the spike density functions (SDFs) of the activity for each of the 8 cue positions are superimposed and aligned on the cue onset. The mean duration of epochs FIX and outward attention is indicated below the SDFs. Neural activity and eye traces are aligned on the cue onset. Scalebar in peri-event time histograms, 70 spikes/s. Binwidth, 40 ms. Eyetraces: scalebar, 60°. Other details as in Figure 2.

Spatial tuning of the outward attention activity was a common finding in our sample of V6A neurons: twenty-six out of 51 cells (51%) resulted significantly spatially tuned (1-way ANOVA, $p<0.05$).

To investigate the direction sensitivity of cells with outward attention activity, we computed a preference index (PI, see Material and methods). Figure 4a shows, separately, the distributions of PIs for excited (red) and inhibited (blue) cells. About half of the excited cells were direction selective, with a PI higher than 0.2. Note that the cell shown in Fig. 3, that was strongly direction-selective, had a PI of 0.44. The inhibited cells were even more sensitive to the direction of covert attention, showing a larger number of cells with a high preference index.

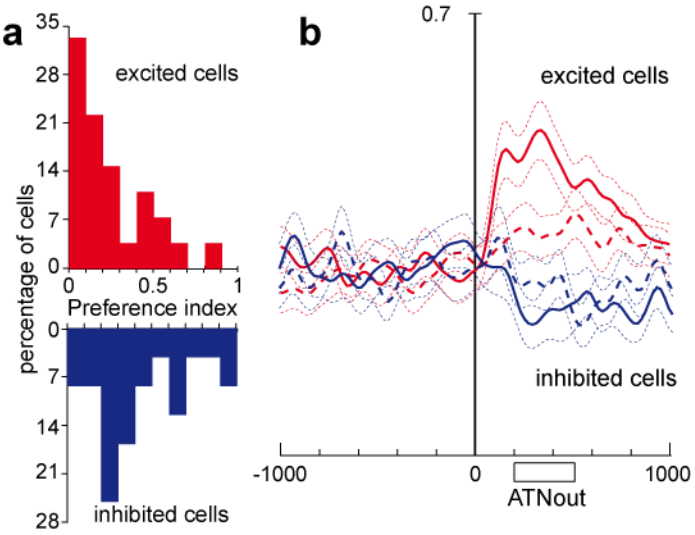

Fig 4. Activity modulation during outward attention epoch. **a**) Distribution of preference index (see Experimental procedures) for cells excited (red histogram) and inhibited (blue histogram) during outward attention epoch. **b**) Effect of the covert dislocation of the spotlight of attention on the activity of V6A cells during outward attention epoch. The average SDF for the excited (red lines) and inhibited (blue lines) cells are shown. Continuous lines represent the average SDF for the cue location evoking the maximal (excited cells) or minimal (inhibited cells) activity, and the dashed line that for the opposite location. Two dotted lines for each SDF indicate the variability band (SEM). The activity of cells in each population is aligned on the cue onset. Scale in abscissa: 200 ms/division; vertical scale 0.7. Other details as in Figure 2.

Figure 4b shows the population activity of V6A cells that were excited (red lines) or inhibited (blue lines) during the epoch of outward attention. The continuous lines represent the average mean activity of cells in trials in which the cue appeared in the position evoking the maximum (excited) or the minimum (inhibited) discharge rate. The dashed lines represents the average mean activity of the cells in trials in which the cue appeared in the opposite position. The plots have been aligned on cue onset.

The discrimination between two opposite spatial positions at population level began around 100 ms after cue onset and peaked around 300 ms (Fig. 4b). This agrees with the time course of the shift of the spotlight of attention as assessed from the behavioral data: a behavioral effect of attention at the cued location was detectable 150 ms after the cue onset and ceased within 450 ms after the cue onset. Also the rapid change of population activity just after cue onset reported in Figure 4**b** well agrees with the fact that the displacement of the spotlight of attention during outward attention epoch is exogenously driven by the cue.

Independently of the effect of outward shift of attention (excitation or inhibition), the number of cells preferring contralateral shifts of covert attention was the same as that preferring ipsilateral shifts. Note that, interestingly, the spatial distribution of visual receptive fields in V6A, mostly contralateral, is significantly different from the spatial selectivity of attentional responses (Chi-squared test, $p<0.0001$), as shown in Figure 5. This fact is against the view that the attentional effect could be the result of a modulation of the visual response, suggesting a functional separation between the two phenomena.

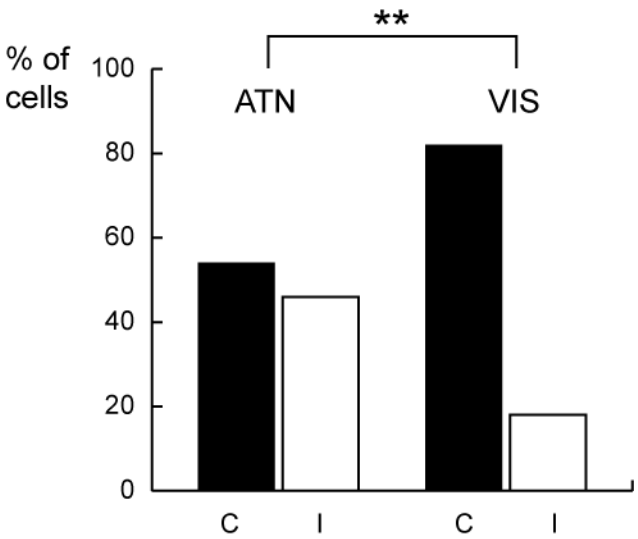

Fig 5. Preferred attentional and visual receptive-field locations in area V6A.
Columns indicate the percentages of neurons modulated during outward attentional epoch (ATN) preferring contralateral (C) or ipsilateral (I) targets, and the percentages of visual cells (VIS) with the receptive-field center in the contralateral (C) or ipsilateral (I) hemifield. ATN and VIS populations include 26 and 684 cells, respectively. The difference between the two distributions was statistically significant, as indicated by the asterisks (Chi-squared test, chi-squared=14.92, p=0.0001).

4.4 Neural responses during inward attention

After target detection (i. e. after button release) the animal was requested to respond to a change in color of the fixation LED that occurred from 1000 to 1500 ms after button release (see Fig. 2a). Thus, it is plausible that, during this period, the focus of attention was brought back to the fixation point (inward attention epoch). Because the fixation LED remained illuminated in the same color throughout the inward attention epoch, and because no further visual stimulation was given after the target presentation and the button release, modulations occurring in the inward attention epoch can not be ascribed to a visual stimulation. They had to be related to endogenously driven shifts of attention towards the fixation point.
Out of the task-related cells, 63 (76%) were significantly modulated during inward attention epoch with respect to the baseline (Student t-test, $p<0.05$): 33% of these cells were excited whereas the majority (67%) were inhibited. Figure 6a shows a cell with a strong discharge during inward attention epoch. This discharge occurred independently of the direction of covert attention during the preceding outward attention epoch (cue location). Most of the excited cells of our population showed this behavior (71%). Figure 6b shows a cell with direction selectivity: its response during inward attention epoch was different for the different cue positions. Neurons like these, showing a change in discharge in periods in which neither the processing of visual information, nor the execution of motor acts is taking place, strongly support the notion that attention modulates V6A neurons.

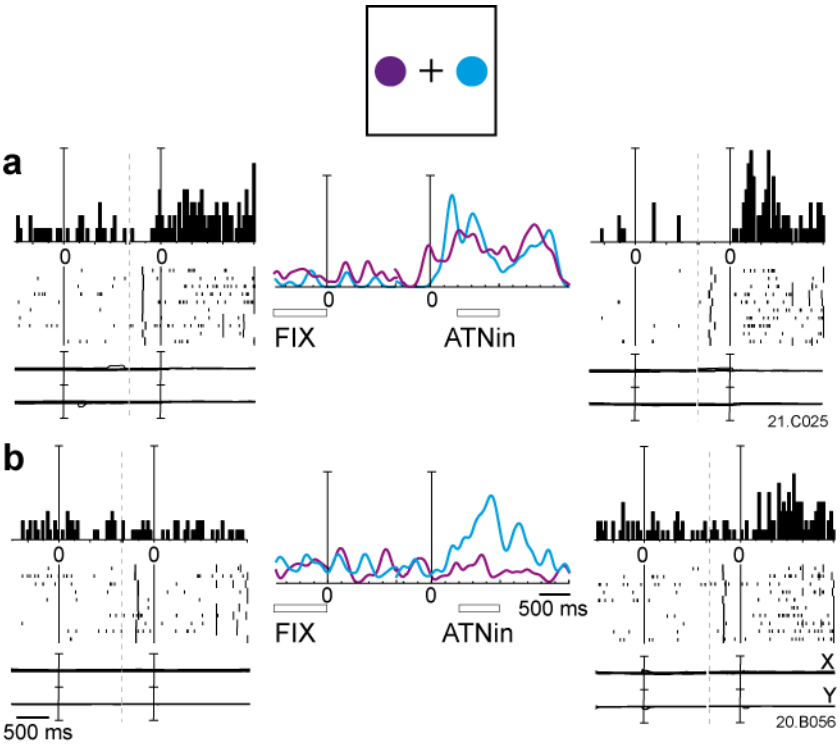

Fig 6 Examples of two neurons excited during inward attention epoch. **a**) Neuron excited during inward attention epoch, insensitive to the direction of the focus of attention. **b**) Neuron excited during inward attention epoch, sensitive to the direction of the focus of attention. Left and right: neural activity, raster dot displays and eye traces are aligned twice, with the cue onset (left) and with the button release (right). Center: SDFs of the two cue positions are superimposed (blue line: right position, purple line: left position). Peri-event time histograms: binwidth, 40 ms; scalebars, 18 spikes/s (**a**), 25 Spikes/s (**b**). Eyetraces: scalebar, 60°. Other details as in Figures 2 and 3.

Selective responses in the different task epochs could be found in combination in individual neurons: 31 cells were driven by both outward and inward shifts of attention, as the example reported in Figure 7. This is a cell whose activity was strongly modulated by the covert shift of attention towards the cue (outward attention epoch), but also by the action of button press, and by the bringing back of attention focus towards the fixation point (inward attention epoch). This last modulation was actually an inhibition. The example of Figure 7 shows that the effect of attention can modulate not only the ongoing activity but also the motor-related activity of single cell. The large majority of V6A cells are of this type.

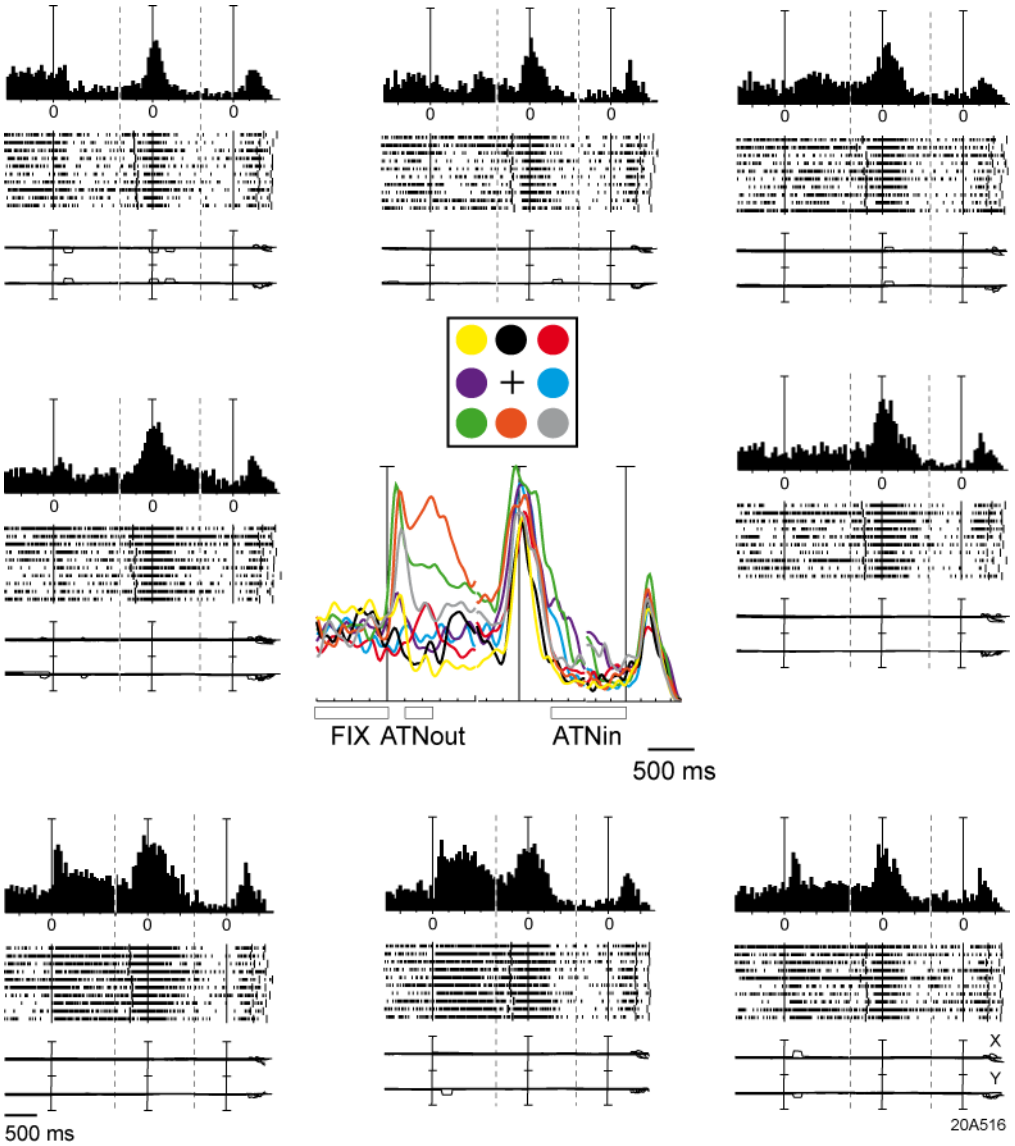

Fig. 7. Example of a cell modulated during outward and inward attention epochs and during button release. This cell was excited during outward attention epoch when attention was covertly directed towards bottom locations, and inhibited during inward attention epoch for all attended locations. Neural activity and eye traces are aligned three times: from left to right: with the cue onset, with the button release and with the change in color of the fixation point. Peri-event time histograms: binwidth, 40 ms; scalebars, 180 spikes/s. Eyetraces: scalebar, 60°. Other details as in Figures 2 and 3.

Spatial tuning for inward attention epoch was less common than for outward attention epoch (17/63, 27%; 1-way ANOVA p<0,05). We calculated the distribution of preference indices separately for the population of excited and inhibited cells. The majority of excited cells (15/21, 71%) showed weak directional selectivity, with PI lower than 0.2 (Fig. 8a, red histogram). The directional selectivity of cells inhibited during inward attention epoch (Fig. 8a, blue histogram) was slightly higher than that of excited cells.

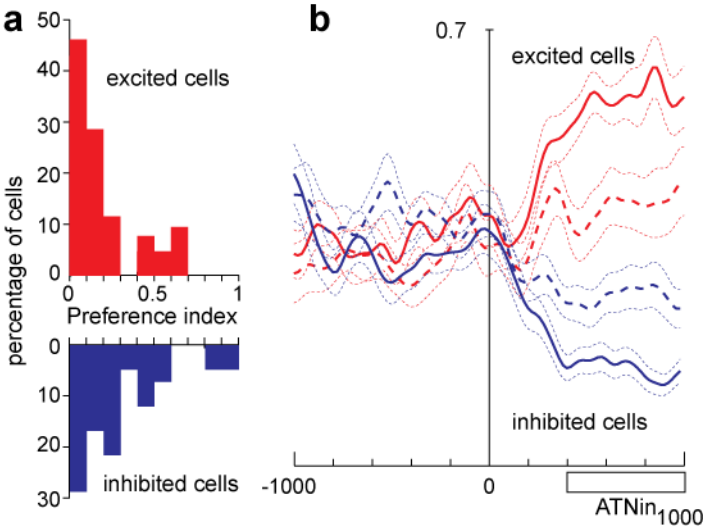

Fig 8. Activity modulation during inward attention epoch. **a**) Distribution of preference indices (see Material and methods) of cells excited (red histogram) and inhibited (blue histogram) during inward attention epoch. **b**) Effect of the increase of the level of attention at the fixation point on the neuronal population activity of V6A cells excited (red lines) or inhibited (blue lines) during inward attention epoch. The average SDF for the cue location evoking the maximal (excited cells) or minimal (inhibited cells) activity and the activity for the opposite cue locations are shown as continuous and dashed lines, respectively. The activity of cells in each population is aligned on the button release. Scale in abscissa: 200 ms/division; vertical scale 0.7. Other details as in Figs. 2 and 4.

Figure 8b shows the population activity of the cells significantly excited (red lines) or inhibited (blue lines) during inward attention epoch (N=21 and 42, respectively). The plots have been aligned on the button release. On average, cell activity changes after the button release, i.e, at a time when attention is redirected to the fixation point in order to detect its upcoming change in color. Cell activity then remained high or low (according to the type of cell) up to the end of the trial. This behavior is in line with a shift of attention to the fixation point and can not be explained by visual stimulation, oculomotor, or any other motor-related activity. The delay of the change in cell discharge is longer than that observed in outward attention epoch (see Fig. 4b), in agreement with the view that the phenomenon is endogenously driven.

5- Discussion

We have recorded responses of cells in monkey area V6A in a task that required covert attention shifts from a central fixation point outward to a peripheral location, and then inward shifts of attention back to the fixation point. The outward shift was exogenously driven by a visual cue while the inward shift was endogenously driven by the learned requirements of the task.
We found that the activity of V6A cells was modulated by the outward shift of covert attention, often in a direction-selective way, with half of the cells excited and half inhibited by the attentional shift. The onset and duration of attentional response correspond well to the typical temporal profile of exogenous attention shifts in humans (Posner, 1980) and to the attentional benefits on reaction times in our monkeys. Because the outward attention shift is driven exogenously by the visual cue signal, the cell response may contain a visual component. However, the latency and duration of attentional responses are clearly different from the typical visual responses in V6A. Visual responses have short latency, small variability between trials, and a duration that matches the duration of the stimulus (see Galletti et al., 1979). Attentional responses have longer latency and higher variability (see for instance rasters of spikes in the bottom part of Fig. 3). In cases where both visual and attentional responses were present in the same cell (e.g. in the bottom insets of Fig. 7), the brief visual response (same duration as the stimulus) was sometimes observed alone (e.g. in the bottom right panel), while in other cases (e.g. in the bottom central and left panels) it was

followed by a tonic (attentional) discharge lasting hundreds of ms after the end of visual stimulation.

The activity of about 35% of V6A cells (63/182) was modulated by inward shifts of attention (inward attention epoch). The majority of the affected cells (about two-thirds) were inhibited, one-third were excited. These activity modulations were usually not spatially tuned, that is they did not vary significantly with the change in location of the cue. This was in agreement with the fact that during inward attention epoch the attention was focused on the same spatial location (the fixation point) regardless of cue location. It is worthwhile to note that contrary to outward shifts, inward shifts were endogenously driven, so they were not prompted by any visual stimulation. Therefore, cell activity during inward attention epoch can not be ascribed to a visual stimulation.

Activity modulations during outward and inward attention epochs may reflect a process representing the spatial location of the focus of attention. The spatial sensitivity of many cells is in line with this view. The excitation observed in the majority of neurons after outward attention shifts might reflect the better responsiveness at the new cued location commonly found in attentional studies. The inhibition observed in the majority of neurons when attention was directed back to the fixation point might reflect the decreasing responsiveness at the formerly cued location. Inhibition at previously cued locations is a common finding in attention research (Posner and Cohen, 1984; Klein, 2000) and an important contribution to the shaping of the 'attentional landscape'. Comparison of the population activities in the outward and inward attention cases (Figs. 4 and 8) shows that the magnitude of the modulation is higher in the inward cases. This could be because in inward cases gaze and attentional focus are aligned, or because the inward attention shift is an endogenous process whereas the outward shift is exogenously driven. It is also possible that the modulation in the outward attention cases is smaller because attention is not maintained at the outward locus long enough to reach the same level of modulation as in the inward case.

It may be argued that the responses observed during the outward and/or inward attention epochs could be related to other cognitive processes, such as the preparation of the monkey to get ready for the button release/press, or arousal, or also the expectation of a later reward. Nevertheless, we believe that, if this were the case, we would have no spatial tuning of the responses, because the arm actions are button presses occurring in a fixed spatial location. Since many cells here are spatially tuned in their attentional shifts, we believe we can rule out other interpretations of the results.

Many studies have focused on the influence of attention on neural activity in different brain areas, namely area LIP (Colby et al., 1996; Gottlieb et al., 1998; Goldberg et al., 2006; Buschman and Miller, 2007; Bisley and Goldberg, 2010; Herrington and Assad, 2010; Lui et al., 2010), superior colliculus (Ignashchenkova et al., 2004; Muller et al., 2005), frontal eye fields (Wardak et al., 2006; Buschman and Miller, 2007), area 7a (Bushnell et al., 1981; Mountcastle, 1981; Constantinidis and Steinmetz, 2001; Raffi and Siegel, 2005; Rawley and Constantinidis, 2010), area DP (Raffi and Siegel, 2005), area MT (Cook and Maunsell, 2002; Herrington and Assad, 2010), area VIP (Cook and Maunsell, 2002). While a large amount of those studies shows that spatial attention modulates the neuronal response to a stimulus (see Desimone and Duncan, 1995 and Constantinidis, 2006 for reviews), our findings provide evidence that spatial attention modulates the ongoing activity of a neuron, and this happens in an area never studied before in the attentional context. Other previous studies have demonstrated that the ongoing activity of cells in a high number of cortical areas, including V6A, is modulated by the direction of gaze (see Galletti et al., 1995; Bremmer et al., 1998). This was generally interpreted as an oculomotor effect. However, since the direction of gaze and the spotlight of attention are usually aligned, the gaze modulation could be the result of an attentional process which modulates the neuronal activity, rather than a direct oculomotor effect. By disengaging the attention from the point of fixation we have shown that this is the case for at least 30% of the neurons in area V6A (outward attentional effect). For these neurons, neural modulation was still present when covert attention was shifted without any concurrent shift of gaze direction, confirming that the modulating factor is the attentional process.

Recent brain imaging studies have shown that in the human medial superior parietal lobe there were transient activations by shifts of covert attention from one peripheral location to another (Chiu and Yantis, 2009; Esterman et al., 2009). The activation was located in the anterior bank of the dorsalmost part of the parieto-occipital sulcus, that is just in front of where area V6 is located in human (Pitzalis et al., 2006). Since in macaque, area V6A is located just in front of area V6, in the anterior bank of the parieto-occipital sulcus, we suggest that the medial superior parietal region described by Chiu and Yantis (2009) is the human counterpart of the macaque area V6A. If this were the case, we could conclude that in both macaque and human, area V6A is modulated by covert shifts of attention.

5.1 Why an attentional modulation in a reaching area?

V6A is an area that contains visual, gaze, and arm movement-related neurons (Galletti et al., 2003). Present results show that V6A neurons are also modulated by covert spatial shifts of attention, and that visual, motor, and attentional responses can co-occur in single V6A cells. We had previously demonstrated that several single V6A cells were particularly sensitive to arm movements directed towards non-foveated objects (Marzocchi et al., 2008). The covert attentional modulations could allow these cells to select the goal of reaching during movement preparation, as well as to maintain encoded, and possibly update, the spatial coordinates of the object to be reached out during movement execution.
Our results have shown a homogeneous spatial tuning of attention. This behavior parallels the homogeneous distribution of preferred gaze and reach directions observed in area V6A (Galletti et al., 1995; Fattori et al., 2005), while it is in contrast with the preferred contralateral representation of the visual field, since the distribution of visual receptive fields in V6A mainly represents the contralateral visual field (Galletti et al., 1999) (see also Fig. 5). In other words, the spatial tuning of attentional preference does not follow the sensory tuning, but rather the oculomotor and arm-reaching tuning found in V6A.
We believe that present results provide crucial support for the hypothesis that spatially-directed attention is linked to motor programming. Our study thus extends previous findings of a connection between attention and eye movement control (Moore et al., 2003; Cavanaugh and Wurtz, 2004; Ignashchenkova et al., 2004; Hamker, 2005; Thompson et al., 2005; Bisley and Goldberg, 2010) to the case of reaching control, and points towards a neural substrate for interactions between attention and reaching that are known from human behavioral data (Castiello, 1996; Deubel et al., 1998).

6- Conclusions

We suggest that area V6A is able to link perception to action in the 3D space through computation of the ocular motor activity (Hadjidimitrakis et al., 2010) and through displacing the spotlight of attention. This area of the medial parieto-occipital cortex has anatomical connections (Gamberini et al., 2009) that, together with these functional data, lead us suggest that V6A can provide to dorsal stream areas these information for producing the link across fragments by coordinating eye- and arm-actions in the 3D space.

The results here presented are the core of a manuscript coauthored by UNIBO and WWU and submitted to peer review for publication in an open-access international journal:
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